COMPETENT AUTHORITY REPORT

CHLOROPHACINONE (PT 14)

Document III-A
Active Substance

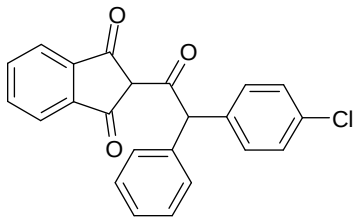
Rapporteur Member State: Spain
July 2008

INDEX

Section A1 Applicant

Annex Point IIA1

1.1	**Applicant**	Name: Liphatech S.A.S Address: Bonnel BP 3, 47480 Pont du Casse, France (Dr Mikaëline Billeret). Telephone: 00 33 5 53 69 XX XX Fax number: 00 33 5 53 69 XX XX E-mail address: : xxxxxxxx@xxxxxxxxxxxx
1.2	**Manufacturer of Active Substance** (if different)	Name: xxxxxxxxxx Address: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx Telephone: xxxxxxxxxxxxxxxxx Fax number: xxxxxxxxxxxxxxxxxxx e-mail address: xxxxxxxxxxxxxxxxxx Location of manufacturing plant: xxxxxxxxx, xxxxxxxx (address as above).
1.3	**Manufacturer of Product(s)** (if different)	See appropriate Section B1 for details of the manufacturer of the biocidal products.

Section A2 Subsection (Annex Point)		Identity of active substance	
			Official use only
2.1	**Common name**	Chlorophacinone	
2.2	**Chemical name**	2-[(4-chlorophenyl)phenylacetyl]-1H-indane-1,3-(2H)-dione	**X1**
2.3	**Manufacturer´s development code number(s)**	LM 91	
2.4	**CAS No and EC numbers**		
2.4.1	**CAS-No**	3691-35-8	
2.4.2	**EC-No**	223-003-0	
2.4.3	**Other**	CIPAC No. 208	
2.5	**Molecular and structural formula, molecular mass**		
2.5.1	**Molecular formula**	$C_{23}H_{15}ClO_3$	
2.5.2	**Structural formula**		
2.5.3	**Molecular mass**	374.82	
2.6	**Method of manufacture of the active substance**	The method of manufacture is confidential to LiphaTech S.A.S. and is presented in the confidential attachment.	
2.7	**Specification of the purity of the active substance, as appropriate**	The main quantitative method for the determination of chlorophacinone in technical chlorophacinone is a titration. This gives the total chlorophacinone plus related organic impurities (those with an acidic hydrogen). The chlorophacinone content is calculated by subtracting the content of the the related impurities (determined by HPLC) from the total value.	**X2**

	g/kg	g/l	% w/w	% v/v
Specification	-	-	>97.8	-
Analytical results			98.8 99.3 99.9 99.2 99.5	

Section A2 Subsection (Annex Point)		Identity of active substance	
		Reference: IIA2.7/01, Schmit, 2003	
2.8	**Identity of impurities and additives, as appropriate**	The identity of impurities and additives is confidential to LiphaTech S.A.S. and is presented in the confidential attachment.	
2.8.1	**Isomeric composition**	Chlorophacinone contains one optically active carbon and therefore exists as two enantiomers. As there is only one optically active carbon there are no diastereomers as is the case for certain other active substances. The ratio of the enantiomers in the active substance is confidential information and is provided in the Confidential Information file.	
2.9	**The origin of the natural active substance or the precursor(s) of the active substance**	Not relevant.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and methods	The applicant's version is adopted. However, there is a mistake in subsection 2.2. (**X1**) This is the CA nomenclature not the IUPAC nomenclature. The correct IUPAC nomenclature is 2-[2-(4-chlorophenyl)-2-phenylacetyl]indan-1,3-dione.
Results and discussion	The applicant's version is adopted
Conclusion	The applicant's version is adopted
Reliability	*Subsection 2.7:* Reliability indicator 1 *The rest of the subsections:* Reliability indicator 0: Not applicable since no studies were performed for these subsections.
Acceptability	Acceptable
Remarks	**X2**: There is an editorial mistake in Section 2.7. The sentence is "This gives the total *bromadiolone* plus related organic…". Bromadiolone must be corrected to chlorophacinone.

Section A2.10 **Annex Point IIA II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p.1) amending Council Directive 67/548/EEC**	
2.10.1 Human exposure towards active substance	--	**Official use only**
2.10.1.1 Production	--	
i) Description of process	The active ingredient is supplied by XXXXXXXXX plant located at XXXXXXXXXXX. A pre-mix of the active substance is prepared by a fully automated process at the XXXXXX plant. The pre-mix consists of: Active substance + XXXXXXXXXXX + Bittering agent. Two types of pre-mix can be prepared: (1) XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX. (2) XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX. The final end-use products (LOGINET SOLIDE and CAID APPATS are non-dusty (see Sections III B1 2.8 and III B2 2.8). LOGINET SOLIDE blocks and CAID APPATS grains are supplied loose and in protective sachets made of LDPE. Packing of the final end used product is automatic for LOGINET SOLIDE blocks and CAID APPATS grains.	
ii) Workplace description	Chlorophacinone is manufactured in the EU at XXXXXXX plant near XXXXXXXXXXX. The manufacturing plant is ISO compliant and Government Approved (a certificate is available). The premix of active substance are manufactured by Liphatech S.A.S plant at Pont du Casse (France). The manufacturing plant is ISO compliant and Government Approved (a certificate is available). Health surveillance and monitoring of all Liphatech personnel is carried out. The health surveillance including blood analysis is done according to the level of exposure for all the personnel involved in the preparation (handling of the actives, the pre-mixes), in the control (laboratory), in administrative tasks in offices located in the plant, or in administrative task out of the plant but having access to the plant (e.g. Liphatech Manager, Marketing and Sells personnel, regulatory affairs etc.). No food or drink are permitted in the factory.	
iii) Inhalation exposure	Handling of the active substances when preparing pre-mixes takes place under strict control procedures, by a restricted number of personnel (2 persons for pure active ingredient; less than 10 persons for pre-mixes). At the end of a production batch and during maintenance, all personnel use full personal protective equipment including closed breathing apparatus and full skin protection in accordance with industrial legislation. No food or drink are permitted in the factory. During packing of the end-used products all personnel (25 persons) use full personal protective equipment (including gloves) in accordance with industrial legislation. Exposure of manufacturing workers is governed by industrial legislation and controlled by the use of automated processes. The active substance is rigorously contained by technical means and exposure of manufacturing workers is prevented. The risk of inhalation exposure of production workers is considered to	

Section A2.10 Annex Point IIA II.2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p.1) amending Council Directive 67/548/EEC	
	be low.	
iv) Dermal exposure	Handling of the active substances when preparing pre-mixes takes place under strict control procedures, by a restricted number of personnel (2 persons for pure active ingredient; less that 10 persons for pre-mixes).. At the end of a production batch and during maintenance, all personnel use full personal protective equipment including closed breathing apparatus and full skin protection in accordance with industrial legislation. No food or drink are permitted in the factory. During packing of the end-used products all personnel (25 persons) use full personal protective equipment (including gloves) in accordance with industrial legislation. Exposure of manufacturing workers is governed by industrial legislation and controlled by the use of automated processes. The active substance is rigorously contained by technical means and exposure of manufacturing workers is prevented. The risk of dermal exposure of production workers is considered to be low.	
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2.10.1.2 Intended use(s)	--	
1. Professional users	--	
i) Description of application process	Professional users (e.g. from private companies and local authorities) are trained operators who handle all product types on a daily basis. They can be expected to wear protective clothing (gloves) when handling all products containing chlorophacinone. After use of most products, unused product is likely to be collected and disposed of in a controlled way except when used in sewers where difficulties of access mean that used product is likely to be left after application.	
ii) Workplace description	Products containing chlorophacinone are used in sewers, in and around buildings, in open areas and in waste dumps. Products are generally used in secured bait points to prevent pets, non-target animals and children from reaching the bait.	
iii) Inhalation exposure	Professional users may be potentially exposed by inhalation when handling products, though chlorophacinone is not volatile and formulated as wax blocks, pellets or paste and so the risk of inhalation exposure is low. The products are non-dusty.	
iv) Dermal exposure	Professional users may be potentially exposed by skin contact when applying products or collecting and disposing of uneaten product.	
2. Non-professional users including the general public	--	
(i) via inhalational contact	All products containing chlorophacinone are supplied loose or in protective sachets. Products are generally used at secured bait points to prevent access by pets or children. Exposure of non-users could occur to baits or to bait from sachets damaged by rodent feeding, but this is expected to be negligible.	
(ii) via skin contact	All products containing chlorophacinone are supplied loose or in protective sachets. Products are generally used in secured bait points to prevent access by pets or children. Exposure of non-users could occur to baits or to bait from sachets are damaged by rodent feeding, but this is expected to be negligible.	
(iii) via drinking water	Products containing chlorophacinone are not expected to come into direct contact with drinking water.	
(iv) via food	Products containing chlorophacinone are not expected to come into direct contact with food.	

Section A2.10 Annex Point IIA II.2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p.1) amending Council Directive 67/548/EEC	
(v) indirect via environment	All products containing chlorophacinone are supplied loose or in protective sachets. Products are generally used at secured bait points to prevent access by pets or children. Exposure of non-users could occur, but this is expected to be negligible.	
2.10.2 Environmental exposure towards active substance	--	
2.10.2.1 Production	--	
(i) Releases into water	No significant releases are made to water.	
(ii) Releases into air	No significant releases are made to air.	
(iii) Waste disposal	No significant releases are made to the environment following safe waste disposal, and waste disposal is governed by industrial legislation.	
2.10.2.2 Intended use(s)	--	
Affected compartment(s):	The potential compartments affected by the use of the products containing chlorophacinone are discussed in more detail in Documents II-B1, B2 and B3.	
water	It is assumed that water will not be affected.	
sediment	It is assumed that sediment will not be affected.	
air	It is assumed that air will not be affected.	
soil	It is assumed that soil will potentially be affected.	
Predicted concentration in the affected compartment(s)	The potential Predicted Environmental Concentrations (PEC's) of in the various compartments following us --	
water	See Document II-B1, B2 and B3 Section 3.3.2.	
sediment	See Document II-B1, B2 and B3 Section 3.3.2.	
air	See Document II-B1, B2 and B3 Section 3.3.3.	
soil	See Document II-B1, B2 and B3 Section 3.3.4.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and methods	The applicant's version is acceptable
Conclusion	The applicant's version is adopted
Reliability	
Acceptability	Acceptable
Remarks	No further remarks

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density								
3.1.1 Melting point								
Melting point 1	OECD 102 (≡ EEC A.1)	Batch XXXXX, purity XXX%	**141-142°C pressure: atmospheric**	Capillary tube method	Y	1	Xxxxxxxx, XXXXx	
Melting point 2	OECD 102 (≡ EEC A.1)	Batch XXXX, purity XX.XX%	**143.0°C pressure: atmospheric**	Capillary tube method	Y	1	Xxxxxx and Xxxxxx,XXXXx	
3.1.2 Boiling point	EEC A.2 (OECD 103)	Batch XXXX, purity XX.XX%	**Melted at 140°C followed by decomposition without boiling that started at 250ºC. pressure: atmospheric, air.**	Differential scanning calorimetry and capillary tube test	Y	1	Xxxxxxxx, XXXX	**X0**
3.1.3 Bulk density/ density								
density	OECD 109/CIPAC MT 3 (≡ EEC A.3)	Batch XXXX, purity XX.XX%	**Density = 1.4301 ± 0.01385 g/mL conducted at 20 °C**	-	Y	1	Xxxxxxxxxx, XXXXx	
Bulk density	Reference method not stated but procedure described is consistent with CIPAC MT 33	Batch XXXX, purity XX.XX%	**Bulk density = 0.35 g/mL conducted at ambient room temperature**	-	Y	1	Xxxxxx and Xxxxxx,XXXXx	
3.2 Vapour pressure	OECD 104 (≡ EEC A.4)	Batch XXXXX, purity XXX%	**Vapour pressure = 4.76 x 10^{-4} Pa temperature: 22.8°C**	Gas saturation method	Y	1	Xxxxxxxx, XXXXx	**X1**
3.2.1 Henry´s Law Constant	Calculation	-	**Measured/calculated: result: 0.013725 Pa.m^3.mol^{-1} Log H: -1.86**	Calculated from vapour pressure of 4.76 x 10^{-4} Pa and water solubility of 13.0 mg/L.	N	1	Xxxx, XXXX	**X2**

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3 Appearance								
3.3.1 Physical state	Visual	Batch XXXXXXX, purity XX.XX%	**Powder**	-	Y	1	Xxxxxxxx, XXXXx	
3.3.2 Colour	Visual (Munsell colour system)	Batch XXXXXXX, purity XX.XX %	**Pale yellow 5Y (9/6)**	-	Y	1	Xxxxxxxx, XXXXx	
3.3.3 Odour	Olfactory - ASTM D1292-80	Batch XXXXXXX, purity XX.XX %	**Odourless**	-	Y	1	Xxxxxxxx, XXXXx	
3.4 Absorption spectra								
UV/VIS	-	Not stated	**All spectra are consistent with the structure of the active substance**	-	N	2	Xxxxxx, XXXX	**X3**
IR	-	Batch XXXXXXX purity not stated		-	N	2	Xxxxxx, XXXX	
NMR	Proton and 13C NMR	Batch XXXXXXX purity not stated		-	N	2	Xxxxxx, XXXX	
MS	HPLC- APCI MS	Batch XXXXXXX purity not stated		-	N	2	Xxxxxx, XXXX	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.5 Solubility in water								
Water solubility 1	OECD 105 (≡EEC A.6)	Batch XXXX, purity XX.XX%	**Water: 13 µg/mL** **pH 4: 1 µg/mL** **pH 7: 344 µg/mL** **pH 10: 459 µg/mL** **temperature: 20°C**	Column elution Column elution Column elution Column elution Test substance shown to be unstable at pH4. Analysis by HPLC.	Y	1	Xxxxxx and Xxxxxxx, XXXXx	**X4**
Water solubility 2	OECD 105 (≡EEC A.6)	Batch XXXXX, purity XXX%	**3.43 µg/mL** **temperature: 25°C** **pH: not stated**	Shake flask method conducted with purified water with no pH control. Analysis by UV spectroscopy.	Y	2	Xxxxxxx, XXXXx	**X5**
3.6 Dissociation constant (-)	OECD 112	Batch XXXX, purity XX.XX %	**pKa = 8.0**	Report describes the results as marginal as the solubility of chlorphacinone is near the limit of applicability for pKa and a co-solvent was required. This would introduce an inaccuracy into the determination.	Y	1	Xxxxxx and Xxxxxxx, XXXXx	
3.7 Solubility in organic solvents, including the effect of temperature on solubility	EPA 40 CFR 158 Subdiv.D, 63.-8 (≡EEC A.6 and OECD 105)	Batch XXXXXXX, purity XXX%	**Hexane: 854 mg/L** **Methanol: 786 mg/L** **temperature: 25°C**	Shake flask method Shake flask method	Y	1	Xxxxxxxxx, XXXX	
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products	Not applicable because the active substance as manufactured does not include an organic solvent and is not formulated in organic solution in the biocidal product.							**X6**

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.9 Partition coefficient n-octanol/water	*including effects of pH (5-9)*							
log Pow 1	OECD 107 (≡EEC A.8)	Batch XXXXX purity XXX%	**Log P_{ow} 1.93 temperature: 23°C**	Shake flask method with no pH control.	Y	1	Loken, 1988	
log Pow 2	OECD 107 (≡ EEC A.8)	Batch XXXX, purity XX.XX%	**pH 4: Log P_{ow} 3.08 pH 7: Log P_{ow} 2.42 pH 9: Log P_{ow} 2.57 temperature: 23°C**	Shake flask method	Y	1	Kramer and Marion, 2002c	
3.10 Thermal stability, identity of relevant breakdown products	OECD 113	Batch XXXXXXXX, Purity XX.XX%	**Apparently stable up to and beyond its melting point.**	Tested using differential scanning calorimetry in an air atmosphere and by capillary tube melting point method.	Y	1	Xxxxxxxxx, XXXXx	
3.11 Flammability, including auto-flammability and identity of combustion products 1	EEC A10 (flammability of solids)	Batch XXXXXXX, Purity XX.XX%	**Not highly flammable**	-	Y	1	Xxxxxxxxx, XXXXx	
2	EEC A16 (auto-ignition)	Batch XXXXXXX, Purity XX.XX%	**Test material does not have a self ignition temperature below its melting point.**	-	Y	1	Xxxxxxxxx, XXXXx	
3.12 Flash-point	Not required for a solid active substance.							
3.13 Surface tension	EEC A5 OECD 115	Batch XXXXXXX, Purity XX.XX%	**68.9 mN/m at 20.6°C at 90% saturated solution**	This value is greater than 60 mN/m and is therefore not considered surface active.	Y	1	Xxxxxxxxx, XXXXx	
3.14 Viscosity (-)	Not applicable because the active substance is a solid.							
3.15 Explosive properties	EEC A14	-	**Not explosive**	Theoretical assessment in compliance with EEC A14.	N	1	Xxxxxxxxx, XXXXx	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.16 Oxidising properties	EEC A17	-	**Not oxidising**	Theoretical assessment in compliance with EEC A17.	N	1	Xxxxxxxxx, XXXXx	
3.17 Reactivity towards container material	Chlorophacinone has been stored in a range of containers (such as plastic bags in metallic containers and plastic containers). No interaction between the active ingredient and the container materials has been observed in the past 20 years of production. Based on results in use and examination of the chemical structure, there are considered to be no problems with reactivity of the active substance towards the container material.							

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Comment	
Evaluation of data submitted under section B3	**3.1.1. Melting point** *Melting point 1* Materials and Method : The applicant's version is adopted. The melting point was determined for a sample of the a.i. with 100 % purity. Results: The applicant's version is adopted. Reliability: 1 Acceptability: Acceptable *Melting point 2* Materials and Method : The applicant's version is adopted. The melting point was determined for a sample of the a.i. with 99.74 % purity. Results: The applicant's version is adopted. Reliability: 1 Acceptability: Acceptable **3.1.2. Boiling point** Materials and Method : The applicant's version is adopted. Results: The applicant's version is adopted. (**X0**): The test substance started to decompose at 250ºC Reliability: 1 Acceptability: Acceptable **3.1.3. Relative density/bulk density** - *Density* Materials and Method : The applicant's version is adopted. The method used for the determination of the density was the pycnometer method. This information was included in the test report included in Doc IVA but not in the corresponding section of Doc IIIA. Results: The applicant's version is adopted. Reliability: 1 Acceptability: Acceptable - *Bulk density* Materials and Method : The applicant's version is adopted. Results: The applicant's version is adopted. Reliability: 1 Acceptability: Acceptable **3.2. Vapour pressure** Materials and Method : (**X1**): The vapour pressure must be studied at two temperatures or a vapour pressure curve must be determined. The applicant has determined the vapour pressure at one temperature (22.4 ºC). Results: The applicant should have determined this parameter at least at two temperatures. Reliability: 2; The applicant has presented the vapour pressure for one temperature. Acceptability: Acceptable with objections **3.2.1. Henry's Law Constant** Materials and Method : (**X2**) Henry's law Constant was calculated using experimental data of the vapour pressure (determined at 23 ºC) and the water solubility (determined at 20 ºC). The applicant should have carried out this calculation using experimental data obtained at the same temperature.

Results: May be acceptable
Reliability: 1
Acceptability: Acceptable with objections

3.3. Appearance

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.4. Absorption spectra, and mass spectrum

(**X3**) The applicant should have indicated the purity of the test substance as indicated in the Guidance on Data Requirements and should have performed the spectra in compliance with GLP.

Materials and Method : The applicant's version is adopted.
Results: The applicant should have included in the test report a summary of the data obtained from the different spectra.
Reliability: 2. The applicant should have indicated the purity of the test sample and followed the GLP protocol.
Acceptability: Acceptable

3.5. Water solubility

Water solubility 1

Materials and Method : The applicant's version is adopted.
Results: The results are acceptable. (**X4**): In Table 4 of this test report it is indicated that the solubility of chlorophacinone at pH 10 is 0.476 g/L however in the summary it is stated that the solubility at this pH is 0.459 g/L. The correct value for the water solubility at pH 10 is 0.476 g/L <> 476 µg/mL
Reliability: 1
Acceptability: Acceptable

Water solubility 2

Materials and Method : The applicant's version is adopted.
Results: The results are acceptable however, (**X5**) the applicant should have included the pH of each sample.
Reliability: 2
Acceptability: Acceptable

3.6. Dissociation constant

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.7. Solubility in organic solvents

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.8. Stability in organic solvents used in b.p.

(X6): The non-submission of data was considered acceptable because the formulation is a solid with a low proportion (1.6%) of organic solvent

3.9 Partition coefficient

Log Pow 1

Materials and Method : The applicant's version is adopted.

Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

Log Pow 2

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.10 Thermal stability

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.11.1. Flammability

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.11.2. Self-Ignition Temperature

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.11. Flammability, including auto-flammability and identity of combustion products

Tests EC A.12 and A.13 were not requested to the applicant because the experience in use indicated that negative results would be obtained.

3.12. Flash point

This property is not required for solid active substances therefore, the non submission of data is justified.

3.13 Surface tension

Materials and Method : The applicant's version is adopted.
Results: The applicant's version is adopted.
Reliability: 1
Acceptability: Acceptable

3.14. Viscosity

This property is not required for solid active substances therefore, the non submission of data is justified.

3.15. Explosive properties

Materials and Method : The applicant's version is adopted.
Results: No experimental determination was performed since the UN Recommendation criteria were applied and the active substance can be considered as non explosive.
Reliability: 1
Acceptability Acceptable

3.16. Oxidizing properties

Materials and Method : The applicant's version is adopted.

Results: No experimental determination was performed since the UN Recommendation criteria was applied and the active substance can be considered as non oxidizing.
Reliability: 1
Acceptability Acceptable

3.17. Reactivity towards the container

The applicant has not presented any experimental data for the reactivity towards container material. The applicant's justification is that chlorophacinone has been stored in a range of containers (such as plastic bags in metallic containers and plastic containers). No interaction between the active ingredient and the container materials has been observed in the past 20 years of production. Based on results in use and examination of the chemical structure, there are considered to be no problems with reactivity of the active substance towards the container material.

Section A4.1/01	**Analytical Methods for Detection and Identification**
Annex Point IIA, IV.4.1	**Note: Details of the analytical methods for determination of impurities and active substance in the technical grade active substance are confidential to LiphaTech S.A.S. and are presented in the confidential attachment.**

			Official use only
		1 REFERENCE	
1.1	**Reference**	-	
1.2	**Data protection**	-	
1.2.1	Data owner	-	
Companies with letter of access		-	
1.2.2	Criteria for data protection	-	
		GUIDELINES AND QUALITY ASSURANCE	
Guideline study		-	
GLP		-	
Deviations		-	
		2 MATERIALS AND METHODS	
2.1	**Preliminary treatment**	-	
2.1.1	Extraction	-	
2.1.2	Cleanup	-	
2.2	**Detection**	-	
2.2.1	Separation method	-	
2.2.2	Detector	-	
2.2.3	Standard(s)	-	
2.2.4	Interfering substance(s)	-	
2.3	**Linearity**	-	
2.3.1	Calibration range	-	
2.3.2	Number of measurements	-	
2.3.3	Linearity	-	
2.4	**Specifity: interfering substances**	-	
2.5	**Recovery rates at different levels**	-	
2.5.1	Relative standard deviation	-	

Section A4.1/01	**Analytical Methods for Detection and Identification**
Annex Point IIA, IV.4.1	**Note: Details of the analytical methods for determination of impurities and active substance in the technical grade active substance are confidential to LiphaTech S.A.S. and are presented in the confidential attachment.**

2.6	**Limit of determination**	-
2.7	**Precision**	-
2.7.1	Repeatability	-
2.7.2	Independent laboratory validation	-
	3 APPLICANT'S SUMMARY AND CONCLUSION	
3.1	**Materials and methods**	-
3.2	**Conclusion**	-
3.2.1	Reliability	-
3.2.2	Deficiencies	-

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and methods	
Conclusion	
Reliability	
Acceptability	Acceptable
Remarks	A complete description of the validated method is given in the Confidential Information and is an acceptable method.

Section A4.2(a) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(a)/01

Chlorophacinone residues in soil

			Official use only
		REFERENCE	
3.1	**Reference**	Xxxx, X. (XXXXx). Development and validation of the residue analytical method for chlorophacinone in soil. XXXXXXXX, unpublished report number XXXXXX, XX Xxxxxxxx XXXX.	
3.2	**Data protection**	Yes.	
3.2.1	Data owner	LiphaTech SAS.	
3.2.2	Companies with letter of access	None.	
3.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		4 GUIDELINES AND QUALITY ASSURANCE	
4.1	**Guideline study**	Yes. SANCO/825/00.	
4.2	**GLP**	Yes.	
4.3	**Deviations**	No.	
		5 MATERIALS AND METHODS	
5.1	**Preliminary treatment**		
5.1.1	Extraction	Soil is shaken with aqueous methanol. The extract is filtered and diluted with water prior to determination.	
5.1.2	Cleanup	None.	
5.2	**Detection**		
5.2.1	Separation method	Reverse-phase HPLC, Luna C-8 column with acetonitrile/water/ ammonium acetate (gradient) mobile phase.	
5.2.2	Detector	Dual mass spectrometer (MS/MS). Ions monitored 373.4/201.2 m/z.	
5.2.3	Standard(s)	External standard.	
5.2.4	Interfering substance(s)	There are no known substances which would interfere with the detection of chlorophacinone using this method.	
5.3	**Linearity**		
5.3.1	Calibration range	0.001 to 0.10 µg/ml.	
5.3.2	Number of measurements	Seven.	
5.3.3	Linearity	Typical $r^2 = 0.9939$.	

Section A4.2(a) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(a)/01

Chlorophacinone residues in soil

5.4 Specifity: interfering substances

There are no substances which would interfere with the detection of chlorophacinone. The method is considered to be specific.

5.5 Recovery rates at different levels

Recovery from fortified soil samples was as follows:

Matrix	Fortification level (mg/kg)	Recovery (%)		
		range	mean	n
soil	0.01	96 - 102	98	5
	0.10	85 - 96	90	5
	overall	85 - 102	94	10

5.5.1 Relative standard deviation

RSD values based on recovery tests were as follows:

Matrix	Fortification level (mg/kg)	RSD (%)	Overall RSD (%)
soil	0.01	2.6	5.4
	0.10	3.3	

5.6 Limit of determination

The limit of determination is 0.01 mg/kg (defined as the lowest concentration at which acceptable recovery has been demonstrated).

5.7 Precision

5.7.1 Repeatability

RSD values are presented above under 3.5.1.

5.7.2 Independent laboratory validation

This data requirement is not applicable to methods for determination of residues in soil.

6 APPLICANT'S SUMMARY AND CONCLUSION

6.1 Materials and methods

Soil is extracted by shaking with aqueous methanol. Determination of the filtered and diluted extract is by reverse-phase LC-MS/MS (monitored ions 373.4/201.2 m/z). A Luna C-8 column is used with acetonitrile/water/ammonium acetate (gradient) mobile phase.

6.2 Conclusion

The method for determination of residues of chlorophacinone in soil has been adequately validated. The method was successfully evaluated and meets the EU criteria with respect to specificity, linearity, accuracy and precision according to the guidance given in SANCO/825/00. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the method is suitable for enforcement purposes.

6.2.1 Reliability

1

6.2.2 Deficiencies

No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date August 2005

Materials and methods The applicant's version is acceptable

Section A4.2(a) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(a)/01

Chlorophacinone residues in soil

Conclusion	The applicant's version is adopted
Reliability	1
Acceptability	The study is considered to be acceptable.
Remarks	The applicant should have explained why matrix matched standards were used since it is stated in 3.2.4. That there are no known substances which would interfere with the detection of chlorophacinone.

Section A4.2(b) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(b)/01

Chlorophacinone residues in air

			Official use only
		1 REFERENCE	
1.1	**Reference**	Xxxx, X. (XXXXx). Development and validation of a residue analytical method for chlorophacinone in air. XXXXXXX., unpublished report number XXXXXX, XX Xxxxxxxxx XXXX.	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	LiphaTech SAS.	
1.2.2	Companies with letter of access	None.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. SANCO/825/00.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Extraction	Air is passed through Tenax absorption tubes. The tubes are eluted with acetonitrile.	
3.1.2	Cleanup	None.	
3.2	**Detection**		
3.2.1	Separation method	Reverse-phase HPLC, Luna C-8 column with acetonitrile/water/ ammonium acetate (gradient) mobile phase.	
3.2.2	Detector	Dual mass spectrometer (MS/MS). Ions monitored 373.4/201.2 m/z.	
3.2.3	Standard(s)	External standard.	
3.2.4	Interfering substance(s)	There are no known substances which would interfere with the detection of chlorophacinone using this method.	
3.3	**Linearity**		
3.3.1	Calibration range	0.0005 to 0.05 µg/ml.	
3.3.2	Number of measurements	Seven.	
3.3.3	Linearity	Typical $r^2 = 0.9968$.	

Section A4.2(b) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(b)/01

Chlorophacinone residues in air

3.4 Specifity: interfering substances

There are no substances which would interfere with the detection of chlorophacinone. The method is considered to be specific.

3.5 Recovery rates at different levels

Recovery from fortified absorption tubes was as follows:

Matrix	Temp/RH (°C/%)	Fortification level (µg/m³)	Recovery (%)		
			range	mean	n
air	21.5/45	0.03	74 - 99	85	5
		0.30	75 - 100	91	5
		overall	74 - 100	88	10
	36/85	0.03	71 - 97	83	5
		0.30	75 - 96	84	5
		overall	71 - 97	83	10

3.5.1 Relative standard deviation

RSD values based on recovery tests were as follows:

Matrix	Temp/RH (°C/%)	Fortification level (µg/m³)	RSD (%)	Overall RSD (%)
air	21.5/45	0.03	12.2	11.3
		0.30	10.8	
	36/85	0.03	11.8	10.1
		0.30	9.3	

3.6 Limit of determination

The limit of determination is 0.03 µg/m³ (defined as the lowest concentration at which acceptable recovery has been demonstrated).

3.7 Precision

3.7.1 Repeatability

RSD values are presented above under 3.5.1.

3.7.2 Independent laboratory validation

This data requirement is not applicable to methods for determination of residues in air.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Air is passed through Tenax absorption tubes which are eluted with acetonitrile. Determination is by reverse-phase HPLC, Luna C-8 column with acetonitrile/water/ ammonium acetate (gradient) mobile phase.

4.2 Conclusion

The method for determination of residues of chlorophacinone in air has been adequately validated. The method was successfully evaluated and meets the EU criteria with respect to specificity, linearity, accuracy and precision according to the guidance given in SANCO/825/00. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the method is suitable for enforcement purposes.

4.2.1 Reliability

1

4.2.2 Deficiencies

No

Section A4.2(b) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(b)/01

Chlorophacinone residues in air

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and methods	The applicant's version is acceptable
Conclusion	The applicant's version is adopted
Reliability	1
Acceptability	The study is considered to be acceptable.
Remarks	No further remarks

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(c)/01

Chlorophacinone residues in water

			Official use only
		1 REFERENCE	
1.1	**Reference**	Xxxx, X (XXXXx). Development and validation of the residue analytical method for chlorophacinone in drinking and surface water. XXXXXXX, unpublished report number XXXXXX, XX Xxxxxxxx XXXX.	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	LiphaTech SAS.	
1.2.2	Companies with letter of access	None.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. SANCO/825/00.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Extraction	Water is partitioned three times with dichloromethane. The organic extract is evaporated to dryness and reconstituted in methanol and water prior to determination.	
3.1.2	Cleanup	None.	
3.2	**Detection**		
3.2.1	Separation method	Reverse-phase HPLC, Luna C-8 column with acetonitrile/water/ ammonium acetate (gradient) mobile phase.	
3.2.2	Detector	Dual mass spectrometer (MS/MS). Ions monitored 373.4/201.2 m/z.	
3.2.3	Standard(s)	External standard.	
3.2.4	Interfering substance(s)	There are no known substances which would interfere with the detection of chlorophacinone using this method.	
3.3	**Linearity**		
3.3.1	Calibration range	0.001 to 0.10 µg/ml.	
3.3.2	Number of measurements	Five.	
3.3.3	Linearity	Typical $r^2 = 0.9960$.	

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(c)/01

Chlorophacinone residues in water

3.4 Specifity: interfering substances

There are no substances which would interfere with the detection of chlorophacinone. The method is considered to be specific.

3.5 Recovery rates at different levels

Recovery from fortified water samples was as follows:

Matrix	Fortification level (µg/L)	Recovery (%)		
		range	mean	n
drinking water	0.05	79 - 92	87	5
	0.50	101 - 107	106	5
	overall	79 - 107	96	10
surface water	0.05	71 - 92	81	5
	0.50	87 - 103	94	5
	overall	71 - 103	87	10

3.5.1 Relative standard deviation

RSD values based on recovery tests were as follows:

Matrix	Fortification level (µg/L)	RSD (%)	Overall RSD (%)
drinking water	0.05	6.2	11.2
	0.50	2.4	
surface water	0.05	9.8	10.9
	0.50	7.0	

3.6 Limit of determination

The limit of determination is 0.05 µg/L (defined as the lowest concentration at which acceptable recovery has been demonstrated).

3.7 Precision

3.7.1 Repeatability

RSD values are presented above under 3.5.1.

3.7.2 Independent laboratory validation

This data requirement is not applicable to methods for determination of residues in water.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Water is extracted by partition into dichloromethane. The extract is evaporated to dryness and reconstituted in aqueous methanol. Determination is by reverse-phase LC-MS/MS (monitored ions 373.4/201.2 m/z). A Luna C-8 column is used with acetonitrile/water/ammonium acetate (gradient) mobile phase.

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(c)/01

Chlorophacinone residues in water

4.2	**Conclusion**	The method for determination of residues of chlorophacinone in water has been adequately validated. The method was successfully evaluated and meets the EU criteria with respect to specificity, linearity, accuracy and precision according to the guidance given in SANCO/825/00. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the method is suitable for enforcement purposes. Acceptable validation data have been generated for determination of chlorophacinone residues in drinking and surface waters. Therefore, it is considered that the method will also be directly applicable to groundwater.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	August 2005
Materials and methods	The applicant's version is acceptable
Conclusion	The applicant's version is adopted
Reliability	1
Acceptability	The study is considered to be acceptable.
Remarks	No further remarks.

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(d)/01

Chlorophacinone residues in blood

			Official use only
		1 REFERENCE	
1.1	**Reference**	Xxxxx, X. (XXXXx). Validation of analytical methodology to determine bromadiolone, chlorophacinone and difethialone in blood. Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx, unpublished report number XXXXXXX, XX Xxxxxxxx XXXX.	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	LiphaTech SAS.	
1.2.2	Companies with letter of access	None.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	SANCO/825/00.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Extraction	Blood is diluted with methanol. Phosphate buffer, a mixture of ethanol/ethyl acetate and trichloroacetic acid solution is added. The sample is shaken and the organic phase removed. The sample is re-extracted with ethanol/ethyl acetate. The combined organic extracts are evaporated to dryness and reconstituted in methanol prior to determination.	
3.1.2	Cleanup	No additional cleanup is required.	
3.2	**Detection**		
3.2.1	Separation method	HPLC, a Thermo Hypersil Keystone column with ammonium acetate/methanol (gradient) mobile phase.	
3.2.2	Detector	MS-MS (two ion transitions monitored 373>201 and 375>203)	
3.2.3	Standard(s)	External standard.	
3.2.4	Interfering substance(s)	There are no known substances which would interfere with the detection of chlorophacinone.	
3.3	**Linearity**		
3.3.1	Calibration range	0.015 to 0.60 µg/mL.	
3.3.2	Number of measurements	Four.	
3.3.3	Linearity	$R^2 = 0.985$.	

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(d)/01

Chlorophacinone residues in blood

3.4 Specifity: interfering substances

There are no substances which would interfere with the detection of chlorophacinone. The use of LC/MS-MS is considered to be highly specific so alternative chromatographic conditions are not required.

3.5 Recovery rates at different levels

Recovery from fortified blood was as follows:

Matrix	Fortification level (mg/L)	Recovery (%)		
		range	mean	n
Blood	0.05	71 - 82	76	5
	0.50	69 - 81	76	5
	overall	69 - 82	76	10

3.5.1 Relative standard deviation

RSD values were as follows:

Matrix	Fortification level (mg/kg)	RSD (%)	Overall RSD (%)
Blood	0.05	6.8	6.4
	0.50	6.7	

3.6 Limit of determination

The limit of determination is 0.05 mg/L (defined as the lowest concentration at which acceptable recovery has been demonstrated).

3.7 Precision

3.7.1 Repeatability

RSD values are presented above under 3.5.1.

3.7.2 Independent laboratory validation

This data requirement is not applicable to methods for determination of residues in blood.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Blood is diluted with methanol. Phosphate buffer, a mixture of ethanol/ethyl acetate and trichloroacetic acid solution is added. Thje sample is shaken and the organic phase removed. The sample is re-extracted with ethanol/ethyl acetate. The combined organic extracts are evaporated to dryness and reconstituted in methanol prior to determination. Determination is by HPLC with a Thermo Hypersil Keystone column and ammonium acetate/methanol (gradient) mobile phase (two ion transitions monitored 373>201 and 375>203).

4.2 Conclusion

The method for determination of residues of chlorophacinone in blood has been adequately validated. The method was successfully evaluated and meets the EU criteria with respect to specificity, linearity, accuracy and precision according to the guidance given in SANCO/825/00. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the method is suitable for enforcement purposes. This conclusion is confirmed in the report by the performing laboratory - which is also the UK monitoring laboratory.

4.2.1 Reliability

1

4.2.2 Deficiencies

No.

Section A4.2(c) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(d)/01

Chlorophacinone residues in blood

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and methods	The applicant's version is acceptable
Conclusion	The applicant's version is adopted
Reliability	1
Acceptability	The study is considered to be acceptable.
Remarks	Matrix matched calibration standards were used for the determination of chlorophacinone in blood samples.

Section A4.2(d) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(d)/02

Chlorophacinone residues in liver

			Official use only
		1 REFERENCE	
1.1	**Reference**	Xxxxx, X. (XXXXx). Validation of analytical methodology to determine bromadiolone, chlorophacinone and difethialone in blood. Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx, unpublished report number XXXXXXX, XX Xxxxxxxx XXXX.	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	LiphaTech SAS.	
1.2.2	Companies with letter of access	None.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	SANCO/825/00.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Extraction	Liver is blended with phosphate buffer (pH 5.5) and a mixture of ethanol and ethyl acetate (1+19, v/v). A solution of trichloroacetic acid is added and the sample is blended again.	
3.1.2	Cleanup	The centrifuged extract is cleaned-up by gel permeation chromatography.	
3.2	**Detection**		
3.2.1	Separation method	HPLC, Thermo hypersil keystone column with ammonium acetate/methanol (gradient) mobile phase.	
3.2.2	Detector	MS-MS (two ion transitions monitored 373>201 and 375>203).	
3.2.3	Standard(s)	External standard.	
3.2.4	Interfering substance(s)	There are no known substances which would interfere with the detection of chlorophacinone.	
3.3	**Linearity**		
3.3.1	Calibration range	0.03 to 1.2 µg/mL.	
3.3.2	Number of measurements	Four.	
3.3.3	Linearity	$R^2 = 0.9903$.	

Section A4.2(d) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(d)/02

Chlorophacinone residues in liver

3.4 Specifity: interfering substances

There are no substances which would interfere with the detection of chlorophacinone. The use of LC/MS-MS is considered to be highly specific so alternative chromatographic conditions are not required.

3.5 Recovery rates at different levels

Recovery from fortified liver was as follows:

Matrix	Fortification level (mg/kg)	Recovery (%)		
		range	mean	n
Liver	0.05	57 - 106	70	5
	0.50	76 - 126	95	5
	overall	57 - 126	82	10

3.5.1 Relative standard deviation

RSD values were as follows:

Matrix	Fortification level (mg/kg)	RSD (%)	Overall RSD (%)
Liver	0.05	29.7	27.7
	0.50	19.8	

3.6 Limit of determination

The limit of determination is 0.05 mg/L (defined as the lowest concentration at which acceptable recovery has been demonstrated).

3.7 Precision

3.7.1 Repeatability

RSD values are presented above under 3.5.1.

3.7.2 Independent laboratory validation

This data requirement is not applicable to methods for determination of residues in liver.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Liver is blended with phosphate buffer (pH 5.5) and a mixture of ethanol and ethyl acetate (1+19, v/v). A solution of trichloroacetic acid is added and the sample is blended again. Clean-up of the centrifuged extract is by GPC. Determination is by HPLC with Thermo hypersil keystone column and ammonium acetate/methanol (gradient) mobile phase (two ion transitions monitored 373>201 and 375>203).

4.2 Conclusion

The method for determination of residues of chlorophacinone in liver has been adequately validated. The method was successfully evaluated and meets the EU criteria with respect to specificity, linearity and accuracy according to the guidance given in SANCO/825/00. Precision falls slightly outside the generally accepted criteria (overall RSD is 27.7 %) but this is not uncommon for methods of analysis of residues in body tissues. The method requires equipment and instrumentation which is commonly available in most well-equipped laboratories. Therefore, the method is suitable for enforcement purposes. This conclusion is confirmed in the report by the performing laboratory - which is also the UK monitoring laboratory.

4.2.1 Reliability

1

4.2.2 Deficiencies

No.

Section A4.2(d) Analytical Methods for Detection and Identification

Annex Point IIA, IV.4.2(d)/02

Chlorophacinone residues in liver

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and methods	The applicant's version is acceptable
Conclusion	The applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	At the fortification level of 0.05 mg/kg, one of the recovery results could have been identified as an outlier. The applicant should have applied an appropriate method to see if this value could have been discarded, and therefore the RSD could have been in the range considered as acceptable. The recovery results obtained for this fortification level are below the acceptable range.

Section A4.3 Analytical Methods for Detection and Identification

Chlorophacinone residues in food and feedingstuff

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Remarks	The applicant considered that an analytical method for the determination of chlorophacinone was not relevant as it is not used for the treatment of food or feedingstuffs. However, this product is going to be used in places where food or feedingstuff are produced or stored. Therefore, it is necessary an analytical method for these matrices. The CEFIC Rodenticide Working Group has developed a multiresidue method for the determination of several rodenticides in food of plant and animal origin. In the case of chlorophacinone, the developed method was acceptable for some of the matrices, however the method has to be optimised , fully validated, and reported. This has to be taken into account before the inclusion of this compound in Annex I.

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)	*Rattus norvegicus* (Norway rat, Brown rat) *Mus musculus* (House mouse)	**X1**
5.2.2 Products, organisms or objects to be protected (IIA5.2)	Chlorophacinone is used for the urban and agricultural control of rodents indoors (i.e. in grain silos, warehouses), in and around farms buildings, in sewers and in open areas. It is used to protect human food and animal feedstuffs and for general hygiene purposes.	**X2**
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 organisms (IIA5.3)	Chlorophacinone is a first-generation anticoagulant rodenticide. It disrupts the normal blood clotting mechanisms resulting in increased bleeding tendency and, eventually, profuse haemorrhage and death. Effectiveness of the active substance depends on exposure (i.e. consumption of the bait by the target organism). Generally, effects can be observed using bait concentrations of 5 mg/kg or more. However, for effective and comprehensive control of rats and mice, a bait concentration of 50 mg/kg is proposed. In the case of tracking powder the target species will ingest relatively small amounts during grooming only and so a higher effective concentration of 2000 mg/kg is proposed. The formulated product type has no significant difference on the effects of the active substance on the target organisms.	
5.3.2 Likely concentrations at which the A.S. will be used (IIA5.3)	-	
PT14	The active substance is used in a range of cereal-based baits (pellets, wax blocks) at a concentration of 50 mg/kg and in tracking powder at a concentration of 2 g/kg..	

Section A5	Effectiveness against target organisms and intended uses	
5.4 Mode of action (including time delay) (IIA5.4)	-	
5.4.1 Mode of action	As with other anticoagulant rodenticides, the active substance is a vitamin K antagonist. It interferes with the regeneration of prothrombin, disturbing the normal blood clotting mechanisms and causing an increased tendency to bleed. The site of action is the liver, where several of the blood coagulation precursors undergo vitamin K dependent post translation processing before they are converted into the respective procoagulant zymogens. The point of action appears to be the inhibition of K1 epoxide reductase.	
5.4.2 Time delay	Rodents usually die within 3 to 6 days of the first consumption. Clinical symptoms may be observed around one to two days before death.	
5.5 Field of use envisaged (IIA5.5)	MG03: Pest control. Product type 14.	
5.6 User (IIA5.6)	-	
Industrial	The active substance, chlorophacinone, is used directly by manufactures to make products which are then sold to professional and non-professional users. The use by professional and non-professional users of the actual products containing the active substance is described in more detail in Sections B1 5.3 and 5.4, B2 5.3 and 5.4 and B3 5.3 and 5.4 for the products supported.	
Professional	The active substance, chlorophacinone, is not used directly by professional users. The use by professional and non-professional users of the actual products containing the active substance is described in more detail in Sections B1 5.3 and 5.4, B2 5.3 and 5.4 and B3 5.3 and 5.4 for the products supported.	
General public	The active substance, chlorophacinone, is not used directly by the general public. The use by professional and non-professional users of the actual products containing the active substance is described in more detail in Sections B1 5.3 and 5.4, B2 5.3 and 5.4 and B3 5.3 and 5.4 for the products supported.	
5.7 Information on the occurrence or possible occurrence	-	

Section A5	Effectiveness against target organisms and intended uses	
of the development of resistance and appropriate management strategies (IIA5.7)		
5.7.1 Development of resistance	To our knowledge there have been no case of resistance to chlorophacinone.	
5.7.2 Management strategies	A management strategy to minimise the likelihood of resistance to the active substance developing in the target species consists of the following three components: Firstly, in general ineffective use of anticoagulant rodenticides is often misdiagnosed as resistance. The success of a control campaign is often dependant on how the control measures are conducted in practice. It is therefore most important to select an appropriate control strategy. An effective control programme needs to consider the following aspects: • Identification of target organism and selection of an appropriate product. • Correct positioning of bait stations. • Attractiveness of bait selected/competition with abundant food sources. • Baiting for an adequate time. • Understanding of the extent and area of the infestation to ensure an adequate amount is used over a sufficient area. • Immigration from neighbouring populations. Further guidance is given in Document IV, A 5.7.2-01. Secondly, to avoid the development of resistance in susceptible rodent populations the following points should be adopted for all control programmes: • Use anticoagulant rodenticides. Ensure that all baiting points are inspected weekly and old bait replaced where necessary • Undertake treatment according to the label until the infestation is completely cleared. • On completion of the treatment remove all unused baits. • Do not use anticoagulant rodenticides as permanent baits routinely. Use permanent baits only where there is a clear and identified risk of immigration or introduction or where protection is afforded to high	

Section A5	Effectiveness against target organisms and intended uses	
	risk areas. • Monitoring of rodent activity should be undertaken using visual survey, through the use of non-toxic placebo monitors or by other effective means. • Record details of treatment. • Where rodent activity persists due to problems other than resistance, use alternate baits or baiting strategy, extend the baiting programme or apply alternate control techniques to eliminate the residual infestation (acute or sub-acute rodenticides, gassing or trapping). • Ensure that complete elimination of the infestation is achieved. • As appropriate during the rodenticide treatment apply effective Integrated Pest Management measures (remove alternate food sources, remove water sources, remove harbourage and proof susceptible areas against rodent access). Thirdly, when resistance to anticoagulants is suspected or identified, the following should be conducted: • Where rodent infestations containing resistant individuals are identified, immediately use an alternate anticoagulant of higher potency. If in doubt, seek expert advice on the local circumstances. • Alternatively use an acute or sub-acute but non anticoagulant rodenticide. • In both cases it is essential that complete elimination of the rodent population is achieved. Gassing or fumigation may be useful in specific situations. • Apply thorough Integrated Pest Management procedures (environmental hygiene, proofing and exclusion). • Do not use anticoagulant rodenticides as permanent baits as routine. Use permanent baits only where there is a clear and identified risk of immigration or introduction or where protection is afforded to high risk areas. • Record details of treatment. Where individual infestations are found to be resistant or contain resistant individuals it is possible that the resistance extends further to neighbouring properties: • Where there are indications that resistance may be more extensive than a single infestation, apply area or	

Section A5	Effectiveness against target organisms and intended uses	
	block control rodent programmes. • The area under such management should extend at least to the area of known resistance and ideally beyond. • There programmes must be effectively co-ordinated and should encompass the procedures identified above. These considerations are discussed in more detail in Document IV, A 5.7.2-02.	
5.8 Likely tonnage to be placed on the market per year (IIA5.8)	The mean total quantity of chlorophacinone active ingredient placed on the market by LiphaTech S.A.S. in the world is less than or equal to XXXXXX/year equivalent active ingredient.	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	December 2005	
Materials and methods	The applicant's version is adopted	
Conclusion	The applicant's version is adopted	
Reliability		
Acceptability	Acceptable taking into account the remarks below	
Remarks	See Effectiveness against target organisms and intended uses in Section 5 of Doc III-B1, Doc III-B2 and Doc III-B3. **X1** (Field 5.2.1): The efficacy tests reported in section 5.10 of the *Loginet Solide* is only referred to Rattus norvegicus (Doc III-B1 Section 5). The efficacy tests reported in section 5.10 of *Caïd Appats* and *Caïd Poudre Concentrée de Piste* are referred to *Rattus norvegicus* and *Mus musculus* (Doc III-B2 Section 5 and Doc III-B3 Section 5). Therefore, the reference to *Rattus rattus* has to be deleted. **X2** (Field 5.2.2): See Intended uses for each product in Document II-B1, Document II-B2 and Document II-B3.	

Section 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*
Rodenticide	MG03, PT14	Chlorophacinone active substance as given in section 2. The active substance was prepared in block baits and cereal baits.	Rats and mice *Rattus norvegicus, Mus musculus,* For laboratory tests, rodents were all laboratory bred either using wild strains. The efficacy against warfarin susceptible animals was investigated.	A total of 7 laboratory tests are reported in the product efficacy dossier. In all cases baits treated with chlorophacinone were used and animals were exposed free choice with untreated feed.	Efficacy: All baits were prepared at 50 mg/kg and made available *ad libitum* for test periods of between 4 and 5 days. Palatability/ attractivity: For a number of the efficacy tests, bait consumption was compared to untreated competition bait.	In all 7 tests, the 50 mg/kg baits were both attractive and palatable enough for test rodents to consume lethal doses. Efficacy (mortality) rates ranged from 90% to 100% in free choice tests, with deaths occurring from 4 to 17 days after the start of exposure to treated baits.	A5.3/01

* References: Refer to main reference list for full details.

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*
Rodenticide	MG03, PT14	Chlorophacinone active substance as given in section 2. The active substance was prepared in tracking powder.	Rats and mice *Rattus norvegicus*, *Mus musculus*, For laboratory tests, rodents were all laboratory bred either using wild strains. The efficacy against warfarin susceptible animals was investigated.	A total of 6 laboratory tests are reported in the product efficacy dossier. In all cases tracking powder treated with chlorophacinone was used. The powder was presented such that the test animals had to walk through it to have access to untreated food.	Efficacy: The powder was prepared at 2000 mg/kg and made available for test periods of 1 and 4 days.	In all 6 tests, enough of the 2000 mg/kg tracking powder was ingested for test rodents to consume lethal doses. Efficacy (mortality) rates ranged from 93% to 100% with deaths occurring from 4 to 21 days after the start of exposure to treated baits. All the one day exposure tests were 100% effective.	A5.3/01

* References: Refer to main reference list for full details.

Section 6: Toxicological and Metabolic Studies

INDEX

Section A 6.1.1-01 **Annex Point IIA VI 6.1.1**		**Oral toxicity** LD50 study in the rat	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxx X., Xxxxxxxxxxx X. (XXXX): LD_{50} Evaluation of Chlorophacinone in Solution in PEG 300 Orally to Rats. XXXXXXXXXXXXXXXX, XXXX, XXXXXX (Dates of experimental work: March 1, XXXX - March 22, XXXX). Unpublished report No.: Xxxxxxxxxx (Xxx XX, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA Guideline 81-1. In accordance with EC Method B.1.	
2.3	**GLP**	Yes	
2.4	**Deviations**	None identified	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone (LM 91)	
3.2.1	Lot/Batch number	XXXXX	
3.2.2	Specification	97-103%, sulphates lower than 1500 ppm	
3.2.2.1	Description	Pale yellow powder	
3.2.2.2	Purity	XXXXX%	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Sprague Dawley Rat	
3.3.2	Strain	IOPS- VAF	
3.3.3	Source	Xxxxxxxxxxxxxx, France	
3.3.4	Sex	Males and females	
3.3.5	Age/weight at study initiation	4 weeks 67-105 g for males 62-94 g for females	
3.3.6	Number of animals per group	10 males and 10 females per group	
3.3.7	Control animals	Yes	

Section A 6.1.1-01 **Annex Point IIA VI 6.1.1**		**Oral toxicity** LD50 study in the rat	
3.4	**Administration/ Exposure**	Oral	
3.4.1	Postexposure period	21 days	
3.4.2	Type	Oesophageal force-feeding - gavage single dose	
3.4.3	Concentration	Doses used: 2.0 mg/kg (lot A); 3.2 mg/kg (lot B); 5.20 mg/kg (lot C); 8.20 mg/kg (lot D); 13.20 mg/kg (lot E); 21.00 mg/kg (lot F)	
3.4.4	Vehicle	PEG 300	
3.4.5	Concentration in vehicle	Solution at 2g chlorophacinone /L PEG 300	
3.4.6	Total volume applied	Lot A - 1.0 ml/kg; Lot B - 1.6 ml/kg; Lot C - 2.6 ml/kg; Lot D - 4.1 ml/kg; Lot E - 6.6 ml/kg; Lot F - 10.5 ml/kg	
3.4.7	Controls	0 mg/kg (10.5 ml/kg vehicle) (lot T)	
3.5	**Examinations**	Clinical observations, mortality, body weight, macroscopic examination at autopsy.	

Section A 6.1.1-01 **Annex Point IIA VI 6.1.1**	**Oral toxicity** LD50 study in the rat	
3.6 Method of determination of LD_{50}	Litchfield and Wilcoxon	
	4 RESULTS AND DISCUSSION	
4.2 Clinical signs	Passive behaviour, almost lethargic in all the lots of treated animals, predominantly in males at the end of the first week; pale mucous membranes for lots B, C, D (3 animals), and E (most of the males and females); discoloured eyes in 3 males from lot D, 1 male in lot E, 2 females in lot E; bristled hair in lot C, D (4 animals), lot E (5 animals) and F- most of the male and female animals; haematomas at the head – for 1 animal in lot C and 3 animals in lot D; traces of blood at the snout and the fore limbs (1 animal in lot A); stiffness of the hind legs for 1 animal in lots C,E,F; panting - 1 animal in lots C and F. The clinical observations very probably demonstrated the consequences of an internal haemorrhage; the signs were more severe and intense as the dose was increased.	
4.3 Pathology	For animals dying during the study: haemothorax, haemorrhagic thymus, intra-cranial haemorrhages, abdominal haemorrhages, haemorrhages located at the urogenital-system, disseminated haematomas at the sub-cutaneous, renal and muscular areas, a discoloration of the thoracic or abdominal organs, almost generalised. For animals autopsied at the end of the study: almost non-existent haemorrhagic signs. One rat (Lot D) had a liquid blood pocket surrounding the right ovary, one female rat (Lot F) had haemorrhagic points in lungs, and uterus filled with blood.	
4.4 Other	Body weight: males – starting with lot C treated at 5.2 mg/kg, a decrease of (–28%) in the first week; the most intense variations appeared at the dose of 8.2 mg/kg (lot D) with (-104 %) decrease in the first week and (–15 %) after 3 weeks. Body weight: females- significant decrease starting with lots E and F treated at 13.2 (- 63 %) and 21 mg/kg (-73%) with a strong recovery of Lot E for weeks 2 and 3. Mortality: Controls- no dead animals. Between days 4 and 9: 2 mg/kg group - 4 males; 3.2 mg/kg – 6 males; 5.2 mg/kg – 4 males and 2 females; 8.2 mg/kg – 8 males and 3 females; 13.2 mg/kg – 10 males and 6 females; 21.0 mg/kg – 9 males and 9 females. Evidence of an increased sensitivity for the males (some of the males died prematurely at all doses, contrary to the females, which all survived at 2.0 and 3.2 mg/kg.); the relationship dose/effect was more evident in the females.	

Section A 6.1.1-01 **Annex Point IIA VI 6.1.1**	**Oral toxicity** LD50 study in the rat	
4.5 **LD_{50}**	Males - 3.15 mg/kg (1.48-6.68) Females - 10.95 mg/kg (6.46-18.57) Males and Females – 6.26 mg/kg (3.96-9.89)	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 **Materials and methods**	The study was performed to establish the LD_{50} after a single oral administration to rats of Chlorophacinone in solution in PEG 300. Seven groups of Sprague Dawley rats (10 females and 10 males in each group) were given a single oral dose (oesophageal force-feeding) of test material at dose levels of 0 mg/kg, 2.0 mg/kg; 3.2 mg/kg; 5.2 mg/kg; 8.2 mg/kg; 13.2 mg/kg or 21 mg/kg. The animals were observed for clinical signs, mortality, and body weight. A macroscopic examination was completed at the scheduled termination and for all interim decedents.	
5.3 **Results and discussion**	The clinical observations very likely demonstrated the consequences of an internal haemorrhage; the signs were more severe and intense as the dose was increased. Significant weight-drop preceding the death of the animals starting at 8.2 mg/kg in the males or at higher doses in females. With the exception of one female (21 mg/kg), all the mortalities were grouped between the 4^{th} and 9^{th} day after treatment. There was evidence of an increased sensitivity among males. The relationship dose/effect was more evident in the females. Autopsy showed mainly a haemothorax, haemorrhage affecting the abdominal or cranial cavities, or located at certain organs (kidney, bladder, thymus, testicles, epididymis), as well as various haematomas. The LD_{50} and 95% confidence interval of the test material were calculated by the method of Litchfield and Wilcoxon to be:	
5.4 **Conclusion**	The LD_{50} and 95% confidence interval of the test material were calculated to be: Males - 3.15 mg/kg (1.48 - 6.68) Females - 10.95 mg/kg (6.46 - 18.57) Males and Females – 6.26 mg/kg (3.96 - 9.89) **The LD_{50} is approximately 3 times lower in males than in females.**	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies were found	

Section A 6.1.1-01 **Annex Point IIA VI 6.1.1**	**Oral toxicity** LD50 study in the rat	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	November 2005 (revised 12 December 2005)
Materials and Methods	Applicant version is adopted
Results and discussion	Applicant version is adopted The clinical and pathological observations consequences of an internal haemorrhage; the signs were more severe and intense as the dose was increased. Significant weight-drop preceding the death of the animals was observed starting at 8.2 mg/kg in the males. Mortalities were mainly grouped between the 4th and 9th day after treatment. Males were more sensitive than females. Mortalities in males were observed from the lowest dose (4 males died at 2 mg/kg bw and 6 at 3.2 mg/kg bw)
Conclusion	**LD_{50} for oral dosing in rats:** **Males - 3.15 mg/kg (1.48 - 6.68)** **Females - 10.95 mg/kg (6.46 - 18.57)** **Males and Females - 6.26 mg/kg (3.96 - 9.89)** **Male were more sensitive than females with LD_{50} at least 3 time lower in males** **High mortality was observed in males at all doses, including** the lowest doses (4 of 10 males died at 2 mg/kg bw and 6 at 3.2 mg/kg bw), and so, the confidence interval go down to 1.48 mg/kg for LD_{50} in males. Mortalities occurred mainly on the 4th and 9th day after treatment.
Reliability	1
Acceptability	Accepted
Remarks	

Table A 6.1.1-1: Table for oral toxicity to rats

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)	Observations
0 Lot T	0/20	NA	Very discrete, almost non-existent haemorrhagic signs at the autopsy
2.0 Lot A	4/20	day 5-6	4 males, 0 females Presence of blood around snout and front paws
3.2 Lot B	6/20	day 4-8	6 males, 0 females Weakness, pale mucous membranes
5.2 Lot C	6/20	day 4-8	4 males, 2 females Lethargy, discoloured eyes, stiffness in front paws, bristled fur, haematoma at the head, panting
8.2 Lot D	11/20	day 4-9	8 males, 3 females Lethargy, discoloured eyes, haematoma at the hind paws, bristled fur, spasmodic breathing, decrease of body weight, haematoma at the head
13.2 Lot E	16/20	day 4-9	10 males, 6 females Weakness, stiffness, bristled fur, spasmodic breathing, discoloured eyes, pale mucous membranes
21.0 Lot F	18/20	day 4-13	9 males, 9 females Lethargy, stiffness, bristled fur, spasmodic breathing, discoloured eyes, pale mucous membranes
LD_{50} value	Males - 3.15 mg/kg (1.48-6.68) Females - 10.95 mg/kg (6.46-18.57) Males and Females – 6.26 mg/kg (3.96-9.89)		

Section A 6.01.1-02 **Annex Point IIA VI.6.1.1**		**Oral toxicity** LD_{50} study in the dog	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxx XX (XXXX): Acute oral LD_{50} of Chlorophacinone in **Beagle Dogs**. XXXXXXXXXXXXXXXXXX, XXX., XXXXX, XX (Dates of Experimental work - August XXXX -September XXXX). Unpublished XXXX study No: XXXXX (Xxxxxxxx XX, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA 86-1. In accordance with EC Method B.1.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deviations were identified.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone	
3.2.1	Lot/Batch number	Lot XXXXX	
3.2.2	Specification	Less than 500 ppm of sulphates	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXX%	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Dogs	
3.3.2	Strain	Purebred Beagle	
3.3.3	Source	XXXXXXXXXXXXXX, XXX., XXXXXXX, XX, USA	
3.3.4	Sex	Males and Females	
3.3.5	Age/weight at study initiation	4 -7 months Males – 5.1-9.0 kg Females – 5.5-7.7 kg	
3.3.6	Number of animals per group	Pre-test study: two females, two males Main Study: four males, four females	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Oral	
3.4.1	Postexposure period	32 days	

Section A 6.01.1-02 **Annex Point IIA VI.6.1.1**	**Oral toxicity** LD_{50} study in the dog	
3.4.2 Type	Single oral dose via gelatine capsule to animals fed a Vitamin K-deficient diet	
3.4.3 Concentration	Dose: Pre-test study: 4.0 mg/kg, 25 mg/kg, 50 mg/kg Dose: Main study: 2.0 mg/kg; 4.6 mg/kg; 10.8 mg/kg, 25.0 mg/kg	
3.4.4 Vehicle	Not applicable	
3.4.5 Controls	No	
3.5 Examinations	Clinical observations and mortality (1, 2, 4 hours post administration, on day 1, twice daily thereafter). Body weight (prior to dosing on day 1 and on days 8, 15, 22, 29, 32). Blood samples from one male and one female at each dose level (at baseline, daily until prothrombin time exceeded 100 seconds and weekly thereafter). Gross necropsy and histopathology	
3.6 Method of determination of LD_{50}	Probit analysis (Finney, DJ, Statistical methods in Biological Assay, second edition. London: Griffin Press, 1971)	
	4 RESULTS AND DISCUSSION	

Section A 6.01.1-02 **Annex Point IIA VI.6.1.1**	**Oral toxicity** LD_{50} study in the dog	
4.2 Clinical signs	Blood around the mouth, blood present in both stools and urine, pale mucous membranes, decreased activity, anorexia, laboured breathing.	
4.3 Pathology	Haemolysed blood in the gastro-intestinal tract, haemorrhagic lungs and thymus, pale liver, kidneys, spleen, pancreas, blood in the cranial, thoracic and/or abdominal cavities and haemorrhagic areas of the brain, oesophagus, heart, kidneys, liver, pancreas, peritoneum and urinary bladder.	
4.4 Other	Body weight: Mean bodyweight for males dosed at 10.8 mg/kg was 100 grams less on day 8 than on day 1; mean bodyweight on day 8 for males dosed at 2.0, 4.6 or 25.0 mg/kg were increased; on day 15 the only surviving males at 4.6 and 10.8 mg/kg had a decreased or same bodyweight as on day 8. Decreased mean bodyweight for females at day 15 compared with day 8, overall decrease by day 32. Haematology: Pre-test study: An increase in prothrombin time occurred the day after the test article was administered in all animals. Values continued to increase and on day 5 exceeded 100 seconds. Main study: Increase in prothrombin time values on day 2, 3, 4; exceeded 100 seconds on day 5; values failed to drop below 100 seconds in the surviving animals for the remainder of the study. Mortality: Pre-test study: All animals Mortality Main study: 4 males and 3 females at dose of 2.0 mg/kg, 4.6 mg/kg, and 25 mg/kg; 4 males and 4 females at dose of 10.8 mg/kg.	
4.5 LD_{50}	Males and females: Less than 2.0 mg/kg body weight	

Section A 6.01.1-02 **Annex Point IIA VI.6.1.1**	**Oral toxicity** LD_{50} study in the dog	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The purpose of the study was to determine the acute oral LD_{50} of Chlorophacinone using purebred Beagle dogs. The test article, Chlorophacinone, was administered via gelatine capsule to each of 4 female and 4 male Beagle dogs at doses of 2.0, 4.6, 10.8, or 25 mg/kg bw. The animals were observed for clinical signs, mortality, and changes in body weight; blood samples, gross necropsy, and histopathology were performed. The study was performed according to FDRL Standard Operating Procedures and Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human And Domestic Animals, November 1982. The method was in accordance with EC Method B.1.	
5.3 Results and discussion	The pharmacotoxic sign noted most frequently was internal bleeding demonstrated by observation of blood around the mouth, blood present in both stools and urine and pale mucous membranes. Other observations noted with increased frequency were decreased activity, anorexia, and laboured breathing. Decreases in body weight were probably the result of not eating and fluid loss due to haemorrhaging. Four males and 3 females in each group dosed at 2.0 mg/kg, 4.6 mg/kg, or 25 mg/kg died. Four males and 4 females dosed at 10.8 mg/kg died. All prothrombin time values exceeded 100 seconds from day 5 and remained elevated through to study termination in surviving animals. Gross pathology: Haemolysed blood in the gastro-intestinal tract, haemorrhagic lungs and thymus, pale liver, kidneys, spleen, pancreas, blood in the cranial, thoracic and/or abdominal cavities and haemorrhagic areas of the brain, oesophagus, heart, kidneys, liver, pancreas, peritoneum, urinary bladder. Oral administration of Chlorophacinone caused an adverse effect on coagulation, which was not reversible after 32 days (study termination) in surviving dogs at the dose levels administered. Based on the results, the acute oral LD_{50} of Chlorophacinone in male and female Beagle dogs, which were fed a vitamin K-deficient diet, is less than 2.0 mg/kg body weight.	
5.4 Conclusion	The acute oral LD_{50} of Chlorophacinone in male and female Beagle dogs is less than 2.0 mg/kg body weight. A more precise value could not be determined from the dosing regimen used in the study.	
5.4.1 Reliability	2	
5.4.2 Deficiencies	The only deficiency appears to be that dose levels were too high to accurately determine an LD_{50} value.	

Section A 6.01.1-02 **Annex Point IIA VI.6.1.1**	**Oral toxicity** LD_{50} study in the dog	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2005 (Revised 22 December 2005)
Materials and Methods	Too high dose.
Results and discussion	Adopted applicant version.
Conclusion	All animals died in pre-study and in **main study: 4/3 males/ females at 2.0, 4.6, and 25 mg/kg; 4/4 males/females at 10.8 mg/kg.** The acute oral LD_{50} of Chlorophacinone in male and female Beagle dogs is less than 2.0 mg/kg body weight. A more precise value could not be determined from the dosing regimen used in the study.
Reliability	3. For information only as LD_{50} cannot be determined (<< 2 mg/Kg)
Acceptability	Not accepted for assessment.
Remarks	However it should be noted that dogs seems to have higher sensitivity than rat by oral exposure. This is a matter of concern in order to do evaluation on the basis of rat data only. So risk assessment based in rat will have to use a higher safety factor to consider uncertainty.

Table A 6.1.1-2: Table for oral toxicity in dogs

Dose (mg/kg)	Number of dead / number of investigated	Time of death (range)	Observations
0	0/4 males and 0/4 females	NA	
2.0	4/4 males and 3/4 females	Day 9 to Day 21	4 males (day 9 - 13) and 3 females (day 11 - 21). Anorexia, blood around mouth, bloody stools, decreased activity, pale mucous membranes, lacrimation, limping, swollen eye and/or sclera haemorrhaging, emaciated, intracutaneous oedema lower abdomen, laboured breathing, swollen limb. Decreased mean body weight, increase in prothrombin time values – over 100 sec after day 5, until day 32.
4.6	4/4 males and 3/4 females	Day 6 to Day 20	4 males (day 6 - 16), 3 females (day 9 - 20) Anorexia, blood present around mouth and on extremities, bloody stools, bloody urine, decreased activity, gasping, pale mucous membranes, laboured breathing, haemorrhaging sclera, emaciation, intracutaneous oedema (abdomen, neck, under tongue); decreased mean body weight; increase in prothrombin time values - over 100 seconds from day 5 until the male animal died prior to day 13, and the female prior to day 26.
10.8	4/4 males and 4/4 females	Day 6 to Day 24	4 males (day 6 - 17), 4 females (day 7 - 24) Ataxia, blood present around mouth and nose, bloody stools, decreased activity, limping, pale mucous membranes, laboured breathing, diarrhoea, emaciation; decreased mean body weight; increase in prothrombin time values - over 100 seconds after day 5 until the male animal died prior to day 20, and the female prior to day 11.
25	4/4 males and 3/4 females	Day 8 to Day 13	4 males (day 8 - 14), 3 females (day 9 – 13). Anorexia, blood present around mouth, nose, and on extremities, bloody stools, bloody urine, decreased activity, hypothermia, pale mucous membranes, laboured breathing, vomiting, diarrhoea, intracutaneous oedema (abdomen); decreased mean body weight; increase in prothrombin time values - over 100 seconds after day 5 until the male animal died prior to day 20, and the female on day 12.
LD_{50} value	Males and females: Less than 2.0 mg/kg		

Section A 6.01.2-01 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Range-finding study for dermal LD_{50} in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx XX, (XXXXx): Single Dose Dermal Toxicity Study (Range Finding I) Chlorophacinone. XXXXXXXXXXXX, XXXXX, XX. (Dates of experimental work: November, 9-November 20, XXXX). Unpublished report No: XXXXXXXX (Xxxxxx XX, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA 81-1. Range-finding study in accordance with requirements of EC Method B.3.	
2.3	**GLP**	Yes	
2.4	**Deviations**	None identified	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone: Chemical name 2- [(p-chlorophenyl) phenylacetyl) 1,3- indandione	
3.2.1	Lot/Batch number	Lot XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXX%	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	New Zealand White Rabbits	
3.3.2	Strain	Not specified	
3.3.3	Source	XXXXXXXXXXXXXX, XXX., XXXXXXX, XX, USA	
3.3.4	Sex	Males	
3.3.5	Age/weight at study initiation	11 weeks Between 2.0-3.0 kg	
3.3.6	Number of animals per group	Two	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Postexposure period	14 days	

Section A 6.01.2-01 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Range-finding study for dermal LD_{50} in rabbits	
3.4.2	Area covered	10% of body surface	
3.4.3	Occlusion	Semi-occlusive	
3.4.4	Vehicle	No, the test substance was applied as received	
3.4.5	Concentration in vehicle	Dose levels: 100, 50, 10, 5, 1 mg/kg	
3.4.6	Duration of exposure	24 hours	
3.4.7	Removal of test substance	The skin was wiped and rinsed with water	
3.4.8	Controls	No	
3.5	**Examinations**	Erythema and oedema following 24 hours of exposure based on the Draize scale, clinical observations, body weight, mortality, gross necropsy.	
3.6	**Method of determination of LD_{50}**	Not specified for rangefinder.	
		4 RESULTS AND DISCUSSION	
4.2	**Clinical signs**	The overt signs of toxicity were limited to lethargy, abnormal breathing, pale ears and eyes, bleeding from the eye orbit and hind limb paralysis.	
4.3	**Pathology**	Lesions associated with internal haemorrhage – unclotted blood surrounding brain, abdominal haemorrhages, haemorrhages located at the urogenital and cardio-vascular system, lungs pale with dark red foci	
4.4	**Other**	Erythema and Oedema: No erythema and oedema following 24 hours of exposure Mortality: All test animals died by **day 11.** Body weight: All of the test animals exhibited a weight loss prior to death. A second range finding test is proposed at 1, 0.5, 0.10, 0.05, and 0.01mg/kg.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The test substance Chlorophacinone was evaluated in a single dose dermal rabbit range finding study at dose levels of 100, 50, 10, 5, 1 mg/kg	
5.3	**Results and discussion**	Both animals at each dose level died during the 14-day post treatment period. The test substance was lethal at all dose levels. A second range finding test is proposed at 1, 0.5, 0.10, 0.05, and 0.01 mg/kg	
5.4	**Conclusion**	Dermal dose levels of 1 mg/kg or higher resulted in lethality in the rabbit.	
5.4.1	Reliability	1	
5.4.2	Deficiencies	None	

Section A 6.01.2-01 Annex Point IIA VI.6.1.2	**Dermal toxicity** Range-finding study for dermal LD_{50} in rabbits	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2005
Materials and Methods	Applicant version is adopted. The test substance Chlorophacinone was evaluated in a single dose dermal rabbit range finding study at dose levels of 100, 50, 10, 5, 1 mg/kg using 2 male animals per group.
Results and discussion	Applicant version is adopted. Both animals at each dose level died during the 11 day post treatment period (Before finishing the scheduled 14 days observation period). Animals died between day 6 to 11 post treatment.
Conclusion	This is a range finding study. Data is not useful for evaluation. Dermal dose levels of 1 mg/kg or higher resulted in lethality in the two rabbits of each group. In contrast with other study (A 6.1.2-02) at which 1 mg/kg bw resulted in no deaths. The test substance was lethal at all dose levels in the range of 1 to 100 mg/kg bw.
Reliability	3
Acceptability	Only for information and range finding
Remarks	All animals died at dose from 1 to 100 mg/kg bw during day 6 to 11 post treatment.

Table A 6.1.2-1: Table for dermal toxicity in the rabbit

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range in days after dosing)	Observations
100	2/2	8-9	Loss of weight, lethargy, pale ears and eyes, abnormal necropsy
50	2/2	8-11	Loss of weight, lethargy, abnormal necropsy
10	2/2	7-9	Loss of weight, abdominal breathing, lethargy, pale ears and eyes, abnormal necropsy
5	2/2	6-11	Loss of weight, lethargy, bleeding from right eye, hind limb paralysis, abnormal necropsy
1	2/2	8-9	Loss of weight, lethargy, abdominal breathing, abnormal necropsy

Section A 6.01.2-02 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Dermal toxicity range finding study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx XX, (XXXXx): Single Dose Dermal Toxicity Study (Range Finding II) Chlorophacinone. Unpublished report No: XXXXXXXXX (xxxxxxx XX, XXXX); XXXXXX XXXXX, XXXXX, XX. (Dates of experimental work: January, XXXX – February XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA 81-1, Range-finding study in accordance with requirements of EC Method B.3.	
2.3	**GLP**	Yes	
2.4	**Deviations**	None identified	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in the report as Chlorophacinone: Chemical name 2- [(p-chlorophenyl) phenylacetyl) 1,3- indandione	
3.2.1	Lot/Batch number	Lot #: XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXX g%	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	White Rabbits	
3.3.2	Strain	New Zealand	
3.3.3	Source	XXXXXXXXXXXXXX, XXX., XXXXXXX, XX, USA	
3.3.4	Sex	Males	
3.3.5	Age/weight at study initiation	11 weeks. Between 2.0 and 3.0 kg	
3.3.6	Number of animals per group	Two	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Postexposure period	14 days	

Section A 6.01.2-02 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Dermal toxicity range finding study in rabbits	
3.4.2	Area covered	10% of body surface or other	
3.4.3	Occlusion	Semi-occlusive	
3.4.4	Vehicle	Corn starch	
3.4.5	Concentration in vehicle	5 mg/kg Dose levels: 1, 0.5, 0.05, 0.01 mg/kg	
3.4.6	Duration of exposure	24 hours	
3.4.7	Removal of test substance	The skin was wiped and rinsed with water	
3.4.8	Controls	No	
3.5	**Examinations**	Erythema and Oedema following 24 hours of exposure based on the Draize scale, clinical observations, body weight, mortality, gross necropsy	
3.6	**Method of determination of LD_{50}**	Not specified for rangefinder.	
		4 RESULTS AND DISCUSSION	
4.2	**Clinical signs**	No overt signs of toxicity were evident during the course of the study in any of the animals.	
4.3	**Pathology**	Various lesions associated with internal haemorrhage - abdominal and thoracic haemorrhages, pale lungs with large dark red foci, pitted kidneys.	
4.4	**Other**	Erythema and Oedema: No erythema and oedema following 24 hours of exposure. Mortality: All test animals survived the study Body weight: All of the test animals exhibited a loss in body weight during the course of the study.	
4.5	**LD_{50}**	Greater than 1.0 mg/kg/day	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The test substance Chlorophacinone was evaluated in a single dose dermal rabbit range finding study at dose levels of 1, 0.5, 0.05, 0.01 mg/kg.	
5.3	**Results and discussion**	Both animals at each dose level survived during the 14-day post treatment period. The test substance was non-lethal at all dose levels. A third range finding test is proposed at 5, 1, 0.5, 0.1 mg/kg.	
5.4	**Conclusion**	The test substance was non-lethal at all dose levels. A third range finding test is proposed at 5, 1, 0.5, 0.1 mg/kg.	
5.4.1	Reliability	1	
5.4.2	Deficiencies	No deficiencies were found.	

Section A 6.01.2-02 **Annex Point IIA VI.6.1.2**	**Dermal toxicity** Dermal toxicity range finding study in rabbits	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2005
Materials and Methods	Applicant version is adopted
Results and discussion	Applicant version is adopted
Conclusion	Applicant version is adopted, summarised as follows: The test substance was non-lethal at all dose levels. Various lesions associated with internal haemorrhage - abdominal and thoracic haemorrhages, pale lungs with large dark red foci, pitted kidneys were observed. The absence of mortality at doses up to 1 mg/kg bw in this study, contrasted with other study (A 6.1.2-01) showing lethality for animals dosed with 1 mg/kg bw or higher. All of the test animals exhibited a loss in body weight during the course of the study.
Reliability	3. Only useful for information
Acceptability	Not accepted for evaluation
Remarks	All animal survived using dose from 0.1 up to 1 mg/kg bw while in another study (A6.1.2-01) all animal died using dose from 1 to 100 mg/kg bw. This is an evidence that the drastic dose-effect relationship of the substance.

Table A 6.1.2-2: Table for dermal toxicity in the rabbit

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)	Observations
1	0/2	NA	No overt signs of toxicity; no signs of erythema or oedema; loss in body weight; blood in thoracic cavity, subcutaneous haemorrhage in thoracic cavity, lungs with dark red foci, pitted kidneys, pericardial sac and thymus contained blood, enlarged atria and thymus
0.5	0/2	NA	No overt signs of toxicity; no signs of erythema or oedema; loss in body weight, pale left lung with black foci, haemorrhaging on the external and internal surface of intestines and stomach, red foci on kidneys
0.1	0/2	NA	No overt signs of toxicity; no signs of erythema or oedema; loss in body weight, pale lungs with dark red foci, haemorrhaging in intestines and stomach
0.05	0/2	NA	No overt signs of toxicity; no signs of erythema or oedema; loss in body weight, lungs with dark red foci, blanched liver, small amount of unclotted blood in duodenum, haemorrhaging on the external and surface stomach
0.01	0/2	NA	No overt signs of toxicity; no signs of erythema or oedema; loss in body weight, subcutaneous haemorrhages in the abdominal area, dark foci in lungs.
LD_{50} value	A range finding test is proposed at 5, 1, 0.5, 0.1 mg/kg.		

Section A 6.01.2-03 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Dermal toxicity range finding study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx XX, (XXXXx): Single Dose Dermal Toxicity Study (Range Finding III) Chlorophacinone. XXXXXXXXXXX, XXXXX, XX. Unpublished report No: XXXXXXX (Xxxxxxx XX, XXXX); Dates of experimental work: April XXXX – May XXXX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA 81-1. Range-finding study in accordance with requirements of EC Method B.3.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deviations were noted	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in the report as Chlorophacinone: Chemical name 2- [(p-chlorophenyl) phenylacetyl) 1,3- indandione	
3.2.1	Lot/Batch number	Lot #: XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXX%	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	Rabbits	
3.3.2	Strain	New Zealand White	
3.3.3	Source	XXXXXXXXXXXXXX, XXX., XXXXXXX, XX, USA	
3.3.4	Sex	Males	
3.3.5	Age/weight at study initiation	11 weeks. Between 2.0-3.0 kg	
3.3.6	Number of animals per group	Two	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Postexposure period	14 days	

Section A 6.01.2-03 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** Dermal toxicity range finding study in rabbits	
3.4.2	Area covered	10% of body surface	
3.4.3	Occlusion	The solution was aliquoted onto separate pieces of Scotch-Pak in accordance with the dose for each animal and the acetone was allowed to evaporate; the pieces of Scotch-Pak were applied to the test sites on each animal test substance side down	
3.4.4	Vehicle	Acetone	
3.4.5	Concentration in vehicle	10 mg/ml	
3.4.6	Total volume applied	Dose levels: 5, 1, 0.5, 0.1, and 0.05 mg/kg	
3.4.7	Duration of exposure	24 hours	
3.4.8	Removal of test substance	The skin was wiped and rinsed with water	
3.4.9	Controls	No	
3.5	**Examinations**	Erythema and Oedema following 24 hours of exposure based on the Draize scale, clinical observations, body weight, mortality, and gross necropsy.	
3.6	**Method of determination of LD_{50}**	Not specified for rangefinder	
		4 RESULTS AND DISCUSSION	
4.2	**Clinical signs**	Signs of toxicity were limited to tachypnea, lethargy, pale ears and eyes, blood in the corner of the eye, blood around the nostril	
4.3	**Pathology**	Various lesions associated with internal haemorrhage - abdominal and thoracic haemorrhages, pale lungs with large dark red foci, pitted kidneys, blood in urine, blood clots on superior side of heart	
4.4	**Other**	Erythema and Oedema: No erythema and oedema following 24 hours of exposure. Mortality: All test animals in the two highest dose groups died; one animal in the middle dose group died and both animals in each of the lowest dose groups survived. Body weight: All of the surviving test animals exhibited a gain in the body weight during the course of the study	
4.5	**LD_{50}**	Proposed dose levels for the LD_{50} main study are 0.75, 0.50 and 0.25 mg/kg	

Section A 6.01.2-03 **Annex Point IIA VI.6.1.2**	**Dermal toxicity** Dermal toxicity range finding study in rabbits	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The test substance Chlorophacinone was evaluated in a single dose dermal rabbit range finding study at dose levels of 5, 1, 0.5, 0.1, and 0.05 mg/kg, according to EPA 81-1.	
5.3 Results and discussion	Both animals at each of the two highest dose levels died during the 14-day post treatment period, one animal in the middle dose group died and both animals in each of the lowest dose groups survived. The test substance was lethal to both animals at 5 and 1 mg/kg and one of two animals at 0.5 mg/kg. The proposed dose levels for the LD_{50} are 0.75, 0.50, and 0.25 mg/kg.	
5.4 Conclusion		
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies were noted.	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2005
Materials and Methods	Applicant version is adopted. Range finding study with 2 animals per group with dose levels of 5, 1, 0.5, 0.1, and 0.05 mg/kg
Results and discussion	Applicant version is adopted. It is summarised as follows: Both animals at 5 and 1 mg/kg and one of 0.5 mg/kg bw died during the 14 day post treatment period. Various lesions associated with internal haemorrhage were observed.
Conclusion	This is a range finding study. Data is not useful for evaluation.
Reliability	3
Acceptability	Only for information and range finding
Remarks	Mortality from 0.5 mg/kg bw, was associated with anticoagulant properties.

Table A 6.1.2-3: Table for dermal toxicity in rabbits

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)	Observations
5	2/2	Day 7-9	Tachypnea, lethargy, pale ears and/or eyes, loss in body weight, blood in corner of the left eye, blood from nose and mouth, haemorrhage in thoracic cavity, pale lungs and kidneys, dark red foci in lungs, large blood clot over superior side of heart.
1	2/2	Day 5-7	Tachypnea, lethargy, pale ears and/or eyes, loss in body weight, blood around nose and mouth, blood in thoracic cavity, large blood clot over superior side of heart, blanched liver, pale lungs and kidneys, blood in urine, salivary glands haemorrhaging.
0.5	1/2	Day 6	Tachypnea, pale ears and/or eyes, kidneys pale with dark red foci, unclotted blood in thoracic cavity, pale lungs with white foci, large blood clot on top of heart, haemorrhaging on exterior lining of stomach, the animal that survived experienced gain in the body weight
0.1	0/2	NA	Pale ears and/or eyes, lungs pale with dark red foci, liver blanched, pitted kidneys, gain in the body weight
0.05	0/2	NA	Pale ears and/or eyes, no pathology at necropsy, gain in the body weight
LD_{50} value	The proposed dose levels for the LD_{50} are 0.75, 0.50 and 0.25 mg/kg		

Section A 6.01.2-04 **Annex Point IIA VI.6.1.2**		**Dermal toxicity** LD 50 dermal toxicity study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx XX., (XXXXx): Single Dose Dermal Toxicity Study (LD_{50} I) Chlorophacinone. Unpublished report No: XXXXXXXX (August 21, 1990); XXXXXX XXXXXXX, Xxxxxx, XX. (Dates of experimental work: May XXXX – June XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes – FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984. EPA 81-1. In accordance with requirements of EC Method B.3.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No GLP deviations were found.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone.	
3.2.1	Lot/Batch number	Lot #: XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXXX%	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	Rabbits	
3.3.2	Strain	New Zealand White	
3.3.3	Source	XXXXXXXXXXXXXX, XXX., XXXXXXX, XX, USA	
3.3.4	Sex	Males	
3.3.5	Age/weight at study initiation	11 weeks. Between 2.0-3.0 kg	
3.3.6	Number of animals per group	Ten	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Postexposure period	21 days	

Section A 6.01.2-04 Annex Point IIA VI.6.1.2		Dermal toxicity LD 50 dermal toxicity study in rabbits	
3.4.2	Area covered	10% of body surface	
3.4.3	Occlusion	The appropriately calculated dosing amount for each animal was placed onto separate pieces of Scotch-Pak and the acetone was allowed to evaporate; the pieces of Scotch-Pak were applied to the test sites on each animal test substance side down.	
3.4.4	Vehicle	Acetone	
3.4.5	Concentration in vehicle	250 mg test substance in 10 ml acetone	
3.4.6	Total volume applied	Dose levels: 0.75, 0.50, 0.25 mg/kg	
3.4.7	Duration of exposure	24 hours	
3.4.8	Removal of test substance	The skin was wiped and rinsed with water	
3.4.9	Controls	No	
3.5	**Examinations**	Erythema and oedema based on the Draize scale following 24 hours of exposure, clinical observations, body weight, mortality and gross necropsy	
3.6	**Method of determination of LD_{50}**	Litchfield and Wilcoxon	
		4 RESULTS AND DISCUSSION	
4.2	**Clinical signs**	Signs of toxicity were limited to lethargy, abdominal breathing, pale ears and eyes, bleeding from the nostrils, discharge from the nostrils, watery stool, tachypnea and somnolence.	
4.3	**Pathology**	Various lesions associated with internal haemorrhage - abdominal and thoracic haemorrhages, pale lungs with large dark red foci, pitted kidneys, blood in urine, blood clots surrounding heart, loose stools mixed with blood, haemorrhages in the digestive and urogenital systems	
4.4	**Other**	Erythema and oedema: No erythema and oedema following 24 hours of exposure. Mortality: 9 animals in the high dose group (0.75 mg/kg) died by day 18; six animals in the middle dose group (0.75 mg/kg) died by day 16 and 4 animals in the low dose group (0.25 mg/kg) died by day 19. Body weight: All of the test animals in the high dose group (0.75 mg/kg) exhibited a loss in the body weight during the course of the study except with one animal, which gained weight. In the middle dose group (0.50 mg/kg), 7 animals exhibited a weight loss and 3 animals gained weight.	
4.5	**LD_{50}**	The median lethal dermal dose to rabbits was 0.329 mg/kg	

Section A 6.01.2-04 **Annex Point IIA VI.6.1.2**	**Dermal toxicity** LD 50 dermal toxicity study in rabbits	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The LD_{50} study was conducted to determine the median lethal dose and its statistical limits and slope using a single 24-hour exposure and a 21-day post-exposure period. The test substance Chlorophacinone was evaluated in a single dose dermal rabbit study utilizing a limit dose of 2 mg/kg, a range finding test at dose levels of 5, 1, 0.5, 0.1, and 0.05 mg/kg, and an LD_{50} study using with final selected doses at 0.75, 0.50, and 0.25 mg/kg.	
5.3 Results and discussion	Signs of toxicity were limited to lethargy, abdominal breathing, pale ears and eyes, bleeding from the nostrils, discharge from the nostrils, watery stool, tachypnea and somnolence Various lesions associated with internal haemorrhage - abdominal and thoracic haemorrhages, pale lungs with large dark red foci, pitted kidneys, blood in urine, blood clots surrounding heart, loose stools mixed with blood, haemorrhages in the digestive and urogenital systems. Erythema and Oedema: No erythema and oedema following 24 hours of exposure. Mortality: 9 animals in the high dose group (0.75 mg/kg) died by day 18; six animals in the middle dose group (0.75 mg/kg) died by day 16 and 4 animals in the low dose group (0.25 mg/kg) died by day 19. Body weight: All of the test animals in the high dose group (0.75 mg/kg) exhibited a loss in the body weight during the course of the study except with one animal, which gained weight. In the middle dose group (0.50 mg/kg), 7 animals exhibited a weight loss and three animals gained weight.	
5.4 Conclusion	The test substance Chlorophacinone elicited limited lethality at all dose levels enabling a determination of the lethal dose LD_{50} of 0.329 mg/kg.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies were found.	

Section A 6.01.2-04 **Annex Point IIA VI.6.1.2**	**Dermal toxicity** LD 50 dermal toxicity study in rabbits	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2005 (Revised 23 December 2005)
Materials and Methods	Applicant version is adopted In short this is LD_{50} study using with final selected doses at 0.75, 0.50, and 0.25 mg/kg for dermal application in rabbit, using 10 male rabbit per group.
Results and discussion	Applicant version is adopted, summarised as follows: Signs of toxicity were limited to lethargy, abdominal breathing, pale ears and eyes, bleeding from the nostrils, discharge from the nostrils, watery stool, tachypnea and somnolence. Various lesions associated with internal haemorrhage were observed including abdominal and thoracic haemorrhages, pale lungs with large dark red foci, pitted kidneys, blood in urine, blood clots surrounding heart, loose stools mixed with blood, haemorrhages in the digestive and urogenital systems. No erythema and oedema following 24 hours of exposure. Mortality: 9/10 by day 18; 6/10 by day 16, and 4/10 by day 19 at 0.75, 0.50 and 0.25 mg/kg bw, respectively. 9/10 animals in the high dose and 7/10 in the middle group exhibited a weight loss.
Conclusion	The test substance Chlorophacinone elicited limited lethality at all dose levels enabling a determination of the **LD_{50} of 0.329 mg/kg**. The main efect were lessions associated with internal haemorrhage.
Reliability	1
Acceptability	Accepted
Remarks	Mortality was observed at all dose level tested, including the lowest dose of 0.25 mg/kg bw.

Table A 6.1.2-4: Table for dermal toxicity for rabbits

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)	Observations
0.75	9/10	Day 5 to 18	Loss in weight, abdominal breathing, lethargy, watery stool, pale ears and eyes, bleeding from nostrils, abnormal necropsy, no signs of erythema or oedema
0.50	6/10	Day 5 to 16	4 survived animals – lost weight, pale ears, abdominal breathing, one of the survived animals had somnolence, bleeding from nostrils, lethargy, no signs of erythema or oedema
0.25	4/10	Day 5 to 19	6 survived animals - lost weight or slightly gained, no clinical and necropsy pathology, no signs of erythema or oedema
LD_{50} value	Median lethal dose for combined sexes estimated to be 0.329 mg/kg		

Section A 6.01.3-01 **Annex Point IIA VI.6.1.3**		**Inhalation toxicity** Acute inhalation toxicity study in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxx XX., (XXXX): Acute inhalation toxicity study of technical Chlorophacinone in rats. Unpublished laboratory report No: XXXXXXX (Xxxx XX, XXXX); XXXXXXX, XXX., XXXXXXXXX, XXXXX (Dates of experimental work – December 11, XXXX-February 15, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 81-3, EPA 540/9-84-014, 1984. EPA 81-2. In accordance with EC Method B.2.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone	
3.2.1	Lot/Batch number	XXXXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXX %	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	Albino rat	
3.3.2	Strain	Sprague-Dawley	
3.3.3	Source	XXXXXXXXXXXXXX, XXXXXX, XXXXX	
3.3.4	Sex	Males and females	
3.3.5	Age/weight at study initiation	Young adult Males – 219-350 g Females – 175-244 g	
3.3.6	Number of animals per group	7-8 males and 7-9 females	
3.3.7	Control animals	No	

Section A 6.01.3-01 **Annex Point IIA VI.6.1.3**		**Inhalation toxicity** Acute inhalation toxicity study in rats	
3.4	**Administration/ Exposure**	Inhalation	
3.4.1	Postexposure period	21 days	
3.4.2	Concentrations	Nominal concentrations : Dose level 1.33 µg /L – 72.3 µg/L Dose level 10.3 µg/L – 88.63 µg/L Dose level 11.5 µg/L – 440 µg/L Dose level 14.5 µg/L – 166 µg/L	
		Analytical concentration Dose level 1.33 µg/L Dose level 10.3 µg/L Dose level 11.5 µg/L Dose level 14.5 µg/L	
3.4.3	Particle size	Concentration 1.33 µg/L: MMAD (mass median aerodynamic diameter)= 0.994 µm± GSD (geometric standard deviation)=3.570 % particles < 1.1 micron: 43.9 Concentration 10.3 µg/L: MMAD (mass median aerodynamic diameter)= 1.118 µm± GSD (geometric standard deviation)=3.960 % particles < 1.1 micron: 44.5 Concentration 11.5 µg/L: MMAD (mass median aerodynamic diameter)=1.868 µm± GSD (geometric standard deviation)=2.752 % particles < 1.1 micron: 22.8 Concentration 14.5 µg/L: MMAD (mass median aerodynamic diameter) =3.092 µm± GSD (geometric standard deviation)=-9.453 % particles < 1.1 micron: 30.1	
3.4.4	Type or preparation of particles	Dust generated with Venturi dust dispersion system sprayed into baffling chamber, diluted with filtered air, and then introduced into chamber.	
3.4.5	Type of exposure	Nose only	
3.4.6	Vehicle	None	
3.4.7	Concentration in vehicle	Not applicable	
3.4.8	Duration of exposure	4 h	
3.4.9	Controls	No	
3.5	**Examinations**	Clinical observations on the day of exposure (at 0.5, 1 and 2.5 hours during the exposure) and at least once daily for 21 days, body weights prior to exposure and on days 7,14,21; mortality and gross necropsy	

Section A 6.01.3-01 **Annex Point IIA VI.6.1.3**	**Inhalation toxicity** Acute inhalation toxicity study in rats	
3.6 Method of determination of LD_{50}	Litchfield and Wilcoxon	
	4 RESULTS AND DISCUSSION	
4.2 Clinical signs	Clinical signs of poisoning were evident only in animals that died - activity decrease, ataxia, blanching, apparent bleeding from ears, corneal opacity, discoloured urine, lacrimation, loss of hind leg use, muscle tremors, piloerection, polyuria, prolapsed penis, ptosis	
4.3 Pathology	Chromodacryorrhea, diarrhoea, lacrimation, nasal discharge and polyuria, apparent bleeding from ears, discoloration of vital organs, lungs swollen, discoloration of the contents of the gastrointestinal tract and bladder, gastrointestinal tract distended with gas, materials in pleural and abdominal cavity, testes drawn into the abdominal cavity.	
4.4 Other	Mortality: Six animals died from suffocation within the first 5 hours. The deaths were considered stress-related, animals showed no clinical signs of haemorrhage or pathology findings at necropsy. No deaths observed in the lowest dose level (1.33 µg/L); 6 out of 14 animals in the 10.3 µg/L level died, 13 out of 14 animals in the 11.5 µg/L died, 5 out of 11 animals died in the 14.5 µg/L group.	
4.5 LD_{50}	Males – 7.0 µg/L (0.83-59.0) Females – 12.0 µg/L (7.80-18.0) Males and Females – 9.3 µg/L (2.30-38.0)	

Section A 6.01.3-01 **Annex Point IIA VI.6.1.3**	**Inhalation toxicity** Acute inhalation toxicity study in rats	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	An acute inhalation toxicity study was conducted on albino rats using test material chlorophacinone. The animals were exposed nose-only to a dust with >=25% of particle size under 1micron generated from the test material (fine powder) for four hours at dose levels of 1.33 µg/L, 10.3 µg/L, 11.5 µg/L, and 14.5 µg/L.	
5.3 Results and discussion	Clinical signs of poisoning were evident only in animals that died - activity decrease, ataxia, blanching, apparent bleeding from ears, corneal opacity, discoloured urine, lacrimation, loss of hind leg use, muscle tremors, piloerection, polyuria, prolapsed penis, ptosis. Mortality: Six animals died from suffocation within the first 5 hours. The deaths were considered stress-related, animals showed no clinical signs of haemorrhage or pathology findings at necropsy. No deaths observed in the lowest dose level (1.33 µg/L); 6 out of 14 animals in the 10.3 µg/L level died, 13 out of 14 animals in the 11.5 µg/L died, 5 out of 11 animals died in the 14.5 µg/L group. Gross pathology - Chromodacryorrhea, diarrhea, lacrimation, nasal discharge and polyuria, apparent bleeding from ears, discoloration of vital organs, lungs swollen, discoloration of the contents of the gastrointestinal tract and bladder, gastrointestinal tract distended with gas, materials in pleural and abdominal cavity, testes drawn into the abdominal cavity. The acute inhalation LC50 with 95 % confidence intervals for technical Chlorphacinone when administered undiluted as a dust to albino rats was calculated to be: Males – 7.00 µg/L (0.83-59.0); Females – 12.00 µg/L (7.80-18.0); Males and Females – 9.30 µg/L (2.30-38.0).	
5.4 Conclusion	The acute inhalation LC50 with 95 % confidence intervals for technical Chlorphacinone when administered undiluted as a dust to albino rats were calculated to be: Males – 7.00 µg/L (0.83-59.0); Females – 12.00 µg/L (7.80-18.0); Males and Females – 9.30 µg/L (2.30-38.0)	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies were identified	

Section A 6.01.3-01 **Annex Point IIA VI.6.1.3**	**Inhalation toxicity** Acute inhalation toxicity study in rats	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2005 (revised 26 December 2005)
Materials and Methods	An acute inhalation toxicity study was conducted on albino rats. The animals were exposed nose-only to a dust with ≥ 25% of particle size under 1micron generated from the test material (fine powder) for four hours at dose levels of 1.33 µg/L, 10.3 µg/L, 11.5 µg/L, and 14.5 µg/L. The interval of exposure from the second to the four dose level were only in the range from 10.39 to 14.5 µg/L.
Results and discussion	Clinical signs of poisoning were evident only in animals that died - activity decrease, ataxia, blanching, apparent bleeding from ears, and other The deaths were considered stress-related, animals showed no clinical signs of haemorrhage or pathology findings at necropsy. Mortality: 0/14, 6/14, 13/14, 5/11 at 1.33, 10.3, 11.5 and 14.5 µg/L group, respectively. So except the lowest dose, all other dose levels showed high mortality with no well established dose-effect relationship. So the LD_{50} values deduced had high uncertainty. Gross pathology: Chromodacryorrhea, diarrhea, lacrimation, nasal discharge and polyuria, apparent bleeding from ears, discoloration of vital organs, lungs swollen, discoloration of the contents of the gastrointestinal tract and bladder, gastrointestinal tract distended with gas, materials in pleural and abdominal cavity, testes drawn into the abdominal cavity.
Conclusion	The acute inhalation LC_{50} with 95 % confidence intervals for technical Chlorphacinone when administered undiluted as a dust to albino rats were calculated to be: Males – 7.00 µg/L (0.83-59.0); Females – 12.00 µg/L (7.80-18.0); Males and Females – 9.30 µg/L (2.30-38.0).
Reliability	1
Acceptability	Accepted
Remarks	The critical lower LD_{50} values (in males) showed high uncertainty with a wide range in infidence interval and with a lower limit of confidence of 0.83 µg/L. This is related with the observation that high mortality (6 out of 14) ocurred from the second tested dose of 10.3 µg/L and short intervals among dose levels from the second to the highest dose level.

Table A 6.1.3-1: Table for inhalation toxicity

Dose [µg/L]	Number of dead / number of investigated	Time of death (range)	Observations
1.33	0/12	NA	No observable abnormalities
10.3	6/14	Day 3-5	4/6 males and 2/8 females died - lost weight, abnormal necropsy – signs of lacrimation, dry dark red material on ears, pale heart, lungs, spleen, kidneys, testes, liver, testes drawn into abdominal cavity, small amount of red mucoid material in gastrointestinal tract; surviving animals - no observable abnormalities
11.5	13/14	Day 4-7	8/8 males and 5/6 females died - lost weight, abnormal necropsy – lacrimation, polyuria, diarrhoea, stomach distended with gas and yellow liquid, urinary bladder full with red and dark liquid, testes drawn into abdominal cavity, red clots in pleural and abdominal cavities, nasal discharge, brown mucoid material in small intestine; one surviving animal no observable abnormalities
14.5	5/11	Day 5-8	2/5 males and 3/6 females died - lost weight, abnormal necropsy – polyuria, chromodacryorrhea, red nasal discharge, gastrointestinal tract distended with red muciod material and gas, urinary bladder full with red liquid, testes drawn into abdominal cavity; one surviving male had pale kidney and slightly mottled.
LC_{50} value	Males – 7.00 µg/L, 95 % confidence limits (0.83-59.0) Females – 12.00 µg/L, 95 % confidence limits (7.80-18.0) Males and Females – 9.30 µg/L, 95 % confidence limits (2.30-38.0)		

Section A 6.01.4-01 **Annex Point IIA VI.6.1.4**		**Acute dermal irritation** Primary dermal irritation study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx XX., (XXXXx): Primary Dermal Irritation Study. Chlorophacinone. Unpublished report No: XXXXXXX (XXXX X, XXX); XXXXXXX XXXXXXX, XXXXXX, XX. (Dates of experimental work: May 13, XXXX-May 16, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes – FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984. EPA 81-4. In accordance with EC Method B.4.	
2.3	**GLP**	Yes	
2.4	**Deviations**	None identified	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone.	
3.2.1	Lot/Batch number	Lot #: XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXX%	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	New Zealand White Rabbits	
3.3.2	Strain	Not specified	
3.3.3	Source	XXXXXXXXXX, XXXXXX, XX, USA	
3.3.4	Sex	Females	
3.3.5	Age/weight at study initiation	11 weeks. Between 2.0-3.0 kg	
3.3.6	Number of animals per group	6 animals	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Application	Non entry field	

Section A 6.01.4-01 **Annex Point IIA VI.6.1.4**	**Acute dermal irritation** Primary dermal irritation study in rabbits	
3.4.1.1 Preparation of test substance	Test substance was used as delivered.	
3.4.1.2 Test site and Preparation of Test Site	Test site: dorsal area of the trunk – clipping the skin free of hair, no abrasions on the skin	
3.4.2 Occlusion	Semi-occlusive	
3.4.3 Vehicle	No	
3.4.4 Concentration in vehicle	A dose of 0.5 g of the test substance per rabbit	
3.4.5 Removal of test substance	Water	
3.4.6 Duration of exposure	4 h	
3.4.7 Postexposure period	72 hours	
3.4.8 Controls	One untreated intact skin site per animal	
3.5 Examinations	Non entry field	
3.5.1 Clinical signs	Yes	
3.5.2 Dermal examination	Yes – signs of erythema or oedema	
3.5.2.1 Scoring system	Draize scale	
3.5.2.2 Examination time points	30 min, 1 hour, 24, 48, 72 hours after the exposure	
3.5.3 Other examinations	Body weight	
	4 RESULTS AND DISCUSSION	
4.2 Average score		
4.2.1 Erythema	Average score for all animals at 24, 48, 72 h = 0	
4.2.2 Edema	Average score for all animals at 24, 48, 72 h = 0	
4.3 Reversibility	NA	
4.4 Other examinations	Clinical: No overt signs of toxicity were evident during the course of the study. Body weights: All of the 6 test animals exhibited a gain in body weight during the course of the study	
4.5 Overall result	No signs of erythema and oedema were evident in any of the animals at any of the observation times.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A 6.01.4-01 **Annex Point IIA VI.6.1.4**	**Acute dermal irritation** Primary dermal irritation study in rabbits	
5.2 Materials and methods	The test substance article, Chlorophacinone, was evaluated for its potential to produce primary dermal irritation after a single topical 4-hour application to the skin of rabbits at dose of 0.5 g. The design was in accordance with FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984 and EC Method B.4.	
5.3 Results and discussion	Clinical: No overt signs of toxicity were evident during the course of the study. Body weights: All of the 6 test animals exhibited a gain in the body weight during the course of the study. The test substance's average score for all animals at 24, 48, 72 h is 0 for erythema and oedema.	
5.4 Conclusion	The test substance is considered non-irritating to the skin of laboratory animals in accordance with the guidelines.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	None identified.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	The Applicant version is adopted. Chlorophacinone was evaluated for its potential to produce primary dermal irritation after a single topical 4 hour application to the skin of rabbits at dose of 0.5 g. (standard dose suggested in guideline) The design was in accordance with FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984 and EC Method B.4	
Results and discussion	The Applicant version is adopted The test substance's average score or irritant properties for all animals at 24, 48, 72 h is 0 for erythema and oedema. At this dose no overt signs of toxicity were evident during the course of the study. Body weights: All of the 6 test animals exhibited a gain in the body weight during the course of the study.	
Conclusion	The Applicant version is adopted. Chlorophacinone was not skin irritant in the skin rabbit test.	
Reliability	1	
Acceptability	Accepted	
Remarks		

Table A 6.1.4-1: Table for skin irritation study

Treated site:

Score (average of six animals investigated)	Time	Erythema	Oedema
Average score (6 animals investigated) Draize scores	60 min	0	0
	24 h	0	0
	48 h	0	0
	72 h	0	0
Average score	24h, 48h, 72h	0	0

Table A 6.1.4-2: Table for skin irritation study

Control (Untreated site):

Score (average of six animals investigated)	Time	Erythema	Oedema
Average score (6 animals investigated) Draize scores	60 min	0	0
	24 h	0	0
	48 h	0	0
	72 h	0	0
Average score	24h, 48h, 72h	0	0

Section A 6.01.4-02 **Annex Point IIA VI.6.1.4**		**Acute eye irritation** Eye irritation	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx XX., (XXXXx): Primary Ocular Irritation Study Chlorophacinone. Unpublished report No: XXXXXXXX (Xxxx X, XXXX); Xxxxxxxxxxxxxxxx, Xxxxxx, XX. (Dates of experimental work: May 15, XXXX – May 18, XXXX).	
1.2	**Data protection**	No data protection claimed	
1.2.1	Data owner	LiphaTech S.A.S	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA Guideline 81-4. In accordance with EC Method B.5.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone	
3.2.1	Lot/Batch number	Lot #: XXXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXX%	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	New Zealand White Rabbits	
3.3.2	Strain	Not specified	
3.3.3	Source	Xxxxxxxxxxxxxxx, Xxxxxx, XX	
3.3.4	Sex	Females	
3.3.5	Age/weight at study initiation	Young adults between 2.0-3.0 kg	
3.3.6	Number of animals per group	6 animals were used in the study	
3.3.7	Control animals	No	

Section A 6.01.4-02 **Annex Point IIA VI.6.1.4**		**Acute eye irritation** Eye irritation	
3.4	**Administration/ Exposure**	Ocular instillation in the left eye	
3.4.1	Preparation of test substance	Test substance was used as delivered.	
3.4.2	Amount of active substance instilled	0.1 g per animal	
3.4.3	Exposure period	24 hours	
3.4.4	Postexposure period	72 hours	
3.5	**Examinations**	Eye examination, fluorescein staining, clinical observations, body weight	
3.5.1	Ophthalmoscopic examination	No	
3.5.1.1	Scoring system	Draize Scale for Ocular Lesions	
3.5.1.2	Examination time points	1, 24, 48, 72 hours	
		4 RESULTS AND DISCUSSION	
4.2	**Clinical signs**	No overt signs of toxicity were evident during the course of the study	
4.3	**Average score**		
4.3.1	Cornea	Average score for all animals at 24, 48, 72 h - 0.00	
4.3.2	Iris	Average score for all animals at 24, 48, 72 h - 0.00	
4.3.3	Conjunctiva	Non-entry field	
4.3.3.1	Redness	Average score for all animals at 24, 48, 72 h - 0.00	
4.3.3.2	Chemosis	Average score for all animals at 24, 48, 72 h - 0.00	
4.4	**Reversibility**	NA	
4.5	**Other**	Body weights: 3 of the 6 test animals exhibited a gain in the body weight during the course of the study, and 3 of the animals exhibited a slight decrease in the body weight	
4.6	**Overall result**	Chlorophacinone showed no potential to elicit ocular irritation or other ocular lesions.	

Section A 6.01.4-02 **Annex Point IIA VI.6.1.4**	**Acute eye irritation** Eye irritation	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The test substance article, Chlorophacinone, was evaluated for its potential to produce an irritating and/or corrosive effect on the ocular tissue of laboratory animals (rabbits) following instillation into the eye in the dose of 0.1 mg. The study design was in accordance with FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984 and met the requirements of EC Method B.5.	
5.3 Results and discussion	The test substance's average score for all animals at 24, 48, 72 h is 0 for the iris and cornea, and for chemosis and redness of the conjunctiva. There were no overt ocular lesions following administration of chlorophacinone.	
5.4 Conclusion	The test substance is considered non-irritating to the ocular tissue of laboratory animals in accordance with the guidelines.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	The Applicant version is adopted	
Results and discussion	The Applicant version is adopted	
Conclusion	The Applicant version is adopted	
Reliability	1	
Acceptability	Accepted	
Remarks		

Table A 6.1.4-3: Results of eye irritation study

Mean scores for six rabbits at each timepoint	**Cornea**	**Iris**	**Conjunctiva**	
			redness	**chemosis**
Score (range of possible scores for each assessment)	0 to 4	0 to 2	0 to 3	0 to 4
60 min	0.0	0.0	0.0	0.0
24 h	0.0	0.0	0.0	0.0
48 h	0.0	0.0	0.0	0.0
72 h	0.0	0.0	0.0	0.0
Average 24h, 48h, 72h	0.0	0.0	0.0	0.0

Section A 6.01.5-01 **Annex Point IIA VI.6.1.5**		**Skin sensitisation** Guinea pig sensitisation: Buehler Test	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxx X., (XXXX): EPA Guinea Pig Sensitisation (Buehler); Unpublished report No: XXXX (Xxxx xx, xxxx); Xxxxxxxxxxxxxxxxxxxxxxxxxxx, XX, (Dates of experimental work – May 7, XXXX –June 14, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA Pesticide Assessment guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, 1984, Acute Exposure, Guinea Pig Sensitisation (Buehler), EPA 81-6. The Buehler design study is accepted as a suitable method in accordance with EC Method B.6.	
2.3	**GLP**	Yes	
2.4	**Deviations**	Deviations from final protocol: Animals were weighed weekly in addition to the intervals outlined in the protocol, to assess toxic effects. During the first week of induction, an unscheduled dose of test material and DNCB was applied due to a technical error. The chambers were removed within an hour of dosing and all sites were carefully cleaned of test material. Only ten animals were allocated to the test group rather than twenty as required by EC test guidelines.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone, Technical grade	
3.2.1	Lot/Batch number	Lot # XXXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXXX %	
3.2.2.3	Stability	Stable	
3.2.2.4	Preparation of test substance for application	<u>For induction:</u> used as delivered, undiluted <u>For challenge:</u> used as delivered, undiluted	
3.2.2.5	Pretest performed on irritant effects	Yes	

Section A 6.01.5-01 **Annex Point IIA VI.6.1.5**		**Skin sensitisation** Guinea pig sensitisation: Buehler Test	
3.3	**Test Animals**		
3.3.1	Species	Guinea pigs	
3.3.2	Strain	Hartley	
3.3.3	Source	Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx, XX, USA.	
3.3.4	Sex	Males	
3.3.5	Age/weight at study initiation	317-400 g	
3.3.6	Number of animals per group	10 animals for the test group, 10 animals for the positive control group, 5 animals for the naïve control group.	
3.3.7	Control animals	Yes	
3.4	**Administration/ Exposure**	Buehler Test.	
3.4.1	Induction schedule	The animals were induced twice a week for 3 weeks – total of 6 inductions	
3.4.2	Way of Induction	Occlusive topical application under Hilltop chambers secured in place with adhesive tape. Dental dam and secure restraint of the animals was not part of the study design.	
3.4.3	Concentrations used for induction	0.003 mg of tested material per site on each induction and challenge occasion.	
3.4.4	Challenge schedule	Day 14 after the 6^{th} induction	
3.4.5	Concentrations used for challenge	0.003 mg of tested material per site	
3.4.6	Rechallenge	No	
3.4.7	Scoring schedule	24h, 48h after challenge	
3.4.8	Removal of the test substance	6 hours after exposure, for induction 1-3 each test site was wiped with a damp cloth, for induction 4-6 test sites were wiped with mineral oil, 95% ethyl alcohol and tap water	
3.4.9	Positive control substance	0.08% Dinitrochlorobenzene (DNCB) in 95% Ethyl Alcohol.	
3.5	**Examinations**		
3.5.1	Pilot study	Yes – Determination of maximum non-irritating dose and maximum non-lethal doses.	
		4 RESULTS AND DISCUSSION	
4.2	**Results of pilot studies**	Maximum non irritant concentration not available. Dose selection was completed on basis of survival. A study conducted with doses of 0.01 g/induction reduced to 0.005 g/induction after two days was terminated due to high test group mortality.	
4.3	**Results of test**	One animal was found dead on day 8 and a second animal on day 13. All other guinea pigs appeared active and healthy. No signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour. All surviving animals gained weight.	

Section A 6.01.5-01 **Annex Point IIA VI.6.1.5**	**Skin sensitisation** Guinea pig sensitisation: Buehler Test	
	All of the positive control animals exhibited varying degrees of erythema at the dose sites 24 and 48 h post challenge – predominantly moderate with one site exhibiting severe erythema and eschar at 48 h. No signs of irritation were observed at any of the challenged sites of any of the naïve animals nor at any of the challenged sites in the test group.	
4.3.1 24h after challenge	No signs of erythema were observed at any of the sites.	
4.3.2 48h after challenge	No signs of erythema were observed at any of the sites.	
4.3.3 Other findings	The results of the positive control study indicated the methods used were reliable and sensitive for detecting a strong/severe sensitiser.	
4.4 Overall result	No signs of irritation were observed at any of the challenged sites of any of the naïve animals and at any of the challenged sites. Chlorophacinone does not require classification for delayed contact hypersensitivity in accordance with EC Classification and labelling guidelines.	

Section A 6.01.5-01 **Annex Point IIA VI.6.1.5**	**Skin sensitisation** Guinea pig sensitisation: Buehler Test	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	A sample of Chlorophacinone, technical grade, was tested as received at the dose of 0.003 mg per site to determine its potential to promote skin sensitisation reaction after repeated topical skin application. A 3 week induction period (2 times a week for a total of 6 inductions) was initiated during which 10 young adult male guinea pigs were treated with the test material and 10 were treated with 0.08% DNCB in 95% ethyl alcohol. 14 days after the 6th induction a challenge dose of 0.003 mg per site was applied to a naïve site of each guinea pig and to 5 naïve animals. 24 and 48 hours later the animals were scored for a sensitisation response. The study was conducted according to the EPA Pesticide Assessment guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, 1984, Acute Exposure, Guinea Pig Sensitisation (Buehler) EPA 81-6 and EC Method B.6.	
5.3 Results and discussion	One animal was found dead on day 8 and a second animal- on day 13. All other guinea pigs appeared active and healthy. No signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour. All surviving animals gained weight. All of the positive control animals exhibited varying degrees of erythema at the dose sites 24 and 48 h post challenge – predominantly moderate with one site exhibiting severe erythema and eschar at 48 h. No signs of irritation were observed at any of the challenged sites of any of the naïve animals or at any of the challenged sites in the test group.	
5.4 Conclusion	No signs of irritation were observed at any of the challenged sites of any of the naïve animals and at any of the challenged sites. Chlorophacinone does not require classification for delayed contact hypersensitivity in accordance with EC Classification and labelling guidelines.	
5.4.1 Reliability	2	
5.4.2 Deficiencies	Minor deficiencies identified in Section 2.3 did not impact study reliability.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	Applicant version is adopted with some remarks: In point 3.3.3 and 3.3.5 as well as in point 5.1, it is reported 0.003 mg where should be 0.003 grams as tested dose per site. The dose was selected on the basis of the maximum dose without lethality and maximum non irritant dose. Dose selection was completed on basis of survival. A study conducted with doses of	

Section A 6.01.5-01 Annex Point IIA VI.6.1.5	Skin sensitisation Guinea pig sensitisation: Buehler Test	
	0.01 g/induction reduced to 0.005 g/induction after two days was terminated due to high test group mortality. Chlorophacinone, (technical grade, 0.003 g per site) was tested to determine its potential to promote skin sensitisation reaction after repeated topical skin application (3 week, 2 times/week, total of 6 inductions) using 10 young adult male guinea pigs (other 10 animals with positive control: 0.08% DNCB in 95% ethyl alcohol). After 14 days of the 6th induction a challenge dose of 0.003 g per site was applied to a naïve site and to 5 naïve animals and scored 24 and 48 hours later. The study was conducted according to EPA 81-6 and EC Method B.6.	
Results and discussion	Two animals died (day 8 and 13). All other guinea pigs appeared active and healthy. No signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour. All surviving animals gained weight. All of the positive control animals exhibited varying degrees of erythema at the dose sites 24 and 48 h post challenge – predominantly moderate with one site exhibiting severe erythema and eschar at 48 h. No signs of irritation were observed at any of the challenged sites of any of the naïve animals or at any of the challenged sites in the test group.	
Conclusion	No signs of irritation were observed at any of the challenged sites of any of the naïve animals and at any of the challenged sites. Chlorophacinone does not require classification for delayed contact hypersensitivity in accordance with EC Classification and labelling guidelines.	
Reliability	2. The guideline required 20 animals and in this study only 10 were used. This a significant deviation but it does not seem to be a severe discrepancy a results seems to be very clearly negative with any animal or sites with response	
Acceptability	Accepted	
Remarks		

Table A 6.1.5-1: Detailed information including induction/challenge/scoring schedule for skin sensitisation test

Skin Irritation Scores – Induction phase 1-7, 24-48 hours after induction dose

	1		2		3	4		5		6		7	
	24	48	24	48		24	48	24	48	24	48	24	48
Chlorophacinone	0	0	0	0	*	0 a	0	0 b	0	0	0	0	0
Positive Control	0-0.5	0-0.5	0-1	0-0.5	*	1-3e	0.5-3e	0.5-1	0.5-1	0.5-2	0.5-2	2-3e	2-3e

*Animals accidentally dosed off schedule

a One animal found dead on day 8

b One animal found dead on day 13

e Eschar

Scoring for Irritation:

0	No reaction
0.5	Very faint erythema, usually non-confluent
1	Faint erythema, usually confluent
2	Moderate erythema
3	Strong erythema with or without oedema

Skin Irritation Scores After Challenge

	Scores after 24 h	Scores after 48 h
Chlorophacinone	0	0
Naïve control	0	0
Positive control	2	2-3, eschar

Table A 6.1.5-2: Sensitization scores

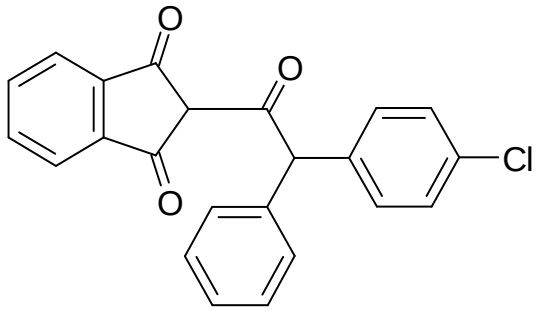
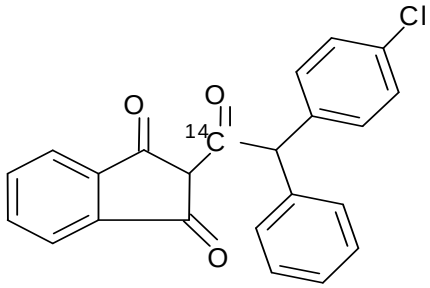
	Number of animals with signs of allergic reactions / number of animals in group	Mean Challenge Scores for 24 and 48 h	Sensitization induced at 24 hours
Chlorophacinone	0/8 ^	0	0
Negative control	0/5	0	0
Positive control	10/10	1.5-2.5	2+

^ 2 animals died during induction

Classification System for Induced Sensitization:

Mean Irritation Score	Degree of sensitization	Classification
0 – 0.9	0	None
1.0 -1.4	1+	Minimal
1.5 - 2.4	2+	Mild
2.5 – 2.9	3+	Moderate
3.0+	4+	Severe

Section A 6.02-01 **Annex Point IIA VI.6.2**		**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxx XX., (XXXX): Absorption, distribution, metabolism and excretion studies in the rat using ^{14}C-labeled Chlorophacinone. Xxxxxxxxxxxxxxxxxxxx, Xxxx, France	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	No. The study was conducted prior to the availability of guidelines for this study type. However, the methodology is similar to US EPA 85-1 guidelines.	
2.3	**GLP**	No	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Chlorophacinone, Rozol LM-91	
3.2.1	Lot/Batch number	117	
3.2.2	Specification	As given in section 2	

Section A 6.02-01 **Annex Point IIA VI.6.2**	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
3.2.2.1 Description	Not stated in report	
3.2.2.2 Purity	Not stated in report	
3.2.2.3 Stability	Not stated in report	
3.2.2.4 Structure	 chlorophacinone 2-[2-(4-chlorophenyl)-2-phenyl-acetyl]-indan-1,3-dione Radiolabelled chlorophacinone 	
3.2.2.5 Specific activity	15 mCi/mMol	
3.3 Test Animals		
3.3.1 Species	Rat	
3.3.2 Strain	Not specified	
3.3.3 Source	Xxxxxxxxxx, France	
3.3.4 Sex	Male	
3.3.5 Age/weight at study initiation	200-250 g	
3.3.6 Number of animals per group	Blood kinetics after 1 dose – 4 rats Determination in the organs 4 h after administration (maximum blood radioactivity) - 2 rats Blood kinetics after 3 doses – 2 rats Urinary, fecal and respiratory elimination – 2 rats Biliary excretion – 2 rats	
3.3.7 Control animals	No	
3.4 Administration/ Exposure	Oral	
3.4.1 Type	Oral gavage	
3.4.2 Concentration/dose	1.0 to 1.43 mg/animal	X
3.4.3 Vehicle	Gum Arabic in ammoniacal alcohol	

Section A 6.02-01 **Annex Point IIA VI.6.2**		**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
3.4.4	Concentration in vehicle	1.5 mg of LM 91/ml of gum Arabic/alcohol per animal	
3.4.5	Total volume applied	1.0 ml per animal	
3.4.6	Duration of treatment	Single dose and repeat dose (3X)	
3.4.7	Post exposure period	Excretion was examined for 48 hours after exposure	
3.4.8	Urine and faeces collection	Yes – every day	
3.4.9	Cage Wash	Yes – every day	
3.4.10	Volatiles	Yes – the CO_2 solvent trap – every day	
3.5	**Sacrifice and pathology**		
3.5.1	Blood Tissues and Carcass	Blood was sampled after 30 min, 1 hr, 2 hr, 4 hr, 6 h, 8 hr, 24 hr and 48 hr. At 48 hours, liver, kidneys, heart, muscle, fat, lungs, carcass were analysed for radiolabel.	
3.6	**Sample processing and analysis**		
3.6.1	Faeces	Daily assays for radiolabel	
3.6.2	Urine and cage washing samples	Daily assays for radiolabel	
3.6.3	Blood	Analyzed for radiolabel after 30 minutes, 1, 2, 4, 6,8, 24 and 48 hr	
3.6.4	Tissues	Single dose animals analysed after 48 hr, repeat dose animals analysed after 8 hr. Biliary excetion assessed hourly for 8 hours (total radiolabel measured).	
3.6.5	Carcass and GI tract	Same as tissues above (3.5.4)	
3.6.6	Plasma	Chromatographed plasma extracts analysed by autoradiography after 4 hrs	
3.6.7	Radioactivity measurement	Not specified	
3.6.8	Statistical analysis and data calculation	Data not statistically analysed	
		4 RESULTS AND DISCUSSION	
4.2.1	Observations		
4.2.2	Clinical signs	Not reported	
4.2.3	Mortality	Animals died 8 to 24 hours after the last dose	
4.3	**Concentrations of radioactivity**		
4.3.1	Faeces	101.6% after 4 days	

Section A 6.02-01 **Annex Point IIA VI.6.2**		**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
4.3.2	Urine	0.75% after 4 days	
4.3.3	Blood	Mean of the maximums was 7.17 µg/ml.	
4.3.4	Tissues and carcass	Liver (2.9 ppm), kidney (1.18 ppm), lung (0.39 ppm, heart (0.16 ppm), muscle (0.097 ppm), fat (0.673 ppm), carcass (0.306 ppm)	
4.4	**Absorption and elimination**		
4.4.1	Absorbed dose	Not calculated. However, biliary excretion after 8 hr is 26%. Less than 1% excreted via urine or CO_2. Maximum blood concentration is reached after 4 hr.	
4.4.2	Excreted dose	100% excretion after 4 days. The blood half-life for elimination is 10 hr. Excretion is predominantly fecal.	
4.5	**Radiolabel recovery**	Not reported	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	C-14 labelled LM 91 was administered orally on a single dose basis and after three daily doses to rats. The absorption, tissue distribution and excretion, as well as tissue residues were studied. Radioactivity was measured after each dose and the quantity received per animal calculated (between 1 and 1.4 mg). The animals were fasted overnight prior to sampling for blood kinetics and food returned 4 hours after sampling. Blood samples were collected from the retro-orbital sinus. At each scheduled termination duplicate organ samples were collected following exsanguination. The remainder of the animal (with head, tail and extremities removed) constituted the carcass which was pulverised in a blender with 50% its weight in water. For the elimination phase, rats were retained in metabolism cages for 4 days with separate collection of urine and faeces. The cages were hermetically sealed and a CO_2 trap was placed at the exit. Biliary elimination was measured hourly on bile-cannulated rats. The study design included assessment of blood kinetics on four rats after a single dose. Samples were collected at 0.5, 1, 2, 4, 6, 8, 24 and 48 hours after administration. Samples of liver, kidneys, heart, muscle, fat, lungs and carcass were retained at 48 hours post-dosing. Maximum blood radioactivity was determined in two rats dosed at 1.26 mg/rat by total blood and plasma assay. Chromatographed plasma extracts were submitted to autoradiography. Samples of liver, kidneys, heart, muscle, fat, lungs and carcass were retained at 48 hours post-dosing.	

Section A 6.02-01 **Annex Point IIA VI.6.2**	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
	Blood kinetics after three doses – two rats dosed at 1.43 mg/day. Blood sampling on third day at 0.5, 1, 2, 4, 6 and 8 hours post-dosing. At termination the liver was assayed. Urinary, faecal and respiratory elimination – two rats dosed at 1.43 and 1.28 mg/rat. Daily assays during four days occupancy of metabolism cages. Biliary excretion – two bile-duct cannulated rats dosed intraduodenally at 1.4 mg/animal. Bile was collected hourly for 8 hours and analysed by TLC and autoradiography before and after hydolysis with glucuronidase.	
5.3 Results and discussion	The single dose studies indicate the T ½ to be 10.2 hours with the maximum blood concentration being attained at 4 hours after administration. After the subchronic administration, the concentrations attained after the third dose are approximately twice the concentration attained after a single dose administration at 4 to 6 hours. **The excretion studies indicate that 90 % of the compound is recovered from faeces within 48 hours after oral administration and 100 % within 4 days**. The study of the degradation of the compound from extracted faeces indicated that the material is mainly excreted unaltered. The urinary and CO_2 elimination is less than 1 %. Studies of the biliary excretion with LM91 indicate that 2 hours after administration, the biliary elimination is constant, and at the end of 8 hours, approximately 26% of the administered radioactivity is eliminated in the bile. These observations, coupled with the concentration of the rodenticide in liver tissue as well indicate that the compound is absorbed, enters the enterohepatic circulation and then is excreted through the faeces. Chromatographic studies of the bile indicate that the rodenticide is present principally as metabolised LM 91. Tissue residue studies on animals sacrificed 48 hours following a single dose of LM 91 show that the liver is the organ with by far the highest concentrations of radioactivity present. This is followed by the kidney with the concentration of LM 91 being five times higher in the liver than in the kidney at 4 hours and approximately 2.8 times higher after 48 hours. The concentration of LM 91 in fat drops within 48 hours to ½ of the concentration at 4 hours. The carcass residues indicate that within 48 hours after a single dose, the levels are quite low. At 96 hours, the level of radioactivity in the carcass continues to fall though it is still detectable.	X

Section A 6.02-01 **Annex Point IIA VI.6.2**	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
5.4 Conclusion	Overall, the administration of chlorophacinone appears to result in rapid absorption. The rodenticide is absorbed from the gut and enters the enterohepatic circulation, 100% being eliminated in the faeces within 96 hours after administration. The highest tissue concentration is found in the liver. Chromatographic evidence indicates that unchanged parent accounted for only a small component of the faecally eliminated radioactivity and some 86% of the faecal extract remained unmoved on the plate. No metabolite identification was undertaken in this study. Further dertails were obtained in later study – see study summarised at section 6.2-02.	X
5.4.1 Reliability	2	
5.4.2 Deficiencies	Only two animals were used for some the analyses. This study was conducted prior to the availability of guidelines or GLP.	

Section A 6.02-01 Annex Point IIA VI.6.2	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

September 2005 (reviewed 20 December 2005)

Materials and Methods

Actual dose in mg/kg bw not indicated Dose: 1 mg/animal. Body weight between 200 and 250 g. Dose: 5-4 mg/kg bw. Dose was some higher than LD_{50} for males 3.15 mg/kg bw (see study A6.1.1-01). It explain that animal.

Detail of animal dosing are not properly detailed. The following studies were actually done:

(1) Blood kinetic after single dose: 4 rat 1 mg/rat blood sampling at 0.5-1-2-4-6-8-24-48 hours and tissues at 48 h (liver, kidney, heart, fat, lungs, carcass)

(2) Organs after 4 hours: 2 rat 1.26 mg/rat. Samples: bled, total blood, plasma, for total radioactivty and chromatographed plasma extracts. Sampling the same organs for total radioactivity.

(3) Blood kinetic after 3 doses: 2 rats, 1.43 mg/day. Sampling ofter 3rd day: blood (0,5-1-2-4-6-8 hours). After death, liver and the rest of animal.

(4) Urinary, fecal and respiratory elimination: 2 rats, 1.43 and 1.28 mg. Daily sampling urine, faeces CO2 during 4 days. The sacrifice and measured radioactivity in blood, organs and carcass. Extraction from urine and faeces, thin layer chromatography and autoradiography.

(5) Biliary excretion: 2 rats, 1.4 mg/animal Intraduodenally. Colection of bile, houly, for 8 hours for total radioactivity. TLC + autoradiography before and after hydrolysis with glucuronidase.

Results and discussion

There is a contradiction in the Applicant report. In results it is said that it "is mainly excreted unaltered" while in conclusion is said that, "unchanged parent accounted for only a small component of the faecally eliminated radioactivity". In the original paper the first sentence is actually: **"the material is excreted as metabolized rodenticide".**

In another study (See Section A 6.2-02) it was demostrated in SD CD-rats that 19.6 % of faecal radioactivity (15 % of total dosed) was unchanged chlorophacinone) and 46 % (36 % of total) was from the main two metabolites (monohidroxylated chlorophacinone) and they remaining are due to unidentified metabolites.

Conclusion

Chlorophacinone appears have a rapid absorption.

The single dose studies indicate the T ½ =10.2 hours with the maximum blood concentration at 4 hours after administration.

After 3 dose administration, the concentrations are approximately twice the concentration after a single dose at 4 to 6 hours.

Excretion of 90 % of the radioactivity is recovered from faeces within 48 hours after oral administration and 100 % within 4 days.

The urinary and CO_2 elimination was less than 1 %.

Biliary excretion at the end of 8 hours is approximately 26% of the administered radioactivity. The highest tissue concentration is found in the liver.

It is concluded that the compound is absorbed, enters the enterohepatic circulation and then is excreted through the faeces via

Section A 6.02-01 Annex Point IIA VI.6.2	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
	bilis. Extracted faeces and extracted bile in TLC indicated that the material is mainly excreted as metabolitzed compounds and that unchanged parent accounted for only a small component of the faecally eliminated radioactivity but the proportions of unchanged substance and metabolites were not quantified in this study. Moreover, no metabolite identification was undertaken in this study. Quantification and metabolite identification are shown in study summarised at section 6.2-02.	
Reliability	2. Only two animals were used for some the analyses. This study was conducted prior to the availability of guidelines or GLP.	
Acceptability	Accepted	
Remarks		

Table A 6.2-1: Mean blood concentrations in µg equiv of LM 91 (chlorophacinone) after single dose

Time after administration (hours)								Half-life in hours
0.5	**1**	**2**	**4**	**6**	**8**	**24**	**48**	
1.421	2.418	4.07	6.419	6.373	5.915	1.818	0.312	9.8

Table A 6.2-2: Mean blood concentrations in µg equiv of LM 91 (chlorophacinone) after three doses

Time after administration (hours)					
0.5	**1**	**2**	**4**	**6**	**8 ##**
7.141	8.943	10.165	11.504	12.224	14.156
## Retro-orbital sinus repeated sampling resulted in continuous external haemorrhage and deterioration in animal haelth resulting in death between 8 and 24 hours after 3rd administration					

Table A 6.2-3: Mean concentrations in organs µg equiv of LM 91 (chlorophacinone)/g organ 4 and 24 hours after single dose

Tissue/organ	4 hours		24 hours	
	Mean concentration (µg equiv of LM 91/g organ)	**Ratio of concentration in organ to concentration in blood**	**Mean concentration (µg equiv of LM 91/g organ)**	**Ratio of concentration in organ to concentration in blood**
Liver	31.124	4.2	2.926	9.4
Kidney	6.589	0.9	1.238	4.0
Lung	4.52	0.6	0.39	1.3
Heart	3.12	0.4	0.16	0.5
Thigh	2.016	0.3	0.097	0.3

muscle Fat Carcass	1.157 5.18	0.15 0.7	0.673 0.306	2.2 1.0

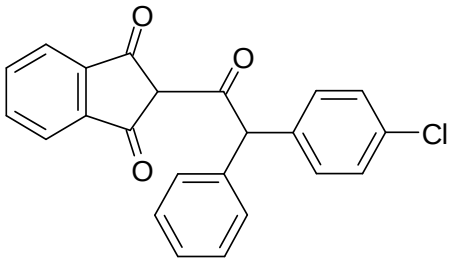
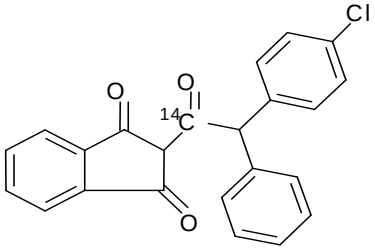
Table A 6.2-4: Biliary excretion in µg equiv of LM 91 (chlorophacinone) after single dose

Rat	Hours after dosing								Total
	1	2	3	4	5	6	7	8	
1	5.54	38.37	54.59	58	64.68	61.29	52.14	48.54	383.15 = 27.7%
2	9.27	32.85	41.13	48.77	51.56	50.17	50.67	52.95	337.37 = 24.3%

Table A 6.2-1: Mean percent of administered dose of radiolabel recovered from rats

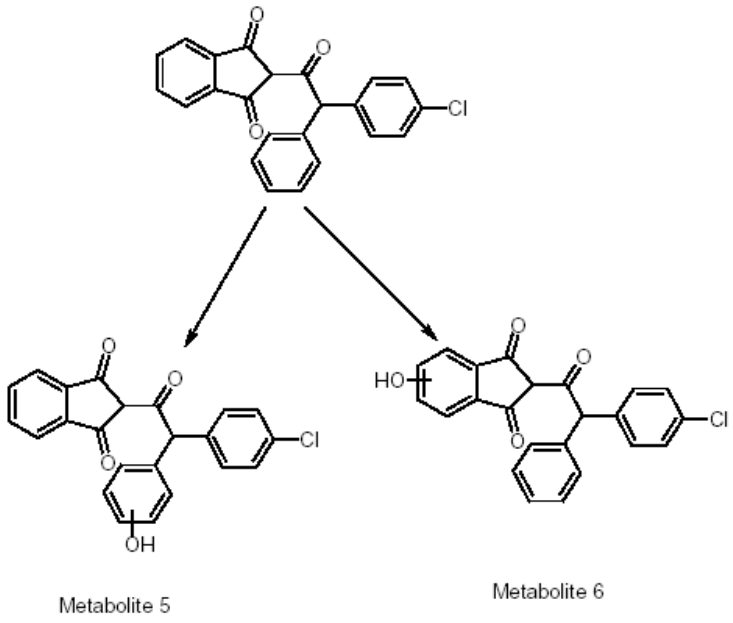
Elimination Route	% of administered dose recovered during time interval (days)				
	Day 1	Day 2	Day 3	Day 4	Total %
Urine	0.383	0.241	0.082	0.052	**0.75**
faeces	37.19	52.54	10.08	1.8	**101.64**
volatiles	0.025	0.013	0.004	0.006	**0.047**

Section A 6.02-02 **Annex Point IIA VI.6.2**		**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxxx, X and Xxxxxxx, X., (XXXX): [^{14}C]-Chlorophacinone: Metabolism in the rat following oral dosing. Xxxxxxxxxxxxxxxxxxx., XX. Laboratory report no. XXXXXXXXXX. Report date March XXXX (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Chlorophacinone and radiolabelled chlorophacinone.	
3.2.1	Lot/Batch number	Non-radiolabelled batch – XXXXXXX Radiolabelled batch – XXXXXXX	
3.2.2	Specification	As given in section 2	

Section A 6.02-02 **Annex Point IIA VI.6.2**	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
3.2.2.1 Description	Pale yellow powder	
3.2.2.2 Purity	XXXX%	
3.2.2.3 Stability	Formulations prepared or use in the study were confirmed to be stable and homogeneous for up to 24 hours.	
3.2.2.4 Structure	 chlorophacinone 2-[2-(4-chlorophenyl)-2-phenyl-acetyl]-indan-1,3-dione Radiolabelled chlorophacinone  Radiochemical purity determined at the laboratory to be XXXXX%	
3.2.2.5 Specific activity	2118.1 MBq/mmol (5.62 MBq/mg)	
3.3 Test Animals		
3.3.1 Species	Rat	
3.3.2 Strain	Crl:CD(SD)IGSBR	
3.3.3 Source	XXXXXXXXXXX, UK.	
3.3.4 Sex	Male	
3.3.5 Age/weight at study initiation	201-226 g	
3.3.6 Number of animals per group	Single group of eight rats	
3.3.7 Control animals	No	
3.4 Administration/ Exposure	Oral	
3.4.1 Type	Oral gavage	
3.4.2 Concentration/dose	Nominal dose of 2 mg/kg administered in nominal dose volume of 4 mL/kg. Nominal concentration 0.5 mg/mL.	
3.4.3 Vehicle	Radiolabelled and non-radiolabelled chlorophacinone were co-dissolved in acetonitrile, the solvent was then removed by nitrogen convection and the dried powder was suspended in 1% aqueous gum Arabic.	
3.4.4 Concentration in	Nominal concentration 0.5 mg/mL.	

Section A 6.02-02 Annex Point IIA VI.6.2		Absorption, distribution, metabolism and excretion study Single oral dose study in the rat	
	vehicle		
3.4.5	Total volume applied	4 mL/kg	
3.4.6	Duration of treatment	Single dose	
3.4.7	Post exposure period	168 hours	
3.4.8	Urine and faeces collection	Yes – samples collected daily and then pooled to provided samples for 0-48 hrs; 48-72 hrs; 72-168 hrs	
3.4.9	Cage Wash	Excreta and cage debris were collected from each cage daily and pooled by animal for the entire study period. Cages were washed with water and then methanol at the completion of the collection phase.	
3.4.10	Volatiles	No	
3.5	**Sacrifice and pathology**		
3.5.1	Blood Tissues and Carcass	No blood or tissue samples were collected. The carcass of each rat was frozen after killing by carbon dioxide overdose and cervical dislocation.	
3.6	**Sample processing and analysis**		
3.6.1	Faeces	Faeces and cage debris was homogenised in deionised water. Aliquots were then solubilised in Soluene 350 and incubated prior to analysis by LSC.	
3.6.2	Urine and cage washing samples	Added directly to scintillation fluid priorto LSC on pooled samples.	
3.6.3	Carcass	Digested in solution of potassium hydroxide in methanol (circa 40% w/v) under reflux. Aliquots were neutralised, added to scintillation fluid and analysed by LSC.	
3.6.4	Radioactivity measurement	Radioactivity measurements taken in duplicate. Radioactivity was measured for 5 minutes using Packard Tri-Carb liquid scintillation counters, to compute quench-corrected disintegrations per minute (dpm).	
3.6.5	Limit of quantification	Twice the mean background disintegration rate.	
		4 RESULTS AND DISCUSSION	
4.2.1	Mortality	Three rats were killed on health grounds 72 hours after dosing. None of the other five showed adverse reactions to dose administration.	
4.3	**Concentrations of radioactivity**		
4.3.1	Faeces	78% after 7 days	
4.3.2	Urine	Less than 1% after 7 days	
4.3.3	Blood	Not investigated	

Section A 6.02-02 **Annex Point IIA VI.6.2**		**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
4.3.4	Carcass	8% of dose was found in the carcass at necropsy, 7 days after dosing indicating excretion was incomplete.	
4.4	**Metabolite identification**		
4.4.1	Metabolite identification	Metabolite identification was carried out on the 0-48 hour faecal samples since these contained the highest concentration of radioactivity. Aliquots were extracted with one of three solvents, methanol, ethyl acetate or methyl tri-isobutyl ether (MTBE). Methanol was most efficient extracting 83.7% of faecal metabolites; ethyl acetate extracted 74.9% and MTBE removed 66.4%. Solvent concentrates were analysed by HPLC, which showed up five metabolites, the major one co-eluting with the chlorophacinone standard. Methanol and MTBE extracts were also applied to TLC plates and developed in three solvent systems. The results confirmed that chlorophacinone was the major component present. Some of the minor metabolites co-eluted with impurities present in the radiolabelled chlorophacinone. Initially faecal extracts in MTBE were prepared although this was the least efficient. The final concentrated extract contained 63.6% of total radioactive residues. HPLC analysis of the concentrate revealed the presence of chlorophacinone but metabolites failed to ionise properly or were suppressed and no identifiable spectra were obtained. An aliquot of faeces was extracted three times in methanol. The combined extract contained 85.6% of total radioactive residues. The samples were subject to further clean-up procedures resulting in an extract concentrate with 90% of total radioactive residues and a further non-extractable 12.5% remaining in the faecal pellet. The metabolite profile was similar to the initial methanol extract profile with chlorophacinone or a degradtion product of chlorophacinone as the major element and some additional polar metabolites each of which accounted for less than 1% of the dosed radioactivity. Aliquots of the methanol extract were evaporated to near dryness to remove organic solvents and incubated overnight with a mixture of β–D-glucuronidase and aryl sulphatase. Incubation was halted by addition of methanol and the results showed no significant difference in metabolite profile suggesting there were no glucuronide or sulphate conjugates present. A second MTBE extract was prepared, concentrated and subjected to extensive clean-up prior to analysis by HPLC/MS. The process produced three ions for the three major radiolablled compounds found in faecal extracts. The three ions had retention times of circa 18.6, 16.9 and	

Section A 6.02-02 **Annex Point IIA VI.6.2**	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
	15.6 minutes. Mass spectrometry and chromatographic data confirmed the first to be chlorophacinone. The second was identifed as an hydroxylated analogue of chlorophacinone with hydroxylation occurring on the indandione portion of the molecule. The third was also an hydroxylated analogue of chlorophacinone with hydroxylation occurring on the biphenyl portion of the molecule.	
	 Metabolite 5 Metabolite 6 Further attempts to identify the minor metabolites were unsuccessful since no meaningful spectra could be obtained.	
4.4.2 Metabolite quantification	Faecal samples from the five animals surviving to termination were pooled and extracted in methanol. The extract contained 81.8% of the faecal radioactivity (equivalent to 64.4% of dosed radioactivity) with 18.2% remaining in the residue (equivalent to 14.3% of dosed radioactivity). The extract was concentrated to low volume under nitrogen and analysed by radio-HPLC. Minior unidentified metabolites eluting before 12 minutes accounted for only 3.4% of theradioactive dose. One metabolite with a retention time of 14-15 minutes acounted for 8.1% of dose but was not identified. The three major metabolites identified were chlorophacinone and hydroxylated products accounting for 80.2% of the radioactivity in the faeces or 51.7% of the administered radioactive dose. Unchanged chlorophacinone accounted for 19.3% of faecal radioactivity and indicated that 15.5% of administered radioactivity was preent in faeces as unchanged parent molecule. The hydroxylated analogues accounted for 36.2% of the	

Section A 6.02-02 **Annex Point IIA VI.6.2**		**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
		administered dose and 46% of radioactivity eliminated in faeces.	
4.5	**Radiolabel recovery**	The overall mean recovery for the eight rats was 91%.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	^{14}C-Chlorphacinone was administered orally on a single occasion to eight rats at a nominal dose of 2 mg/kg in a nominal dose volume of 4 mL/kg. Excreta and associated cage debris were collected for measurement of elimination. Metabolite identification and quantification was conducted using the 0-48 hour faecal samples that contained the greatest amount of radioactivity. Methods are detailed above.	
5.3	**Results and discussion**	A single dose of chlorophacinone was administered to eight male rats at 2 mg/kg in a dose volume of 4 mL/kg. Excretion was not complete within 168 hours with 8% of radioactivity detected in the carcass at termination. The major route of elimination was via faeces (78% of the dose) with less than 1% detected in urine. Overall recovery was 91%. Despite extensive investigation by various methods and using at least three extraction solvents, only three major metabolites could be identified. The three ions had retention times of circa 18.6, 16.9 and 15.6 minutes. Mass spectrometry and chromatographic data confirmed the first to be chlorophacinone. The second was identifed as an hydroxylated analogue of chlorophacinone with hydroxylation occurring on the indandione portion of the molecule. The third was also an hydroxylated analogue of chlorophacinone with hydroxylation occurring on the biphenyl portion of the molecule. Unchanged chlorophacinone accounted for 19.3% of faecal radioactivity and indicated that 15.5% of administered radioactivity was present in faeces as unchanged parent molecule. The hydroxylated analogues accounted for 36.2% of the administered dose and 46% of radioactivity eliminated in faeces. Further attempts to identify the minor metabolites were unsuccessful since no meaningful spectra could be obtained.	
5.4	**Conclusion**	Excretion was incomplete 168 hours after a single oral dose at 2 mg chlorophacinone/kg to male rats. Faecal elimination was major route of excretion, urine accounted for less than 1% of administered dose. Unchanged chlorophacinone was eliminated in the faeces (19.3% of faecal radioactivity). Two major metabolites, accounting for 46% of faecal radioactivity, were identified as mono-hydroxylated analogues of chlorophacinone.	

Section A 6.02-02 **Annex Point IIA VI.6.2**	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
5.4.1 Reliability	1	
5.4.2 Deficiencies	None	

Section A 6.02-02 Annex Point IIA VI.6.2	**Absorption, distribution, metabolism and excretion study** Single oral dose study in the rat	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

September (revised 26 december 2005)

Materials and Methods

^{14}C-Chlorphacinone was administered orally on a single dose to eight rats at a nominal dose of 2 mg/kg in a nominal dose volume of 4 mL/kg. Excreta and associated cage debris were collected for measurement of elimination. Metabolite identification and quantification was conducted using the 0-48 hour faecal samples that contained the greatest amount of radioactivity. Identification were done using HPLC/MS/MS.

Results and discussion

Applicant version is adopted with some remarks indicated in Conclusion

A 77.56 % of total dosed radioactivity was recovered in faeces.
Less than 1% radioactivity was detected in urine.
For metabolite analysis, extract were done in methanol, ethylacetate and MTBE. Methanol extract had the highest efficacy for extraction. However for metabolite identification the extract in MTBE were used because it was containing less endogenous material (a more "clean" extract) but for quantification the methanol extract were used.

Quantification of the metabolites of chlorophacinone in the rat.

All of the faecal samples from animals 102M and 105-108M, those that survived until 168 h, were pooled. This pool accounted for 78.8% of the dosed radioactivity for these animals. An aliquot (ca 2.5 g) was extracted using methanol (3 x 10 mL). The extracts were pooled and, together with the residue, analysed for radioactive content. The extract contained 81.8% of the total radioactivity in the faeces (**64.4% of dosed radioactivity**), and the residue 18.2% (14.3% of dosed radioactive).

The extract was concentrated to low volume under nitrogen, and analysed by radio-HPLC. The sample was run twice and the results from the two runs were averaged. The chromatograms (Figure 11) showed that when the whole 0-168 h faeces was analysed, there was a significant decrease in the level of the minor polar metabolites eluting before 12 min. These now accounted for only 3.4% of the dose. A metabolite (peak number 4) at Rt 14-15 min accounted for 8.1% of the dose but was not identified.

About 24 % of of the assigned peaks (19.6 % of faecal radioactivity) was from unchanged chlorophacinone (equivalent to 15% of dosed radioactivity. Two major metabolites (5 and 6) represented 27 and 29 % assigned peaks, accounting for 45% of faecal radioactivity (equivalent to 36 % of total dosed radioactivity).

It is important to note that a peak representing 12.49 % of assigned peaks (representing about 8 % of dosed radioactivity) was detected but not identified.

Metabolite	% assigned peaks	% faecal radioactivity	% dosed radioactivity
1	0.88	0,72	0.56
2	2.39	1,96	1.54
3	2.00	1,64	1.29
4	12.49	10,22	8.05

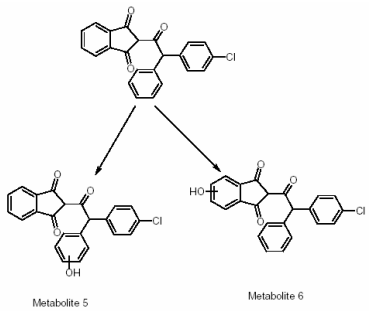
Section A 6.02-02 Annex Point IIA VI.6.2	Absorption, distribution, metabolism and excretion study Single oral dose study in the rat			
	5	**27.07**	**22,14**	**17.44**
	6	**29.13**	**23,83**	**18.77**
	7 Chlorophacinone	**24.01**	**19,64**	**15.47**
	8	1.16	0,95	0.74
	Total	**99.11**	**81,09**	**63.87**

The two major metabolites and parent compound were identified (peak 4. 5, 6. They accounted for 80.2% of the assigned peaks, 66% of the faecal radioactivity, 51.7% of the dosed radioactivity (Table 3 in original study, Table A6.2-9 above).

Metabolite 5 and 6 were identified as mono-hydroxylated metabolites of chlorophacinone. The three main idenfied excreted compounds in feces (parent compoiund plus monohydroxylated metabolites) accounted for 80.2 % of assigned peaks (66% of the faecal radioactivity) equivalent to 51.76 % of dosed radioactivity.

So about 34% of the faecal radioactivity remained unidentified due to other minor unidentified metabolites.

The metabolite profile of chlorophacinone is given in Figure. It is likely that in Metabolite 5 the position of hydroxylation is para to the carbon-phenyl bond.



Conclusion

In this study, dosing 2 mg/kg b, excretion was incomplete 168 hours after a single oral dose at 2 mg ^{14}C-chlorphacinone /kg bw to male rats with 8% of dosed radioactivity residues found in carcase at necropsy.

However in a previous study (see Section A 6.2-01), excretion was reported estimatedto be 100%, 96 hours after a single dose of 1 mg/kg bw. The discrepancy is not explained.

Faecal elimination was major route of excretion, urine accounted for less than 1% of administered dose with 91 % recovery of total radioactivity.

A 77.56 % of total dosed radioactivity was recovered in feces.

About 19.6% of of the faecal radioactivity was from unchanged chlorophacinone (equivalent to 15% of dosed radioactivity. Two major metabolites represented, accounting for 45% of faecal radioactivity (equivalent to 36.2 % of total dosed radioactivity)

The three idenfied excreted compounds in feces (parent compoiund plus monohydroxylated metabolites) accounted for 66% of the faecal radioactivity,

Section A 6.02-02 Annex Point IIA VI.6.2	Absorption, distribution, metabolism and excretion study Single oral dose study in the rat	
	being the remainding 34% due to other minor unidentified metabolites. It is important to note that a peak representing 12.49 % of assigned peaks (representing about 8 % of dosed radioactivity) was detected but not identified.	
Reliability	2 A significant peak representing 12.49 % of chromatographed peak of faecal extract (8% of total dosed radioactivity) was detected but not identified.	
Acceptability	Aceppted	
Remarks		

Table A 6.2-6: Dosing details for radiolabelled chlorophacinone - single oral dose

Identification number	Initial bodyweight (g)	Dose administered (mL)	Dose administered (mg/kg)	Radioactivity administered (MBq)
101M	226	0.8881	2.0084	2.3082
102M	220	0.8341	1.9377	2.1678
103M	219	0.8681	2.0260	2.2564
104M	205	0.8878	2.0256	2.3074
105M	205	0.8073	2.0127	2.0982
106M	201	0.8006	1.9959	2.0807
107M	223	0.7972	2.0272	2.0721
108M	224	0.8611	1.9736	2.2381

Table A 6.2-7: Mean excretion of radioactivity

Sample	Collection interval	Mean % of administered radioactivity
Urine	0-48 h	0.421
	48-72 h	0.152
	72-168 h	0.161
	Subtotal	**0.733**
Faeces	0-48 h	59.83
	48-72 h	11.91
	72-168 h	5.816
	Subtotal	**77.56**
Faeces extract	168 h	1.915
Faeces residue	168 h	0.422
Cage debris	168 h	0.419
Final cage wash	168 h	0.081
Cage was	168 h	0.177
Carcass	168 h	9.366
	Subtotal	**10.04**
	Total	**90.67**

NS no sample
three animals died after 120 hours – terminal excretion collectins forthese animals wer completed at 120 hours
** The 168 hour figures include data from the 120 hour terminated animals

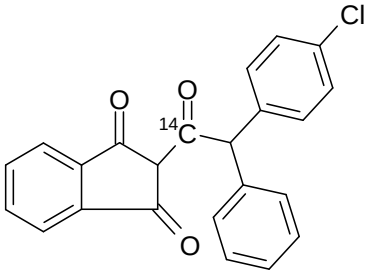
Table A 6.2-8: Extraction of faecal metabolites of chlorophacinone

Sample	Percentage of faecal metabolites *(radioactivity)* following extraction with:		
	Methanol	**Ethyl acetate**	**MTBE**
Extract Residue Total	**83.7** 21.4 105.1	74.9 31.3 106.2	66.4 43.0 109.4
Concentrated extract	**78.5**	68.2	60.5

Table A 6.2-9: Quantification of radioactive components in pooled 0-168 h faecal samples

Metabolite	Retention time (minutes)		% assigned peaks	% dosed radioactivity
	Run 1	**Run 2**		
1 2 3 4 5 6 Chlorophacinone 8	6.5 8.6 12 14.9 16.7 18.1 20.0 23.4	5.6 8.4 11.4 14.4 16.1 17.4 19.2 22.4	0.88 2.39 2.00 12.49 **27.07** **29.13** **24.01** 1.16	0.56 1.54 1.29 8.05 **17.44** **18.77** **15.47** 0.74
Total			99.11	63.87

Section A 6.02-03 Annex Point IIA VI.6.2		Percutaneous absorption (*in vitro* test)	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxx, X. and Xxxxxx, X. (2003). [^{14}C]-Chlorophacinone: Rates of penetration through human skin using a flow through *in vitro* system. Xxxxxxxxxxxxxx XXX. Laboratory report number XXXXXXXXXX. Report date 23 December XXXX (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. OECD draft guideline 428: Skin absorption: *in vitro* method December 2000.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No major deviations from protocol. The recovery results were reported for a range of 80 –120% rather than 90-110% but this was not anticipated to affect study integrity.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2 for unlabelled material and ^{14}C-Chlorophacinone	
3.2.1	Lot/Batch number	Radiolabelled batch no XXXXXXX Non labelled batch XXXXXXX	
3.2.2	Specification	As given in section 2. Deviating from specification given in section 2 as follows: The test material was radiolabelled.	

Section A 6.02-03 **Annex Point IIA VI.6.2**		**Percutaneous absorption (*in vitro* test)**	
3.2.2.1	Description	Unlabelled material - pale yellow solid (CAS number 3691-35-8)	
3.2.2.2	Purity	Unlabelled material purity XXXXX%.	
3.2.2.3	Stability	Not stated.	
3.2.2.4	Radiolabelling	^{14}C. Specific activity 2118.1 MBq/mmol, 5.62 MBq/mg. Radiochemical purity >97% structural location of radio labelling  	
3.3	**Skin samples**	Non-entry field	
3.3.1	Human	Full thickness skin samples (dorsal region) were obtained from cadavers by the Pennsylvania Regional Tissue Bank (USA). The samples were transhipped on ice to the laboratory. Only samples with intact epidermis at time of excision were accepted. The tissues were stored at <-10°C on arrival.	
3.3.2	Split thickness samples	Frozen skin samples were removed from storage. The samples were cleaned of subcutaneous fat and a strip of skin (circa 45 mm) was placed flat on a corkboard and briefly frozen to <-50°C to attach sample to baord. A section was cut at circa 400 µm with a dermatome. The epidermal section was flaoted in deionised water and stored at between 1 and 10°C.	
3.4	**Administration/ Exposure**	*In vitro* flow through diffusion cell	
3.4.1	Preparation of test membranes	Each skin membrane prepared as detailed in section 3.2.2 was positioned on the diffusion cell receptor chamber and the donor chamber was then tightened onto the membrane. Excess skin was trimmed and the exposed surface area of skin was demarcated by the donor chamber as 0.64cm^2 (0.9 cm diameter). The prepared cells, after completion of the integrity check were placed in heated manifold to maintain the skin at approximately 32ºC.	
3.4.2	Diffusion cell apparatus	An automated flow through cell was used. Peristaltic pump attached to the afferent port and receptor fluid effluent collected into scintillation vials. The exposed skin surface area was 0.64 cm^2. The flow rate through the cell was maintained at circa 1.5 mL/h.	
3.4.3	Receptor fluid	Ethanol and water (1:1 v/v)	

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	
3.4.4 Barrier integrity evaluation	Tritiated water (18 µl, equivalent to approximately 5.4 KBq) was applied to the surface of the prepared human skin samples and penetration of tritiated water assessed by collecting samples from the receptor fluid at 0-0.5; 0.5-1 and 1-2 hour post application. The fractions were then analysed by liquid scintillation counting for amount penetrated within 2 hours. Permeability coefficients were calculated and any skin sample with a value greater than 10 x 10^{-4} cm/h was excluded from the subsequent test material evaluation on presumption of altered membrane integrity.	
3.4.5 Dose formulation preparations	Group A represented the tracking powder (concentration 2g/kg) and was applied as a slurry 50:50 w/w in water to provide a 1 g/kg formulation. Radiolabelled and non-labelled chlorophacinone were co-dissolved in methanol and then solvent removed by nitrogen convection. The formulation was made up to weight with a suspension of talc in water (1:1w/w). Group B represented the wheat bait formulation (concentration 50 mg/kg) and was also applied as wet slurry to represent possible contact with wet skin. The concentration of the final wheat bait was doubled to ensure sufficient radioactivity was applied to enable measurements to be recorded, due to low specific activity of labelled ^{14}C-Chlorophacinone. ^{14}C-Chlorophacinone was dissolved in 320 µL of propylene glycol and 100 µL of PEG 300, warmed and sonicated to aid dissolution. The mixture was then coated onto wheat grains, which were dried and homogenised to a fine powder. The powder was then added to water (1:1 w/w) and re-homogenised to form starch/water paste.	
3.4.6 Application and exposure period	Each dose formulation was applied to the upper suface of the prepared skin membranes. The weight ofeach dose applied was calculated from weight differences before and after application. The skin was exposed for 6 hours and then washed with a soap solution and rinsed with deionised water. Skin washes were retained for LSC analysis.	
3.4.7 Sampling time	Receptor fluid fractions were collected for an hour prior to application of the dose, at 15 minute intervals for the first 2 hours following dosing and then over hourly intervals from 3 to 24 hours after dosing. At the end of the 18 hour post-exposure observation period (after the last receptor fluid collection at 24 hours), the diffusion cell was dismantled. The treated skin surface was tape stripped five times. This procedure is intended to remove the epidermis from lower layers of skin. {However, there are indications in results that the starch/water "glue" that constituted the dose formulation for wheatflour was not	

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	
	removed by this process and residual test material remained stuck to the surface artificially enhancing apparent absorption values.} After tape stripping the skin was solubilised in Soluene 350. Tape strips were immersed for 72 hours in Emulsifier Safe and then analysed by LSC. The cell (donor and receptor chambers) was immersed in ethanol for 24 hours and washings retained for analysis.	
3.4.8 Analysis of Samples	Liquid samples (receptor fluid, surface washings, diffusion cell washings, solubilised skin and tape strip washings) were all analysed directly in scintillation fluid by LSC. Radioactivity was measured for 5 minutes using Packard Tri-Carb liquid scintillation counters with quench correction. The limit of quantification was twice the background disintegration rate.	
	4 RESULTS AND DISCUSSION	
4.2 Calculations	Various calculations were performed using the experimental data: suface area of skin = A[cm^2] = 0.64 total volume of receptor fluid or weight of sample = T[mL or g] $\frac{\text{(weight of cell and receptor fluid) – (weight empty cell)}}{\text{density of receptor fluid}}$ Volume of receptor fluid analysed = V[mL] Weight of sample analysed = W[g] Radioactivity (sample dpm-background dpm) in receptor fluid aliquot or sample analysed = R[dpm] Concentration of radioactivity in receptor fluid = C=R/V[dpm/mL] Specific radioactivity of test substance = S[MBq/mg] Weight of substance applied to each preparation = D[mg/cm^2] Radioactive dose administered to each preparation = S x D x A[MBq] Time period for rate of penetration = Δt[hrs] Rate of penetratiion taken from linear portion of graph (ng equivalents absorbed/cm^2/time) = ΔP From these the rates of penetration were calculated (ng equivalents/cm^2/h) = J = ΔP/Δt And percentage recovery/sample = $\frac{K \times T}{10 \times D \times V \times A}$ And Permeability Coefficient (K_p) = J/ΔC where ΔC is the	

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	
	test material concentration difference across the skin membrane.	
4.3 Recovery of labelled compound		
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The study was conducted in accordance with OECD Guideline for testing of chemicals, draft new guideline 428: skin absorption: *in vitro* method (December 2002). Two test preparations of ^{14}C-Chlorophacinone were prepared as the tracking powder (equivalent to the dry concentrate) and as a wheat bait formulation (equivalent to the liquid concentrate) to achieve target doses of 0.01 mg/cm^2 or 0.0005.0 mg/cm^2 respectively. Mean dose weights actually applied were 0.0116 mg/cm^2 (0.0430MBq/cm^2) for the tracking powder formulation and 0.00127 mg/cm^2 (0.0430MBq/cm^2) for the wheat bait formulation.	
	The radiochemical purity of the ^{14}C-Chlorophacinone sample used was 99.57% and the reported specific radioactivity was 5.62 MBq/mg. Dose weights were applied to human split thickness skin samples for an exposure period of six hours. The samples were mounted *in vitro* on flow through diffusion cells and receptor fluid samples were collected prior to dosing, at 15 minute intervals for two hours and then over hourly intervals to 24 hours after completion of exposure. The skin samples were washed after six hours to remove test material residues. The skin samples were tapestripped at the conclusion of the study to remove epidermal layers and provide information about non-absorbed radioactivity in the stratum corneum. All liquid samples were retained for LSC analysis. Samples were analysed by liquid scintillation counting and skin permeability and absorption values calculated.	
5.3 Results and discussion	^{14}C- Chlorophacinone was applied in two test formulations to human split thickness skin membranes mounted *in vitro* in flow through diffusion chambers. The two preparations were applied to achieve application rates of circa 0.01 mg/cm^2 for the tracking powder and 0.001 mg/cm^2. <u>^{14}C- Chlorophacinone – tracking powder formulation applied to human skin</u> The mean maximum rate of absorption was 1.6 ng/cm^2/hour. The mean rate of absorption was 0.498 ng/cm^2/hour. The lag phase, (period prior to absorption of radioactivity), was circa 1.7 hours.	

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	
	Absorption was steady throughout the study and showed no plateau effects. Mean permeability coefficient (Kp) was 0.001 cm/h. Absorbed radioactivity in the receptor fluid accounted for < 0.1% of the applied dose at terminal timepoint, equivalent to a mean of 11.5 ng equiv/cm^2. The majority of radioactivity was in the skin washings – the dislodgeable dose included 92% in skin washing; 0.3% in tape strips and 1.3% radioactivity extracted from the diffusion chamber. Solubilisation of the skin sample provided only 1% of the dose in the skin. The dermal delivery (absorbed dose) made up of receptor fluid, residual skin levels and tape strips accounted for no more than 1.4% of the applied dose. ^{14}C- Chlorophacinone – wheat bait formulation applied to human skin The mean maximum rate of absorption was 1.2 ng/cm^2/hour. The mean rate of absorption was 0.237 ng/cm^2/hour. The lag phase, (period prior to absorption of radioactivity), was circa 0.25 hours. Absorption was proportional to time for six hours and then rate slowed until termination. Mean permeability coefficient (Kp) was 0.002 cm/h. Absorbed radioactivity in the receptor fluid accounted for < 0.5% of the applied dose at terminal timepoint, equivalent to a mean of 6.3 ng equiv/cm^2.	
	The majority of radioactivity was in the skin washings (48%±54%) or following solubilisation of the skin sample (55%±51%). 0.2% radioactivity was in the tape strips and 0.1% was extracted from the diffusion chamber. The dermal delivery (absorbed dose) made up of receptor fluid, residual skin levels and tape strips accounted for circa 56% of the applied dose of which the majority was in the residual skin sample. The discrepancy between absorption of chlorophacinone in tracking powder and chlorophacinone in wheat flour was marked when the dermal delivery amounts are compared. However the amount reaching the receptor fluid was similar – a ten fold increase in concentration resulted in only a two-fold increase in penetration (6.3 to 11.5 ng/equivalents/cm^2) which, taken together with the maximum absorption rates which were similar for both formulation, indicated the routes of absorption had been saturated at the higher dose. Minimal amounts of radioactivity were removed by tape stripping. For the wheat flour formulation this was probably due to the effective presence of a starch/water glue covering the skin surface.	

Section A 6.02-03 **Annex Point IIA VI.6.2**	**Percutaneous absorption (*in vitro* test)**	
	The skin washing accounted for almost all of the recovered dose for the tracking powder formulation and dermal absorption was minimal. For the wheat flour formulation the amounts removed by washing were highly variable. The report authors confirmed that the final formulation applied contained large pieces of wheat that were not rendered to a fine powder and not removed in the homogenisation process. The formulation applied was therefore not homogeneous in the small aliquots applied to the skin samples. This was exacerbated by the fragility of the skin samples which made removal of the wheat pieces difficult, particularly when held in place by the "flour and water" glue that constituted the dose formulation. It would appear that much of the residual radioactivity associated with the skin samples at termination of the test may be attributable to glued on pieces of wheat or dose residues glued onto the skin surface that were not removed by washing. Since the fomulation of a flour/water glue is not an appropriate scenario for application of chlorophacinone as a wheat bait, the absorption values can only be assessed using the receptor fluid values to indicate the actual amounts of radioactivity absorbed.	
5.4 Conclusion	Topical application of ^{14}C-Chlorophacinone as a tracking powder formulation or wheatflour bait to human split thickness skin samples maintained *in vitro* resulted in similar rapid rates of absorption with radioactivity appearing within 1.7 or 0.25 hours respectively. The amount reaching the receptor fluid was similar – a ten fold increase in concentration resulted in only a two-fold increase in penetration (6.3 to 11.5 ng/equivalents/cm^2) which, taken together with the maximum absorption rates which were similar for both formulations, indicated the routes of absorption had been saturated at the higher dose. The majority of the applied dose of ^{14}C-Chlorophacinone as a tracking powder formulation was removed by washing (92%) but the amount of the wheat flour formulation removed was highly variable (mean 48%). This was accounted for by pieces of wheat in the formulation adhering to the skin and providing a large amount of radioactivity which, while associated with the skin, did not appear to have been absorbed into the stratum corneum or lower layers. The dermal absorption of chlorophacinone has therefore been calculated using receptor fluid levels only as an estimate of dermal penetration excluding any possible delayed absorption from residual amounts associated with the stratum corneum. The receptor fluid level as a measure of absorption of ^{14}C-	

Section A 6.02-03 **Annex Point IIA VI.6.2**	**Percutaneous absorption (*in vitro* test)**	
	Chlorophacinone (tracking powder) was 0.093% and for the wheat flour formulation was 0.44% in human skin. Total absorption of ^{14}C- Chlorophacinone (tracking powder) including residual skin levels and tapestripping values was 1.4%. The total absorption for the wheatflour formulation, excluding residual skin values which were artificially enhanced, was 0.676%. If it is assumed that a similar residual skin value is appropriate then total absorption is circa 1.7%.	
5.4.1 Reliability	2	
5.4.2 Deficiencies	No	

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date September 2005 (revised December 2005)

Materials and Methods

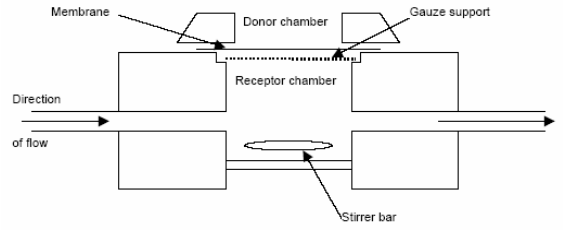
Applicant version is adopted with some remarks and summarised as follows:

The study was conducted in accordance with OECD Guideline for testing of chemicals, draft new guideline 428: skin absorption: *in vitro* method (December 2002).

Two test preparations of ^{14}C-Chlorophacinone were prepared: (A) the tracking powder (concentration 2g/kg) applied as a slurry 50:50 w/w in water to provide a 1 g/kg formulation; radiolabelled and non-labelled chlorophacinone were co-dissolved in methanol and then solvent removed by nitrogen convection. The formulation was made up to weight with a suspension of talc in water (1:1w/w). (B) wheat flour bait formulation (concentration 50 mg/kg) and was also applied as wet slurry to represent possible contact with wet skin. The concentration of the final wheat bait was doubled to ensure sufficient radioactivity.

Nominal intended doses were 0.01 and 0.0005 mg/cm^2 but mean dose weights actually applied were 0.0116 mg/cm^2 (tracking powder) and 0.00127 mg/cm^2 (wheat bait).

Dose weights were applied to human split thickness skin samples for an exposure period of six hours. The samples were mounted *in vitro* on flow through diffusion cells and receptor fluid samples were collected prior to dosing, at 15 minute intervals for two hours and then over hourly intervals to 24 hours after completion of exposure. The skin samples were washed after six hours to remove test material residues. The skin samples were tapestripped at the conclusion of the study to remove epidermal layers and provide information about non-absorbed radioactivity in the stratum corneum. All liquid samples were retained for LSC analysis. Samples were analysed by liquid scintillation counting and skin permeability and absorption values calculated.



Radioactivity measurement used to estimate dermal absorption:

- *Aliquots from the receptor chamber obtained at different time and total collected sample estimated*
- *Tape strips used to get material in epidermis*
- *Residual skin(solubilised) This was not technically possible with wheat flour formulation, due to adhesion of not absorbed particles]*

Washing liquids of skin and chamber were also measured to check the appropriate total radioactive recovery.

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	
	Applicant version is adopted with some remarks and summarised as follows:	

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	

Results and discussion

Results

The table below, summarises the key mean absorption parameters of [^{14}C]-Chlorophacinone through human skin membranes.

Sample	Mean recovery of radioactivity (% applied dose)			
	Tracking powder 0.01 mg/cm^2		Wheat bait 0.0005 mg/cm^2	
	Mean	SD	Mean	SD
Receptor Fluid	0.093	0.125	0.440	0.631
Residual Skin	1.006	1.602	55.15	51.38
Skin Wash	92.29	7.118	47.81	53.60
Tape Strip (epidermis)	0.301	0.154	0.236	0.180
Cell Wash	1.277	2.191	0.123	0.275
Total	94.97	5.433	103.8	14.66
	Absorption Kinetics			
Maximum rate of penetration (ng/cm^2/h)	1.631	1.931	1.232	2.103
Mean rate of penetration (ng/cm^2/h)	0.498	0.607	0.237	0.274
Permeability coefficient (cm/h)	0.001	0.001	0.002	0.003
Lag Time (hours)	1.685	2.703	0.249	0.323

Topical application of ^{14}C-Chlorophacinone as a tracking powder formulation or wheatflour bait to human split thickness skin samples maintained *in vitro* resulted in similar rapid rates of absorption with radioactivity appearing within 1.7 or 0.25 hours respectively but absorption was minimal and less than 0.1 or 0.5 % were detected in the receptor fluid.

The amount reaching the receptor fluid was similar – a ten fold increase in concentration resulted in only a two-fold increase in penetration (6.3 to 11.5 ng/equivalents/cm^2) which, taken together with the maximum absorption rates which were similar for both formulations, indicated the routes of absorption had been saturated at the higher dose.

For the powder formulation, the mean maximum rate of absorption was 1.6 ng/cm^2/hour. The mean rate of absorption was 0.498 ng/cm^2/hour. The lag phase, (period prior to absorption of radioactivity), was circa 1.7 hours. Absorption was steady throughout the study and showed no plateau effects. Mean permeability coefficient (Kp) was 0.001 cm/h. **Absorbed radioactivity in the receptor fluid accounted for < 0.1% of the applied dose** at terminal timepoint, equivalent to a mean of 11.5 ng equiv/cm^2.

For the wheat flour formulation, the mean maximum rate of absorption was 1.2 ng/cm^2/hour. The mean rate of absorption was 0.237 ng/cm^2/hour. The lag phase, (period prior to absorption of radioactivity), was circa 0.25 hours. Absorption was proportional to time for six hours and then rate slowed until termination. Mean permeability coefficient (Kp) was 0.002 cm/h. Absorbed radioactivity in the **receptor fluid accounted for < 0.5% o**f the applied dose at terminal timepoint, equivalent to a mean of 6.3 ng equiv/cm^2. Total final radioactive absorption for estimation dermal absorption:

Tracking powder:

Receptor fluid: 0.093%

Tape strips: 0.301 %

Section A 6.02-03 Annex Point IIA VI.6.2	Percutaneous absorption (*in vitro* test)	

Solubilised skin: 1.006%
Total absorption 1.4%

Wheat flour grains:
Receptor fluid: <0.5% (0.440%)
Tape strips: 0.236 %
Solubilised skin: (Not valid data)

If it is assumed that actual residual skin is similar to that obtained by solubilization in the test with powder (1.006%), then:

Total absorption is estimated to be 1.7%

Conclusion

Applicant version is adopted and summarised as follows:

Topical application of ^{14}C-Chlorophacinone as a tracking powder formulation or wheatflour bait to human split thickness skin samples maintained *in vitro* resulted in similar rapid rates of absorption with radioactivity appearing within 1.7 or 0.25 hours respectively but absorption was minimal and less than 0.1% (powder) or 0.5 % (bait) were detected in the receptor fluid.

Tape strips accounted for 0.3% (powder) and 0.23% (bait). Residual skin was about 1 % with tracking powder and data in weat flour baits was not used due to high amount of adhesive particles. It was assumed that similar value of 1% can be applied.

Total absorption in human skin is estimated to be not more than 1.7%, deduced in in vitro test using in vitro test of topical application of ^{14}C-Chlorophacinone as a tracking powder formulation or wheatflour bait to human split thickness skin samples maintained *in vitro*, considering total absortion including radioactivity measured in receptor fluid, tape stripping and residual skin values.

Reliability 2

Acceptability Accepted

Remarks

Table A6.2-2: Table for percutaneous absorption (*in vitro* test)

	^{14}C-Chlorophacinone			
	Tracking powder aqueous slurry 0.01 mg/cm^2 (Actual dose: 0.0116 mg/cm^2)		Slurry in propylene glycol and PEG 300 coated on wheat grains 0.0005 mg/cm^2 (Actual dose: 0.00127 mg/cm^2)	
	Mean	SD	Mean	SD
Receptor fluid	0.093	0.125	0.440	0.631
Residual skin	1.006	1.602	55.15	51.38
Skin wash	92.29	7.118	47.81	53.60
Tape strip (epidermis)	0.301	0.154	0.236	0.180
Cell wash	1.277	2.191	0.123	0.275
Total	94.97	5.433	103.8	14.66

The shaded values for residual skin levels were highly variable – attributed to the sticky nature of the formulation and presence of large pieces of wheat grain in non-homogeneous formulations applied to some cells.				
Maximum rate of penetration (ng/cm^2/hr)	1.631	1.931	1.232	2.103
Mean rate of penetration (ng/cm^2/hr)	0.498	0.607	0.237	0.274
Permeability coefficient	0.001	0.001	0.002	0.003
Lag time (hours)	1.685	2.703	0.249	0.323

Section A 6.03.1-01 Annex Point IIA, 6.3.1	Repeated dose toxicity (oral)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A 28-day short-term toxicity study was not included in the dossier since this study is not required when a sub-chronic toxicity study is available. Data from a 13-week subchronic study in rats was presented in Section 6.4 and this provides the information for Section 6.3 endpoints. There are special considerations for rodenticides in relation to long term exposure since the target species is also the test model in long term rodent studies. The implications for longterm exposure were particularly discussed in the dossier in relation to chronic studies and multigeneration reproduction toxicity or carcinogenicity investigations. However, a subchronic study in the rat was condcted over an 11 week dosing period although all rats dosed at 80 µg/kg bw/day or higher died within 16 days of starting dose administration and animals dosed at 40 µg/kg bw/day died sporadically throughout the study. Full results are presented for the subchronic investigation under point 6.4.1-01. A summary of findings is presented below indicating the changes observed after repeated oral administration of chlorophacinone to the rat were consistent with the known mode of action for an anti-coagulant rodenticide including observation of haemorrhagic developments and delayed deaths. Subchronic study findings: Mortality was noted in all dosage groups above 10 µg/kg. No mortality noted at 5 µg/kg over the 11 weeks of study. The frequency of mortality as well the survival time of animals was related to the administered dose. Males were more affected than females. At 20 µg/kg, the 4 deaths involved only males that died within the last weeks of the study; in groups 40 µg/kg, all the males died within 82 days, compared to 4 out of 10 females that died during the 3-4-th month. In the 80 and 160 µg/kg groups, death appeared very quickly, within 16 days, and with no clear difference between sexes. The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of chlorophacinone. Animals appeared weakened with decreased mobility and hemorrhages both externally and internally. Males were more sensitive to the effects of chlorophacinone than females. In those animals surviving at the end of the study, growth was unaffected by administration of the test article. Food and water consumption were also unaffected. With the exception of the coagulation time, haematological parameters were similar to controls. Coagulation time was significantly increased at all doses examined in a dose-related fashion. The lowest dosage examined was 10µg/kg where increases, while minimal were significantly different from controls. Increases were notably pronounced in groups C (20 µg/kg) and D (40 µg/kg). Males were more affected	

Section A 6.03.1-01 Annex Point IIA, 6.3.1	Repeated dose toxicity (oral)
	than females. Clinical chemistry parameters were generally unaffected by chlorophacinone at the lowest levels examined. However, at 10 and 20 µg/kg, increases in urea and creatinine levels, bilirubin, cholesterol, triglycerides, and ASAT and ALAT, were suggestive of hepatic and renal disorders. Macroscopic examination revealed extensive hemorrhagic lesions in all dosage groups above 20 µg/kg. A few were noted in the 10 µg/kg group with none noted in the 5 µg/kg group. Gross and microscopic examinations of tissues and organs were consistent with the clinical observations of hemorrhagic activity.
	LOAEL = 10 µg/kg b.w. /day NOAEL = 5 µg/kg b.w. /day (11 weeks administration)
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	Data not required, as a subchronic study is available. Applicant have presented comments about the findings and conclusions in the subchronic study which is discussed in detal in Study summary in Section A 6.4.1.-01
Conclusion	Accepted justification
Remarks	

Section A 6.03.2-02 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** **21-day dermal toxicity study in rabbits – <u>dose rangefinder</u>**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxx XX, (XXXXx): Repeated Dose Dermal Toxicity (21-Day) Study – New Zealand Albino Rabbits (Chlorophacinone). Unpublished report No: XXXXXX (November 11, XXXX); XXXXXXXXXXX, XXXXX, XX. (Dates of experimental work: September 24, XXXX – October 22, XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes – FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984 EPA Pesticide Assessment Guidelines Subdivision F, Series 82-2, 1984. Dose range-finding study in accordance with requirements of EC Method B.9.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone	
3.2.1	Lot/Batch number	Lot No: XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXX %	
3.2.2.3	Stability	Stable	
3.3	**Test Animals**		
3.3.1	Species	Albino rabbits	
3.3.2	Strain	New Zealand	
3.3.3	Source	XXXXXXXXXXXXXXXXXXX, XXXXX, XX	
3.3.4	Sex	Male and Female	
3.3.5	Age/weight at study initiation	11 weeks. 2.00 to 3.00 kg	
3.3.6	Number of animals per group	2 (1 M and 1 F)	

Section A 6.03.2-02 Annex Point IIA VI.6.3		**Subchronic dermal toxicity** **21-day dermal toxicity study in rabbits – <u>dose rangefinder</u>**	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Duration of treatment	3 weeks	
3.4.2	Frequency of exposure	6h daily, 5 days/week for three weeks	
3.4.3	Postexposure period	1 week	
3.4.4	**<u>Dermal</u>**		
3.4.4.1	Area covered	Approximately 10% of the body surface	
3.4.4.2	Occlusion	Semi-occlusive	
3.4.4.3	Vehicle	Acetone	
3.4.4.4	Concentration in vehicle	Not specified. Dosage levels: 1 mg/kg; 0.3 mg/kg, 0.1 mg/kg, 0.03 mg/kg, 0.01 mg/kg, 0.003 mg/kg	
3.4.4.5	Total volume applied	Not specified.	
3.4.4.6	Duration of exposure	6 hours daily, 5 days a week	
3.4.4.7	Removal of test substance	The test site was wiped with USP water for injection	
3.4.4.8	Controls	No	
3.5	**Examinations**		
3.5.1	Observations	Daily	
3.5.1.1	Clinical signs	Yes – daily	
3.5.1.2	Mortality	Yes	
3.5.2	Body weight	Yes - once weekly	
3.5.3	Food consumption	Yes - once weekly	
3.5.4	Water consumption	No	
3.5.5	Ophthalmoscopic examination	No	
3.5.6	Haematology	No	
3.5.7	Clinical Chemistry	No	
3.5.8	Urinalysis	No	
3.6	**Sacrifice and pathology**		
3.6.1	Organ Weights	No	
3.6.2	Gross and histopathology	Gross necropsy all dose groups. Examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their	

Section A 6.03.2-02 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** **21-day dermal toxicity study in rabbits – <u>dose rangefinder</u>**	
		contents.	
3.6.3	Statistics	No statistical analyses were performed.	
		4 RESULTS AND DISCUSSION	
4.2	**Observations**		
4.2.1	Clinical signs	Two of the females that died during this study showed signs of lethargy, unusual locomotion (swaying) and catalepsy. One of the females that survived showed signs of unusual locomotion during the post-treatment period (days 23 to 25).	
4.2.2	Mortality	Observed in the three highest dose levels (5/12 animals). All deaths occurred during the dosing period. Both the female and the male rabbits in the lowest dose levels survived the entire observation period.	
4.3	**Body weight gain**	All the surviving animals lost body weight. All the animals that died during the study either lost weight or had only a slight gain in weight	
4.4	**Sacrifice and pathology**		
4.4.1	Organ weights	Not measured	
4.4.2	Gross and histopathology	The necropsy of the dead animals revealed blood in the thoracic cavity, subcutaneously in the neck region, liver, stomach, bladder, brain, and the small intestine. No unusual lesions were noted in any of the surviving animals.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The test substance Chlorophacinone was evaluated for its potential to produce death following topical 6-hour application for 5 days/week, for 3 weeks at 6 dose levels 1 mg/kg; 0.3 mg/kg, 0.1 mg/kg, 0.03 mg/kg, 0.01 mg/kg, 0.003 mg/kg in New Zealand Albino Rabbits. The study was conducted according to FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984; EPA Pesticide Assessment Guidelines Subdivision F, Series 82-2, 1984. The method used as a range-finding investigation was in compliance with EC Method B.9.	
5.3	**Results and discussion**	Both male and females died at the 2 highest doses (1 mg/kg and 0.3 mg/kg) and one female died at the 0.1 mg/kg dose level.	
5.4	**Conclusion**	The test substance was defined toxic and an LD_{50} study with dose levels of 0.01, 0.1, and 0.2 mg/kg/day was recommended.	
5.4.1	LO(A)EL	Not applicable	
5.4.2	NO(A)EL	Not applicable	
5.4.3	Reliability	1	

Section A 6.03.2-02 **Annex Point IIA VI.6.3**	**Subchronic dermal toxicity** **21-day dermal toxicity study in rabbits – <u>dose rangefinder</u>**	
5.4.4 Deficiencies	No deficiencies were identified.	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November 2005	
Materials and Methods	The Applicant version is adopted summarised as follows: Chlorophacinone (100% purity) was topically applied for 6-hour for 5 days/week, for 3 weeks at 6 dose levels (1 , 0.3, 0.1, 0.03, 0.01 and 0.003 mg/kg bw/day in New Zealand Albino Rabbits in two animal/group (1 male, 1 female), using acetone as vehicle applying in approximately 10% of the body surface using semi-occlusive dressing. The study was a **range-finding** investigation in compliance with EC Method B.9.	
Results and discussion	The Applicant version is adopted summarised as follows: Mortalities: Both male and females at the 2 highest doses (1 and 0.3 mg/kg/day) and one female at the 0.1 mg/kg/day dose level died (total 5/12 animals). All deaths occurred during the dosing period. Both the female and the male rabbits in the lowest dose levels survived the entire observation period. All the surviving animals lost body weight. All the animals that died during the study either lost weight or had only a slight gain in weight Two of the females that died during this study showed signs of lethargy, unusual locomotion (swaying) and catalepsy. One of the females that survived showed signs of unusual locomotion during the post-treatment period (days 23 to 25). The necropsy of the dead animals revealed blood in the thoracic cavity, subcutaneously in the neck region, liver, stomach, bladder, brain, and the small intestine. No unusual lesions were noted in any of the surviving animals.	
Conclusion	Mortalities occurred from the dose of 0.1 mg/kg/day. Clinical signs were reduced to lethargy, unusual locomotion (swaying) and catalepsy. Necropsy showed several signs of bleeding related with the anticoagulant properties of the substance. The test substance was defined toxic and an LD_{50} study with dose levels of 0.01, 0.1, and 0.2 mg/kg/day was recommended.	
Reliability	3 For information only. Range finding study	
Acceptability	Acceptable but not usable for assessment	
Remarks		

Table A 6.3.2-2: Results of subchronic toxicity rangefinding study – New Zealand Albino Rabbits

Parameter	0.003 mg/kg		0.01 mg/kg		0.03 mg/kg		0.10 mg/kg	
	M	F	M	F	M	F	M	F
No. of animals examined	1	1	1	1	1	1	1	1
Mortality	0	0	0	0	0	0	0	1
Clinical signs	None	None	None	None	None	Unusual locomotion	None	Death day 20
Body weight	Loss -0.03	Loss -0.20	Loss -0.13	Loss -0.21	Loss -0.16	Loss -0.40	Loss -0.18	Loss -0.67
Gross pathology	N	N	N	N	N	Ab	Ab	Ab

Parameter	0.30 mg/kg		1.00 mg/kg	
	M	F	M	F
No. of animals examined	1	1	1	1
Mortality	1	1	1	1
Clinical signs	Dyspnea Unusual locomotion Death day 8	Lethargy, Catalepsy, Dyspnea Death day 11	Tachypnea Death day 8	Lethargy Unusual locomotion Death day 9
Body weight	Gain 0.05	Same	Same	Gain 0.04
Gross pathology	Ab	Ab	Ab	Ab

Ab = Abnormal necropsy – blood in thoracic cavity, subcutaneously in the neck region, liver, stomach, bladder, brain, and the small intestine.
N = No gross abnormalities observed

Section A 6.03.2-03 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21-day dermal range finding toxicity in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxx X. (XXXXx): 21-Day Dermal Rangefinding Toxicity Study in Rabbits with Chlorophacinone; Unpublished report No: XXXXXXXXX (February 6, XXXX); XXXXXXXXXXXXXXXXXXXXXX (Dates of experimental work 30, XXXX to 10, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA 82-2. In accordance with EC Method B.9.	
2.3	**GLP**	Yes	
2.4	**Deviations**	Minor GLP deviations were noted. Necropsies were to be performed within two hours after animals were found dead according to the protocol; the following animals were not necropsied within the designated time: Group 4 No. 2316 and Group 5 No. 2320. It cannot be verified that pre-treatment clinical pathology results were given to the study director four days after collection. A reserve sample of the test material was not taken at the initiation of the study. The sample was taken three days prior to the study start.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Rozol Tracking powder (clay chlorophacinone mixture)	
3.2.1	Lot/Batch number	Lot No: XXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Greenish powder	
3.2.2.2	Purity	**XX%**	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Rabbit	
3.3.2	Strain	New Zealand White (Hra☹NZW)SPF)	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Male	
3.3.5	Age/weight at study	12.5 weeks. 2116 to 2450g	

Section A 6.03.2-03 Annex Point IIA VI.6.3		**Subchronic dermal toxicity** 21-day dermal range finding toxicity in rabbits	
	initiation		
3.3.6	Number of animals per group	3	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Duration of treatment	3 weeks	
3.4.2	Frequency of exposure	6h daily, 5days/week, for 3 weeks	
3.4.3	Postexposure period	0 days	
3.4.4	**Dermal**		
3.4.4.1	Area covered	Group 1 = 7.5 x 4.5 cm; Group 2 = 10.5 x 6.0 cm, Group 3 = 14.0 x 9.0 cm, Group 4 = 15.0 x 9.5 cm, Group 5 = 15.0 x 13.0 cm, Group 6= 15.0 x 18.0 cm.	
3.4.4.2	Occlusion	Semi-occlusive	
3.4.4.3	Vehicle	Distilled water to moisten the test material (powder)	
3.4.4.4	Concentration in vehicle	Either 1 or 3 mls of distilled water was applied to moisten the test material. Dosage level: Group 1 (Low-1) – 0.41 mg/kg/day; Group 2 (Low-2) – 0.81 mg/kg/day, Group 3 (Mid-1) – 1.63 mg/kg/day, Group 4 (Mid-2) – 3.25 mg/kg/day, Group 5 (High-1) – 6.50 mg/kg/day, Group 6 (High-2) – 13.00 mg/kg/day	
3.4.4.5	Total volume applied	The volume of test material applied varied with the dosage level.	
3.4.4.6	Duration of exposure	6 hours daily	
3.4.4.7	Removal of test substance	The test site was wiped with dry gauze	
3.4.4.8	Controls	No	
3.5	**Examinations**		
3.5.1	Observations	Rabbits were observed twice daily for mortality and moribundity.	
3.5.1.1	Clinical signs	Clinical/cage side observations twice daily. Thorough physical examinations – once each week. Dermal observations daily.	
3.5.1.2	Mortality	Yes –twice daily	
3.5.2	Body weight	Yes – one week prior to treatment and weekly thereafter	
3.5.3	Food consumption	Yes – one week prior to treatment and weekly thereafter	
3.5.4	Water consumption	No	
3.5.5	Ophthalmoscopic examination	No	

Section A 6.03.2-03 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21-day dermal range finding toxicity in rabbits	
3.5.6	Haematology	Prothrombin time – two times prior to treatment (Weeks –1 and 0) and at study termination (week 3) for survivors or at times of death if possible	
3.5.7	Clinical Chemistry	No	
3.5.8	Urinalysis	No	
3.6	**Sacrifice and pathology**		
3.6.1	Organ Weights	No	
3.6.2	Gross and histopathology	Gross pathology – all animals	
3.6.3	Statistics	Repeated measures analysis of variance/covariance.	
		4 RESULTS AND DISCUSSION	
4.2	**Observations**		
4.2.1	Clinical signs	The only dermal observations were slight oedema for one animal in Group 6 and compound residue for all animals. Signs observed at the weekly physical examinations included apparent haematoma to the right and left lateral-abdominal region for one Gp 3 animal and one Gr.4 animal (considered due to the dosing procedure- body wrap); anorexia or hypoactivity for one Gr. 6 animal; few faeces for one Gr. 6 animal; soft faeces for one Gr. 4 animal; crust on left ear for one Gr. 1 animal and one Gr. 4 animal; urine stains for one Gr. 4 animal and one Gr. 6 animal. The signs observed at the AM And PM cageside observations were similar to the signs observed at the weekly physical examinations.	
4.2.2	Mortality	One Gr. 4 animal was found dead on day 20, two Gr. 5 animals were found dead on days 18 and 19, two Gr. 6 animals were found dead on days 10 and 11, and one Gr. 6 animal was sacrificed in a moribund condition on day 9. All other animals survived to the scheduled sacrifice.	
4.3	**Body weight gain**	Animals in all groups lost weight during the first week of the study; the greatest mean weight loss was in Gr. 1,2, and 6 (-91, -79, -251 g respectively). This change was considered due to the dosing procedure (body wrap and collaring required for dermal exposure). In group 6 however, it was considered due to a combination of the dosing procedure and the compound. By the end of the week 2, weight gain for Gr.1-Gr. 5 was normal.	
4.4	**Food consumption and compound intake**	Mean food consumption and mean total food consumption were normal for all groups with one exception – food consumption for Gr. 6 was decreased due to the compound.	
4.5	**Blood analysis**		
4.5.1	Haematology	Mean prothrombin time: Increase in prothrombin time was noted over time in the surviving animals of groups 1	

Section A 6.03.2-03 **Annex Point IIA VI.6.3**	**Subchronic dermal toxicity** 21-day dermal range finding toxicity in rabbits	
	through 5. However, the prolongation at week 3 was not dose-related; the mean increase in group 1 and 3 exceeded that of the surviving animal in group 5. One surviving animal in groups 1 and 4 maintained a normal prothrombin time after week 3. Increasing values were extremely variable over the three lower dose groups, there was no dose-relationship in these groups.	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	No	
4.6.2 Gross and histopathology	Several compound-related findings: dark tissues or dark areas in a tissue; enlarged tissues; fluid in cavities; pale tissues; mottled tissues; depressed area; adhesion; firm tissues; thickened wall; material in lumen; intussuscepted colon. These findings were observed in the lung; liver; kidneys; abdominal cavity; colon; muscle; lymph node; heart; thymus; glandular stomach; pancreas; spleen; subcutaneous tissue; thoracic cavity. A few compound-related findings were noted in groups 1,2,3 and 5 at the terminal sacrifice: dark material or dark areas in a tissue; mottled tissues; adhesion in lungs, muscle, heart, abdominal cavity, and subcutaneous tissue. No other compound-related findings were noted at the scheduled sacrifice.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The toxicity of Chlorophacinone 0.2 % (tracking powder) formulation when applied to New Zealand White rabbits dermally daily for 6 hours, 5 days a week for 3 weeks was evaluated. The dose levels were 0.41 mg/kg/day, 0.81 mg/kg/day, 1.63 mg/kg/day, 3.25 mg/kg/day, 6.50 mg/kg/day, and 13.00 mg/kg/day. The study was performed according to EPA 82-2 guidelines. The methods were compliant with requirements of EC Method B.9.	
5.3 Results and discussion	Doses of 3.25, 6.50, and 13.0 mg/kg/day produced death after 15, 13-14, and 6-8 doses respectively. Physical/cageside observations considered compound-related in these animals were anorexia, few faeces, pale appearance (eyes, body), hypoactivity, cold-to-touch, dyspnoea, and red-coloured urine stains. A general decline in body weight and food consumption was also seen in animals exposed to 3.25 and 6.50 mg/kg/day. An increase in prothrombin time was seen in the animals that survived to the study termination which were exposed to 3.25 and 6.50 mg/kg/day. Evidence of haemorrhage was seen at the necropsy of each of these animals. Doses of 0.41, 0.81, and 1.63 mg/kg/day did not produce	

Section A 6.03.2-03 **Annex Point IIA VI.6.3**	**Subchronic dermal toxicity** 21-day dermal range finding toxicity in rabbits	
	any compound-related physical/cage side observations, body weight effects or food consumption changes. **An increase in prothrombin time was seen during the third week of the study. A few signs of haemorrhage were seen at necropsy of at least one animal at each dose level**. Chlorophacinone applied dermally in the 0.2 % tracking powder form at doses of 3.25 mg/kg/day, 6.50 mg/kg/day, 13.00 mg/kg/day five days per week for 3 weeks can produce death. Doses of 0.41 mg/kg/day, 0.81 mg/kg/day, and 1.63 mg/kg/day did not produce death, but did produce an increase in prothrombin time values indicative that exposure to Chlorophacinone had occurred. A range of dose levels recommended for the subsequent study to achieve definitive effect and no-effect dose levels should be: Less than 0.1, 0.1 to 0.5, 0.5 to 2.5 mg/kg/day of chlorophacinone for the low, mid and high-dose groups, respectively.	
5.4 Conclusion	Dermal application of 0.2% tracking powder produced signs of toxicity. It was recommended that 0.2% tracking powder be tested in a subsequent rangefinding study.	
5.4.1 LO(A)EL	Not applicable	
5.4.2 NO(A)EL	Not applicable.	
5.4.3 Reliability	1	
5.4.4 Deficiencies	No deficiencies were noted.	

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	Applicant version is adopted
Results and discussion	Applicant version is adopted
Conclusion	Dermal application of 0.2% tracking powder produced signs of toxicity. It was recommended that 0.2% tracking powder be tested in a subsequent range finding study. Dose of 0.4 and 0.8 mg/kg/d so some signs of haemorrhage without mortality
Reliability	3 Range finding study for information only
Acceptability	Acceptable but not usable for assessment, only as range finding study
Remarks	

Table A 6.3.2-3: Results of subchronic toxicity study

Parameter	Dose mg/kg/day					
	0.41	**0.81**	**1.63**	**3.25**	**6.50**	**13**
Number of animals examined	3	3	3	3	3	3
Mortality	0	0	0	1	2	3
Clinical signs*	No abnormal dermal observ.	No abnormal dermal observ	No abnormal dermal observ; apparent hematoma to the right and left lateral-abdominal region.	Anorexia, pale eyes, crust on left ear, few feces, apparent hematoma on ventral-cervical abdominal region	Anorexia, hypoactivity, few feces, apparent hematoma to the right and left lateral-abdominal region	Cold to touch, entire body pale,anorexia, hypoactivity, few feces, dyspnea, urine stains
Body weight	Loss -91g 1 week; normal week 2	Loss -79 g 1 week; normal week 2	Loss -54 g 1 week; normal week 2	Loss -14 g 1 week; normal week 2	Loss -68 g 1 week; normal week 2	Loss -251 g 1 week; not normalised
Food consumption	N	N	N	N	N	decreased
Haematology Prothrombin time	↑	↑	↑	↑	↑	↑
Gross pathology	No pathology findings	Not remarkable pathology findings One animal-mottled atrium, one animal– adhesion of abdominal cavity	Not remarkable pathology findings One animal-mottled atrium	Terminal sacrifice - one animal – dark areas in muscles; one animal - dark material in subcutaneous tissue Necropsy – dark areas in lings, pale area in liver, pale kidney, fluid in abdominal and thoracic cavity, dark enlarged thymus	Necropsy – dark muscles, dark area in ventricle, dark enlarged thymus, stomach- pale mucosa, dark areas, gelatinous subcutaneous tissues, fluid and adhesion in thoracic cavity	Necropsy - dark area in lungs, liver –pale, prominent reticular pattern, pale kidneys, fluid in abdominal cavity, dark adipose tissue, colon-dark serosa, intussusceptions, dark enlarged thymus, gelatinous muscles, mottled atrium and ventricles, fluid in pericardiac sac, pale pancreas and spleen, fluid in thoracic cavity.

Section A 6.03.2-04 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxx X. (XXXXx): **21-Day Dermal Toxicity Study in Rabbits** with Chlorophacinone; Unpublished report No: XXXXXXXXX (February 6,XXXX); XXXXXXXXXXXX XXXXXXXXXXX (Dates of experimental work July 1, XXXX – July 23, XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA 82-2. In accordance with EC Method B.9.	
2.3	**GLP**	Yes	
2.4	**Deviations**	Several minor deviations from the protocol were noted. Necropsies were to be performed within two hours after animals were found dead according to the protocol; the following animals were not necropsied within the designated time: Group 4 No. 02538 and Group 4 No. 02364. Liver with gallbladder weight was to be taken at necropsy, as stated by protocol; Group 4 animal No. 02363 had no liver weight due to technician error. On July 3-5, 1991, Group 4 animal No.02363 was dosed with 2.7702 grams of test material, instead of the calculated dose of 2.7700 g. Food consumption was to be performed weekly as stated by protocol, on July 15, 1991, three Group 1 males inadvertently had no empty feeder weight taken. Therefore, during that week there were no food consumption values for those animals. Body weights were to be performed weekly as stated by protocol, but were collected on Day 21, instead on Day 22. It was necessary to collect body weights prior to fasting the animals for terminal sacrifice, in order to have accurate terminal body weights and organ weights. These deviations did not impact the integrity of the study.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone 0.2% Tracking powder (clay chlorophacinone mixture)	
3.2.1	Lot/Batch number	Lot No: XXXXX	
3.2.2	Specification	Not specified	

Section A 6.03.2-04 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits	
3.2.2.1	Description	Light green powder	
3.2.2.2	Purity	**XXX %**	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Rabbit	
3.3.2	Strain	New Zealand White Hra:(NW) SPF)	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Males and females	
3.3.5	Age/weight at study initiation	17.5 weeks Males 2283-2585g Females 2255-2891g	
3.3.6	Number of animals per group	**Ten: 5 males and 5 females**	
3.3.7	Control animals	Yes	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Duration of treatment	3 weeks	
3.4.2	Frequency of exposure	5 days per week	
3.4.3	Postexposure period	0 days	
3.4.4	**Dermal**		
3.4.4.1	Area covered	Group 2 = 2 x 2 cm; Group 3 = 4x 4 cm, Group 4 = 8 x 8 cm.	
3.4.4.2	Occlusion	**Semi-occlusive**	
3.4.4.3	Vehicle	Distilled water to moisten the test material (powder)	
3.4.4.4	Concentration in vehicle	A vehicle was not used. Dosage level: Group 1 (Control) – 0 mg/kg/day; Group 2 (Low) – 0.08 mg/kg/day, Group 3 (Mid) – 0.40 mg/kg/day, Group 4 (High) – 2.00 mg/kg/day	
3.4.4.5	Total volume applied	The volume of water used to moisten the test material varied with the dose level. One ml of distilled water was applied to all groups except Group 4. Two mls of test material was applied to Group 4. The dose was applied as a powder, by weight, based on most recent body weight .	
3.4.4.6	Duration of exposure	6 hours	
3.4.4.7	Removal of test substance	The test site was wiped with dry gauze	
3.4.4.8	Controls	Distilled water	
3.5	**Examinations**		

Section A 6.03.2-04 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits	
3.5.1	Observations	The rabbits were observed for mortality and moribundity twice daily.	
3.5.1.1	Clinical signs	Twice daily	
3.5.1.2	Mortality	Yes	
3.5.2	Body weight	Yes – one week prior to treatment and weekly thereafter (exception week 3)	
3.5.3	Food consumption	Yes – one week prior to treatment and weekly thereafter (exception week 3)	
3.5.4	Water consumption	No	
3.5.5	Ophthalmoscopic examination	No	
3.5.6	Haematology	Samples for prothrombin time were collected three times prior to treatment from all animals. Blood samples for haematology were collected from all surviving animals at study termination. Parameters studied: Erythrocyte count (RBC), haematocrit (HCT), haemoglobin (Hb), platelet count, mean cell volume, mean cell haemoglobin, prothrombin time, mean cell haemoglobin concentration MCHC, leukocyte count, corrected leukocyte count, differential leukocyte count, activated partial thromboplastin time.	
3.5.7	Clinical Chemistry	Yes Parameters: sodium, potassium, glucose, albumin, globulin, total bilirubin, A/G ratio, aspartate aminotransferase, alanine aminotransferase, urea nitrogen, calcium, inorganic phosphorus, creatinine.	
3.5.8	Urinalysis	No	
3.6	**Sacrifice and pathology**		
3.6.1	Organ Weights	Yes – liver with drained gallbladder, kidneys, testes with epididymis, ovaries, uterus	
3.6.2	Gross and histopathology	Yes - all dose groups Organs: skin –treated and untreated, lungs, ovaries, uterus, liver with gallbladder, kidneys, testis with epididymis.	
3.6.3	Statistics	Absolute body weights, body weight change, food consumption, clinical pathology data and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups.	
		4 RESULTS AND DISCUSSION	
4.2	**Observations**		
4.2.1	Clinical signs	The only dermal observations were compound residue for all animals in all treatment groups. Signs observed at the weekly physical examinations included pale eyes for one Gr.4 animal; anorexia for one Gr. 2 and two Gr. 4 animals; lacrimation for one Gr. 1 animal;	

Section A 6.03.2-04 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits	
		dyspnea for two Gr. 4 animals; few faeces for one Gr. 2 and one Gr. 4 animals; urine stains for one gr. 4 animals; sores on the right dorsal cervical region for one Gr. 2 animal; sores on the left ear for one Gr. 1 animal and one Gr.3 animal. Cage side observations: pale eyes for three Gr.4 animals PM and one Gr.4 animal AM; anorexia for five Gr. 4 and one Gr. 2 animals PM and for two Gr. 4 and one Gr. 2 animals AM; hypoactivity for two Gr.4 animals PM; dyspnea for three Gr. 4 animals PM and two Gr. 2 animals AM	
4.2.2	Mortality	Five Gr. 4 animals were found dead - one male each on day 14,15,16, 18, and one female on day 21. All other animals survived to the scheduled sacrifice.	
4.3	**Body weight gain**	Animals in all groups lost weight during the first week of the study; the greatest mean weight loss was in Gr. 1 and 4 males, and in Gr. 2 and 4 females (-88, -86, -113, and –110 grams respectively). This change was considered mainly due to the dosing procedure - body wrap and collaring required for dermal exposure. All animals appear to have recovered body weight by the second week. Overall, data were comparable among all groups.	
4.4	**Food consumption and compound intake**	Data were generally comparable among all groups.	
4.5	**Blood analysis**		
4.5.1	Haematology	Prothrombin and activated partial thromboplastin time: In the male animals, no statistically significant differences, however, dose-related increase (not statistically significant) in mean values for prothrombin time was observed in Gr. 3 and 4. Female data showed significantly prolonged mean values for prothrombin time in all groups and prolonged activated partial thromboplastin time in mid- and high-dose groups. The mean prothrombin time in low dose females was slightly increased based on the mean of the concurrent controls, at week 3, and was comparable to the mean of the three pre-treatment intervals for each of those animals. Female animals in the concurrent control for the week 3 intervals showed a statistically significant decrease based on their own three pre-treatment values. This gave rise to the statistical significance in the Gr. 2 females value. The subsequent statistical analyses indicated no significant increase in the female prothrombin time values at the low dose.	
4.5.2	Clinical chemistry	Mild but significant decrease in the glucose values for female in Gr. 3 and 4. Mild decrease in the albumin values without change in the albumin/globulin ratio. No statistically significant changes for the males during week 3.	

Section A 6.03.2-04 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	There were no statistically changes noted, organ weight data were comparable in all groups.	
4.6.2	Gross and histopathology	Gross pathology: Several findings at necropsy of the animals found dead: dark areas in regions of the muscle, kidneys, thymus, lungs, lymph nodes, eyes; enlarged, mottled thymus; pale tissues in liver, kidneys, heart, eyes; fluid in the lumen of trachea, thoracic cavity, pericardial sac; adhesions in thoracic cavity; prominent reticular pattern in the liver, gelatinous subcutaneous tissue. Findings at terminal sacrifice most in Gr. 4: dark areas in regions of the muscle, urinary bladder, glandular stomach, thymus, lungs, lymph nodes, mottled heart, pale or pale areas in liver, kidneys, eyes, depressed areas in liver and kidneys, enlarged thymus and lymph nodes, firm lymph nodes, prominent reticular pattern in the liver. Histopathology: Primary treatment-related effects occurred in the liver, described as a "prominent reticular pattern" (centrilobular liver necrosis) of a moderate to severe degree in three males and one female of the high-dose group. This finding was not seen in animals of the mid- and low-dose groups.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The subchronic dermal toxicity of Chlorophacinone 0.2 % (tracking powder) formulation when applied to New Zealand White rabbits dermally 5 days a week for 3 weeks was evaluated. The dose levels were 0.08 mg/kg/day, 0.40 mg/kg/day, and 2 mg/kg/day. The study was conducted according to EPA 82-2 guidelines and the method was compliant with EC Method B.9.	
5.3	**Results and discussion**	The 2.00 mg/kg/day dose produced death after 10 to 15 doses. Five Gr. 4 animals were found dead - one male each on day 14,15,16, 18, and one female on day 21. Widespread fresh haemorrhages into parenchymal organs and body cavities were observed, no haematological indications of a response to a blood loss – suggestive that haemorrhages occurred prior to death and were likely the foremost cause of death. Evidence of haemorrhage and moderate to moderately severe centrilobular liver necrosis was seen at the necropsy of each of the animals. Cage side/clinical compound-related signs included anorexia, few faeces, pale eyes, hypoactivity, and dyspnea. The 0.40 mg/kg/day dose did not produce any clinical/clinical compound-related signs, mortality, body weight and food consumption changes, nor changes in gross	

Section A 6.03.2-04 **Annex Point IIA VI.6.3**	**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits	
	pathology or histopathology. All animals lost weight during first week but had recovered it by the second week. Compound related increase in prothrombin values in the males and females of the mid- and high-dose groups. In the male animals, no statistically significant differences, however, dose-related increase (not statistically significant) in mean values for prothrombin time was observed in Gr. 3 and 4. Female data showed significantly prolonged mean values for prothrombin time in all groups and prolonged activated partial thromboplastin time in mid- and high-dose groups. The mean prothrombin time in low dose females was slightly increased based on the mean of the concurrent controls, at week 3, and was comparable to the mean of the three pre-treatment intervals for each of those animals. Female animals in the concurrent control for the week 3 intervals showed a statistically significant decrease based on their own three pre-treatment values. This gave rise to the statistical significance in the Gr. 2 females value. The subsequent statistical analyses indicated no significant increase in the female prothrombin time values at the low dose. The low dose (0.08 mg/kg/day) was considered to be the no effect level for both males and females.	
5.4 Conclusion	A dose of 0.40 mg chlorophacinone/kg/day did not produce any clinical/clinical compound-related signs, mortality, body weight and food consumption changes, nor changes in gross pathology or histopathology. At higher doses death occurred as a result of a developing haemorrhagic syndrome seen clinically as signs of ataxia, hypoactivity, pallor and pale eyes with elongated prothrombin times. The effects were confirmed by necropsy observation of widespread haemorrhages, body cavities containing free fluid and pale organs.	
5.4.1 LO(A)EL	0.40 mg/kg/day	
5.4.2 NO(A)EL	0.08 mg/kg/day	
5.4.3 Reliability	1	
5.4.4 Deficiencies	No significant deficiencies were noted.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2005 (Revised 27 December 2005)	

Section A 6.03.2-04 Annex Point IIA VI.6.3	Subchronic dermal toxicity 21 day dermal toxicity study in rabbits	
Materials and Methods	The Applicant version is adopted with some remarks: It was used the formulation tracking power containing 0.2 % of active substance. It was applied to New Zealand White rabbits dermally 5 days a week for 3 weeks to 10 animals (5 males and 5 females) per group. The dose levels of active substance were 0.08, 0.40 and 2 mg/kg/day. The study was conducted according to EPA 82-2 guidelines and compliant with EC Method B.9	
Results and discussion	The Applicant version is adopted Mortalities: At 2.00 mg/kg/day dose 4 males died on day 14, 15, 16, 18, and one female on day 21. Widespread fresh haemorrhages into parenchymal organs and body cavities were observed and were likely the foremost cause of death. Evidence of haemorrhage and moderate to moderately severe centrilobular liver necrosis was seen at the necropsy of each of the animals. Cage side/clinical compound-related signs included anorexia, few faeces, pale eyes, hypoactivity, and dyspnea. A dose of 0.40 mg chlorophacinone/kg/day did not produce any clinical/clinical compound-related signs, mortality, body weight and food consumption changes, nor changes in gross pathology or histopathology. All animals lost weight during first week but had recovered it by the second week. Compound related increase in prothrombin values were observed in the males and females of the 0.4 and 2 mg/kg/day dose groups. The low dose (0.08 mg/kg/day) was considered to be the no effect level for both males and females.	
Conclusion	The study allows getting **NOAEL by dermal exposure as 0.08 mg/kg/d** in rabbit dosed as tracking power formulation being the most sensitive observation the alteration of prothrombin times which was observed at 0.4 and 2 mg/kg/day. The mid dose of 0.4 mg/kg bw did not produce compound related clinical signs mortality nor histopathological changes. The highes dose of 2 mg/kg/day caused hig mortality (4/5 males and 1/5 females). The substance was used in “tracking powder” formulation (0.2 % Chlorophacinone).	
Reliability	2 The conclusion has not general value but only for the formulation used as tracking powder	
Acceptability	Acceptable	
Remarks	The relevant of the study is conditioned to the use of the formulation used. It is needed to do comparison with study done with active substance to confirm the general value of this study.	

Table A 6.3.2-4: Results of subchronic dermal toxicity study in rabbits

Parameter	Control		0.08 mg/kg/day		0.04 mg/kg/day		2.00 mg/kg/day		dose-response +/-	
	M	F	M	F	M	F	M	F	M	F
Number of animals examined	5	5	5	5	5	5	5	5		
Mortality	0	0	0	0	0	0	4	1		
Clinical signs	Lacrimation right eye	Sore on left ear	Sores dorsal-cervical	Anorexia few feces	Sores on left ear	Normal	Anorexia, Dyspnea, Pale eyes, Hypoactive, Few feces	Pale eyes, Anorexia, Few feces, Dyspnea, Urine stains, Hypoactive	+	+
Body weight	Loss -88g week 1; recover week 2	Loss -65g week 1; recover week 2	Loss -51g week 1; recover week 2	Loss -113g week 1; recover week 2	Loss -85g week 1; recover week 2	Loss -65g week 1; recover week 2	Loss -86g week 1; recover week 2	Loss -110g week 1; recover week 2	-	-
Food consumption*										
Clinical chemistry	Normal	Normal	Normal	Decrease glucose	Normal	Decrease albumin	Normal	Decrease glucose and albumin	_	_
Haematology	Normal	Normal	Normal	Normal	No statistically different changes	Significantly increased Prothrombin time and activated thromboplastin time	No statistically different changes	Significantly increased Prothrombin time and activated thromboplastin time	-	+
Organ weights *										
Gross pathology	Normal	Normal	Not remarkable pathology findings, one animal-small testis	Not remarkable pathology findings	Not remarkable pathology findings	Not remarkable pathology findings	**	**	+	+
Histopathology	Normal	Normal	Normal	Normal	Normal	Normal	***	***	+	+

* data comparable among groups

**dark areas in regions of the muscle, kidneys, thymus, lungs, lymph nodes, eyes; enlarged, mottled thymus; pale tissues in liver, kidneys, heart, eyes; fluid in the lumen of trachea, thoracic cavity, pericardial sac; adhesions in thoracic cavity; gelatinous subcutaneous tissue

*** prominent reticular pattern (centrilobular liver necrosis) of a moderate to severe degree. Evidence of hemorrhage observed in several tissues.

Section A 6.03.2-05 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits – dose rangefinding study	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxx X. (XXXXx): 21-Day Dermal Rangefinding Toxicity Study in Rabbits with Chlorophacinone; Unpublished report No: XXXXXXXXX (February 6, XXXX); XXXXXXXXXXXXXXXXXXXXX (Dates of experimental work February, XXXX – May, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA 82-2. In accordance with EC Method B.9.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deficiencies were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in the study as: Phase One: Chlorophacinone-Liphadione 0.00051% pelleted end-use product ground into a powder Phase Two: Chlorophacinone 2% dry concentrate (a cornstarch-chlorophacinone mixture). Chlorophacinone 0.2% Tracking powder (clay chlorophacinone mixture)	
3.2.1	Lot/Batch number	Chlorophacinone-Liphadione - lot No: XXXX Rozol tracking powder – lot No: XXXX Rozol 2% dry concentrate lot No: XXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Chlorophacinone 0.00051% Green pellets Rozol tracking powder –brownish powder Rozol 2% dry concentrate - off-white brownish powder	
3.2.2.2	Purity	Chlorophacinone – Liphadione - 0.0051% Rozol tracking powder – 0.2% Rozol 2% dry concentrate – 1.92%	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Rabbit	
3.3.2	Strain	New Zealand White (Hra:(NZW) SPF)	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Male	

Section A 6.03.2-05 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits – dose rangefinding study	
3.3.5	Age/weight at study initiation	14 to 16 weeks. 2006 to 2992g	
3.3.6	Number of animals per group	3	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Dermal	
3.4.1	Duration of treatment	3 weeks	
3.4.2	Frequency of exposure	6h daily, 5days/week	
3.4.3	Postexposure period	0 days	
3.4.4	**Dermal**		
3.4.4.1	Area covered	Phase one: Group 1 = 7.5 x 4.5 cm; Group 2 = 10.5 x 6.0 cm, Group 3 = 14.0 x 9.0 cm, Group 4 = 15.0 x 9.5 cm, Group 5 = 15.0 x 13.0 cm, Group 6= 15.0 x 18.0 cm. Phase two: 15.0 x 18.0 cm	
3.4.4.2	Occlusion	Semi-occlusive	
3.4.4.3	Vehicle	Distilled water to moisten the test material (powder).	
3.4.4.4	Concentration in vehicle	Phase one Chlorophacinone-Liphadione 0.00051% pelleted end-use product dosage levels: Group 1 (Low-1) – 0.001 mg/kg/day; Group 2 (Low-2) – 0.003 mg/kg/day, Group 3 (Mid-1) – 0.01 mg/kg/day, Group 4 (Mid-2) – 0.03 mg/kg/day, Group 5 (High-1) –0.1 mg/kg/day, Group 6 (High-2) – 0.3 mg/kg/day Phase two dosage levels: Group 1 - Rozol tracking powder 0.2% - 13 mg/kg/day Group 2 - Rozol 2% dry concentrate – 125 mg/kg/day	
3.4.4.5	Total volume applied	Dose applied as moistened powder by weight adjusted for bodyweight.	
3.4.4.6	Duration of exposure	6 h	
3.4.4.7	Removal of test substance	The test site was wiped with dry gauze	
3.4.4.8	Controls	No	
3.5	**Examinations**		
3.5.1	Observations		
3.5.1.1	Clinical signs	Dermal and clinical signs – twice daily	
3.5.1.2	Mortality	Yes – twice daily	
3.5.2	Body weight	Yes – upon receipt, at randomisation, and weekly thereafter	
3.5.3	Food consumption	Yes – one week prior to treatment and weekly thereafter	

Section A 6.03.2-05 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits – dose rangefinding study	
3.5.4	Water consumption	No	
3.5.5	Ophthalmoscopic examination	No	
3.5.6	Haematology	Yes - prothrombin time (phase one only) two times prior to treatment from all animals and at study termination from all animals.	
3.5.7	Clinical Chemistry	No	
3.5.8	Urinalysis	No	
3.6	**Sacrifice and pathology**		
3.6.1	Organ Weights	No	
3.6.2	Gross and histopathology	Yes - all dose groups; examination of the external surface, all orifices, cranial cavity, carcass, external surface of the brain, the spinal cord, cut surfaces of the spinal cord and the brain, nasal cavity and paranasal sinuses, cervical tissues and organs, thoracic, abdominal and pelvic cavities and their viscera, signs of hemorrhage.	
3.6.3	Statistics	Body weight, prothrombin time, and food consumption values were analysed by repeated measures analysis of variance/covariance.	
		4 RESULTS AND DISCUSSION	
4.2	**Observations**		
4.2.1	Clinical signs	Phase one: The only dermal observations were slight erythema for one animal in Groups 3, 4, 6, slight edema for one animal in each of Groups 2, 3, 4, and compound residue for all animals. Signs observed at the weekly physical examinations included lacrimation from both eyes of one Gr. 3 animal; apparent hematoma in the right lateral-abdominal region for two Gr. 1 animals, one Gr. 5 animal and one Gr. 6 animal; apparent hematoma in the left lateral-abdominal region for two Gr. 1 animals, one Gr. 2 animal, two Gr. 3 animals, two Gr. 4 animals, one group 5, and two Gr.6 animals; apparent hematoma in the dorsal region for one Gr. 2 animal and one Gr. 3 animal; and apparent hematoma in the lumbar region for one Gr. 3 animal (these signs considered due to the dosing procedure-body wrap). Cage side observations similar to clinical signs. Phase two: The only dermal observation was compound residue for all animals exposed to tracking powder. Signs observed at the weekly physical examinations included lacrimation from both eyes of one Gr. 1 animal; apparent hematoma in the right and left lateral-abdominal region for one Gr. 1 animal; cold to the touch for one Gr. 1 animal; entire body pale for one Gr.1 and one Gr. 2 animals;	

Section A 6.03.2-05 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits – dose rangefinding study	
		anorexia or hypoactivity for one Gr. 1 and Gr. 2 animal; few or soft faeces for one Gr. 1 animal. Cage side observations similar to clinical signs.	
4.2.2	Mortality	Mortality: Phase one: All animals survived to the scheduled sacrifice. Phase two: Two animals exposed to the tracking powder (after the first 9 doses administered) were found dead on days 11 and 15, two animals exposed to the dry concentrate (after the first 5 doses administered) died on days 6 and 7, and one animal exposed to the dry concentrate was sacrificed in a moribund condition on day 8.	
4.3	**Body weight gain**	Body weight: Phase one: Animals in all groups lost weight over the entire study; the greatest weight loss was in Gr. 3,5,6 (-107, -107, and –173 grams respectively). The change considered due to the dosing procedure - body wrap and collaring required for dermal exposure. Most weight loss occurred during the first week of study; thereafter body weight loss was notably less. Phase two: All animals lost weight except for the Gr. 1 animals, which survived to termination of the study. The change in body weight was considered due to a combination of the dosing procedure and the compound.	
4.4	**Food consumption and compound intake**	Food consumption: Phase One: Mean data indicated some statistically significant differences among the groups without any particular pattern. This was considered due to the dosing procedure (body wrap and collaring required for dermal exposure). Phase two: Due to the number of deaths and the feed spillage, there was no data to evaluate.	
4.5	**Blood analysis**		
4.5.1	Haematology	Mean prothrombin time: Values at study termination were similar to pre-treatment values for the phase one animals, no data for phase two.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Not performed	
4.6.2	Gross and histopathology	Phase one: Crusty material on the treated skin of one Gr. 1, one Gr. 2., one Gr. 5 and two Gr.6 animals; thickened skin on one Gr. 1, one Gr.2, one Gr. 3 and two Gr. 4 animals. Finding considered due to the wrapping procedure. No other gross lesions were observed. Phase two: Several compound-related gross lesions - dark tissues or dark areas in a tissue; enlarged tissues; fluid in cavities; pale tissues; crusty material, sore; adhesion; prominent reticular pattern in liver; and gelatinous muscles. These findings were observed in treated skin, thymus,	

Section A 6.03.2-05 **Annex Point IIA VI.6.3**		**Subchronic dermal toxicity** 21 day dermal toxicity study in rabbits – dose rangefinding study	
		thoracic cavity, heart, lung; liver; muscle, lymph node, thoracic aorta, abdominal cavity, urinary bladder, glandular stomach; and kidneys of the Gr. 1 and 2 animals.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The dermal toxicity of Chlorophacinone in three formulations was evaluated: Chlorophacinone-Liphadione 0.00051 % pelleted end-use product, Chlorophacinone 2% dry concentrate, and Chlorophacinone 0.2% Tracking powder when applied to New Zealand White rabbits dermally 5 days a week for 3 weeks. The dose levels for the Phase one Chlorophacinone-Liphadione 0.00051 % pelleted end-use product were 0.001 mg/kg/day, 0.003 mg/kg/day, 0.01 mg/kg/day, 0.03 mg/kg/day, and 0.1 mg/kg/day, 0.3 mg/kg/day. Phase two dosage levels: Rozol 0.2% - 13 mg/kg/day; Rozol 2% dry concentrate – 125 mg/kg/day. The study was performed according to EPA 82-2 guidelines and was in accordance with requirements of EC Method B.9.	
5.3	**Results and discussion**	In Phase One of the study, no-compound-related changes occurred in survival, dermal/clinical/cage side observations, body weight, and food consumption, prothrombin time or necropsy findings. In Phase Two of the study, two animals exposed to the tracking powder (after the first 9 doses administered) and three animals exposed to the dry concentrate (after the first 5 doses administered) died. Compound-related changes in dermal /clinical/cage side signs, body weight, food consumption, necropsy were seen in the animals that died. One animal exposed to 15 doses of the tracking powder survived to the study end and did not exhibit compound-related changes. Chlorophacinone, applied dermally in the 0.2 % Tracking powder and the 2% dry concentrate forms produced signs of toxicity. It is recommended that the 0.2 % Tracking powder be tested in a subsequent rangefinding study.	
5.4	**Conclusion**	Chlorophacinone, applied dermally in the 0.2% Tracking powder and the 2% dry concentrate forms produced signs of toxicity. It is recommended that the 0.2 % Tracking powder be tested in a subsequent rangefinding study.	
5.4.1	LO(A)EL	Not applicable for rangefinding study	
5.4.2	NO(A)EL	Not applicable for rangefinding study	
5.4.3	Reliability	2	
5.4.4	Deficiencies	No	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2005 (revised 21 December 2005)	
Materials and Methods	The description of the applicant version is accepted but summarised as follows: Three formulations was evaluated containing chlorophacinone as active substance: Three animals per groups were applied to New Zealand White rabbits dermally 5 days a week for 3 weeks, 6 hours daily Phase one Chlorophacinone-Liphadione 0.00051% pelleted end-use product Dose level: 0.001, 0.003, 0.01, 0.03, 0.1 and 0.3 mg/kg/day. Phase two dosage levels: Group 1. Rozol tracking powder 0.2%: 13 mg/kg/day Group 2. Rozol 2% dry concentrate: 125 mg/kg/day The study was performed according to EPA 82-2 guidelines and was in accordance with requirements of EC Method B.9. No control applied.	
Results and discussion	The description of the applicant version is accepted. Phase one with Liphadione in dose range of 0.001 to 0.3 mg/kg/day All animals survived to the scheduled sacrifice. Animals in all groups lost weight over the entire study and showed some significant difference in food consumption. The changes were considered due to the dosing procedure (body wrap and collaring required for dermal exposure). Most weight loss occurred during the first week of study; thereafter body weight loss was notably less. Some alteration in skin interpreted as due to the wrapping procedure. No other gross lesions and clinical signs were observed. Phase two- Group 1 "Rozol tracking powder 0.2%" (dose level of 13 mg/kg/day). Two of three animals exposed to the tracking powder died on days 11 and 15 (after the first 9 doses administered). Clinical signs included lacrimation from both eyes, apparent hematoma in the right and left lateral-abdominal region, cold to the touch; entire body pale anorexia or hypoactivity few or soft faeces. Phase two- Group 2" Rozol 2% dry concentrate (dose level of 125 mg/kg/day). Two animals exposed to the dry concentrate (after the first 5 doses administered) died on days 6 and 7, and the other was sacrificed in a moribund condition on day 8. Clinical signs included anorexia, hypoactivity, entire body pale, urine stains, apparent hematoma in the right lateral-abdominal region. In both groups, lost weight were observed and considered due to a combination of the dosing procedure and the compound.	

Conclusion	A dose range finding study none using the active substance but three formulated preparations. Doses of active substance at 0.3 mg/kg/day or lower applying " Chlorophacinone-Liphadione 0.00051% pelleted end-use product" is not causing lethality and other significan toxicity. Dose level of 13 mg/kg/day applying dermally Rozol tracking powder 0.2% and dose level of 125 mg/kg/day or Rozol 2% dried concentrate are causing severe effect with lethality. As no controls are applied it cannot be dilucidated from this study if effect are due to active substance or to other components of the formulation, although the observed effect are in acordance with toxicity of active substance in other studies.
Reliability	3. This is a range finding study.
Acceptability	Not accepted for assessment
Remarks	

Table A 6.3.2-5: Phase One - Chlorophacinone-Liphadione 0.00051% pelleted end-use product

Parameter	0.001 mg/kg/day	0.003 mg/kg/day	0.01 mg/kg/day	0.03 mg/kg/day	0.1 mg/kg/day	0.3 mg/kg/day
Number of animals examined	3	3	3	3	3	3
Mortality	0	0	0	0	0	0
Clinical signs	Apparent hematoma Lateral abdominal-right, left	Apparent hematoma Lateral abdominal-right, left	Apparent hematoma Lateral abdominal-right, left, dorsal, lumbar Lacrimation Swollen penis	Apparent hematoma Lateral abdominal-right	Apparent hematoma Lateral abdominal-right	Apparent hematoma Lateral abdominal-right. Swollen penis
Body weight	-88g week 1	-65g week 1	-71g week 1	-47g week 1	-156g week 1	-135g week 1
Food consumption^	^	^	^	^	^	^
Haematology*	*	*	*	*	*	*

Gross pathology	crusty material, thickened skin, no other remarkable pathology findings	crusty material, thickened skin, no other remarkable pathology findings	thickened skin, no other remarkable pathology findings	thickened skin, no other remarkable pathology findings	crusty material on the skin, no other remarkable pathology findings	crusty material on the skin, no other remarkable pathology findings

^ Mean data indicated some statistically significant differences among the groups without any particular pattern. This was considered due to the dosing procedure (body wrap and collaring required for dermal exposure).

***** Mean prothrombin time: Values at study termination were similar to pre-treatment values for the phase one animals

Table A 6.3.2-6: Phase Two - Rozol 0.2% and Rozol 2% dry concentrate

Parameter	**Rozol 0.2 % - 13 mg/kg/day**	**Rozol 2% dry concentrate - 125 mg/kg/day**
Number of animals examined	3	3
Mortality	2	3
Clinical signs	Lacrimation from both eyes, apparent hematoma in the right and left lateral-abdominal region, cold to the touch; entire body pale anorexia or hypoactivity few or soft faeces	Anorexia, hypoactivity, entire body pale, urine stains, apparent hematoma in the right lateral-abdominal region
Body weight	Loss -39 g week 1	Loss -255g week 1
Food consumption*		
Gross pathology	Enlarged dark thymus, dark material in thoracic cavity, pale ventricle and atria, pale liver, prominent reticular pattern, pale kidney, dark material in abdominal cavity, dark adipose tissue Terminal sacrifice – sore skin, thickened area, no other abnormalities.	Sore skin, crusty material, enlarged dark thymus, fluid and adhesions in thoracic cavity, pale ventricle and atria, dark lung, dark, gelatinous muscles, pale liver, prominent reticular pattern, dark material in abdominal cavity

* Due to the number of deaths and the feed spillage, there were no data to evaluate

Section A 6.03.3-01 Annex Point IIA, 6.3.3	Repeated dose inhalation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	A repeat dose inhalation study is not required. An acute inhalation study showed that the molecule is acutely toxic. The LC50 for male and female rats was – 9.3 µg/L. Appropriate protection measures (6.12.1) ensure no exposure to the (powdered) technical material or to the products during the production process. The active ingredient is not volatile. The acutely toxic nature of the material combined with its potential for hepatic accumulation, is such that repeated exposure to lower doses will result in death by induction of a haemorhagic syndrome with associated acute clinical signs of reaction to treatment (see 6.1.1, 6.1.2 or 6.1.3 for indications of haemorrhagic syndrome). The mechanism of clotting inhibition caused by hydroxy coumarin-type anticoagulant rodenticides is dependent on inhibition of vitamin K epoxide or vitamin k reductases and is unaffected by route of application. Therefore specific repeat dose studies would not provide any additional useful information. As the outcome of such a study can be predicted from the knowledge on mode of action and acute inhalation exposure, performing a repeat administration study would contravene Directive 86/609/EC which militates against unnecessary testing using animals	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2004	
Evaluation of applicant's justification	Arguments are reasonable but there are some concerns. Directive requires repeated dose study and the TNG of data requirement (introduction to point 6.3) indicate that the "required route of administration is the oral route". So inhalation study is not obliged as a primary required route "unless it can be justified that an alternative route is more appropriate". Point 6.3.3 state that alternative or additional inhalation route is required "for volatile substances (vapour pressure >1x 10 Pa) or in cases where the potential inhalation exposure is significant", and "in some cases (e.g. aerosols and dusts/particulate matter)" The study is not required if a 90 days study are available. This is the case for oral but not for inhalation. So justification should be by "scientifically unjustified" or "technically not feasible"	

Section A 6.03.3-01 Annex Point IIA, 6.3.3	**Repeated dose inhalation**
	Applicant argues the non-submission of **repeated dose inhalation** study as scientifically unjustified on the basis of: (a) "Compound is not volatile". However if product is used in powder then the potential inhalation exposure is depending of particle size (<50 um?), and this data is not indicated. (b) "As a results of acutely toxicity nature the repeated dose will results in death of animals at the "lowest dose" Which is the lowest dose? Under general consideration in point 1 is said: "The study is technically not possible to perform. The intrinsic physico-chemical or other (e.g. toxicological) properties of the rodenticidal active substances are such that specific route of exposure cannot be tested or not all tests can be performed". The "technical difficulty" argued is that it is high acutely toxic by inhalation. This can overcome either testing lower relevant doses or to choice appropriate relevant species. To accept non-submission then should be proved that expected exposure are actually much lower than the lowest dose that is reasonable to be tested.
Conclusion	**Accepted** Arguments are reasonable but there are some concerns. So justification could be provisionally accepted depending of further detail evaluation of the following data: (a) potential exposure by inhalation and indication of particle size, (b) inhalation acute toxicity of other related chemicals, oral repeated dose and subchronic oral study
Remarks	Under the "Addendum to the TNsG on Data Requirements" for rodenticides waiving of repeated dose study is not considered and there is not specific indication for inhalation assay.

Section A 6.04.1-01 **Annex Point IIA VI.6.4**		**Subchronic oral toxicity** 3 month toxicity study on rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	XXXX X., XXXXXXXXX., XXXX X. (XXX): 3 Month Toxicity Study on Rats by Oral Method Chlorophacinone (LM-91). Unpublished report No: XXXXXXXXXX (December 18, XXXX). XXXXXXXXXXXXXXXXX, France (Dates of experimental work June 1984 – November XXXX). Reformat prepared by XXXXXXXXXXXXXXXXXXX XXXXXXXXXXXXXX.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA Pesticide Assessment Guidelines, Subdivision F, 82-1. Study design was in accordance with EC Method B.27.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No GLP deviations were identified.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone (2-((4-chlorophenyl) phenyl acetyl) 1H-indane-1,3(2H)-dione) [also known as (2-((p-chlorophenyl)phenyl acetyl)-1,3-indanedione]	
3.2.1	Lot/Batch number	Various lot numbers were identified in the Certificates of Analysis: E6071; E6072; E6073; E6074; E6079; E6086; E6091; E6093; E6098 for 100 mg/mL formulation; E6100; E6101; E6102; E6103; E6115; E6142 for 10 mg/mL formulation;;	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Stock solution of chlorophacinone dissolved in corn oil.	
3.2.2.2	Purity	Not specified	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Rats	
3.3.2	Strain	Sprague Dawley OFA IOPS	
3.3.3	Source	XXXXXXX, France	
3.3.4	Sex	Males and females	
3.3.5	Age/weight at study	5 weeks. Mean body weights for groups of males ranged	

Section A 6.04.1-01 Annex Point IIA VI.6.4		Subchronic oral toxicity 3 month toxicity study on rats	
	initiation	from 124 to 126 g, mean body weights for groups of females ranged from 114-122 g	
3.3.6	Number of animals per group	10	
3.3.7	Control animals	Yes, 10/sex	
3.4	**Administration/ Exposure**	Gavage (oral intubation)	
3.4.1	Duration of treatment	A period ranging from 11 to approximately 16 weeks	
3.4.2	Frequency of exposure	7 days per week, daily for 11 weeks (5 µg/kg bw group) 7 days per week, daily for 16 weeks (all dosage groups scheduled to receive 10 µg/kg bw and above)	
3.4.3	Postexposure period	0	
3.4.4	**Oral**		
3.4.4.1	Type	Gavage	
3.4.4.2	Concentration	Dosages: 0 (groups T, T1, T2), 5 (group A), 10 (group B), 20 (group C), 40 (group D), 80 (group E), 160 (group F) µg/kg bw	
3.4.4.3	Vehicle	Corn oil	
3.4.4.4	Concentration in vehicle	Concentration in vehicle ranged from 0.1 mg/ml to 0.8 mg/ml	
3.4.4.5	Total volume applied	5 ml/kg bw	
3.4.4.6	Controls	Vehicle (corn oil)	
3.5	**Examinations**		
3.5.1	Observations	Daily clinical and cage side	
3.5.1.1	Clinical signs	Yes, daily	
3.5.1.2	Mortality	Yes	
3.5.2	Body weight	Yes, weekly	
3.5.3	Food consumption	Yes, weekly	
3.5.4	Water consumption	Yes, weekly	
3.5.5	Ophthalmoscopic examination	No	
3.5.6	Haematology	Yes, on ten of each sex at the end of the experiment Performed at weeks: Week 16 – group T (0 µg/kg), B (10 µg/kg), C (20 µg/kg) males only Week 17 – group T (0 µg/kg), C (20 µg/kg) Week 17 – group T1 (0 µg/kg), D (40 µg/kg) Parameters studied: Haematocrit (HCT), haemoglobin (Hb), erythrocytes (RBC) – average globular concentration in	

Section A 6.04.1-01 **Annex Point IIA VI.6.4**		**Subchronic oral toxicity** 3 month toxicity study on rats	
		hemoglobin MCHC, average globular volume MCV, average globular content in hemoglobin MCH, total and differential leukocyte count, platelet count, coagulation (quick) time.	
3.5.7	Clinical Chemistry	Performed at weeks: Week 16 – group T (0 µg/kg), B (10 µg/kg), C (20 µg/kg) males only Week 17 – group T (0 µg/kg), C (20 µg/kg), T1 (0 µg/kg), D (40 µg/kg) Parameters studied: alanine aminotransferase, aspartate aminotransferase, total bilirubin, calcium, chloride, cholesterol, creatinine, glucose, magnesium, alkaline phosphatase, inorganic phosphorus, potassium, total proteins, sodium, truglycerides, urea.	
3.5.8	Urinalysis	Yes, performed individually, at week 16 on ten animals of each sex - groups T (0 µg/kg), B (10 µg/kg), C (20 µg/kg), T 1(0 µg/kg), C (20 µg/kg); examinations for volume, pH, protein, reductase substances, glucose, blood, urobilinogen, bilirubin, ketone body, crystals, epithelial cells, leukocytes, reticulocytes, organisms, cylinders, abnormal constituents.	
3.6	**Sacrifice and pathology**		
3.6.1	Organ Weights	Yes: suprarenals, brain, heart, liver, kidneys, ovaries, hypophysis, thymus, spleen, testes, thyroid, uterus, prostate.	
3.6.2	Gross and histopathology	Gross pathology: Yes: all dose groups; Histopathology: Yes: on 2 male and 5 female animals of group D (40 µg/kg), on 2 males and 1 female of group E (80 µg/kg), on 2 males and two females of group F (160 µg/kg), and on 5 males and 5 females of the corresponding control groups Organs: aorta, cecum, heart, diaphragm, duodenum, stomach, liver, mammary gland, salivary glands, hypophisis, small and large intestines, tongue, skeletal muscle, lymphatic ganglions, oesophagus, ovaries, pancreas, skin, lungs, eyes (including optic nerve), prostate spleen, sternum, suprarenals, testicles, thyroid, abnormal tissue, trachea, vagina, bladder.	
3.6.3	Statistics	The weight of the animals, the results of the haematological examinations and blood chemistry, weight of organs – t-test (Student and Fisher), or by U-test (Mann and Whitney) Food and water consumption – bifactorial analysis (time and treatment)	
		4 RESULTS AND DISCUSSION	
4.2	**Observations**		
4.2.1	Clinical signs	The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of	

Section A 6.04.1-01 Annex Point IIA VI.6.4	Subchronic oral toxicity 3 month toxicity study on rats	
	chlorophacinone. Between 1 and 7-10 days and inversely related to the dose, the animals showed some weakness, lack of energy, increased until complete immobility, the hair was bristled and back arched. Other signs: nose bleeding, dyspnea (indicative of pulmonary and/or thoracic hemorrahages), swollen and bluish-colored testicles (testicular hemorrhage), black fecal matter or emission of clear blood (intestinal hemorrhage), sub-cutaneous hemorrhages with the formation of hematomas at the ears and the limbs, paresis paralysis of the limbs (indicative of internal hemorrahages), bleeding at bites, puncture points, pale mucous membranes, lifeless eyes. Signs of intoxication: weakness to immobilisation, frizzled hair, epistaxis, dyspnea indicative of pulmonary and/or thoracic hemorrahages, black fecal matter or emission of clear blood (intestinal hemorrhage), sub-cutaneous hemorrhages at the ears and the limbs, paresis paralysis of the hind limbs (indicative of internal hemorrahages), swollen and bluish-colored testicles (testicular hemorrhage), intense paleness of the mucous membranes, pale eyes, almost translucent few tremors before death.	
4.2.2 Mortality	Noted in all dosage groups above 10 µg/kg. No mortality noted at 5 µg/kg over the 11 weeks of study. Two animals of group B (10 µg/kg) died following intubation errors. The frequency of mortality as well the survival time of animals was related to the administered dose. Males were more affected than females. At 20 µg/kg, the 4 deaths involved only males that died within the last weeks of the study; in groups 40 µg/kg, all the males died within 82 days, compared to 4 out of 10 females that died during the 3-4th month. In the 80 and 160 µg/kg groups, death appeared very quickly, within 16 days, and with no clear difference between sexes. Nevertheless, it was noted that death was spread over 3 days for males, over 4 days for females.	
4.3 Body weight gain	In males, a higher weight gain was noted in groups A (5 µg/kg), B (10 µg/kg), and C (20 µg/kg), relative tothe controls (+7, +3, + 9 % respectively), lower in group D (40 µg/kg)(-21 %). In this group males were quickly affected and began die starting the 5-th week. During the days preceding death, the weight loss increased. In groups E (80 µg/kg) and F (160 µg/kg), death started within the first week and no valid curve could be established. For females the weight gains of groups A (5 µg/kg), B (10 µg/kg), and C (20 µg/kg) were lower than those of the	

Section A 6.04.1-01 **Annex Point IIA VI.6.4**		**Subchronic oral toxicity** 3 month toxicity study on rats	
		controls (-11, -8, -8 % respectively). This was directly correlated to decrease in food consumption. In group E (80 µg/kg) a slightly higher weight gain than that of the controls was noted. The females of this group were notably less affected than the males. In groups E (80 µg/kg) and F (160 µg/kg), death started within the first week and no valid curve could be established.	
4.4	**Food consumption and compound intake**	In the males of groups A (5 µg/kg), B (10 µg/kg), and C (20 µg/kg), daily food consumption was slightly higher than that of the controls. In group D (40 µg/kg), food consumption remained close to that of the controls up to the 9-th week. Afterwards, it decreased progressively and precipitously dropped at the 12th week. For groups E (80 µg/kg) and F (160 µg/kg), because of the rapid mortality and anorexia preceding death, food consumption could not be compared with that of controls. In the females, food consumption of group A (5 µg/kg) remained close to that of controls. It decreased slightly for groups B (10 µg/kg) and C (20 µg/kg) starting at the 8-9 week. In group D (80 µg/kg), food consumption was identical to that of controls, which showed that females were clearly less affected than the males. For groups E (80 µg/kg) and F (160 µg/kg) food consumption dropped. Food consumption variations were not correlated with administered dose, and not in agreement for both sexes.	
4.5	**Ophthalmoscopic examination**	Not performed	
4.6	**Blood analysis**		
4.6.1	Haematology	At the 16th week, differences were rare in groups T (0 µg/kg), B (10 µg/kg) and C (20 µg/kg). In both males and females, significant increases of the coagulation (quick) time were noted at the threshold of 1 % in the males of groups B (10 µg/kg) and C (20 µg/kg), and the threshold of 5 % in the groups B (10 µg/kg) females. At 16 weeks, increases in the average globular concentration in hemoglobin were noted ($p<=0.01$) in the group C (20 µg/kg) males and in the number of platelets in the group B (10 µg/kg) females, ($p<=0.01$) and in the males of this same group. A slight increase in the number of WBC ($p<0.05$) was noted in the males of group B (10 µg/kg). At the 17th week, analyses were made on the controls on the only one male rat (the samples had to be stopped because of bleeding at the puncture site and death of animals) from group C (20 µg/kg). A clear increase of the coagulation time relative to the controls was noted, although no	

Section A 6.04.1-01 **Annex Point IIA VI.6.4**		**Subchronic oral toxicity** 3 month toxicity study on rats	
		statistical study was performed. For the females a low but significant increase of the coagulation time was noted, very significant increase of Hb and RBC-s, and a significant increase in hematocrit and a lowering of globulin content. For group D (40 µg/kg) a marked increase of coagulation time was observed, also a slight rise in platelet count. The only parameter having toxicological relevance in both sexes was an increase in coagulation (quick) time. These in creases were minimal in group B (10 µg/kg) and were notably pronounced in groups C (20 µg/kg) and D (40 µg/kg). The other few variations, which were not dose related, were considered to be of no toxicological significance.	
4.6.2	Clinical chemistry	At the 16th week, in groups T (0 µg/kg), B (0 µg/kg), and C (20 µg/kg), the following differences were noted: Males: Group B (10 µg/kg) – decrease in bilirubin, phosphorus, magnesium, potassium, ASAT levels Group C (20 µg/kg) – increase in urea content, bilirubin, creatinine (not significant), triglycerides Females: Group B (10 µg/kg) – decrease in bilirubin, triglycerides, and ASAT; increase in creatinine, cholesterol, and total proteins. At the 17th week, biochemical observations for the females of group C (20 µg/kg) – decrease in glucose, phosphorus, magnesium, ASAT, small increase in sodium and ALAT. Results for one male in group C (20 µg/kg) – not interpretable. In group D (40 µg/kg) females - noticeable increases (p<= 0.01 or P<= 0.001) in cholesterol, triglycerides, phosphorus, magnesium, potassium, and ALAT; low but significant (P< 0.05) increase in creatinine, calcium, and a non-significant increase in ASAT. Parameters were extremely variable, and for a good number of them, did not correlate with sex or with dose. Some differences found at the highest dose groups, i.e. increases in urea and creatinine levels, bilirubin, cholesterol, triglycerides, and ASAT and ALAT, were suggestive of hepatic and renal disorders.	
4.6.3	Urinalysis	No differences were noted between groups	
4.7	**Sacrifice and pathology**		
4.7.1	Organ weights	In general, for all the groups, there were no dose-related weight differences (in absolute or relative value). The difference noted was either of no toxicological significance, or were related to the body weight loss preceding death.	
4.7.2	Gross and	Gross pathology:	

Section A 6.04.1-01 **Annex Point IIA VI.6.4**	**Subchronic oral toxicity** 3 month toxicity study on rats	
histopathology	Group A 5 µg/kg – no hemorrhagic lesions. Group B 10 µg/kg – some hemorrhagic lesions of weak to moderate severity found at the thymus and hypothesis in small number of rats (10 %). Group C 20 µg/kg –hemorrhagic lesions of average intensity were found on all subjects: thymus haemorrhages (90 % of the animals), less frequently hemothorax, hemoperitoneum, and haemorrhages at the stomach, the digestive tract, and the hypophysis. They were of average intensity with the exception of some very significant hemothorax. No deaths induced. Group D 40 µg/kg –hemorrhagic lesions of average intensity were observed on 90 % of the animals: frequent and sometimes intense at the thymus, the testicles, and the lungs with hemothorax, less frequent in the stomach, the digestive tract. No deaths induced. Group E 80 µg/kg and group F 160 µg/kg – haemorrhages often significant at the thymus were found on almost all subjects. Frequent haemorrhages in the testicles and prostate. Few haemorrhages in the salivary glands, trachea were noted. Histopathology: Ordinary lesions which existed on the controls and on the treated animals: interstitial pneumonia, emphysema, atelectases in lungs, calcium salt precipitates in the kidneys of females, acidophiles granulations in the nephrocytes in males, hyperplasia of the mesenteric ganglion, inflammatory infiltrates in the liver, moderate congestion of various organs. Lesions that existed only in treated animals: hemorrhagic lesions of varying localization and extent, especially found in the testicle, the ovary, the meninges, the brain, the thymus, the periaortic and peritracheal areas. In group D (40 µg/kg) animals, especially males, lesions of hepatic degeneration and coagulation necrosis, comparable to necrosis lesions of ischemic origin were noted. Diffuse lesions of lymphoid hyperplasia of the splenic pulp were also found in the males, practically non-existent in the other tested rats, especially the females.	
4.8 Other	Water consumption: There were no differences between the groups for the males and females of groups T (0 µg/kg), A (5 µg/kg), and B (10 µg/kg) and for the females of group C (20 µg/kg). A slight increase of water consumption was noted in the males of group C (20 µg/kg). In the group D (40 µg/kg, the consumption was identical to that of the controls for the females. It was slightly lowered for the males starting from the 10^{th} week. For groups E (80 µg/kg) and F (160 µg/kg), death occurred too early to	

Section A 6.04.1-01 **Annex Point IIA VI.6.4**	**Subchronic oral toxicity** 3 month toxicity study on rats	
	permit accurate comparisons with the control groups.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The subchronic oral study was designed to determine the toxic effects associated with repeated oral exposure for a period exceeding 90 days and to identify potential target organs and systems. Chlorophacinone (2-((4-chlorophenyl) phenyl acetyl) 1H-indane-1,3(2H)-dione) [or (2-((p-chlorophenyl)phenyl acetyl)-1,3-indanedione] CAS # 3691-35-8, dissolved in corn oil, was administered by gavage (oral intubation) to rats, 7 days per week for a period ranging from 11 to 16 weeks at dosages of 0, 5, 10, 20, 40, 80, 160 µg/kg bw per day. The low dose group was terminated after 77 days due to complete absence of any toxicological effects. Daily clinical cageside observations were performed, body weight, food and water consumption were registered, and haematological and clinical biochemical analyses were performed, as well as urinanalysis. Gross and microscopic examination of all tissues and major organs were performed. The study was conducted according to EC Method B.27 guidelines and EPA Pesticide Assessment Guidelines, Subdivision F, 82-1.	
5.3 Results and discussion	Mortality was noted in all dosage groups above 10 µg/kg. No mortality noted at 5 µg/kg over the 11 weeks of study. The frequency of mortality as well the survival time of animals was related to the administered dose. Males were more affected than females. At 20 µg/kg, the 4 deaths involved only males that died within the last weeks of the study; in groups 40 µg/kg, all the males died within 82 days, compared to 4 out of 10 females that died during the 3-4-th month. In the 80 and 160 µg/kg groups, death appeared very quickly, within 16 days, and with no clear difference between sexes. The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of chlorophacinone. Animals appeared weakened with decreased mobility and hemorrhages both externally and internally. Males were more sensitive to the effects of chlorophacinone than females. In those animals surviving at the end of the study, growth was unaffected by administration of the test article. Food and water consumption were also unaffected. With the exception of the coagulation time, haematological parameters were similar to controls. Coagulation time was significantly increased at all doses examined in a dose-related fashion. The lowest dosage examined was 10µg/kg where increases, while minimal were significantly different	

Section A 6.04.1-01 **Annex Point IIA VI.6.4**		**Subchronic oral toxicity** 3 month toxicity study on rats	
		from controls. Increases were notably pronounced in groups C (20 µg/kg) and D (40 µg/kg). Males were more affected than females. Clinical chemistry parameters were generally unaffected by chlorophacinone at the lowest levels examined. However, at 10 and 20 µg/kg, increases in urea and creatinine levels, bilirubin, cholesterol, triglycerides, and ASAT and ALAT, were suggestive of hepatic and renal disorders. Macroscopic examination revealed extensive hemorrhagic lesions in all dosage groups above 20 µg/kg. A few were noted in the 10 µg/kg group with none noted in the 5 µg/kg group. Gross and microscopic examinations of tissues and organs were consistent with the clinical observations of hemorrhagic activity.	
5.4	**Conclusion**		
5.4.1	LO(A)EL	10 µg/kg b.w. /day	
5.4.2	NO(A)EL	5 µg/kg b.w. /day based on results from 11 weeks administration.	
5.4.3	Reliability	1. With the exception of limited microscopic examination (described below) and limitations in the reporting of clinical signs, no major deviations from the protocol or relevant test guidelines were found.	
5.4.4	Deficiencies	Histopathological examinations were conducted on fewer animals than is required by the relevant test guidelines. However, the extent of examination is considered to be sufficient to characterize the dose-response pattern and does not impact the NOAEL determination or study reliability. A second deficiency is that clinical signs were not reported for each dose group.	
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		September 2005 (revised December 2005)	
Materials and Methods		Applicant version is adopted and summarised as follows: The subchronic oral study for a period exceeding 90 days, Chlorophacinone, dissolved in corn oil, was administered by gavage (oral incubation) to rats, 7 days per week at dosages of 0, 5, 10, 20, 40, 80, 160 µg/kg bw per day for a period ranging from 11 to 16 weeks. The low dose group was terminated after 11 weeks (77 days) due to complete absence of any toxicological effects. Daily clinical cageside observations were performed, body weight, food and water consumption were registered, and haematological and clinical biochemical analyses were performed, as well as urinanalysis. Gross and microscopic examination of all tissues and major organs were performed. The study was conducted according to EC Method B.27 guidelines and EPA Pesticide Assessment Guidelines, Subdivision F, 82-1. Not all animal were treated simulteneously and the lowest dose of 5 µg/kg/d was terminated at shortertime. The test programmes was initiated for dosing up to 20 µg/kg/day but due to low mortality after 1 month another higher dose group of 40	

Section A 6.04.1-01 Annex Point IIA VI.6.4	Subchronic oral toxicity 3 month toxicity study on rats	

µg/kg/day (and new control) were initiated, and after a month later with limited mortality two additional groups of 80 and 160 µg/kg/day and new controls were initiated. After 77 days on study, it was decided to terminate the lowest dosage group of 5 µg/kg/day, because complete absence of notable toxicity. Consequently no haematological examination of coagulation parameter were measured at this dose.

TEST PROGRAM

The initial protocol (see Segment 13) called for 10 rats of each sex to be treated by gavage seven days per week at dosages of 0, 5, 10 and 20 µg/kg b.w./day. After approximately one month, when no effects were noted at the lowest dose and mortality was minimal in the two higher groups, an additional group (40 µg/kg) was initiated with a concurrent control. After 77 days on study, it was decided to terminate the lowest dosage group because of complete absence of notable toxicity in the study and to perform gross, macroscopic analysis of tissues. No other analyses were made on the lowest dosage group. Again, because of limited mortality within the first month of study in the 40 µg/kg group, an additional two dosage groups were initiated; 80 and 160 µg/kg and another concurrent control. Protocol amendments are documented in Segment 13 of this report.

Results and discussion

Applicant version is adopted with some remarks:

Mortality

No mortality was noted at 5 µg/kg over the 11 weeks of study. One male and one female of 10 µg/kg died but was interpreted as due to intubation error. Mortality was noted in all dosage groups above 10 µg/kg. The frequency of mortality as well the survival time of animals was related to the administered dose.

Males were more affected than females.

At 20 µg/kg 4 out of 10 males died during days 105-111 and no female died. At 40 µg/kg all the males died within 82 days (range 29-82 days), compared to 4 out of 10 females that died during days 69-111. All animals died in the 80 µg/kg groups during 7-16 days and in the 160 µg/kg group during 5-8 days, and with no clear difference between sexes.

Macroscopic examination. Group dosed at 5 µg/kg showed no hemorrhagic lesions. Thymus haemorrhages were observed in some 10% animals dosed at 10 µg/kg and in most animals (≥90%) of groups at 20, 40 and 160 µg/kg and frequent haemorrhages were also noted in hemothorax, hemoperitoneum, and haemorrhages at the stomach, the digestive tract, and the hypophysis, and at 20 µg/kg. At 40 and 80 µg/kg also in lungs, testicles, prostate and other.

Clinical signs. The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of chlorophacinone. Animals appeared weakened with decreased mobility and hemorrhage both externally and internally.

Clinical chemistry parameters were generally unaffected by chlorophacinone at the lowest levels examined (the level without mortality). However, at 10 and 20 µg/kg, increases in urea and creatinine levels, bilirubin, cholesterol, triglycerides, and ASAT and ALAT, were suggestive of hepatic and renal disorders.

Haematology-Coagulation.: with the exception of the coagulation time, haematological parameters were similar to controls. The only parameter having toxicological relevance in both sexes was an increase in coagulation (quick) time

Section A 6.04.1-01 Annex Point IIA VI.6.4	Subchronic oral toxicity 3 month toxicity study on rats	
	which was notably pronounced in groups 20 and 40 µg/kg and was minimal in group 10 µg/kg. The other few variations, which were not dose related, were considered to be of no toxicological significance. Surprisingly, the animals of the lowest dosage group (5 µg/kg) were not examined for coagulation time and was not continued for the entire 16 weeks of study as they were stoped at week 11 (day 77). It was justified because the absent of any sign of toxicity. So a full evaluation cannot be made of this dosage group. The author of the study and the Applicant deduced that the NOAEL for this study is 5 µg/kg on the basis of no clinical, pathological and histopthological effect observed during the 77 days of the study at this dose (although no hemathological studies on coagulation time were performed at this dose level). The dose level of 10 µg/kg might consider at the LOAEL. As the dose of 10 µg/kg only caused minimal increase in coagulation time, it is reasonable to accept that in the complete absent of clinical, pathological alterations the dose tested of 5 µg/kg to be accepted as NOAEL. However this involves some uncertainty in this conclusion and consequently, to be considered for risk assessment.	
Conclusion	High mortality is observed at dose 20 µg/kg/d of higher for males and 40 µg/kg/day or higher for females. Males were more sensitive to the lethal effects of chlorophacinone than females. (At 20 µg/kg 4 males died. At 40 µg/kg all the males died within 82 days (range 29-82 days), compared to 4 out of 10 females that died during days 69-111. The dominant clinical signs were related to the anticoagulant activity of Chlorophacinone and were responsible for death of animals. Macroscopic examination revealed extensive haemorrhagic lesions in all dosage above 20 µg/kg/day, with a few haemorrhages in the 10 µg/kg/day group and none noted at 5 µg/kg/day. Gross and microscopic examinations of tissues/organs were consistent with the visual observation of hemorrhagic activity and with the known anticoagulant properties of Chlorophacinone. Coagulation (quick) time which were notably pronounced in groups 20 and 40 µg/kg and were minimal in group 10 µg/kg but significantly different from controls. It is concluded that for subchronic oral toxicity NOAEL value of 5 µg/kg bw/day can be stablished based on results from 11 weeks (77 days) administration. An uncertainty is mantained on this conclusion as no coagulation time were measured at this dose and this group were terminated before the 90 days, justified by the complete absent of toxicological effects. A LOAEL of 10µg/kg/day is stablished on the basis of 16 weeks dosing period with minimal increase but statistically significant in coagulation time and other biochemical parameters alteration which are suggestive of hepatic and renal disorders. **This uncertainty must be considered for risk assessment.**	

Section A 6.04.1-01 Annex Point IIA VI.6.4	Subchronic oral toxicity 3 month toxicity study on rats	
Reliability	2 The high mortality inherent to the substance toxicity involve some uncertainty in the meaning of the NOAEL With the exception of limited microscopic examination (described below) and limitations in the reporting of clinical signs, no major deviations from the protocol or relevant test guidelines were found. Histopathological examinations were conducted on fewer animals than is required by the relevant test guidelines. However, the authors of the study claim that "the extent of examination is considered to be sufficient to characterize the dose-response pattern and does not impact the NOAEL determination or study reliability". A second deficiency is that clinical signs were not reported for each dose group. A main deficiency for a definitive adoption of NOAEL/LOAEL is that coagulation activity were not monitored at the lowest dose of 5 µg/kg bw/day proposed for NOAEL as the study in this group was terminated earlier on day 77 (week 11). This test programme is justified by the authors but in any case some uncertainty is maintained.	
Acceptability	Acceptable (with the uncertainties commented)	
Remarks	The high mortality is inherent to the rodenticide family and the addendum TNG for rodenticides should be considered. The uncertainty in the estimation of NOAEL/LOAEL will have to be considered for risk assessment.	

Table A 6.4.1-1: Results of subchronic toxicity study – Groups A, B, C

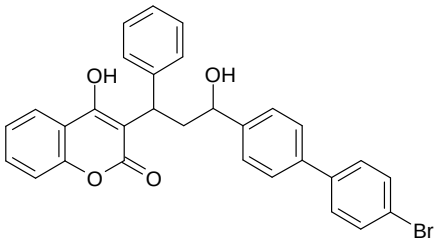
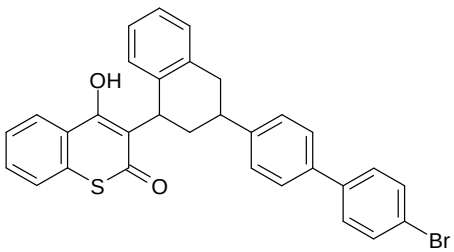
Parameter	Control T 0 µg/kg bw		Gr. A 5 µg/kg bw		Gr.B 10 µg/kg bw		Gr.C 20 µg/kg bw		dose-response +/-	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals examined	10	10	10	10	10	10	10	10		
Mortality	0/10	0/10	0/10	0/10	1/10*	1/10*	4/10, time to death 105-111 days	0/10	+	+
Body weight	Gain 310g	Gain 170g	+7 %	-11%	+3%	-8%	+9%	-8%	+	+
Food consumption	19.2	15.9	21.8	16.0	20.3	14.6	20.8	14.7	-	-
Clinical chemistry**										
Haematology*	12.0	11.4	Not measured		15.3 Min. ↑ coag. (quick)t p<= 0.01	12.1 Min. ↑ coag. (quick)t p<= 0.05	39.3 Pronounced ↑ coagulation (quick)time p<= 0.01		+	+
Urinalysis**									-	-
Thymus										
organ weight***									-	-
gross pathology	N	N	N	N	1 male with low significance degeneracy and 1 male with haemorrhage		90% of animals - low significance haemorrhage		+	+
microscopic pathology	N	N	N	N	hemorrhagic lesions – moderate intensity		hemorrhagic lesions – average intensity		+	+
Lungs	N	N	N	N	N		2 males with very great significant hemothorax		+	-
microscopic pathology	interstitial pneumonia		interstitial pneumonia		interstitial pneumonia		interstitial pneumonia		-	-
Other organs	N	N	No hemorrhagic lesions		No hemorrhagic lesions		Hemorrhagic lesions of average intensity found on all subjects: hemothorax, hemoperitoneum, and hemorrhages at the stomach, the digestive tract, and the hypophysis		+	+
microscopic pathology	interstitial pneumonia		interstitial pneumonia, emphysema, atelectases in lungs		interstitial pneumonia, atelectases		interstitial pneumonia, emphysema, atelectases in lungs		-	-

* Presumed intubation error resulting in death of one male and 1 female
** No differences between groups
*** No dose-related weight differences
N- No pathology findings related to test article

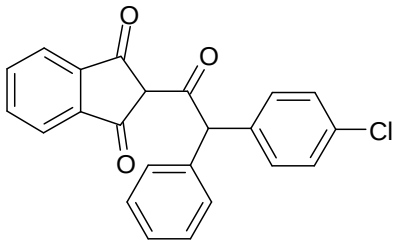
Parameter	**Control T1 0 µg/kg bw**		**Gr.D 40 µg/kg bw**		**Control T2 0 µg/kg bw**		**Gr.E 80 µg/kg bw**		**Gr.F 160 µg/kg bw**		**dose-resp.+/-**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals examined	10	10	10	10	10	10	10	10	10			
Mortality	0/10	0/10	10/10 time to death 29-82 days	4/10 time to death 69-111 days	0/10	0/10	10/ 10 time to death 7-13 days	10/ 10 time to death 9-16 days	10/ 10 time to death 5-7 days	10/10 time to death 5-8 days	+	+
Body weight	Gain 362g	Gain 170g	-21 %	+8%	Gain 162g	Gain 43g	-63%	-60%	-100%	-100%	+	+
Food consumption	22.5	15.2	19.7	15.4	27.1	16.0	17.1	9.1	17.6	12.6	-	-
Clinical chemistry							↑ urea, creatinine, bilirubin, cholesterol, triglycerides, ASAT and ALAT				-	-
Haematology	12.2	11.1	29.3 for females Marked ↑ coagulation (quick)time p<= 0.01		Not measured						+	+
Urinalysis**												
Thymus												
Organ weight***												
Gross pathology	N	N	90% of animals – average to great significance haemorrhage		N	N	90% of animals – great significance haemorrhage		95% of animals – great to very great significance haemorrhage		+	+
Microscopic pathology	N	N	hemorrhagic lesions – average intensity		N	N	perilobular hemorrhages		Significant intra- and perilobular hemorrhages + autolysis		+	+
Lungs			8 animals with low to average significant hemorrhages in lungs				low significant hemothorax in most animals		Almost all animals – hemothorax with different intensity and hemorrhages in lungs		+	+
Microscopic pathology	interstitial pneumonia		interstitial pneumonia, emphysema, atelectases in lungs		interstitial pneumonia, atelectases		interstitial pneumonia, emphysema, atelectases in lungs		interstitial pneumonia, emphysema, atelectases in lungs		-	-

Other organs	N	N	hemorrhagic lesions of average intensity were observed on 90 % of the animals: the testicles, brain, kidneys	N	N	Frequent hemorrhages in the testicles and prostate. Few hemorrhages in the salivary glands, trachea, cranium	Frequent hemorrhages in the testicles. Few hemorrhages in the salivary glands, trachea, cranium, liver
Microscopic pathology	N		males, lesions of hepatic degeneration and coagulation necrosis, comparable to necrosis lesions of ischemic origin	N		hemorrhagic lesions of varying localization and extent, especially found in the testicle, the ovary, the meninges, the brain, the periaortic and peritracheal areas.	

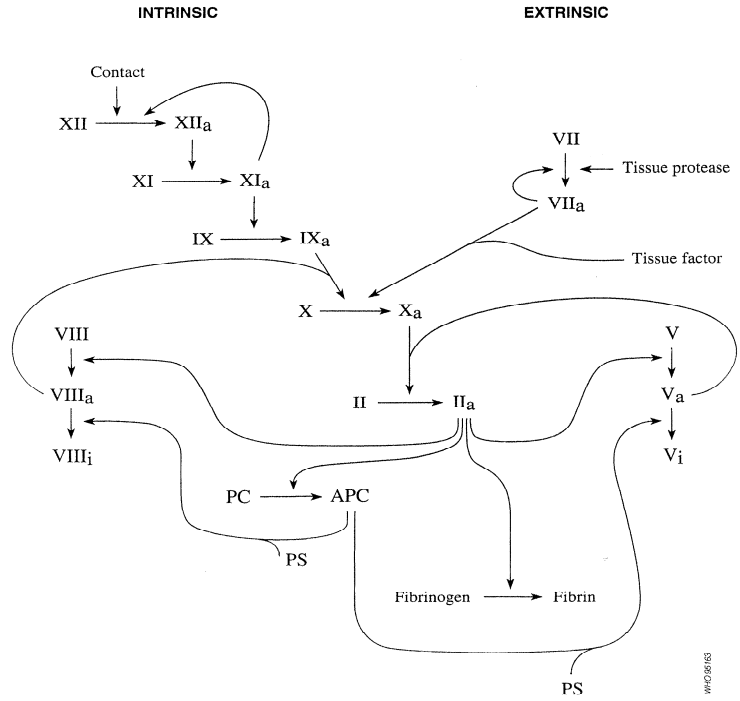
N- No pathology findings related to test article
** No differences between groups
*** No dose-related weight differences
Haematology analysis not performed on gr. E, F

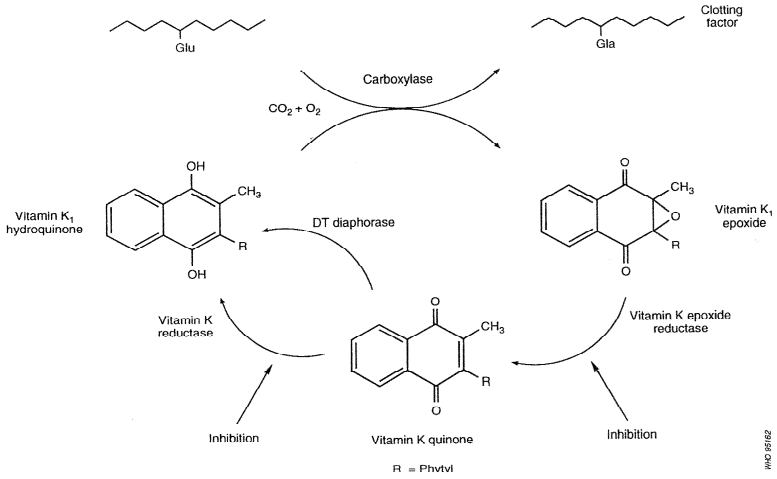
Section A 6.04.1-02 Annex Point IIA 6.4.1	Subchronic dose oral toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A 90-day short-term toxicity study in a second, non-rodent, species was not included in the dossier since there was data available for such a study based on results from two similar molecules supported by the same Notifier. There are special considerations for rodenticides in relation to long term exposure since the target species is also the test model in long term rodent studies. The implications for longterm exposure were particularly discussed in the dossier in relation to chronic studies and multigeneration reproduction toxicity or carcinogenicity investigations in the rat. These discussions formed the basis for comparison of results between three rodenticide molecules with similar action and similar toxicity profiles. Since the outcome of a subchronic study in the dog with chlorophacinone can be broadly predicted from knowledge of the mode of action and from results in non-target and target test models with the two molecules, bromadialone and difethialone, performing a repeat administration study in the dog may be seen to contravene Directive 86/609/EC which militates against unnecessary testing using animals. While the chemical structure for the coumarin derivatives bromadialone and difethialone is somewhat different to the indanone derivative, chlorophacinone, the mode of action for the three related rodenticides supported by LiphaTech S.A.S. is identical. This, together with a comparison of test results in the dog and rat conducted for the three molecules, may support the non-submission of a specific test in the dog for chlorophacinone. Structure for bromadiolone  Sturcture for difethialone  Sturcture for chlorophacinone	

Section A 6.04.1-02 Subchronic dose oral toxicity
Annex Point IIA 6.4.1



The clotting cascade affected by anticoagulant rodenticides and the mode of action are presented below:





Results from the subchronic study in rats with chlorophacinone can be summarised as :

Section A 6.04.1-02 Annex Point IIA 6.4.1	Subchronic dose oral toxicity	
	Mortality was noted in all dosage groups above 10 µg/kg. No mortality noted at 5 µg/kg over the 11 weeks of study. The frequency of mortality as well the survival time of animals was related to the administered dose. Males were more affected than females. At 20 µg/kg, the 4 deaths involved only males that died within the last weeks of the study; in groups 40 µg/kg, all the males died within 82 days, compared to 4 out of 10 females that died during the 3-4-th month. In the 80 and 160 µg/kg groups, death appeared very quickly, within 16 days, and with no clear difference between sexes. The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of chlorophacinone. Animals appeared weakened with decreased mobility and hemorrhages both externally and internally. Males were more sensitive to the effects of chlorophacinone than females. In those animals surviving at the end of the study, growth was unaffected by administration of the test article. Food and water consumption were also unaffected. With the exception of the coagulation time, haematological parameters were similar to controls. Coagulation time was significantly increased at all doses examined in a dose- related fashion. The lowest dosage examined was 10µg/kg where increases, while minimal were significantly different from controls. Increases were notably pronounced in groups C (20 µg/kg) and D (40 µg/kg). Males were more affected than females. Clinical chemistry parameters were generally unaffected by chlorophacinone at the lowest levels examined. However, at 10 and 20 µg/kg, increases in urea and creatinine levels, bilirubin, cholesterol, triglycerides, and ASAT and ALAT, were suggestive of hepatic and renal disorders. Macroscopic examination revealed extensive hemorrhagic lesions in all dosage groups above 20 µg/kg. A few were noted in the 10 µg/kg group with none noted in the 5 µg/kg group. Gross and microscopic examinations of tissues and organs were consistent with the clinical observations of hemorrhagic activity. Results from the subchronic study in rats with difethialone can be summarised as : Doses of 16 µg/kg bw/day or above, repeatedly administered to rats by oral gavage, resulted in the death of all treated animals within 8 weeks of treatment. In the main study, lower dose levels, 2, 4 or 8 µg/kg bw/day, were investigated. Three of the ten rats treated for 13 weeks in the high dose group died on day 91 or 92. Ante-mortem signs indicative of internal haemorrhage included pallor, dull eyes, weakness, epistaxis, haematuria and shallow respiration. Bodyweight gains, food consumption and water	

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	consumption showed no effects of treatment at any of the dose levels throughout the study. Haematological investigations at week 6 and 14 (termination) indicated elongated Quick time and thrombotest times, particularly among males; normochromic anaemia among males suggesting marked blood loss and neutrophilia in the rats with the poorest clinical condition. Biochemical investigations showed changes consequent to blood loss e.g. hypoproteinaemia and several marked disturbances primarily among those rats that subsequently developed anaemia (changes in urea, glucose, creatinine, phosphorus, cholesterol, sodium and transaminases). Urinalysis at week 5 indicated diuresis and increased urinary pH. Necropsy revealed haemorrhage in the abdominal and thoracic cavities and at the location of many organs and tissues including thymus, encephalon, genitalia, salivary glands and tracheo-oesophageal musculature at 8 µg/kg bw/day. There were no notable changes in organ weights. Microscopic examination revealed only haemorrhagic lesions, confirming macroscopic observations. There were no notable clinical signs following dosing at 2 or 4 µg/kg bw/day and none of the rats died. None of the parameters investigated during the in-life phase showed any treatment-related effects. Necropsy revealed haemorrhagic lesions, primarily at the thymus, and haemorrhages were more prominent among the females. There were no histopathological changes of note in organs or tissues examined for animals of the 2 or 4 µg/kg bw/day groups. Difethialone was shown to have anticoagulant activity in the rat at doses of 8 µg/kg bw/day or above and to cause death among rats at these dose levels. Results from the subchronic study in dogs with bromadialone can be summarised as : There were no deaths in either the control or low dose groups but all dogs dosed at 50 µg/kg bw/day died within 21 to 32 days and there were severe effects in the intermediate group (20 µg/kg bw/day) such that four animals were sacrificed on humane grounds before completing the dosing phase (sacrificed on days 64, 71, 75 or 84). No clinical signs were evident for dogs of the control or low dose group. Clinical signs of reaction to treatment at 20 µg/kg bw/day that were critical in the decision to sacrifice animals included vesical, vaginal, gingival, sublingual, gastrointestinal, subcutaneous and internal haemorrhages. Other ante-mortem signs observed included progressive exhaustion, difficulty getting up or walking, lateral decubitus, partial anorexia until haemorrhagic signs appeared when the animals were too weak to feed, pale mucosa, cold extremities or hypothermia. The signs were	

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	evident for between 3-4 and 11 or 21 days prior to death. Similar effects were evident in the high dose group, all of whom showed evidence of severe haemorrhagic disorder. The onset and course of the haemorrhagic syndrome was rapid proceeding to exhaustion and partial or total anorexia and then to death. The rapidity of death meant that generally changes in weight loss were not evident. Other signs observed included paresis, progressive hindquarter paralysis, lateral decubitus, pale mucosae and hypothermia. Death was confirmed to have resulted from massive internal and external haemorrhage. Bodyweights and weight gains were generally similar to controls in low dose group. In the intermediate group (20 µg/kg bw/day) there were weight losses recorded for those animals where anorexia was not rapidly followed by death. Generally in the high dose group the period between onset and development of severe signs, including anorexia and death was too short to record weight loss. At week 4, the erythrocyte count was slightly lower than control for the males but this was not considered to be a treatment related effect because of the similar low values recorded among male dogs prior to dose administration. There were no other changes in comparison with controls for haematological parameters for the low dose group animals that were considered to be treatment related. Males dosed at 20 µg bromadiolone/kg bw/day also had significantly lower ($p<0.01$) erythrocyte counts at week 4. At the same time point, dogs of both sexes had significantly increased coagulation and prothrombin times (males $p<0.05$; females $p<0.01$) and the effect became more pronounced in samples collected at day 45 and day 60. By the end of the study surviving males showed a 5-fold increase in prothrombin time and a 3-fold increase in coagulation time; the effect was greater in females with 25-fold and 8-fold increases respectively. Only one female, dosed at 50 µg bromadiolone/kg bw/day, survived to the initial blood sampling point after 4 weeks of dose administration. Results for this animal indicated marked increases in coagulation and prothrombin times, reduced erythrocyte counts and haemoglobin levels and a marked increase in leukocyte numbers. There were no other changes in haematological parameters that were considered to be toxicologically significant. There were no changes in biochemical parameters that were considered to be an effect of treatment. There were no intergroup differences in urinalysis results that were considered to be treatment related. There were no effects of treatment on organ weights at any of the dose levels in either sex. Necropsy of the control and low dose animals revealed no notable or treatment-related macroscopic changes. There were no histopathological	

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	lesions considered attributable to treatment in the low dose group tissues and organs examined microscopically. Necropsy of the animals that died or were sacrificed during the treatment phase in the intermediate and high dose groups and surviving dogs of the intermediate group all had extensive, non-specific haemorrhages with development of haemothorax, haemoperitoneum and haemopericardium. The highest incidence was of subcutaneous, sublingual, gingival, thymic, pulmonary and vesical haemorrhagic. In addition there was evidence of early developing subcutaneous haematomas. Microscopic changes confirmed the haematological effects and gross evidence of haemorrhages and haematomas seen in the high and intermediate dose groups. Diffuse haemorrhages were seen at various sites and at various stages of progression affecting numerous sites including myocardium, aorta, lymph nodes, spleen, thymus, stomach submucosa, liver and muscle. Results from the subchronic study in dogs with difethialone can be summarised as : There were no deaths at any of the three dose levels, 5, 10 or 20 µg difethialone/kg bw/day. Pale gums were noted for two high dose animals in the last two weeks of the study. No other clinical signs of reaction to treatment were observed. Bodyweights and weight gains were generally similar to controls in both sexes at all dose levels throughout the study. The only other indication of a possible effect of treatment was one high dose male that lost weight in the final week of treatment. Administration of Difethialone had no effect on food consumption. Ophthalmic examination prior to dosing and then in week 13 revealed no treatment related ocular abnormalities. Male dogs dosed at 20 µg difethialone/kg bw/day had significantly reduced haemoglobin levels during week 13 and packed cell volume and erythrocyte counts had not risen from pre-dose levels. The additional coagulation tests conducted on samples for PT and APTT showed no clear effects except for markedly elevated values for one high dose male during week 13 only. There were no other changes considered to be toxicologically significant. There were no changes in biochemical parameters that were considered to be an effect of treatment. There were no intergroup differences in urinalysis results that were considered to be treatment related. There were no effects of treatment on organ weights at any of the dose levels in either sex. Necropsy revealed only two animals, both in the high dose group, with possible treatment-related macroscopic abnormalities. The female had a depression on the capsule of the spleen. The male had multiple firm, blood-filled nodules on the thymus,	

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	congestion of the serosal surface of the oesophagus, dark discolouration and multiple firm white nodules on one lobe of the lungs and red adhesions to mediastinum on a second lobe. The male was the same animal showing pale gums, weight loss and increased PT and APTT. Histopathological changes considered to be treatment-related were limited to the thoracic cavity of one high dose male. Microscopic changes included haemorrhage in the lungs, pleural fibrosis, pleural adhesion, haemorrhage and fibrosis of the thymus and haemorrhage in the mediastinum adjacent to oesophageal serosal surface. While oral administration of difethialone to dogs for 13 weeks at dose levels of 5 or 10 µg/kg bw/day resulted in no toxicologically significant effects, the high dose elicited some reactions after 13 weeks of treatment which were consistent with the test substance mode of action as an anticoagulant rodenticide and haemorrhagic events were evident macroscopically and microscopically. The high dose, 20 µg/kg bw/day, did not cause death in the dog under the conditions ofthis test. However, given the cumulative nature of the test material it is not unreasonable to conclude that if treatment had continued beyond 13 weeks or if the high dose level had been slightly higher, all of the high dose group would have shown signs consistent with anticoagulant toxicity The 90 day rat LOAEL for difethialone was 4 µg/kg bw/day based on haemorrhagic changes seen at necropsy. The 90 day rat NOAEL for difethialone was found to be 2 µg/kg bw/day. The values for chlorophacinone were LOAEL 10 µg/kg bw/day based on haemorrhagic changes seen at necropsy and a NOEL of 5 µg/kg bw/day. The 90 day dog LOAEL was 20 µg/kg bw/day based on haemorrhagic changes seen at necropsy. This was the same as the value for Bromadialone. The consistent pattern of response in rodents and non-rodents to the repeated administration of an anticoagulant rodenticide was for an initial increase in coagulation time with a correlated change in poor clinical condition and , where measured, disruption of haemoglobin and erythrocyte parameters. If no Vitamin K antidote was administered, the cumulative effects of the material results in death by non-specific diffuse haemorrhage. In many studies, in various models, this pattern was identified as a haemorrhagic syndrome almost always resulting in death or severely compromised clinical condition. Since the mode of action, consistency of response (fatal haemorrhagic syndrome) and similarity of results for LOAEL or NOEL values has been established in rodent and non-rodent models in studies of varying duration, it is not considered scientifically justifiable	

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	to investigate this molecule further by means of animal testing. The highly toxic nature of the material is such that repeated administration studies result in death at high doses – in the rat a high dose is anything greater than 4 µg/kg bw/day and in the non-rodent model, the value was any dose greater than 20 µg/kg bw/day. The highly cumulative nature of the material means that lower doses, administered over several days, can also be predicted to cause death. In all cases death was caused by the specific pharmacological action of the molecule, inducing fatal haemorrhage. The mechanism of clotting inhibition caused by hydroxy coumarin-type and indanone anticoagulant rodenticides is dependent on inhibition of vitamin K epoxide or vitamin k reductases and is unaffected by route of application. Therefore repeating the study in the dog rather than rat would have provided no additional useful information. This approach is consistent with the guiding principles with regard to data requirements set out in Chapter 1, section 1.2 (8) of thetechnical notes for guidance in support of Directive 98/8 concerning the placing of biocidal products on the market.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2004	
Evaluation of applicant's justification	Justification is based on comparison of data of chlorophacinone with two other rodenticides (diphethialone and bromadialone) with similar toxicological profile and identical mode of action. There are available data of oral subchronic studies in rats for the three substances and in dog for two of them but not for chlorophacinone. Applicant shows and compares summary data which are consistent and allow predicting the effect of chlorophacinone in dogs, concluding that a study in dog will not provide additional useful information. So it is concluded that a new subchronic study in a non-rodent species is not required.	
Conclusion	**Accepted**. Applicant presents a consistent justification. However it is supported with data of two rodenticides also notified (diphethialone and bromadiolone) and assigned to other Member State as Reporteur for evaluation. So the suggested conclusion is critically depending of the evaluation of these other substances and will be validated only after the evaluation of the toxicological data of diphethialone and bromadiolone will be concluded	
Remarks	As the arguments for no providing a non-rodent subchronic study and the possibilities of evaluating the subchronic toxicity of chlorophacinone is so critically depending of the data about subchronic toxicity in rat and dog of these other rodenticides, it would be convenient that the **Notifier would provide to this Reporteur Member State the original data and summaries of the subchronic studies presented in the notification of diphethialone and bromadialone.**	

Section A 6.04.3-01 Annex Point IIA, 6.4.3	Subchronic dose inhalation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	A repeat dose inhalation study is not required. An acute inhalation study showed that the molecule is acutely toxic. The LD_{50} for male and female rats was 9.3 µg/L. Appropriate protection measures (6.12.1) ensure no exposure to the (powdered) technical material or to the products during the production process. The active ingredient is not volatile and none of the products have the potential to generate a toxic inhalable atmosphere. The acutely toxic nature of the material combined with its potential for hepatic accumulation, is such that repeated exposure to lower doses will result in death by induction of a haemorhagic syndrome with associated acute clinical signs of reaction to treatment (see 6.1.1, 6.1.2 or 6.1.3 for indications of haemorrhagic syndrome). The mechanism of clotting inhibition caused by hydroxy coumarin-type anticoagulant rodenticides is dependent on inhibition of vitamin K epoxide or vitamin k reductases and is unaffected by route of application. Therefore specific repeat dose studies would not provide any additional useful information. As the outcome of such a study can be predicted from the knowledge on mode of action and acute inhalation exposure, performing a repeat administration study would contravene Directive 86/609/EC which militates against unnecessary testing using animals	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2004	
Evaluation of applicant's justification	Directive requires repeated dose study and the TNG of data requirement (introduction to point 6.3) indicate that the "required route of administration is the oral route". So inhalation study is not obliged as a primary required route "unless it can be justified that an alternative route is more appropriate". Point 6.3.3 state that alternative or additional inhalation route is required "for volatile substances (vapour pressure >1x 10 Pa) or in cases where the potential inhalation exposure is significant", and "in some cases (e.g. aerosols and dusts/particulate matter)" Applicant justify the non-submission of subchronic 90 days inhalation toxicity study on the basis of: (a) "Compound is not volatile". However if product is used in powder then the potential inhalation exposure is depending of particle size (<50 um?), and this data is not indicated. (b) "As a results of acutely toxicity nature the repeated dose will results in	

Section A 6.04.3-01 Annex Point IIA, 6.4.3	Subchronic dose inhalation
	death of animals at the "lower dose" Which is the lower dose? This could overcome testing lower relevant doses or choosing appropriate relevant species. However the Addendum of the TNG indicate possible waiving of subchronic data on the basis of "low toxicity" in repeated dose in non-rodents and mechanistic information suggesting that the main effect is not relevant to human.
Conclusion	**Accepted.** Arguments are reasonable but there are some concerns. So justification could be provisionally accepted depending of further detail evaluation of the following data: (a) potential exposure by inhalation and indication of particle size, (b) inhalation acute toxicity, oral repeated dose and subchronic oral study. It should be considered the additional difficulty for evaluating the chemical due to the no submission of a subchronic oral study in non-rodent species
Remarks	Under the "Addendum to the TNsG on Data Requirements" waiving of subchronic study may be considered but there is not specific indication in the Addendum for inhalation assay. The "technical difficulty" argued is that it is high acutely toxic. If accepted then should be proved that expected exposure is actually lower than the lowest dose that is reasonable to be tested

Section A 6.05-01 Annex Point 6.5	Long term toxicity in rats	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	**Waiver for carcinogenicity/toxicity studies in rodents on Chlorophacinone.** The following is a series of rationales to waive the requirement to perform carcinogenicity/chronic toxicity studies on the anticoagulant rodenticide active substance Chlorophacinone under the Biocidal Products Directive 98/8/EEC. **1 INTRODUCTION.** The Biocidal Products Directive (98/8/EEC 'the Directive') requires long-term testing in rodents as part of the suite of toxicology tests in order to assess the possible adverse consequences of chronic exposure (i.e., chronic toxicity and carcinogenicity) to the biocidal active substance Chlorophacinone. It is a unique feature of the rodenticides that the test species used in long-term toxicity and carcinogenicity studies is also the target species, and that the active substances are lethal in the target species at very low levels. This gives rise to several questions: Is it relevant to consider the possible use of long term rodent studies to predict possible effects of rodenticides in humans. Is it scientifically feasible? Can the data be derived using other species? Given that at one rodenticide molecule has been used for over forty years in human medicine, are there data in the human that are more relevant than animal data would be? Are there other data that demonstrate the potential, or lack of potential, carcinogenic properties of active substances used as rodenticides? The Directive states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". A more detailed waiver concept is given in the TNsG on data requirements. The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". The TNsG recommendations were further refined in an Addendum to the TNsG entitled Refined waiving concept for rodenticides (TMII03-item9a-CA-Jun03-Doc9-	

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	TNsG.doc). These include: The study is technically not possible to perform, Use of other data, Data evaluated with regard to agricultural use Read-across from data on related substances Evaluation of acceptable human data, The study is not scientifically necessary The choice of species is not appropriate The study is not necessary owing to limited exposure and toxicity profile The Notifier has prepared a scientific justification based on this guidance to waive the requirement for these studies. Before the waiving arguments are given, it will be useful to review the way the coagulation system works in mammals and the mechanism by which the anticoagulant rodenticides function. **2 FUNCTION** Anticoagulant rodenticides such as Chlorophacinone function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver. Blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin to the insoluble fibrous protein fibrin, which binds platelets and blood cells to form a solid mass referred to as a blood clot, sealing the site of the haemorrhage and preventing further blood loss. Fibrinogen is present in the blood, but thrombin is not. Thrombin factor IIa in the scheme below) is formed at the site of injury from prothrombin (factor II), which is present in the blood. Conversion of prothrombin to thrombin occurs via the coagulation cascade, in which the blood clotting factors are employed. Without these blood factors clotting cannot take place, and the haemorrhage will not be controlled by clot formation. If the blood vessel is large and/or serves a vital organ, the haemorrhage will be fatal. The synthesis of a number of blood coagulation factors (factors II [prothrombin], VII [proconvertin] IX [Christmas factor], X [Stuart-Prower factor] and the coagulation inhibiting proteins C and S) is dependent upon vitamin K, which acts as a co-enzyme.	

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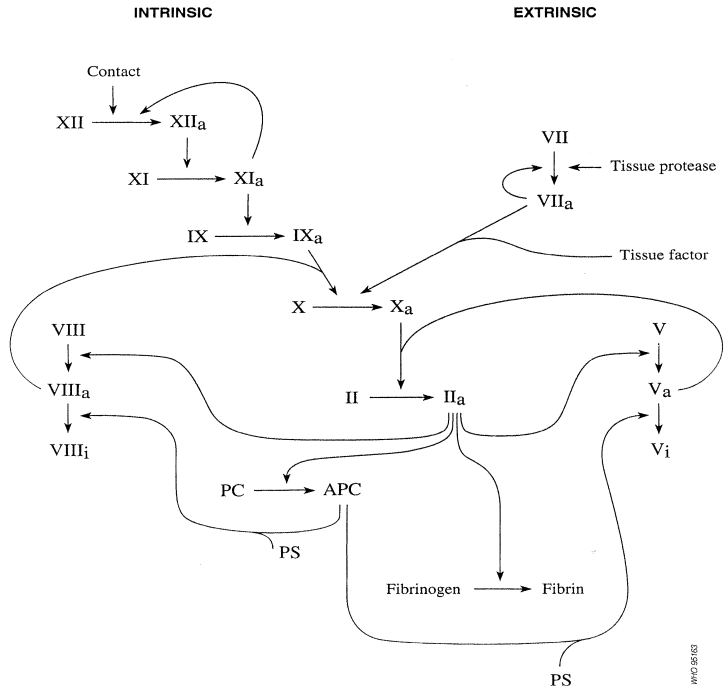
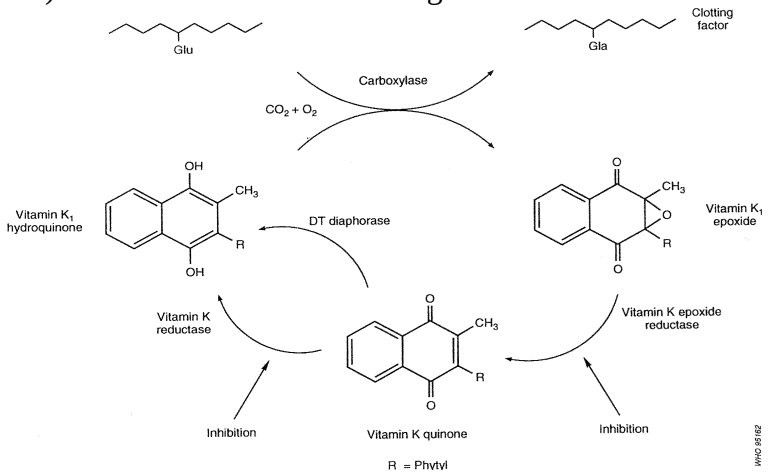


Vitamin K hydroquinone is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide provides the energy required for the carboxylation reaction where glutamate (Glu) in the precursor is converted to γ-carboxyglutamate (Gla) to make the activated clotting factor.



The anticoagulant rodenticide active substances such as Chlorophacinone work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place.
The action is cumulative, increasing levels of the anticoagulant leading to increased clotting times, such that in the event of a significant haemorrhage, death occurs. The amount of vitamin K in the body is finite, and progressive blocking of the regeneration of vitamin K will lead to an

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increasing probability of a fatal haemorrhage. In general terms, progressive intake of anticoagulants results in death. The active substances are highly toxic and bioaccumulative. The oral LD_{50} of Chlorophacinone is 6.26 mg/kg. Rodenticide baits generally contain 50 ppm Chlorophacinone and are fatal after one to three meals.

3 TECHNICAL FEASIBILITY

Carcinogenicity/toxicity studies seek to determine the consequences of long-term (near life-span) exposure to the active substance by the daily, dietary administration for two years of (typically) three increasing doses to groups of rats or mice, and observing their effects in comparison to a similar group of untreated animals (the control group).

3.2 Dose-setting and the Maximum Tolerated Dose

In order to demonstrate the validity of long-term carcinogenicity/toxicity study, the highest dose should induce some form of toxicity. This toxic effect is not necessarily carcinogenicity *per se* but should be a difference from the control group that can be demonstrated experimentally (e.g. reduced body-weight gain, altered enzyme levels, changes in function of an organ exhibited by either weight change or histopathology). This measurable indicator of toxicity should be present in the high dose level, ideally at a level that does not affect the animals sufficiently to affect survival adversely over the length of the study. This high dose level referred to as the Maximum Tolerated Dose (MTD) and, conventionally, should not cause more than 10% mortality above that observed in the control group. Studies without an MTD are considered invalid by many regulatory authorities. The intention is to administer sufficient test material such that the animal has to respond to the chemical burden i.e. it is placed under toxic stress. The implication is that if the animal does not respond to the stress by showing increased incidence of tumours, then the chemical is considered unlikely to be carcinogenic in man. Secondly, if the animal is not stressed sufficiently to show MTD response, it has not been stressed sufficiently to demonstrate the potential to cause increased incidence of tumours.

A difficulty in the administration of an MTD in a two-year study is caused by the fact that the anticoagulants are not excreted rapidly. Terminal half-lives in the liver are relevant, as the liver is the site of vitamin K regeneration, and these half-lives are very long (See Table 6.5-1). Warfarin has the lowest half-life at 42 hours in human plasma. Human liver data are not available (because liver biopsy is too hazardous for routine investigation in humans), but the liver half life is predicted to be several days, where

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'several' is probably greater than ten but less than one hundred). Absorbed doses accumulate, and lethality occurs when a threshold dose is exceeded. This may occur after one or two large doses, or several smaller doses.
It is feasible to conduct short-term animal studies with these substances because it is possible to ensure that the accumulated dose does not exceed lethal levels. However, the LD_{50} of these molecules is very low and, since the level for low lethality (e.g. LD_{10}) will be lower still, the amount to be administered daily over a two year study, in order to deliver (but not to exceed) an LD_{10}, would technically impossible to achieve. For example, for bromadiolone, the LD_{50} in rats is >0.56 mg/kg but < 0.84 mg/kg. A reasonable estimate of the LD_{10} (a value that would theoretically induce 10% mortality allowed in a long-term rodent study) is 0.6 mg per animal during the study. Using excretion data for bromadiolone, and computer software it can be shown that over the 730 days of a typical rat carc/tox study, to reach the LD_{10} by termination would require daily doses (at food intake of 25 g/rat/day) of 0.2 ppm. This is not a feasible level of dietary inclusion.

3.3 Route of Administration of the Test Substance

Dietary admixture is the only practical long-term route for administration of the test substance. It is not feasible accurately to prepare homogenous rodent test diets (to the standards required by GLP and Guidelines) at the very low concentrations needed for the MTD (i.e. 0.2 ppm as shown above). Even lower concentrations would be required for the other dose levels and these would approach the analytical method limit of detection of 0.02 ppm. It may be argued that a regulator would not expect accurate formulations, but that a study should be performed anyway. However, if inhomogeneous diet were administered, some rats would be given a feed ration that contained too much active substance, which could simply be fatal to that entire cage of five rats. Even if the rats were housed singly, the risk of fatality over a two-year period would be too great to anticipate enough animals surviving to the end of the study to provide meaningful data.
An alternative to dietary administration is the use of oral gavage. However, handling for gavage can lead to minor haemorrhage in the nasal passages (shown as brown facial staining), and the act of introducing the plastic or rubber gavage tube or steel cannula may cause minor haemorrhage in the buccal cavity and oesophagus. The use of this procedure daily for two years is considered unfeasible for an anticoagulant. Injection is also not worth considering for similar reasons. The active substances are mostly only sparingly soluble in water, so that administration in drinking

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water is not feasible. (See Table 6.5-2)
Similarly, inhalation is not feasible. Whole body exposure would lead to oral intake from grooming, resulting in death, and nose-only administration is not feasible because the increased handling and restraint of the test animals would promote the likelihood of haemorrhage. Dermal administration is also not feasible: rats need to be shaved frequently to expose the skin. Shaving is inevitably associated with minor cuts and haemorrhage.

3.4 Choice of species

Rodents are used in safety testing because they are small (easy to handle and house), readily available (large numbers can be bred in captivity), and they have a relatively short life span (studies are of shorter duration than with longer-lived species). In the case of rodenticides, designed to kill the wild form of the test species at low doses, long-term testing of the target species is inherently difficult. It is logical to see if there are alternative species, suitable for long-term tests that are less sensitive to these active substances. A comparison of LD_{50} values in other mammals shows that for each active substance the range of tolerance between species is generally one order of magnitude, and all are very low in absolute terms. (See Table 6.5-3).
It has been shown above that a dose intended to achieve LD_{10} in two years for Bromadialone would be equivalent to 0.2ppm in the diet. A slightly less sensitive species such as the dog would need a dose of 2 ppm (by simple pro-rata increase of the dose in proportion to the ratio of LD_{50}s) to reach LD_{10}. Dietary concentrations of 2 ppm are still very difficult to achieve accurately.
There are also practical considerations in performing carcinogenicity studies in large animals such as dogs, pigs or cats. In theory, a carcinogenicity study should be performed over the life span of an animal. This is two years in the rat, but is seven to ten years in the dog and pig, and ten to fifteen years in the cat. Studies of one year duration are performed on pesticides in the dog, but these are considered extensions of the 90-day subchronic study, rather than chronic studies. Dogs are amenable to laboratory housing over lengthy periods; cats are not. They require frequent handling if they are not to revert to feral behaviour and they do not respond well to being caged.
There is also the statistical power of such a study. The EC Guidelines for carcinogenicity (B.32, B.33, Directive 87/302/EEC) recommend 100 rodents per group (50 male and 50 female), with at least three treated groups plus one control. One year dog studies are typically performed with four males and four females per group.
The following statistical proof (from Quantics Consulting,

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	2004, based on 'The design and analysis of long term animal experiments', Gart JJ, Krewski D, Lee PN, Tarone RE, Wahrendorf J.1986. IARC Scientific Publications no 79. IARC, Lyon) shows that unless there are approximately 50 animals per group, it would not be possible to detect excess tumour incidences of less than 20%. If there are N animals in each of four treatment groups: control and 3 doses. Per organ at post mortem examination, the number of animals with at least one tumour in that organ is counted. Incidence in that group is percentage of animals with at least one tumour. Each treated group is compared with the control group in turn. (See Table 6.5-4). It can be seen that with a background incidence of 5%, at least 46 animals would be needed per group to detect an excess of 25% (i.e. total incidence of 30%) in the treated group. Such studies are not feasible in larger (non-rodent) mammals. In addition, there would be virtually no background control tumour incidence data on the species chosen, as such studies are rarely if ever performed in the larger mammals. European legislation militates against the use of animals in unnecessary experimentation; the use of large mammals in such studies, particularly cats and dogs, would be considered unethical in most jurisdictions. **3.5 Antidotal treatment** Studies are presented in the dossier which administer vitamin K as an 'antidote'. These studies variously show that it is possible to use vitamin K in the treatment of low single doses of anticoagulants. For Chlorophacinone, rats were given approximately 5 mg/kg bw/day for 24, 48 or 72 hours, via the diet, and vitamin K administered for 14 days. All rats given chlorophacinone for 24 hours survived, and 3/5 rats given Chlorophacinone for 48 hours survived but all rats treated for 72 hours died (reference A 6.10-01). The anticoagulant active substances are highly lipophilic. They have been shown to accumulate in the liver. The inhibition of the regeneration of vitamin K occurs by blocking, i.e. competitive binding of the active substance and the vitamin K reductase enxyme (see above) to form a lipophilic complex, which will accumulate in the liver in the same manner as the active substance. Long term co-administration of vitamin K as an antidote, would result in the accumulation in the liver of the lipophilic complex; not the active substance. As there would be no free active substance present the test would not be valid.	

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3.6 Absence of carcinogenic risk

The anticoagulant action is the sole pharmacological action of the materials. The mode of action has been described in detail. It is difficult to demonstrate that this is the sole mode of action, as administration is acutely lethal, but it is supported by the available short-term toxicology data. The absence of any other toxic effect indicates that the probability of a physiological effect (such as chronic irritation of gut walls leading to hyperplasia, or adaptive proliferation of liver or kidney cells in response to increased workload) leading to non-genotoxic carcinogenesis is low. Indeed the very long half-lives and accumulation within the liver indicate that the liver is unable actively to excrete the active substances, further indicating that a proliferative or adaptive response is unlikely in that organ. The 90-day rat study showed no indications of any adverse hyperplasia or hypertrophy in the target organ, the liver, at near-lethal levels of administration.

The absence of carcinogenic potential is further supported by the fact that mutagenicity studies on the active substances are negative. Given that the materials are not mutagenic/genotoxic, the likely mechanisms of carcinogenicity are limited to those resulting from effects such as hepatic hypertrophy, or irritance, and short-term studies show that there are no responses of that nature. It is reasonable to conclude that the active substances have no carcinogenic potential. This is supported by human data (see below).

4 USE OF OTHER DATA

4.2 Data evaluated with regard to agricultural use

Chlorophacinone is registered for agricultural uses. All of the available data are presented in the BDP dossier: no other data have been derived specifically to defend agricultural uses.

4.3 Long-term human data

There is long term experience in humans with warfarin, widely used in anti-clotting therapy in humans for over forty years, with no association with increased incidence of cancer.

Warfarin was the first of the anti-vitamin K rodenticides. The anticoagulant rodenticides fall into two categories: inandones, such as chlorophacinone, and hydroxycoumarins such as warfarin, bromadialone and difethialone.

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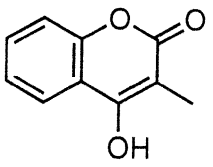
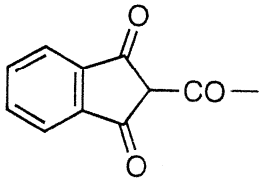
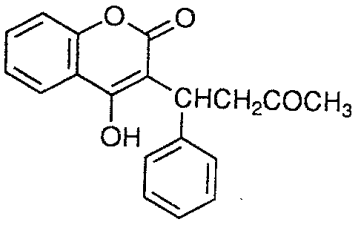
- hydroxycoumarins:



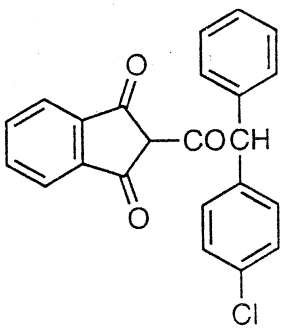
- indandiones:



The molecules all have significant structural similarity to the forms of vitamin K shown in Section 2 above. It can be seen that this structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K. The major differences in the active substances lie in the 'tail', which has varying degrees of lipophilicity. In general, the longer, and more lipophilic the 'tail' the longer the half-life, and more potent the active substance.



Warfarin



Chlorophacinone

It has been established that the molecules are structurally similar, and all have the same mode of action. It is therefore appropriate to use information in humans in one molecule, warfarin, to support the risk assessment of Chlorophacinone.

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	This 'bridging' is an acceptable strategy under the TNG Risk assessment for human health (Section 3.2.2.5 '(Quantitative) structure-activity relationships ((Q)SARs)'). Warfarin is the most frequently prescribed oral anticoagulant human drug. It is the eleventh most frequently prescribed drug in the USA (EU figures not available), with annual sales of $500 million. It is used in stroke prevention, in treatment of vascular heart disease and deep vein thrombosis. For stroke and heart disease, including patients with prosthetic heart valves, duration is 'lifelong' i.e. the patient takes the drug for the rest of their life. (Horton, J., Bushwick, B.M., Warfarin therapy: Evolving strategies in anticoagulation. American Family Physician, February 1, 1999). Doses employed in humans are typically 3 – 9 mg/person/day (dose equivalent to 0.05 – 0.15 mg/kg/day for a 60 kg human [British National Formulary, March 2002]), with most doses being in the 4 – 6 mg/person/day range (Horton op cit). Treatment is associated with increased risk of bleeding episodes, but long-term use in predominantly elderly humans over forty years has not been associated with any increased risk of tumours. The sole long-term effect is bone protein depletion in female humans after 10-12 years of continuous use (WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995). The absence of adverse effects in millions of humans following four decades of long term warfarin therapy is considered sufficient evidence that warfarin is not carcinogenic. The structural similarity of Chlorophacinone to warfarin, together with the negative results in the guideline mutagenicity tests, indicates that Chlorophacinone is not carcinogenic. **4.4 Exposure** The predominant use of anticoagulant rodenticides is at bait points, (varying in design for given situations to provide on a case-by-case basis for protection from enviromental factors such as sunlight or moisture, to prevent access to or interference by non-target animals/children/humans or to incorporate more formal physical obstruction e.g, enclosed boxes designed to be 'tamper-proof'), protected such that members of the general public cannot easily gain access to the baits within. This minimises the chances of secondary exposure, and reduces risk. Where sale to the general public is permitted, block baits (and some pelleted and grain baits) are sold in plastic (LDPE) sachets, such that the user is not directly exposed to the bait. In theory, exposure could occur when partly used baits are cleared up. In this case, exposure should again be minimal, because the user should wear protective equipment	

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	(rubber gloves) to guard against rodent-born disease, such as leptospirosis and hepatitis. Amateur use is intermittent, typically occurring at a maximum of three times a year. This does not constitute long term exposure. In terms of long-term risk, manufacturers regularly monitor the health of personnel, including regular assessment of clotting times. This immediately provides a warning if exposure is occurring, and allow for both vitamin K administration (if necessary to remedy the individual condition) and implementation of measures to prevent further exposure. Pest control operators are advised to wear protective clothing, not only because of the inherent acute toxicity of the active substances, but principally because the wild rodents themselves are significant disease vectors. **5 CONCLUSION** In conclusion, a waiver for long-term rodent studies on anticoagulant rodenticides is scientifically justified, based on lack of mutagenic/genotoxic effects, absence of any other effects that may lead to non-genotoxic carcinogenesis, and the absence of any carcinogenic effects following long-term administration of a closely-related molecule in humans. A waiver of the studies is further supported by the practical difficulties of performing a study, and the low risk of exposure in manufacturing and use. The practical difficulties of long-term administration of anticoagulants are such that an attempt at a study would be certain to fail; knowing this in advance is unethical and contrary to Directive 86/609/EEC. For the Biocidal Products Directive 98/8/EEC, a waiver for the requirement to submit rodent carcinogenicity/toxicity studies under Annex IIA, Section 6.7 is requested.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2004	
Evaluation of applicant's justification	The applicant justify non-submission of long term toxicity (and carcinogenicity) on the basis that (a) the usual specie (rat) is not suitable due it high toxicity (b) technical difficulties in the so low dose that had to be used (c) no relevant other possible species (d) known mechanism of toxicity (e) use of other data from other related chemicals (f) history of human use of related anticoagulant rodenticides. For that, arguments are done with mechanistic based in the specific mode of action of anti-vitamin K anticoagulants, data of the dose needed for the study and experimental and human toxicological properties of related anticoagulant chemicals. Curiously subchronic data are mentioned for argument where non rodent data are submitted. The TNG and directive recommendation of minimise unnecessary animal testing is also argued.	

Section A 6.05-01 Annex Point 6.5	Long term toxicity in rats
	The TNG of data requirement indicate: "The test is required for one rodent and one other mammalian species. The long-term-toxicity of an active substance may not be required where a full justification demonstrates that these tests are not necessary based on the sub-chronic toxicity test and demonstrated reversibility in the same species". The Addenda TNG for refining waiving for rodenticidas made a more flexible criteria for waiving due to the difficulties that "rodenticides designed to kill the wild form of the recommended test species, reproduction or long-term testing of the target species may be inherently difficult". There are significant weaknesses of some of the arguments: (a) The technical reasons might be overcome. (b) The reason that other species are not appropriate are reasonable but not sufficient to wave by itself as the Addendum is just indicating that then the "other" species should be consider the first choice specie and study in other species are also no submitted, and also no submitted subchronic study in other species. (c) The low toxicity argued in human is based with data with other chemical with order of magnitude of different acute toxicity in the rat. In spite of these weaknesses, globally there are strong reasons supporting the waiving due to the difficulties to do long term toxicity study and the strong recommendation of minimise unnecessary animal experimentation.
Conclusion	Justification of non-submission may be **provisionally accepted** to be reconsidered after the detail evaluation of other related data which are used for the justification
Remarks	Data of expected exposure, evaluation of the subchronic submitted data will have to be considered for definitively confirm if appropriate toxicological evaluation and human risk assessment can be done without data of chronic study. Data of related chemicals are used to justify non submission, so evaluation should have to be done considering the worse case (i.e. considering data of the highest toxic related chemicals). The "technical difficulty" argued is that it is high acutely toxic. If accepted then should be proved that expected exposure is actually lower than the lowest dose that is reasonable to be tested.

Table 6.5-1: Comparison of various rodenticide hepatic half-lives

Rodenticide	Terminal Half-life*	Species
Brodifacoum	130 days	Rat (liver)
Brodifacoum	282 days+	Rat (liver)
Bromadiolone	318 days+	Rat (liver)
Difenacoum	120 days	Rat (liver)
Difethialone	126 days	Rat (liver)
Diphacinone	~8 days	Rat
Flocoumafen	220 days +	Rat (liver)
Warfarin	42 hours	Human (plasma)

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)
+ LiphaTech (unpublished 1986)

Table 6.5-2: Comparison of rodenticide water solubility

Rodenticide	Water solubility mg/L 20°C* (+ = 25°C)
Brodifacoum	<10
Bromadialone	19
Chlorophacinone	100
Coumachlor	0.5
Coumatetralyl	425
Difenacoum	<10
Difethialone	0.39+
Diphacinone	0.3
Flocoumafen	1.1 (22°C)
Pindone	18+
Warfarin	insoluble

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)

Table 6.5-3: Comparison of acute median lethal doses for various rodenticides in seven mammalian species

Rodenticide	Acute oral (LD_{50} mg/kg) in species*:						
	Rat	Guinea-pig	Rabbit	Dog	Cat	Sheep	Pig
Brodifacoum	0.26	2.78	0.29	0.25-3.56	~25	>25	0.5-2
Bromadiolone	>0.56-<0.84	2.8	1.0	10+	>25+	-	3
Chlorophacinone	6.26						
Difenacoum	1.8	50	2	~50	100	100	80-100
Difethialone	0.56	-	0.75	11.8@	>16@	-	2-3@
Diphacinone	3.0	-	35	3-7.5	14.7	-	150
Flocoumafen	0.46	>10	0.7	0.075-0.25	>10	>5	~60
Warfarin	58.0	-	800	20-50	6-40	-	1-5

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)
Bromadiolone rat data: LiphaTech (unpublished 1987)

\+ MTD

@ LiphaTech data

Table 6.5-4: Number of animals required to detect a percentage increase in tumour rate

Background incidence:	***Number per group required to detect excess of*:***					
	1%	*5%*	*10%*	*15%*	*20%*	*25%*
0%	1051	206	100	65	47	37
1%	2729	270	115	71	51	39

5%	9101	514	173	95	63	46
10%	16294	788	237	122	77	54

* alpha 5%, power 90%. ONE sided test

Section A 6.06.1-01 **Annex Point IIA VI.6.6.1**		**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxxxxxxxx X (XXXX): Research on the Mutagenic Potential of Chlorophacinone Using the Ames Test. July 31, XXXX. XXXXXXXXXXXXXXXX, XXXXX XXXXXX, XXXX, France	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	EPA 84-2a. In accordance with EC Method B.13/14.	
2.3	**GLP**	No	
2.4	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone (Analysis No. 1750)	
3.2.1	Lot/Batch number	XXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	XXXX %	
3.2.2.2	Purity	Not specified	
3.2.2.3	Stability	Not specified	
3.3	**Study Type**	Bacterial reverse mutation test	
3.3.1	Organism/cell type	Salmonella typhimurium strains: TA 98, TA 100, TA 1535, TA 1537, TA 1538 in culture suspension (about 10^9 bacteria per ml)	
3.3.2	Deficiencies / Proficiencies	Mutation at the histidine operon (his-) checked by assaying growth with and without histidine in bottom agar. Presence of deep rough mutation (rfa), loss of lipopolysaccharide membrane on bacterial cell surface checked for with crystal violet sensitivity. Strains TA 98 and TA 100 contain the ampicillin-resistant plasmid (R factor: pkm 101)	
3.3.3	Metabolic activation system	Species and cell type: Female Sprague-Dawley rat, liver Induced with phenobarbital and β-naphtoflavone 0.5 ml of S9 mix contains 150 µl liver homogenate.	
3.3.4	Positive control	TA 1535: β-propiolactone – 2, 10, 50, 250 µg per petri plate with or without metabolic activation. TA 1537: dantrolene - 2, 10, 50, 250 µg per petri plate with or without metabolic activation. TA 1538: dantrolene - 2, 10, 50, 250 µg per petri plate with	

Section A 6.06.1-01 **Annex Point IIA VI.6.6.1**		**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
		or without metabolic activation. TA 98: niridazole – 0.05, 0.1, 0.5 µg per petri plate with or without metabolic activation. TA 100: niridazole - 0.05, 0.1, 0.5 µg per petri plate with or without metabolic activation.	
3.4	**Administration / Exposure; Application of test substance**	Non entry field	
3.4.1	Concentrations	Test doses - 2, 10, 50, 250 µg per petri plate with or without metabolic activation	
3.4.2	Way of application	Dissolved in medium	
3.4.3	Pre-incubation time	Incubation for approximately 48 hours at approximately 37^{o}C	
3.5	**Examinations**		
3.5.1	Number of cells evaluated	Not specified	
		4 RESULTS AND DISCUSSION	
4.2	**Genotoxicity**		
4.2.1	without metabolic activation	No	
4.2.2	with metabolic activation	No	
4.3	**Cytotoxicity**	Yes – at dose 250 µg without S9 for TA 1535; 250 µg with or without S9 for TA 1537, 1538 TA 100, 50 µg and 250 µg without S9 for TA 98, and 50 µg without S9 for TA 1538	

Section A 6.06.1-01 **Annex Point IIA VI.6.6.1**	**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	Chlorophacinone was tested to evaluate the potential for mutagenicity in an *in vitro* bacterial mutagenicity test using *Salmonella typhimurium* strains: TA 98, TA 100, TA 1535, TA 1537, TA 1538. Metabolism was simulated using an S-9 preparation from female rat liver induced with phenobarbital and β-naphtoflavone. The dose levels in the study were 2, 10, 50, 250 µg per petri plate with or without metabolic activation. Positive controls were as follows: TA 1535: β-propiolactone – 2, 10, 50, 250 µg per petri plate with or without metabolic activation TA 1537: dantrolene - 2, 10, 50, 250 µg per petri plate with or without metabolic activation TA 1538: dantrolene - 2, 10, 50, 250 µg per petri plate with or without metabolic activation TA 98: niridazole – 0.05, 0.1, 0.5µg per petri plate with or without metabolic activation TA 100: niridazole - 0.05, 0.1, 0.5µg per petri plate with or without metabolic activation Negative control plates received the solvent of the test article. Triplicate plates were prepared for each dose. The study was conducted according to EPA 84-2a and EC Method B13/14 test guidelines.	
5.3 Results and discussion	Chlorophacinone did not induce an increase in revertant colonies per plate in any of the *Salmonella typhimurium* strains tested, TA 98, TA 100, TA 1535, TA 1537 or TA1538, either with or without metabolic activation. The performance of the test was validated by appropriate positive and negative control responses.	
5.4 Conclusion	Chlorophacinone did not induce an increase in revertant colonies per plate in any of the Salmonella typhimurium strains tested, TA 98, TA 100, TA 1535, TA 1537 or TA1538, either with or without metabolic activation. Chlorophacinone did not induce a mutagenic effect.	
5.4.1 Reliability	2	
5.4.2 Deficiencies	Although this study predated GLPs no serious deficiencies were found.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 2005 (revised December 2005)	
Materials and Methods	The Applicant version is adopted summarised as follows: Chlorophacinone was tested for mutagenicity by evaluating increase in revertant colonies per plate in the Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 or TA1538, either with or without metabolic activation at 2, 10, 50, 250 µg per petri plate in strain TA 1535, TA 1537 or TA1538 and with 0.05, 0.1, 0.5 µg per petri plate in strain TA 98, TA 100, with and without metabolic activation. The performance of the test was validated by appropriate positive and	

Section A 6.06.1-01 Annex Point IIA VI.6.6.1	In-vitro gene mutation in bacteria Bacterial reverse mutation test	
	negative control responses.	
Results and discussion	The Applicant version is adopted: Chlorophacinone did not induce an increase in revertant colonies per plate in any of the Salmonella typhimurium strains tested, TA 98, TA 100, TA 1535, TA 1537 or TA1538, either with or without metabolic activation. The performance of the test was validated by appropriate positive and negative control responses.	
Conclusion	The Applicant version is adopted: Chlorophacinone did not induce an increase in revertant colonies per plate in any of the Salmonella typhimurium strains tested, TA 98, TA 100, TA 1535, TA 1537 or TA1538, either with or without metabolic activation. Chlorophacinone did not induce a mutagenic effect.	
Reliability	2	
Acceptability	Accepted. Although this study predated GLPs no serious deficiencies were found.	
Remarks		

Table A 6.6.1-1 Number of revertant colonies per plate and mean values TA 1535

Chemical	Dose µg /plate	Average number of revertant colonies per plate; SE		Proportion	
		+ S9	- S9	+S9	-S9
Chlorophacinone	0	19.7; 0.9	27; 1.7		
	2	12.7; 2.2	24; 2.1	0.6	0.9
	10	20.7; 1.2	29.3; 2.9	1.1	1.1
	50	14; 1.5	18; 2.7	0.7	1.1
	250	9.7; 0.3	TE	0.5	-
Standard β-propiolactone	0	19.7; 0.9	27; 1.7		
	2	57.3; 2.4	50; 5	2.9	1.9
	10	181; 3.8	115; 2.9	9.2	4.3
	50	795; 8.7	335; 7.9	40.4	12.4
	250	>1000	= 1000	>50.8	= 37

Proportion: number of mutants in presence of chemical/number of spontaneous mutants
TE: Toxic effect

Table A 6.6.1-2. Number of revertant colonies per plate and mean values TA 1537

Chemical	Dose µg /plate	Average number of revertant colonies per plate; SE		Proportion	
		+ S9	- S9	+S9	-S9
Chlorophacinone	0	10.3; 0.9	4.7; 0.7		
	2	6.7; 0.9	4.3; 1.2	0.7	0.9
	10	6.3; 0.7	3; 0.6	0.6	0.6
	50	7.3; 0.7	3.3; 0.3	0.7	0.7
	250	TE	TE	-	-
Standard dantrolene	0	10.3; 0.9	4.7; 0.7		
	2	14; 1.2	9.7; 1.2	1.4	2.1
	10	27.7; 1.5	22.3; 1.2	2.7	4.7
	50	48.3; 2.6	TE	4.7	-
	250	TE	TE	-	-

Proportion: number of mutants in presence of chemical/number of spontaneous mutants
TE: Toxic effect

Table A 6.6.1-3. Number of revertant colonies per plate and mean values TA 1538

Chemical	Dose µg /plate	Average number of revertant colonies per plate; SE		Proportion	
		+ S9	- S9	+S9	-S9
Chlorophacinone	0	32.7; 0.9	21.3; 2.3		
	2	10.7; 1.5	11.3; 1.8	0.3	0.5
	10	36.3; 1.2	9.3; 1.2	1.1	0.4
	50	36.7; 5.2	TE	1.1	-
	250	TE	TE	-	-
Standard dantrolene	0	32.7; 0.9	21.3; 2.3		
	2	=1000	=1000	30.6	46.9
	10	>1000	>1000	>30.6	>46.9

	50	>>1000	TE	>>30.6	-
	250	TE	TE	-	-

Proportion: number of mutants in presence of chemical/number of spontaneous mutants
TE: Toxic effect

Table A 6.6.1-4. Number of revertant colonies per plate and mean values TA 98

Chemical	**Dose µg /plate**	**Average number of revertant colonies per plate; +/- SE**		**Proportion**	
		+ S9	**- S9**	**+S9**	**-S9**
Chlorophacinone	0	23.7; 0.9	13.7; 2.4		
	2	30; 2.5	13.7; 1.2	1.3	1
	10	27.7; 3.3	13; 2.1	1.2	0.9
	50	15.7; 0.3	TE	0.7	-
	250	11;0.6	TE	0.5	-
Standard niridazole	0	23.7; 0.9	13.7; 2.4		
	0.05	100; 7.6	82.3; 4.3	4.2	6
	0.1	234.7; 9	194; 5.9	9.9	14.2
	0.5	TE	TE	-	-

Proportion: number of mutants in presence of chemical/number of spontaneous mutants
TE: Toxic effect

Table A 6.6.1-5. Number of revertant colonies per plate and mean values TA 100

Chemical	**Dose µg /plate**	**Average number of revertant colonies per plate; SE**		**Proportion**	
		+ S9	**- S9**	**+S9**	**-S9**
Chlorophacinone	0	139; 3.8	113.7; 5.8		
	2	126.7; 10.7	113; 8.7	0.9	1
	10	126; 7.8	109.3; 13.3	0.9	1
	50	104; 8.1	77.3; 7.3	0.7	0.7
	250	TE	TE	-	-
Standard niridazole	0	139; 3.8	113.7; 5.8		
	0.05	920; 15,3	656.7; 18.6	6.6	5.8
	0.1	>1000	TE	>7.2	-
	0.5	TE	TE	-	-

Proportion: number of mutants in presence of chemical/number of spontaneous mutants
TE: Toxic effect

Section A 6.06.1-02 **Annex Point IIA VI.6.6.1**		**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
		1 REFERENCE	**Official use only**
1.1	**Reference**	XXXX XX., (XXXX): Mutagenicity Test with Chlorophacinone in the *Salmonella – Escherichia coli*/ Mammalian-Microsome Reverse Mutation Assay with a confirmatory Assay. Unpublished report No: XXXXXXX-XXXX (June 1, XXXX). XXXXXXXXXXXXXX., XXXX, XXXXX. (Dates of experimental work: February 2, 1994 – May 27, XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA 84-2; In accordance with EC Method B.13/14.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone	
3.2.1	Lot/Batch number	Lot # XXXXX	
3.2.2	Specification	Pale yellow powder	
3.2.2.1	Description	XXX%	
3.2.2.2	Purity	Not specified	
3.2.2.3	Stability	Not specified	
3.3	**Study Type**	Bacterial reverse mutation test	
3.3.1	Organism/cell type	Salmonella typhimurium strains: TA 98, TA 100, TA 1535, TA 1537, in culture suspension (about 10^9 bacteria per ml) Escherichia coli strains : WP2uvrA	

Section A 6.06.1-02 **Annex Point IIA VI.6.6.1**		**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
3.3.2	Deficiencies / Proficiencies	**Strain** / **Histidine mutation** / **Other genetic markers** TA 1535 / his G46 / rfa, uvrB TA 1537 / his C3076 / rfa, uvrB TA 1538 / his D3052 / rfa, uvrB TA 98 / his D3052 / rfa, uvrB TA 100 / his G46 / rfa, uvrB Mutation uvrB causes deficiency in the repair system by excision, enhancing sensitivity of these strains to some mutagens, extension of uvrB deletion through the bio gene means that all of these strains require the vitamin biotin for growth. The mutation rfa confers greater permeability to certain compounds (large ring systems e.g. benzo(a)pyrene) resulting from loss of one enzyme responsible for synthesis of part of the lipopolysaccharide barrier that forms the surface of the bacterial cell wall. The his mutants can form colonies in the absence of histidine. Strains TA 1535 and TA 100 respond to mutagens inducing base pair substitution. Ta 1537 respond to mutagens inducing base pair additions and strains TA 1538 and TA 98 to mutagens inducing base pair suppression. Strains TA98 and TA100 also contain the R-factor plasmid, pKM101, which further increases sensitivity to some mutagens by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.	
3.3.3	Metabolic activation system	Species and Cell type: Male Sprague-Dawley rat, liver induced with Aroclor 1254 at 500 mg/kg	
3.3.4	Positive control	TA 98: 2-aminoanthracene with S9 mix - 2.5 µg per plate TA 98: 2-nitrofluorene without S9 mix - 1.0 µg per plate TA 100: 2-aminoanthracene with S9 mix - 2.5 µg per plate TA 100: sodium azide without S9 mix - 2.0 µg per plate TA 1535: 2-aminoanthracene with S9 mix - 2.5 µg per plate TA 1535: sodium azide without S9 mix - 2.0 µg per plate TA 1537: 2-aminoanthracene with S9 mix - 2.5 µg per plate TA 1537: ICR-191 without S9 mix - 2.0 µg per plate WP2uvrA: 2-aminoanthracene with S9 mix - 25 µg per plate WP2uvrA: 4-nitroquinoline-N-oxide without S9 mix - 10.0 µg per plate	
3.4	**Administration / Exposure; Application of test substance**		
3.4.1	Concentrations	Doses tested for the Salmonella typhimurium strains without S9 mix: 100, 50.0, 10.0, 5.00, 1.00, and 0.500 µg per plate. Doses tested for the Salmonella typhimurium strains with S9 mix: 500, 100, 50.0, 10.0, 5.00, 1.00 µg per plate.	

Section A 6.06.1-02 **Annex Point IIA VI.6.6.1**		**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
		For strain TA 98 an additional dose of 0.500 µg per plate Doses tested for the Escherichia coli strains without S9 mix: 5,000, 1,000, 200, 50.0, 10.0, and 5.00 µg per plate. Doses tested for the Escherichia coli strains with S9 mix: 5,000, 1,000, 500, 100, 50.0, 10.0 µg per plate.	
3.4.2	Way of application	The test material, tester strain and vehicle were added to molten top agar. After vortexing, the mixture was overlaid onto bottom agar.	
3.4.3	Pre-incubation time	Incubation for 48 +/- 8 hours at approximately 37°C +/- 2 °C	
3.4.4	Other modifications	Criteria for TA 98, TA 100, and WP2uvrA: The test article will be considered mutagenic if it produces at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase has to be accompanied by a dose-response to increasing concentrations of the test article. Criteria for TA 1535 and TA 1537: The test article will be considered mutagenic if it produces at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase has to be accompanied by a dose-response to increasing concentrations of the test article.	
3.5	**Examinations**		
3.5.1	Number of plates evaluated	Two per concentration	
		4 RESULTS AND DISCUSSION	
4.2	**Genotoxicity**		
4.2.1	without metabolic activation	No	
4.2.2	with metabolic activation	No	
4.3	**Cytotoxicity**	Cytotoxicity was seen in the rangefinding study. Strain TA 100 showed cytotoxicity at concentrations of 100 µg /plate (S9+) and 33.3 µg /plate (S9-). WP2uvrA showed cytotoxity at 100 µg /plate (S9-). No cytoxicity with WP2uvrA was seen up to the highest level that could be tested (3,300 µg /plate).	

Section A 6.06.1-02 **Annex Point IIA VI.6.6.1**	**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	Chlorophacinone was tested to evaluate the potential for mutagenic activity in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay With A Confirmatory Assay. This study evaluates the test article and/or its metabolite to induce reverse mutaions in the genome *of Salmonella typhimurium* strains: TA 98, TA 100, TA 1535, TA 1537, and *Escherichia coli* WP2uvrA strain. Metabolism was simulated using an S-9 preparation from male rat liver induced with Aroclor 1254 at 500 mg/kg. The dose levels in the study were as follows: Doses tested for the Salmonella typhimurium strains without S9 mix: 100, 50.0, 10.0, 5.00, 1.00, and 0.500 μg per plate. Doses tested for the Salmonella typhimurium strains with S9 mix: 500, 100, 50.0, 10.0, 5.00, 1.00 μg per plate. For strain TA 98 an additional dose of 0.500 c Doses tested for the Escherichia coli strains without S9 mix: 5,000, 1,000, 200, 50.0, 10.0, and 5.00 μg per plate. Doses tested for the Escherichia coli strains with S9 mix: 5,000, 1,000, 500, 100, 50.0, 10.0 μg per plate. Positive controls were as follows: TA 98: 2-aminoanthracene with S9 mix - 2.5 μg per plate TA 98: 2-nitrofluorene without S9 mix - 1.0 μg per plate TA 100: 2-aminoanthracene with S9 mix - 2.5 μg per plate TA 100: sodium azide without S9 mix - 2.0 μg per plate TA 1535: 2-aminoanthracene with S9 mix - 2.5 μg per plate TA 1535: sodium azide without S9 mix - 2.0 μg per plate TA 1537: 2-aminoanthracene with S9 mix - 2.5 μg per plate TA 1537: ICR-191 without S9 mix - 2.0 μg per plate WP2uvrA: 2-aminoanthracene with S9 mix - 25 μg per plate WP2uvrA: 4-nitroquinoline-N-oxide without S9 mix - 10.0 μg per plate. After incubation of the prepared plates, the bacterial colonies on triplicate plates were counted manually for the test and vehicle control and electronically for the positive control plates. The performance of the test was validated by appropriate positive and negative control responses. The test design was based on that developed by Ames, B.N. and complied with the principles of the test detailed in EC Method B.14.	
5.3 Results and discussion	<u>Experiment 16030-B1:</u> The data generated with TA98 in the presence of S9 did not meet the criteria for a valid assay due to an insufficient number of non-cytotoxic doses. However, all remaining data were acceptable and no positive increases in the	

Section A 6.06.1-02 **Annex Point IIA VI.6.6.1**	**In-vitro gene mutation in bacteria** Bacterial reverse mutation test	
	number of revertants per plate were observed with any of the of the remaining tested strain/activation combinations. Experiment 16030-C1: An additional lower dose was tested with TA 98 in the presence of S9 (0.500 µg per plate). All data were acceptable and no positive increases in the number of revertants per plate were observed with any of the of the remaining tested strain/activation combinations. However, there was a technical error in the dilution scheme, so the entire assay was retested in Experiment 16030-D2. Experiment 16030-D1: TA 98 was retested in the presence of S9 using an additional lower dose (0.5 µg per plate). All data generated with TA 98 were acceptable and no positive increases in the number of revertants per plate were observed. Experiment 16030-D2: All data were acceptable and no positive increases in the number of revertants per plate were observed with any of the remaining tested strains with or without activation. The results indicate that under the conditions of this study, the test article, Chlorophacinone, did not cause a positive increase in the number of revertants per plate with any of the tested strains in the presence or absence of activation.	
5.4 Conclusion	Under the conditions of the *Salmonella – Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay, Chlorophacinone did not cause a positive increase in the numbers of revertants per plate in any of the tester strains either in presence or absence of metabolic activation.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies were noted.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 2005 (revised December 2005)	
Materials and Methods	Chlorophacinone was tested to evaluate the potential for mutagenic activity in the Salmonella and Escherichia coli. Reverse Mutation Assay with and without metabolic activation. Salmonella typhimurium strains: TA 98, TA 100, TA 1535, TA 1537, and Escherichia coli WP2uvrA strain were used. Metabolism was simulated using an S-9 preparation from male rat liver induced with Aroclor 1254 at 500 mg/kg. The dose levels in the study were as follows: Doses tested were: S. typhimurium strains without S9 mix: **100**, 50, 10, 5, 1, and **0.5** µg/plate. S. typhimurium strains with S9 mix: **500**, 100, 50, 10, 5, and **1** µg/plate (for TA98 an additional dose of 0.5 5 µg/plate) E. coli without S9 mix: **5,000**, 1,000, 200, 50, 10, and **5** µg/plate. E. coli with S9 mix: **5,000**, 1,000, 500, 100, 50 and **10** µg per plate. The performance of the test was validated by appropriate positive and negative control responses. The test design was based on that developed by Ames, B.N. and complied with the principles of the test detailed in EC Method B.14.	
Results and discussion	Applicant version is adopted	

Section A 6.06.1-02 Annex Point IIA VI.6.6.1	In-vitro gene mutation in bacteria Bacterial reverse mutation test	
Conclusion	Under the conditions of the Salmonella – Escherichia coli Reverse Mutation Assay, Chlorophacinone did not cause a positive increase in the numbers of revertants per plate in any of the tester strains either in presence or absence of metabolic activation.	
Reliability	1	
Acceptability	Accepted	
Remarks		

Table A 6.6.1-6. Mutagenicity Assay Results – mean revertants per plate with standard deviation.
Strains TA 98, TA 100, TA 1535, TA 1537

Chemical	Dose µg per plate	TA 98		TA 100		TA 1535		TA 1537		Background lawn *
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
With S9:										
Vehicle		25	2	119	5	11	2	11	5	1
Test article	**1.00**	25	2	111	15	10	5	10	4	1
	5.00	20	6	116	4	11	4	12	3	1
	10.0	12-	2	104	7	11	3	11	1	1
	50.0	0+	0	103	9	9	2	5	1	1
	100	0+	0	93	15	12	4	5	1	2
	500	0	0	20	5	10	2	0	0	3
Positive control**		1000	73	1096	47	138	19	162	6	1
Without S9:										
Vehicle		14	4	99	3	12	1	9	5	1
Test article	**0.500**	12	3	103	16	10	2	8	3	1
	1.00	13	4	90	7	11	5	6	4	1
	5.00	12	3	97	9	12	1	8	1	1
	10.0	8-	3	99	9	8	3	3	2	1
	50.0	0	1	75	11	8	4	4	3	3
	100	0	0	55	3	7	2	0	1	3
Positive control***		131	5	524	45	447	16	897	221	1

** TA 98: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 100: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 1535: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 1537: 2-aminoanthracene with S9 mix - 2.5 µg per plate

*** TA 98: 2-nitrofluorene without S9 mix - 1.0 µg per plate
TA 100: sodium azide without S9 mix - 2.0 µg per plate
TA 1535: sodium azide without S9 mix - 2.0 µg per plate
TA 1537: ICR-191 without S9 mix - 2.0 µg per plate

* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced
(-) - Background Lawn evaluated as slightly reduced for TA 98 only
(+) - Background Lawn evaluated as moderately reduced for TA 98 only

Table A 6.6.1-7. Mutagenicity Assay Results – mean revertants per plate with standard deviation.
Strain WP2uvrA

Chemical	Dose µg per plate	WP2uvrA		Background lawn *
		Mean	SD	
With S9:				
Vehicle		23	4	1
Test article	**10.0**	21	4	1
	50.0	22	3	1
	100	20	7	1
	500	19	3	1
	1000	16	4	2
	5000	10	1	6mp
Positive control**		315	38	1
Without S9:				
Vehicle		13	6	1
Test article	**5.00**	18	9	1
	10.0	17	0	1
	50.0	13	6	1
	200	22	1	1
	1000	9	3	2mp
	5000	11	3	6mp
Positive control***		1221	112	1

**WP2uvrA: 2-aminoanthracene with S9 mix - 25 µg per plate
***WP2uvrA: 4-nitroquinoline-N-oxide without S9 mix - 10.0 µg per plate
* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced, 4 – extremely reduced, 5 – absent, 6 – obscured by precipitate
sp – slight precipitate mp – moderate precipitate hp - heavy precipitate

5.4.3 Experiment 16030-C1

Table A 6.6.1-8 Mutagenicity Assay Results – mean revertants per plate with standard deviation.
Strains TA 98, TA 100, TA 1535, TA 1537

Chemical	Dose µg per plate	TA 98		TA 100		TA 1535		TA 1537		Background lawn *
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
With S9:										
Vehicle		34	3	120	9					1
	0.500	25	3							
Test article	**1.00**	36	5	122	8	14	4	8	3	1
	5.00	39	12	117	13	12	3	6	3	1
	10.0	23	4	111	10	14	2	8	1	1;2 for TA 98
	10.0 ^	23	2	106	11	9	3	8	3	1;2 for TA 98
	100	24	6	90	4	14	2	3	2	2
	500	11	3	15	2	7	1	1	1	3
Positive control**		867	175	844	37	113	5	107	7	1
Without S9:										
Vehicle		14	6	113	12	10	6	8	2	1
Test article	**0.500**	19	3	127	20	7	4	6	3	1
	1.00	17	9	105	4	14	6	7	2	1
	5.00	20	8	109	12	11	3	6	2	1
	10.0	18	5	99	19	12-	2	4	1	2
	10.0 ^	16	3	106	11	12-	4	5	1	2
	100	2	2	61	9	5	2	4	1	3
Positive control* **		183	7	564	74	489	34	965	266	1

** TA 98: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 100: 2-aminoanthracene with S9 mix - 2.5 c
TA 1535: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 1537: 2-aminoanthracene with S9 mix - 2.5 µg per plate

*** TA 98: 2-nitrofluorene without S9 mix - 1.0 µg per plate
TA 100: sodium azide without S9 mix - 2.0 µg per plate
TA 1535: sodium azide without S9 mix - 2.0 µg per plate
TA 1537: ICR-191 without S9 mix - 2.0 µg per plate

* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced
(-) - Background Lawn evaluated as normal for TA 1535 only
^ Due to a technical error in the dilution scheme fir this assay, two doses of 10 µg per plate
were plated. For this reason, the assay was repeated in Experiment 16030-D2.

Table A 6.6.1-9.. Mutagenicity Assay Results – mean revertants per plate with standard deviation.
Strain WP2uvrA

Chemical	Dose µg per plate	WP2uvrA		Background lawn *
		Mean	SD	
With S9:				
Vehicle		12	3	1
Test article	**10.0**	24	1	1
	10.0 ^	14	3	1
	100	21	2	1
	500	16	6	1
	1000	19	4	1
	5000	16	4	6hp
Positive control**		470	9	1
Without S9:				
Vehicle		15	4	1
Test article	**5.00**	18	3	1
	10.0	22	4	1
	10.0 ^	19	2	1
	200	17	3	2
	1000	14	3	2
	5000	10	2	6hp
Positive control***		993	194	1

**WP2uvrA: 2-aminoanthracene with S9 mix - 25 µg per plate
***WP2uvrA: 4-nitroquinoline-N-oxide without S9 mix - 10.0 µg per plate
* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced, 4 – extremely reduced, 5 – absent, 6 – obscured by precipitate
sp – slight precipitate mp – moderate precipitate hp - heavy precipitate

^ Due to a technical error in the dilution scheme fir this assay, two doses of 10 µg per plate were plated. For this reason, the assay was repeated in Experiment 16030-D2.

Experiment 16030-D1

Table A 6.6.1-10. Mutagenicity Assay Results – mean revertants per plate with standard deviation. Strain TA98

Chemical	Dose µg per plate	TA98		Background lawn *
		Mean	SD	
With S9:				
Vehicle		21	3	1
Test article	**0.500**	19	6	1
	1.00	24	1	1
	5.00	18	3	1
	10.0	22	3	1
	50	22	5	1
	100	16	2	2
	500	0	0	3
Positive control**		927	96	1

**TA 98: 2-aminoanthracene with S9 mix - 25 µg per plate
* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced, 4 – extremely reduced, 5 – absent, 6 – obscured by precipitate
sp – slight precipitate mp – moderate precipitate hp - heavy precipitate

Experiment 16030-D2

Table A 6.6.1-11. Mutagenicity Assay Results – mean revertants per plate with standard deviation. Strains TA 98, TA 100, TA 1535, TA 1537

Chemical	Dose µg per plate	TA 98		TA 100		TA 1535		TA 1537		Background lawn *
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
With S9:										
Vehicle		17	2	111	11	14	2	7	2	1
	0.500	25	2							
Test article	**1.00**	25	7	106	12	9	4	4	3	1
	5.00	21	7	97	13	11	5	6	3	1
	10.0	18	5	98	11	11	2	6	1	1
	50.0	18	5	87	9	5	2	4	2	2
	100	13	5	74	9	9	6	7	3	2
	500	4	3	3	1	7	2	1	1	3
Positive control**		1673	113	1743	104	189	11	231	27	1
Without S9:										
Vehicle		17	3	70	10	13	4	4	1	1
Test article	**0.500**	13	3	70	8	7	3	5	2	1
	1.00	11	6	86	8	9	2	4	2	1
	5.00	13	5	90	12	7	4	4	1	1
	10.0	11	3	62-	4	7	1	4	2	1;2 for TA98
	50.0	7	5	49	5	6	3	4	3	2
	100	1	0	29	7	7+	1	0	0	3
Positive control* **		155	34	573	29	460	27	701	120	1

** TA 98: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 100: 2-aminoanthracene with S9 mix - 2.5 c
TA 1535: 2-aminoanthracene with S9 mix - 2.5 µg per plate
TA 1537: 2-aminoanthracene with S9 mix - 2.5 µg per plate

*** TA 98: 2-nitrofluorene without S9 mix - 1.0 µg per plate
TA 100: sodium azide without S9 mix - 2.0 µg per plate
TA 1535: sodium azide without S9 mix - 2.0 µg per plate
TA 1537: ICR-191 without S9 mix - 2.0 µg per plate
* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced
(-) - Background Lawn evaluated as slightly reduced for TA 98 only
(+) - Background Lawn evaluated as slightly reduced for TA 98 only

Table A 6.6.1-12.. Mutagenicity Assay Results – mean revertants per plate with standard deviation.
Strain WP2uvrA

Chemical	Dose µg per plate	WP2uvrA		Background lawn *
		Mean	SD	
With S9:				
Vehicle		17	5	1
Test article	**10.0**	14	4	1
	50.0	21	6	1
	100	9	4	1
	500	13	1	1
	1000	19	4	1
	5000	12	3	6mp
Positive control**		557	27	1
Without S9:				
Vehicle		16	2	1
Test article	**5.00**	12	5	1
	10.0	12	4	1
	50.0	14	3	1
	200	10	3	1
	1000	7	3	2
	5000	10	3	6hp
Positive control***		1081	114	1

**WP2uvrA: 2-aminoanthracene with S9 mix - 25 µg per plate
***WP2uvrA: 4-nitroquinoline-N-oxide without S9 mix - 10.0 µg per plate
* Background Lawn Evaluation Code:
1 – normal, 2 - slightly reduced, 3 – moderately reduced, 4 – extremely reduced, 5 – absent, 6 – obscured by precipitate
sp – slight precipitate mp – moderate precipitate hp - heavy precipitate

Section A 6.06.2-01 **Annex Point IIA VI.6.6.2**		**In-vitro gene mutation in mammalian cells** Induction of gene mutation in CHO cells	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx X., (XXXX): Test to evaluate the Induction of Genic Mutations in CHO Cells (HGPRT Locus) Chlorophacinone. Unpublished report No: XXXX (July 9, XXXX). XXXXXX XXXXX, France	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	OECD 476 (1984; EEC 67/548 (1967) – 79/831(1979) – 83/467 (1983 – 84/449 (1984)) – 88/302 (1988). EPA 84-2. In accordance with EC Method B.17.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No GLP deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone	
3.2.1	Lot/Batch number	Batch No: XXXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Pale yellow powder	
3.2.2.2	Purity	Not specified	
3.2.2.3	Stability	Not specified	
3.3	**Study Type**	In vitro mammalian cell gene mutation test	
3.3.1	Organism/cell type	mammalian cell lines: Chinese Hamster Ovary (CHO)	
3.3.2	Deficiencies / Proficiencies	The cell line was proficient in Hypoxanthine Guanine Phosphoribosyl Transferase.	
3.3.3	Metabolic activation system	S9 mix. Aroclor 1254 was administered through intraperitoneal injection 5 days before killing to male Sprague Dawley rat at a dose level of 500 mg/kg.	
3.3.4	Positive control	Ethyl methane sulphonate without S9 mix at a final concentration of $5x10^{-1}$ mg/ml; Methyl-cholanthrene with S9 mix at a final concentration of $5x10^{-3}$ mg/ml	
3.4	**Administration / Exposure; Application of test substance**		
3.4.1	Concentrations	$5x10^{-3}$, 10^{-2}, $5x10^{-2}$, 10^{-1}, $2x10^{-1}$ mg/ml	

Section A 6.06.2-01 **Annex Point IIA VI.6.6.2**		**In-vitro gene mutation in mammalian cells** Induction of gene mutation in CHO cells	
3.4.2	Way of application	The cells were cultured in medium for 24 hours. Test material was then added to the flasks at a constant volume of 200 microliters.	
3.4.3	Pre-incubation time	Twenty four hours	
3.5	**Examinations**		
3.5.1	Number of cells evaluated	1.5 x 10^6 cells were cultured in flashes to allow the expression of mutations. 2 x 10^5 cells were plated onto Petri dishes containing medium and 6-thioguanine. Two cells per dish were plated in the cytotoxicity assessment.	
		4 RESULTS AND DISCUSSION	
4.2	**Genotoxicity**		
4.2.1	without metabolic activation	No	
4.2.2	with metabolic activation	No	
4.3	**Cytotoxicity**	Yes – at concentration of $2x10^{-1}$mg/ml in both the presence and the absence of S9.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The test article Chlorophacinone was tested in vitro to provide evidence of the induction of genetic mutations in CHO cells (HGPRT locus). The five concentrations chosen ($5x10^{-3}$, 10^{-2}, $5x10^{-2}$, 10^{-1} , $2x10^{-1}$ mg/ml) were tested with and without metabolic activation. The results were confirmed in a second test performed independently from the first. The study was performed according to guidelines OECD 476 (1984; EEC 67/548 (1967) – 79/831(1979) – 83/467 (1983 – 84/449 (1984)) – 88/302 (1988). A negative control (solvent) and a positive control (standard mutagen) were included in each test.	
5.3	**Results and discussion**	Under the experimental conditions employed, the test article Chlorophacinone (Batch XXXXXXX) did not induce mutagenic effects in CHO cells (HGPRT) locus with or without metabolic activation.	
5.4	**Conclusion**	Chlorophacinone did not induce mutagenic effects in CHO cells (HGPRT) locus with or without metabolic activation.	
5.4.1	Reliability	1	
5.4.2	Deficiencies	One deficiency was noted in the comparison with the protocol. In test 2, in the absence of metabolic activation, the spontaneous mutant frequency was slightly higher than required by the protocol. This was not considered to affect the study reliability.	

Section A 6.06.2-01 **Annex Point IIA VI.6.6.2**	**In-vitro gene mutation in mammalian cells** Induction of gene mutation in CHO cells	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

July 2005 (reviewed 29 December 2005)

Materials and Methods

Chlorophacinone was tested in vitro for induction of genetic mutations in CHO cells (HGPRT locus) at concentrations of $5x10^{-3}$, 10^{-2}, $5x10^{-2}$, 10^{-1}, $2x10^{-1}$ mg/ml for 4 hours, with and without metabolic activation. The results were confirmed in a second test performed independently from the first. The study was performed according to guidelines OECD 476, in accordance with EC Method B.17.

A negative control (solvent) and a positive control (standard mutagen) were included in each test. Citotoxicity was tested either in preliminary study and in colonies in the main study with treated with test compounds and with negative and positive controls.

A preliminary study in the absence of metabolic activation at 10^{-3}, $5x10^{-3}$, 10^{-2}, $5x10^{-2}$, 10^{-1}, $5x10^{-1}$ and 1 mg/ml was tested in order to evalate cytotoxicity and to deduce appropriate dose for the main study. Citotoxicity was evaluated as a reduction in the capacity of treated cells to form clones or reduction in clonning efficiency.

As the concentration in the main study had to provoke a reduction of relative cloning efficiency between 0-90%, the dosed were then adopted as indicated for the main study. A dose of $2x10^{-1}$ mg/ml was added as an intermediary dose between $5x10^{-1}$ (very toxic) and 10^{-1} mg/ml (slightly toxic). So tested concentration were causing from no citotoxicity to high citotoxicity.

Results and discussion

All the results were confirmed in a second study independent from the first.

One criteria of conformity in negative control was not satisfied in test 2 (spontaneous mutant frequency was $18x10^{-6}$, not lower than $15x10^{-6}$ required). As this was a very slight deviation and all other criteria were conformed, the study was accepted. No incident was observed affecting quality of results.

All results were not significant at $p<0.01$ in presence of the test substance in absence of metabolic activation.

In the presence of metabolic activation were also no significant with one exception at $2x10^{-1}$ mg/plate in the first experiment and 10^{-1} in the second one. In the two cases, the observed number of mutant is in the limit of significant and no dose-response relation was observed. So these individual data were not taken into account.

In view of the experimental results, it may be concluded that Chlorophacinone did not induced mutagenic effects in CHO cells (HGPRT locus) in absence or in presence of metabolic activation.

Conclusion

Chlorophacinone did not induce mutagenic effects in CHO cells (HGPRT locus) with or without metabolic activation.

Reliability

1

Acceptability

Accepted

Remarks

Table A 6.6.2-1: Table for gene mutation assay

CHO/HGPRT Test – Colony count 1-st test

Test Article	Concentration mg/ml of medium	S9 mix	Number of mutants/dish	Total mutants	Mutants per 10^6 cells
Solvent	0	-	0 2 0 0 0 0 0 0 0 0 0 0	2	1.1
Ethyl methane sulphonate	$5x10^{-1}$	-	26 26 32 20 36 37 35 33 31 38 48 30	392	267.8*
Chlorophacinone	$5x10^{-3}$	-	1 0 0 0 0 0 0 0 0 0 1 0	2	1.2
Chlorophacinone	10^{-2}	-	2 1 0 0 0 1 2 0 1 1 1 1	10	6.1
Chlorophacinone	$5x10^{-2}$	-	0 1 0 0 0 0 0 0 1 0 0 1	3	2.3
Chlorophacinone	10^{-1}	-	0 1 2 0 0 0 1 0 0 0 0 2	6	3.8
Chlorophacinone	$2x10^{-1}$	-	0 0 0 0 0 0 0 0 **	0	0.0**
Solvent	0	+	0 0 0 0 0 0 0 0 1 0 0 0	1	0.9
Methyl-cholanthrene	$5x10^{-3}$	+	12 7 17 8 6 7 13 7 8 10 5 15	115	129.5*
Chlorophacinone	$5x10^{-3}$	+	0 0 0 0 1 1 0 1 0 1 1 0	5	4.9
Chlorophacinone	10^{-2}	+	3 1 0 1 0 1 0 0 1 0 2 0	9	7.7
Chlorophacinone	$5x10^{-2}$	+	1 1 0 0 1 0 1 0 0 0 0 1	5	5.1
Chlorophacinone	10^{-1}	+	0 1 0 1 3 0 0 1 0 0 0 0	6	5.2
Chlorophacinone	$2x10^{-1}$	+	0 0 0 0 1 2 1 5 0 0 2 0	11	8.4**

* Significant increase at p<= 0.01

** 2.5×10^4 cells seeded in 8 dishes only (due to the toxicity of the test article)

Table A 6.6.2-2: Table for gene mutation assay
CHO/HGPRT Test – Colony count 2nd test

Test Article	Concentration mg/ml of medium	S9 mix	Number of mutants/dish	Total mutants	Mutants per 10^6 cells
Solvent	0	-	0 0 0 3 0 3 3 2 5 1 2 3	22	10.0
Ethyl methane sulphonate	$5x10^{-1}$	-	30 26 36 33 21 28 25 28 30 25 23 37	342	307.1*
Chlorophacinone	$5x10^{-3}$	-	3 1 2 5 2 6 3 2 2 1 2 3	32	30.5
Chlorophacinone	10^{-2}	-	5 5 3 6 3 3 5 5 3 1 2 6	47	
Chlorophacinone	$5x10^{-2}$	-	2 2 1 2 5 0 2 1 0 2 1 1	19	21.1
Chlorophacinone	10^{-1}	-	0 0 0 1 0 0 0 0 **	1	0.9
Chlorophacinone	$2x10^{-1}$	-	0 0 0 0 0 0 0 0 **	0	0.0
Solvent	0	+	2 0 0 5 0 1 2 0 2 0 1 1	14	9.8
Methyl-cholanthrene	$5x10^{-3}$	+	15 18 13 16 7 17 13 20 16 23 11 13	182	152.0*
Chlorophacinone	$5x10^{-3}$	+	0 3 1 2 1 1 0 1 3 3 0 0	15	8.6
Chlorophacinone	10^{-2}	+	0 2 2 0 0 1 2 2 3 1 0 0	13	9.2
Chlorophacinone	$5x10^{-2}$	+	0 0 0 0 0 1 1 2 1 1 0 3	9	5.4
Chlorophacinone	10^{-1}	+	1 1 3 5 2 3 5 5 2 0 3 3	33	21.3*
Chlorophacinone	$2x10^{-1}$	+	0 0 0 0 0 0 0 2 0 **	2	14.6

* Significant increase at p<= 0.01

** 2.5×10^4 cells seeded in 8 dishes only (due to the toxicity of the test article)

Section A 6.06.3-01 **Annex Point IIA VI.6.6.3**		**In-vitro gene mutation in mammalian cells** *In-vitro* mammalian chromosome aberration test	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxxxxx XX (XXXX): Structural Chromosomal Aberration Assay in Human Lymphocytes with Chlorophacinone (CPN). Unpublished report No: XXXXXXXXXXX (August 16, XXXX). Xxxxxxxxx XXXXXXXXXXXX, XXXXXXXXX (Dates of experimental work June XXXX – November XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Guidelines for in vitro chromosome aberration assays by OECD Guideline 473, US EPA Guideline 84-2. In accordance with EC Method B.10.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No major deficiencies were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone (CPN, technical)	
3.2.1	Lot/Batch number	Lot No: XXXXXXX	
3.2.2	Specification	No specifications given	
3.2.2.1	Description	Yellow powder	
3.2.2.2	Purity	XXXXX%	
3.2.2.3	Stability	Not specified	
3.3	**Study Type**	*In vitro* mammalian chromosome aberration test	
3.3.1	Organism/cell type	mammalian cell lines: human lymphocytes cultured from healthy human donor	
3.3.2	Metabolic activation system	Aroclor 1254-induced male Sprague-Dawley rat liver homogenate	
3.3.3	Positive control	Mitomycin C without metabolic activation Cyclophosphamide with metabolic activation	
3.4	**Administration / Exposure; Application of test substance**		
3.4.1	Concentrations	6.25, 12.5, 25, 50 µg/ml	
3.4.2	Way of application	Test article was diluted in solvent and added to lymphocyte culture/medium mixture.	
3.4.3	Pre-incubation time	Lymphocyte cultures were sedimented 24 hours after mitogen stimulation.	

Section A 6.06.3-01 **Annex Point IIA VI.6.6.3**		**In-vitro gene mutation in mammalian cells** *In-vitro* mammalian chromosome aberration test	
3.4.4	Other modifications	Incubation times differed from those in the OECD guidelines.	
3.5	**Examinations**		
3.5.1	Number of cells evaluated	100 metaphase cells per culture. Two replicates were used and the total number of cells scored per dose level was therefore 200.	
		4 RESULTS AND DISCUSSION	
4.2	**Genotoxicity**		
4.2.1	without metabolic activation	No	
4.2.2	with metabolic activation	No	
4.3	**Cytotoxicity**	Yes. Severe cytotoxicity was seen at the highest concentration. Only 20 metaphase cells could be scored at that concentration.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**		
5.3	**Results and discussion**	Although the high concentration was associated with a statistically significant increase in aberrations/cell in the original assay, this finding was not replicated in the repeat assay.	
5.4	**Conclusion**	The test material was considered to be nongenotoxic with or without metabolic activiation.	
5.4.1	Reliability	1. Minor deviations from generally accepted test guidelines were observed but these deviations did not affect the quality of the results.	
5.4.2	Deficiencies	No major deficiencies were found.	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2005 (revised 29 December)	
Materials and Methods	Cultures with metabolic activation were incubated with test substance for 24 hours and cultures without metabolic activation were incubated for 48 hours. Colcemid was added 70 hours after initial treatment and cells were harvested 73 hours after initial treatment for get cells in methaphase. About 100 metaphase cells per culture were scored. The methodology was generally consistent with EEC 5.4.1, EPA 84-2 and OECD 473. The concentration used were 6.25, 12.5, 25, 50 µg/ml. Concentrations were based on a citotoxicity screen and the highest concentration was selected to achieve citotoxicity. Severe cytotoxicity was seen at the highest concentration. Only 20 metaphase cells could be scored at that concentration; this introduced difficulties in statistical fluctuations.	

Results and discussion	In the first (original chromosome aberration study) a statistically significant increase in aberrations/cells in cultures treated 24 hours after stimulation for 5 hours using concentration of 50 µg/ml with S0. Also a significant increase of the proportion of aberrant cells were observed at 25 and 50 µg/ml ($p<0.05$ and $p<0.01$). However this increase at 25 50 µg/ml was within acceptable historical control values (the control in this study was lower than usually. This group showed extreme cytotoxicity for 50 µg/ml (only 20 metaphase cells could be evaluated). Other groups with test substance showed similar or lower values than controls. Polyploid incidence for all substance treated groups were similar to control values. Confirmatory assay, under identical conditions, showed that the test substance did not caused statistically significant increase in the proportion o aberrant cells or aberration/cell at any concentration in any treatment group and polyploid incidence again aproximated those observed in concurrent negative controls. The slight increase in the first assay was considered to be a statistical aberration due to random fluctuation of the spontaneous aberration frequency, probably caused or related to the severe toxicity and the small sample available for scoring assesment. No such increase was confirmed in the second independent confirmatory assay. The results indicate that technical chlorophacinone (technical) was negative in Structural Chromosomal Aberration Assay in Human Lymphocytes.
Conclusion	The results indicate that technical chlorophacinone (technical) was negative in Structural Chromosomal Aberration Assay in Human Lymphocytes.
Reliability	1. Minor deviations from generally accepted test guidelines were observed but these deviations did not affect the quality of the results.
Acceptability	Accepted
Remarks	

Table A 6.6.3-1: Table for assay

Concentration [µg/ml]	Number of mutant cells (%)		Comments
	— S9	+ S9	
0	0.5, 0.5	1.0, 1.0	
6.25	0.5, 1.0	2.0, 0.0	
12.5	1.5, 2.0	1.0, 0.0	
25	0.0, 1.5	3.0, 0.5	
50	0.5, 1.0	10.0*, 0.0	Severe cytotoxicity. First replicate statistically significant at $p < 0.05$ by chi-square Test
CP	49.0, 15.0	-, 29.0	
DMSO	0.5, 0.5	0.0, 0.0	

Table A 6.6.3-2: Table for cytogenetic *in-vitro* test: chromosomal analysis (+S9, 24 hours incubation)

		control	**6.25**	**12.5**	**25**	**50**
cytotoxicity		No	No	No	No	Yes
Chromatid aberrations	gaps	2, 0	0, 0	0, 0	0, 0	0, 0
	deletions	2, 1	4, 0	2, 0	5, 1	2, 0
	interchanges	0, 0	0, 0	0, 0	0, 0	0, 0
Chromosome aberrations	Deletions	0, 1	0, 0	0, 0	1, 0	0, 0
	Ring	0, 0	0, 0	0, 0	0, 0	0, 0

	Dic	0, 0	0, 0	0, 0	0, 0	0, 0
Polyploidy		0, 0	0, 0	0, 0	0, 0	0, 0
endoreduplication		0, 0	0, 0	0, 0	0, 0	0, 0

Table A 6.6.3-3: Table for cytogenetic *in-vitro* test: chromosomal analysis (-S9, 24 hours incubation)

		control	**6.25**	**12.5**	**25**	**50**
cytotoxicity		No	No	No	No	No
chromatid aberrations	gaps	1, 1	1, 1	0, 1	0, 0	0, 0
	deletions	0, 1	2, 0	3, 0	0, 4	1, 3
	interchanges	0, 0	0, 0	0, 0	0, 0	1, 0
Chromosome aberrations	Deletions	0, 0	0, 0	0, 0	0, 0	0, 0
	Ring	0, 0	0, 0	0, 0	0, 0	0, 0
	Dic	0, 0	0, 0	0, 0	0, 0	0, 0
polyploidy		0, 0	0, 0	0, 0	0, 0	0, 0
endoreduplication		0, 0	0, 0	0, 0	0, 0	0, 0

Section A 6.06.4-01 **Annex Point IIA VI.6.6.4**		**In-vivo mutagenicity (bone marrow)** Micronucleus Assay	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx X., (XXXX): Mutagenicity test on Chlorophacinone in an *in vivo* mouse micronucleus assay. Unpublished report No: XXXXXXXXXXXXX (June 20, XXXX); XXXXXXXXXXXXXX. XXXXX, XXXX (Dates of experimental work January 26, XXXX – March 22, XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA 84-2; In accordance with EC Method B.12.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in Section 2. Referred to in report as Chlorophacinone Technical	
3.2.1	Lot/Batch number	Lot # XXXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Light yellow powder	
3.2.2.2	Purity	XXX%	
3.2.2.3	Stability	Not specified	
3.2.2.4	Maximum tolerable dose	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Mouse	
3.3.2	Strain	CD-1 (ICR)	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Male and female	
3.3.5	Age/weight at study initiation	Micronucleus assay: 8 weeks and 1 day Males 25.2 – 34.9 g; Females 20.6-28.3 g	
3.3.6	Number of animals per group	5m + 5f additional 5m+5f for mid-range dose additional 15m+15f for highest dose	
3.3.7	Control animals	Yes – positive controls and vehicle controls	
3.4	**Administration/ Exposure**	Intraperitoneal injections	

Section A 6.06.4-01 **Annex Point IIA VI.6.6.4**	**In-vivo mutagenicity (bone marrow)** Micronucleus Assay		
3.4.1 Number of applications	3		
3.4.2 Interval between applications	24 h		
3.4.3 Postexposure period	24h after treatment		
3.4.4 Vehicle	Corn oil		
3.4.5 Concentration in vehicle	0.375, 0.75, and 1.5 mg/ml		
3.4.6 Total volume applied	10 ml/kg		
3.4.7 dose applied	3.75 mg/kg; 7.5 mg/kg; 15 mg/kg		
3.4.8 Substance used as Positive Control	Cyclophosphamide 80 mg/kg administered on the third day of the test material administration		
3.4.9 Controls	Vehicle – Corn oil administered concurrently with the test article at the volume 10 ml/kg		
3.5 Examinations			
3.5.1 Clinical signs	Yes, including mortality assessment		
3.5.2 Tissue	Bone marrow		
	Number of animals:	5m+5f from each group	
	Number of cells:	1000	
	Time points:	24h after treatment	
	Type of cells	Erythrocytes in bone marrow	
	Parameters:	Polychromatic/normochromatic erythrocytes ratio	
		Frequency of micronucleated polychromatic erythrocytes	
	4 RESULTS AND DISCUSSION		
4.2 Clinical signs	All animals in the vehicle and positive control group appeared normal after dosing and remained healthy until the harvest time. All test article dosed animals appeared normal and healthy immediately after dosing on the first and second days. Approximately 46 hours after first dosing, 2 males from the 15 mg/kg group were languid and had few faeces, indicating less intake of food. All other animals were normal at this time. Immediately after the third dosing, 2 males from the 15 mg/kg group were languid and had fewer faeces, and one had a distended abdomen. Three females from the 15 mg/kg group were bleeding excessively from ear-tag		

Section A 6.06.4-01 **Annex Point IIA VI.6.6.4**	**In-vivo mutagenicity (bone marrow)** Micronucleus Assay	
	site. Approximately 72 h after the first dosing, 2 males and 3 females from the 15 mg/kg group and one female from the 7.5 mg/kg group were found dead. One female from the 15 mg/kg group was bleeding excessively from ear-tag site. All surviving animals from the 15 mg/kg group were languid. All other animals from the 7.5 and 3.75 mg/kg group appeared normal at this time.	
4.3 Haematology / Tissue examination	The mean % micronucleated bone marrow polychromatic erythrocytes (PCE-s) for the vehicle control were 0.10 +/- 0.04 for males, 0.06 +/- 0.04 for females, total 0.08+/- 0.03. The positive control induced significant increase in micronucleated PCE-s in both sexes, with means and standard errors of 1.90% +/- 0.24 and 2.4 %+/- 0.45 for the males and females respectively. The total mean % micronucleated PCE-s for the 3.75 mg/kg dose was 0.04 +/-0.02; for the 7.5 mg/kg dose – 0.06 +/- 0.02; for the 15 mg/kg dose 0.05 +/- 0.02 The test article induced no significant increase in micronucleated PCEs over the levels observed in the vehicle controls for males and females.	
4.4 Genotoxicity	No	

Section A 6.06.4-01 **Annex Point IIA VI.6.6.4**	**In-vivo mutagenicity (bone marrow)** Micronucleus Assay	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The ability of the test article, Chlorophacinone Technical, to induce micronuclei in bone marrow polychromatic erythrocytes of mice, was evaluated in an *in vivo* assay. The test substance was suspended in corn oil and administered intraperitoneally for 3 consecutive days in doses of 3.75 mg/kg; 7.5 mg/kg; 15 mg/kg. Animals were observed for clinical signs and mortality, and euthanatized approximately 24 h after the last administration. Slides were prepared of the bone marrow and scored for frequency of micronucleated cells and PCE/NCE ratio was determined by scoring the first 1000 erythrocytes observed at random in the optic field. Appropriate positive and vehicle control groups were used to validate the test results. The test was conducted in accordance with the EPA 84-2 and EC Method B.12.	
5.3 Results and discussion	The mean % micronucleated bone marrow polychromatic erythrocytes (PCE-s) for the vehicle control are 0.10 +/- 0.04 for males, 0.06 +/- 0.04 for females, total 0.08+/- 0.03. The positive control induced significant increase in micronucleated PCE-s in both sexes, with means and standard errors of 1.90% +/- 0.24 and 2.4 %+/- 0.45 for the males and females respectively. The total mean % micronucleated PCE-s for the 3.75 mg/kg dose is 0.04 +/-0.02; for the 7.5 mg/kg dose – 0.06 +/- 0.02; for the 15 mg/kg dose 0.05 +/- 0.02. The test article induced no significant increase in micronucleated PCEs over the levels observed in the vehicle controls for males and females.	
5.4 Conclusion	Under the conditions of this assay the test article, Chlorophacinone Technical, is considered negative in the mouse bone marrow micronucleus test.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No deficiencies were noted.	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2005 (revised December 2005)	
Materials and Methods	Applicant version is adopted and summarised as follows: Chlorophacinone Technical was tested for induction of micronuclei in bone marrow polychromatic erythrocytes in an in vivo assay in mice, administered intraperitoneally in corn oil for 3 consecutive days in doses of 3.75, 7.5 and 15	

	mg/kg. Animals were observed for clinical signs and mortality, and euthanatized approximately 24 h after the last administration. Slides were prepared of the bone marrow and scored for frequency of micronucleated cells and PCE/NCE ratio was determined by scoring the first 1000 erythrocytes observed at random in the optic field. Appropriate positive and vehicle control groups were used. The test was conducted in accordance with the EPA 84-2 and EC Method B.12.
Results and discussion	Applicant version is adopted summarised as follows: <u>Clinical signs</u> Some animals (2 males at 46 h and 2 males after third dosing) of the 15 mg/kg were languid and had few faeces, indicating less intake of food. A few animals (3 females at 48 h and and 3 females at 72 h) were bleeding excessively from ear-tag site. All surviving animals from the 15 mg/kg group were languid. All other animals from the 7.5 and 3.75 mg/kg group appeared normal at this time. <u>Micronucleous scoring</u> The mean % micronucleated bone marrow polychromatic erythrocytes (PCE-s) were as follows: Vehicle control: 0.10 ± 0.04 (males), 0.06±0.04 (females), total 0.08±0.03. The positive control 1.90±0.24 (males), 2.4±0.45 (females) (significant increase). Test group 3.75 mg/kg: 0.04±0.02 (total); Test group 7.5 mg/kg: 0.06±0.02 (total); Test group 15 mg/kg: 0.05±0.02 (total). The test article induced no significant increase in micronucleated PCEs over the levels observed in the vehicle controls for males and females.
Conclusion	Applicant version is adopted. Under the conditions of this assay the test article, Chlorophacinone Technical, is considered negative in the mouse bone marrow micronucleus test.
Reliability	1
Acceptability	Accepted
Remarks	

Table A 6.6.4-1: Table for micronucleus test in-vivo

Treatment	**Dose (mg/kg)**	**H***	% Micronucleated PCE's Mean +/- SE					
			Males		**Females**		**Total**	
			Mean	SE	Mean	SE	Mean	SE
Vehicle control Corn Oil	10ml/kg	24	0.10	0.04	0.06	0.04	0.08	0.03
Positive control (CP)	80	24	1.90	0.24**	2.04	0.45**	1.97	0.24 **
Test article	3.75	24	0.06	0.04	0.02	0.02	0.04	0.02
	7.5	24	0.08	0.04	0.04	0.02	0.06	0.02
	15	24	0.06	0.02	0.04	0.02	0.05	0.02

* The test article and the vehicle were administered by intra-peritoneal injections for 3 consecutive days and the animals were euthanatized approximately 24 h after the last

administration. CP was administered once orally and the animals were euthanatized approximately 24 h later.
** Significantly greater than the corresponding vehicle control, p<0.05

Table A 6.6.4-2: Table for micronucleus test *in-vivo*

Treatment	**Dose (mg/kg)**	**H***	**Ratio PCE: NCE Mean +/- SE**			
			Males		**Females**	
			Mean	SE	Mean	SE
Vehicle control Corn Oil	10ml/g	24	0.69	0.11	0.56	0.11
Positive control (CP)	80	24	0.47	0.11	0.58	0.09
Test article	3.75	24	0.56	0.20	0.56	0.10
	7.5	24	0.73	0.23	1.47	0.40
	15	24	0.85	0.34	0.70	0.11

* The test article and the vehicle were administered by intra-peritoneal injections for 3 consecutive days and the animals were euthanatized approximately 24 h after the last administration. CP was administered once orally and the animals were euthanatized approximately 24 h later.

Section A 6.06.5-01 Annex Point IIA, 6.6.5	Additional in vivo studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	The Technical Notes for Guidance relating to section 6.6.5 indicates that a second *in vivo* study to investigate mutagenicity or evidence of DNA damage in tissue other than bone marrow should be undertaken if results for tests conducted at 6.6.4 are negative but the *in vitro* tests detailed in 6.6.1; 6.6.2 and 6.6.3 are positive. Chlorophacinone did not meet these criteria and consequently additional testing is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 2004	
Evaluation of applicant's justification	The Aplicant version is adopted	
Conclusion	The Aplicant version is adopted	
Remarks	Accepted	

Section A 6.06.6-01 Annex Point IIA, 6.6.6	Additional in vitro studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	The Technical Notes for Guidance relating to section 6.6.6 indicates that a test for possible germ cell effects may be required if the result of the test in 6.6.4 is positive. Chlorophacinone did not meet this criteria and consequently additional testing is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 2004	
Evaluation of applicant's justification	The Aplicant version is adopted	
Conclusion	The Aplicant version is adopted	
Remarks	Accepted	

Section A 6.06.7-01 Annex Point IIA, 6.6.7	Additional genotoxicity studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [] Limited exposure [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Results for *in vitro* bacterial gene mutation (6.6.1) and *in vitro* mammalian cell gene mutation (6.6.3) tests were negative. The mouse micronucleus test (6.6.4) was also negative. The Technical Notes for Guidance state that if these studies are negative then further testing is normally only required if there are metabolites of concern formed in mammals. The studies presented in Section 6.2 indicate that faecal elimination (the only significant route of elimination) of unchanged chlorophacinone and two hydroxylated metabolites account for at least 80% of radioactivity. The hydroxylated metabolites can be assumed to have similar toxicity to the parent molecule, since both metabolites closely resemble the parent. One other metabolite (unidentified) was present representing 8.1% of radioactive dose and other minor metabolites represented a further 3.4%. Given that none of these metabolites is likely to be of greater toxicity than the parent (given the intended use of the parent, the Notifier would have selected the most toxic from any candidate molecules identified during research), further genotoxicity testing of metabolites is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	June 2004	
Evaluation of applicant's justification	The justification that metabolites will have similar “toxicity” than parent compounds are not specifically justified with experimental in vitro or in vivo studies with this or other related chemicals. Moreover there are about 30 % metabolities without identification and one metabolite which are about 12% of excreted material (not 8 % as claimed by applicant, see metabolism Section (A 6.2-02).	
Conclusion	It may be accepted BUT concern is maintained about evaluation of metabolites.	
Remarks	Metabolite properties will need further attentions.	

Section A 6.07-01 Annex Point 6.7	Carcinogenicity in rats	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	

Detailed justification:

Waiver for carcinogenicity/toxicity studies in rodents on Chlorophacinone.

The following is a series of rationales to waive the requirement to perform carcinogenicity/chronic toxicity studies on the anticoagulant rodenticide active substance Chlorophacinone under the Biocidal Products Directive 98/8/EEC.

1 INTRODUCTION.

The Biocidal Products Directive (98/8/EEC 'the Directive') requires long-term testing in rodents as part of the suite of toxicology tests in order to assess the possible adverse consequences of chronic exposure (i.e., chronic toxicity and carcinogenicity) to the biocidal active substance Chlorophacinone.

It is a unique feature of the rodenticides that the test species used in long-term toxicity and carcinogenicity studies is also the target species, and that the active substances are lethal in the target species at very low levels. This gives rise to several questions: Is it relevant to consider the possible use of long term rodent studies to predict possible effects of rodenticides in humans. Is it scientifically feasible? Can the data be derived using other species? Given that at one rodenticide molecule has been used for over forty years in human medicine, are there data in the human that are more relevant than animal data would be? Are there other data that demonstrate the potential, or lack of potential, carcinogenic properties of active substances used as rodenticides?

The Directive states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". A more detailed waiver concept is given in the TNsG on data requirements.

The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*".

The TNsG recommendations were further refined in an Addendum to the TNsG entitled Refined waiving concept for rodenticides (TMII03-item9a-CA-Jun03-Doc9-

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	TNsG.doc). These include: The study is technically not possible to perform, Use of other data, Data evaluated with regard to agricultural use Read-across from data on related substances Evaluation of acceptable human data, The study is not scientifically necessary The choice of species is not appropriate The study is not necessary owing to limited exposure and toxicity profile The Notifier has prepared a scientific justification based on this guidance to waive the requirement for these studies. Before the waiving arguments are given, it will be useful to review the way the coagulation system works in mammals and the mechanism by which the anticoagulant rodenticides function. **2 FUNCTION** Anticoagulant rodenticides such as Chlorophacinone function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver. Blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin to the insoluble fibrous protein fibrin, which binds platelets and blood cells to form a solid mass referred to as a blood clot, sealing the site of the haemorrhage and preventing further blood loss. Fibrinogen is present in the blood, but thrombin is not. Thrombin factor IIa in the scheme below) is formed at the site of injury from prothrombin (factor II), which is present in the blood. Conversion of prothrombin to thrombin occurs via the coagulation cascade, in which the blood clotting factors are employed. Without these blood factors clotting cannot take place, and the haemorrhage will not be controlled by clot formation. If the blood vessel is large and/or serves a vital organ, the haemorrhage will be fatal. The synthesis of a number of blood coagulation factors (factors II [prothrombin], VII [proconvertin] IX [Christmas factor], X [Stuart-Prower factor] and the coagulation inhibiting proteins C and S) is dependent upon vitamin K, which acts as a co-enzyme.	

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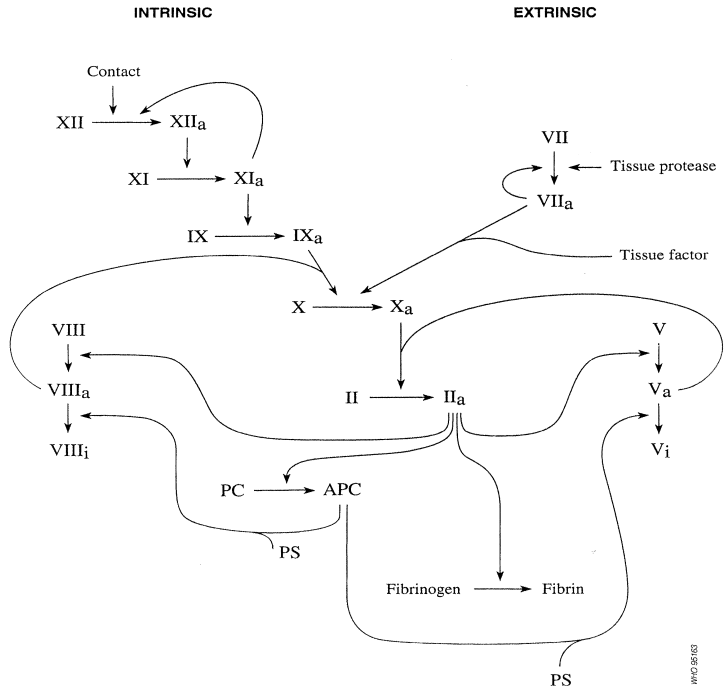
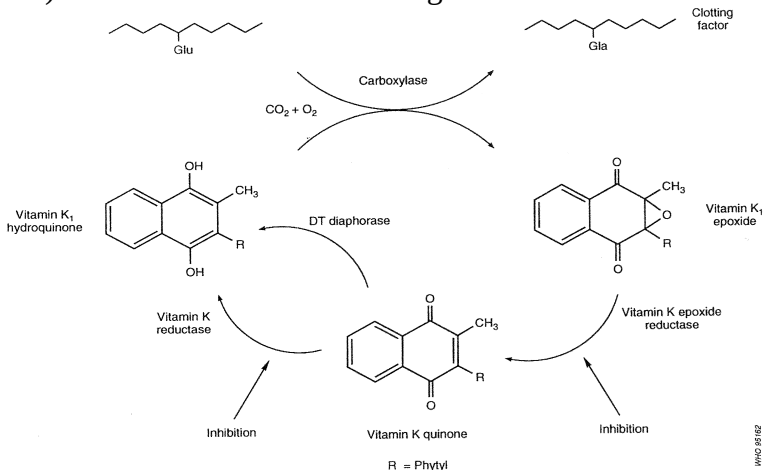


Vitamin K hydroquinone is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide provides the energy required for the carboxylation reaction where glutamate (Glu) in the precursor is converted to γ-carboxyglutamate (Gla) to make the activated clotting factor.



The anticoagulant rodenticide active substances such as Chlorophacinone work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place.
The action is cumulative, increasing levels of the anticoagulant leading to increased clotting times, such that in the event of a significant haemorrhage, death occurs. The amount of vitamin K in the body is finite, and progressive blocking of the regeneration of vitamin K will lead to an

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increasing probability of a fatal haemorrhage. In general terms, progressive intake of anticoagulants results in death. The active substances are highly toxic and bioaccumulative. The oral LD_{50} of Chlorophacinone is 6.26 mg/kg. Rodenticide baits generally contain 50 ppm Chlorophacinone and are fatal after one to three meals.

3 TECHNICAL FEASIBILITY

Carcinogenicity/toxicity studies seek to determine the consequences of long-term (near life-span) exposure to the active substance by the daily, dietary administration for two years of (typically) three increasing doses to groups of rats or mice, and observing their effects in comparison to a similar group of untreated animals (the control group).

3.2 Dose-setting and the Maximum Tolerated Dose

In order to demonstrate the validity of long-term carcinogenicity/toxicity study, the highest dose should induce some form of toxicity. This toxic effect is not necessarily carcinogenicity *per se* but should be a difference from the control group that can be demonstrated experimentally (e.g. reduced body-weight gain, altered enzyme levels, changes in function of an organ exhibited by either weight change or histopathology). This measurable indicator of toxicity should be present in the high dose level, ideally at a level that does not affect the animals sufficiently to affect survival adversely over the length of the study. This high dose level referred to as the Maximum Tolerated Dose (MTD) and, conventionally, should not cause more than 10% mortality above that observed in the control group. Studies without an MTD are considered invalid by many regulatory authorities. The intention is to administer sufficient test material such that the animal has to respond to the chemical burden i.e. it is placed under toxic stress. The implication is that if the animal does not respond to the stress by showing increased incidence of tumours, then the chemical is considered unlikely to be carcinogenic in man. Secondly, if the animal is not stressed sufficiently to show MTD response, it has not been stressed sufficiently to demonstrate the potential to cause increased incidence of tumours.

A difficulty in the administration of an MTD in a two-year study is caused by the fact that the anticoagulants are not excreted rapidly. Terminal half-lives in the liver are relevant, as the liver is the site of vitamin K regeneration, and these half-lives are very long (See Table 6.7-1). Warfarin has the lowest half-life at 42 hours in human plasma. Human liver data are not available (because liver biopsy is too hazardous for routine investigation in humans), but the liver half life is predicted to be several days, where

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'several' is probably greater than ten but less than one hundred). Absorbed doses accumulate, and lethality occurs when a threshold dose is exceeded. This may occur after one or two large doses, or several smaller doses.
It is feasible to conduct short-term animal studies with these substances because it is possible to ensure that the accumulated dose does not exceed lethal levels. However, the LD_{50} of these molecules is very low and, since the level for low lethality (e.g. LD_{10}) will be lower still, the amount to be administered daily over a two year study, in order to deliver (but not to exceed) an LD_{10}, would technically impossible to achieve. For example, for bromadiolone, the LD_{50} in rats is >0.56 mg/kg but < 0.84 mg/kg. A reasonable estimate of the LD_{10} (a value that would theoretically induce 10% mortality allowed in a long-term rodent study) is 0.6 mg per animal during the study. Using excretion data for bromadiolone, and computer software it can be shown that over the 730 days of a typical rat carc/tox study, to reach the LD_{10} by termination would require daily doses (at food intake of 25 g/rat/day) of 0.2 ppm. This is not a feasible level of dietary inclusion.

3.3 Route of Administration of the Test Substance

Dietary admixture is the only practical long-term route for administration of the test substance. It is not feasible accurately to prepare homogenous rodent test diets (to the standards required by GLP and Guidelines) at the very low concentrations needed for the MTD (i.e. 0.2 ppm as shown above). Even lower concentrations would be required for the other dose levels and these would approach the analytical method limit of detection of 0.02 ppm. It may be argued that a regulator would not expect accurate formulations, but that a study should be performed anyway. However, if inhomogeneous diet were administered, some rats would be given a feed ration that contained too much active substance, which could simply be fatal to that entire cage of five rats. Even if the rats were housed singly, the risk of fatality over a two-year period would be too great to anticipate enough animals surviving to the end of the study to provide meaningful data.
An alternative to dietary administration is the use of oral gavage. However, handling for gavage can lead to minor haemorrhage in the nasal passages (shown as brown facial staining), and the act of introducing the plastic or rubber gavage tube or steel cannula may cause minor haemorrhage in the buccal cavity and oesophagus. The use of this procedure daily for two years is considered unfeasible for an anticoagulant. Injection is also not worth considering for similar reasons. The active substances are mostly only sparingly soluble in water, so that administration in drinking

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water is not feasible. (See Table 6.7-2)
Similarly, inhalation is not feasible. Whole body exposure would lead to oral intake from grooming, resulting in death, and nose-only administration is not feasible because the increased handling and restraint of the test animals would promote the likelihood of haemorrhage. Dermal administration is also not feasible: rats need to be shaved frequently to expose the skin. Shaving is inevitably associated with minor cuts and haemorrhage.

3.4 Choice of species

Rodents are used in safety testing because they are small (easy to handle and house), readily available (large numbers can be bred in captivity), and they have a relatively short life span (studies are of shorter duration than with longer-lived species). In the case of rodenticides, designed to kill the wild form of the test species at low doses, long-term testing of the target species is inherently difficult. It is logical to see if there are alternative species, suitable for long-term tests that are less sensitive to these active substances. A comparison of LD_{50} values in other mammals shows that for each active substance the range of tolerance between species is generally one order of magnitude, and all are very low in absolute terms. (See Table 6.7-3).
It has been shown above that a dose intended to achieve LD_{10} in two years for Bromadialone would be equivalent to 0.2ppm in the diet. A slightly less sensitive species such as the dog would need a dose of 2 ppm (by simple pro-rata increase of the dose in proportion to the ratio of LD_{50}s) to reach LD_{10}. Dietary concentrations of 2 ppm are still very difficult to achieve accurately.
There are also practical considerations in performing carcinogenicity studies in large animals such as dogs, pigs or cats. In theory, a carcinogenicity study should be performed over the life span of an animal. This is two years in the rat, but is seven to ten years in the dog and pig, and ten to fifteen years in the cat. Studies of one year duration are performed on pesticides in the dog, but these are considered extensions of the 90-day subchronic study, rather than chronic studies. Dogs are amenable to laboratory housing over lengthy periods; cats are not. They require frequent handling if they are not to revert to feral behaviour and they do not respond well to being caged.
There is also the statistical power of such a study. The EC Guidelines for carcinogenicity (B.32, B.33, Directive 87/302/EEC) recommend 100 rodents per group (50 male and 50 female), with at least three treated groups plus one control. One year dog studies are typically performed with four males and four females per group.
The following statistical proof (from Quantics Consulting,

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	2004, based on 'The design and analysis of long term animal experiments', Gart JJ, Krewski D, Lee PN, Tarone RE, Wahrendorf J.1986. IARC Scientific Publications no 79. IARC, Lyon) shows that unless there are approximately 50 animals per group, it would not be possible to detect excess tumour incidences of less than 20%. If there are N animals in each of four treatment groups: control and 3 doses. Per organ at post mortem examination, the number of animals with at least one tumour in that organ is counted. Incidence in that group is percentage of animals with at least one tumour. Each treated group is compared with the control group in turn. (See Table 6.7-4). It can be seen that with a background incidence of 5%, at least 46 animals would be needed per group to detect an excess of 25% (i.e. total incidence of 30%) in the treated group. Such studies are not feasible in larger (non-rodent) mammals. In addition, there would be virtually no background control tumour incidence data on the species chosen, as such studies are rarely if ever performed in the larger mammals. European legislation militates against the use of animals in unnecessary experimentation; the use of large mammals in such studies, particularly cats and dogs, would be considered unethical in most jurisdictions. **3.5 Antidotal treatment** Studies are presented in the dossier which administer vitamin K as an 'antidote'. These studies variously show that it is possible to use vitamin K in the treatment of low single doses of anticoagulants. For Chlorophacinone, rats were given approximately 5 mg/kg bw/day for 24, 48 or 72 hours, via the diet, and vitamin K administered for 14 days. All rats given chlorophacinone for 24 hours survived, and 3/5 rats given Chlorophacinone for 48 hours survived but all rats treated for 72 hours died (reference A 6.10-01). The anticoagulant active substances are highly lipophilic. They have been shown to accumulate in the liver. The inhibition of the regeneration of vitamin K occurs by blocking, i.e. competitive binding of the active substance and the vitamin K reductase enxyme (see above) to form a lipophilic complex, which will accumulate in the liver in the same manner as the active substance. Long term co-administration of vitamin K as an antidote, would result in the accumulation in the liver of the lipophilic complex; not the active substance. As there would be no free active substance present the test would not be valid.	

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3.6 Absence of carcinogenic risk

The anticoagulant action is the sole pharmacological action of the materials. The mode of action has been described in detail. It is difficult to demonstrate that this is the sole mode of action, as administration is acutely lethal, but it is supported by the available short-term toxicology data. The absence of any other toxic effect indicates that the probability of a physiological effect (such as chronic irritation of gut walls leading to hyperplasia, or adaptive proliferation of liver or kidney cells in response to increased workload) leading to non-genotoxic carcinogenesis is low. Indeed the very long half-lives and accumulation within the liver indicate that the liver is unable actively to excrete the active substances, further indicating that a proliferative or adaptive response is unlikely in that organ. The 90-day rat study showed no indications of any adverse hyperplasia or hypertrophy in the target organ, the liver, at near-lethal levels of administration.

The absence of carcinogenic potential is further supported by the fact that mutagenicity studies on the active substances are negative. Given that the materials are not mutagenic/genotoxic, the likely mechanisms of carcinogenicity are limited to those resulting from effects such as hepatic hypertrophy, or irritance, and short-term studies show that there are no responses of that nature. It is reasonable to conclude that the active substances have no carcinogenic potential. This is supported by human data (see below).

4 USE OF OTHER DATA

4.2 Data evaluated with regard to agricultural use

Chlorophacinone is registered for agricultural uses. All of the available data are presented in the BDP dossier: no other data have been derived specifically to defend agricultural uses.

4.3 Long-term human data

There is long term experience in humans with warfarin, widely used in anti-clotting therapy in humans for over forty years, with no association with increased incidence of cancer.

Warfarin was the first of the anti-vitamin K rodenticides. The anticoagulant rodenticides fall into two categories: inandones, such as chlorophacinone, and hydroxycoumarins such as warfarin, bromadialone and difethialone.

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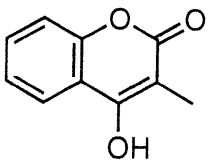
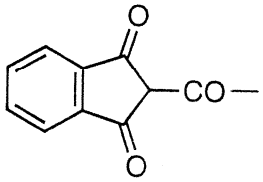
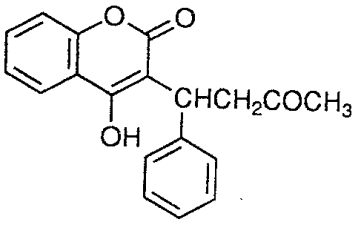
- hydroxycoumarins:



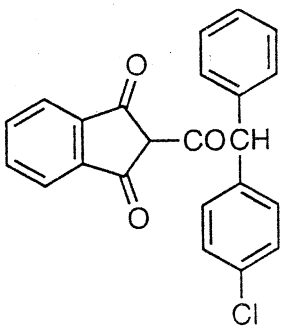
- indandiones:



The molecules all have significant structural similarity to the forms of vitamin K shown in Section 2 above. It can be seen that this structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K. The major differences in the active substances lie in the 'tail', which has varying degrees of lipophilicity. In general, the longer, and more lipophilic the 'tail' the longer the half-life, and more potent the active substance.



Warfarin



Chlorophacinone

It has been established that the molecules are structurally similar, and all have the same mode of action. It is therefore appropriate to use information in humans in one molecule, warfarin, to support the risk assessment of Chlorophacinone.

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	This 'bridging' is an acceptable strategy under the TNG Risk assessment for human health (Section 3.2.2.5 '(Quantitative) structure-activity relationships ((Q)SARs)'). Warfarin is the most frequently prescribed oral anticoagulant human drug. It is the eleventh most frequently prescribed drug in the USA (EU figures not available), with annual sales of $500 million. It is used in stroke prevention, in treatment of vascular heart disease and deep vein thrombosis. For stroke and heart disease, including patients with prosthetic heart valves, duration is 'lifelong' i.e. the patient takes the drug for the rest of their life. (Horton, J., Bushwick, B.M., Warfarin therapy: Evolving strategies in anticoagulation. American Family Physician, February 1, 1999). Doses employed in humans are typically 3 – 9 mg/person/day (dose equivalent to 0.05 – 0.15 mg/kg/day for a 60 kg human [British National Formulary, March 2002]), with most doses being in the 4 – 6 mg/person/day range (Horton op cit). Treatment is associated with increased risk of bleeding episodes, but long-term use in predominantly elderly humans over forty years has not been associated with any increased risk of tumours. The sole long-term effect is bone protein depletion in female humans after 10-12 years of continuous use (WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995). The absence of adverse effects in millions of humans following four decades of long term warfarin therapy is considered sufficient evidence that warfarin is not carcinogenic. The structural similarity of Chlorophacinone to warfarin, together with the negative results in the guideline mutagenicity tests, indicates that Chlorophacinone is not carcinogenic. **4.4 Exposure** The predominant use of anticoagulant rodenticides is at bait points, (varying in design for given situations to provide on a case-by-case basis for protection from enviromental factors such as sunlight or moisture, to prevent access to or interference by non-target animals/children/humans or to incorporate more formal physical obstruction e.g, enclosed boxes designed to be 'tamper-proof'), protected such that members of the general public cannot easily gain access to the baits within. This minimises the chances of secondary exposure, and reduces risk. Where sale to the general public is permitted, block baits (and some pelleted and grain baits) are sold in plastic (LDPE) sachets, such that the user is not directly exposed to the bait. In theory, exposure could occur when partly used baits are cleared up. In this case, exposure should again be minimal, because the user should wear protective equipment	

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	(rubber gloves) to guard against rodent-born disease, such as leptospirosis and hepatitis. Amateur use is intermittent, typically occurring at a maximum of three times a year. This does not constitute long term exposure. In terms of long-term risk, manufacturers regularly monitor the health of personnel, including regular assessment of clotting times. This immediately provides a warning if exposure is occurring, and allow for both vitamin K administration (if necessary to remedy the individual condition) and implementation of measures to prevent further exposure. Pest control operators are advised to wear protective clothing, not only because of the inherent acute toxicity of the active substances, but principally because the wild rodents themselves are significant disease vectors. **5 CONCLUSION** In conclusion, a waiver for long-term rodent studies on anticoagulant rodenticides is scientifically justified, based on lack of mutagenic/genotoxic effects, absence of any other effects that may lead to non-genotoxic carcinogenesis, and the absence of any carcinogenic effects following long-term administration of a closely-related molecule in humans. A waiver of the studies is further supported by the practical difficulties of performing a study, and the low risk of exposure in manufacturing and use. The practical difficulties of long-term administration of anticoagulants are such that an attempt at a study would be certain to fail; knowing this in advance is unethical and contrary to Directive 86/609/EEC. For the Biocidal Products Directive 98/8/EEC, a waiver for the requirement to submit rodent carcinogenicity/toxicity studies under Annex IIA, Section 6.7 is requested.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004 (revised december 2004)
Evaluation of applicant's justification	The applicant justifies non-submission by presenting just a copy of the same argument for non-submission of long-term toxicity in rats. Some specific arguments are presented try to demonstrate the absence of carcinogenic risk on the basis of: (a) Anticoagulant action is the sole pharmacological action (supported by data of acute and short term toxicity data) (b) mutagenicity studies are negative (c) Supported by human data The TNG of data requirement indicate: “One rodent and one other mammalian species should be tested”… The carcinogenicity of an active substance may not be

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	required where a full justification demonstrates that these tests are not necessary". The Addenda TNG for refining waiving for rodenticides made a more flexible criteria for waiving due to the difficulties that "rodenticides designed to kill the wild form of the recommended test species, reproduction or long-term testing of the target species may be inherently difficult". There are significant weaknesses of the general arguments: (d) The technical reasons might be overcome. (e) The reason that other species are not appropriate is reasonable but not sufficient to waive by itself. Addendum is just indicating that the "other" species should be considered the first choice specie but study in other species are no submitted, and also no submitted subchronic study in other species. (f) The low toxicity argued in human is based with data from chemical with order of magnitude of different acute toxicity in rat. The specific argument for absent of carcinogenicity risk has also some weakness: Short term toxicity can not easily demonstrate that other mode of action might be relevant for low dose in long term toxicity and carcinogenicity. In spite of this weakness, globally there are strong reasons supporting the waiving due to the difficulties to do long term toxicity study, the doubts to do more animal experiments for potentially usefulness conclusions.
Conclusion	Justification of non-submission may be provisionally accepted to be reconsidered after the detail evaluation of other related data which are used for the justification.
Remarks	

Table 6.7-1: Comparison of various rodenticide hepatic half-lives

Rodenticide	Terminal Half-life*	Species
Brodifacoum	130 days	Rat (liver)
Brodifacoum	282 days+	Rat (liver)
Bromadiolone	318 days+	Rat (liver)
Difenacoum	120 days	Rat (liver)
Difethialone	126 days	Rat (liver)
Diphacinone	~8 days	Rat
Flocoumafen	220 days +	Rat (liver)
Warfarin	42 hours	Human (plasma)

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)
+ LiphaTech (unpublished 1986)

Table 6.7-2: Comparison of rodenticide water solubility

Rodenticide	Water solubility mg/L 20°C* (+ = 25°C)
Brodifacoum	<10
Bromadialone	19
Chlorophacinone	100
Coumachlor	0.5
Coumatetralyl	425
Difenacoum	<10
Difethialone	0.39+
Diphacinone	0.3
Flocoumafen	1.1 (22°C)
Pindone	18+
Warfarin	insoluble

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)

Table 6.7-3: Comparison of acute median lethal doses for various rodenticides in seven mammalian species

Rodenticide	Acute oral (LD_{50} mg/kg) in species*:						
	Rat	Guinea-pig	Rabbit	Dog	Cat	Sheep	Pig
Brodifacoum	0.26	2.78	0.29	0.25-3.56	~25	>25	0.5-2
Bromadiolone	>0.56-<0.84	2.8	1.0	10+	>25+	-	3
Chlorophacinone	6.26						
Difenacoum	1.8	50	2	~50	100	100	80-100
Difethialone	0.56	-	0.75	11.8@	>16@	-	2-3@
Diphacinone	3.0	-	35	3-7.5	14.7	-	150
Flocoumafen	0.46	>10	0.7	0.075-0.25	>10	>5	~60
Warfarin	58.0	-	800	20-50	6-40	-	1-5

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)
Bromadiolone rat data: LiphaTech (unpublished 1987)

\+ MTD
@ LiphaTech data

Table 6.7-4: Number of animals required to detect a percentage increase in tumour rate

Background incidence:	***Number per group required to detect excess of*:***					
	1%	*5%*	*10%*	*15%*	*20%*	*25%*
0%	1051	206	100	65	47	37
1%	2729	270	115	71	51	39
5%	9101	514	173	95	63	46
10%	16294	788	237	122	77	54

* alpha 5%, power 90%. ONE sided test

Section A 6.08.1-01 **Annex Point IIA VI.6.8.1**		**Teratogenicity study** Developmental toxicity study in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxx XX., Xxxx XX., Xxxx X., (XXXXx): Developmental toxicity Evaluation of Chlorophacinone Administered by Gavage to CD Sprague-Dawley Rats. Unpublished report No: XXXXXXXXXX (July 21, XXXX). Reproductive and Developmental Toxicology Laboratory, Research Triangle Institute, NC (Dates of experimental work September XXXX – March XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	US EPA 83-3, 1988. In accordance with EC Method B.31.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No GLP deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone (2-((4-chlorophenyl) phenyl acetyl) 1H-indane-1,3(2H)-dione) CAS # 3691-35-8	
3.2.1	Lot/Batch number	Lot # XXXXXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow odourless powder	
3.2.2.2	Purity	XXXXXX%	
3.2.2.3	Stability	Test material stability not specified. The range of dosing solutions used in the study was tested for homogeneity, stability and achieved concentration prior to starting the study.	
3.3	**Test Animals**		
3.3.1	Species	Rats	
3.3.2	Strain	VAF CD Sprague- Dawley	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Females – nulliparous. 120 males of same strain obtained as breeders	
3.3.5	Age/weight at study initiation	10 weeks. 211.1-262.2 g on gestational day 0 (gd 0)	
3.3.6	Number of animals per group	25 females	
3.3.7	Control animals	Yes	
3.3.8	Mating period	Males and females were paired until sperm positive	

Section A 6.08.1-01 **Annex Point IIA VI.6.8.1**		**Teratogenicity study** Developmental toxicity study in rats		
3.4	**Administration/ Exposure**	Oral – gavage		
3.4.1	Duration of exposure	10 consecutive days		
		Rat	day 6-15	post mating
3.4.2	Postexposure period	5 days		
3.4.3	Type	Gavage		
3.4.4	Concentration	Gavage at doses of 0.0, 12.5, 25.0, 50.0, 100.0 µg/kg/day		
3.4.5	Vehicle	Corn oil		
3.4.6	Concentration in vehicle	0.0, 6.25, 1112.5, 25.0, 50.0 µg/ml		
3.4.7	Total volume applied	2 ml/kg		
3.4.8	Controls	Vehicle and historical control data set		
3.5	**Examinations**			
3.5.1	Body weight	Yes - maternal weights recorded on gestation days 0, 6, 9, 12, 15, 18 and 20		
3.5.2	Food consumption	Yes – maternal food consumption recorded over intervals of gestation day 0-6, 6-9, 9-12, 12-15, 15-18 and 18-20		
3.5.3	Clinical signs	Yes – once daily on gestational day (gd) 0 to 5 prior to dosing, and on gd 16 to 20 after the dosing period. Signs recorded twice daily, at dosing and 1 hour after dosing throughout the dosing period (gd 6 to 15)		
3.5.4	Examination of uterine content	Gravid uterine weight		
		Number of corpora lutea		
		Number of implantations		
		Number of resorptions		
3.5.5	Examination of foetuses			

Section A 6.08.1-01 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rats	
3.5.5.1 General	Number of foetuses per litter, Foetal Weight, Sex and Sex Ratio, Number of dead foetuses, Number of live foetuses, External malformations and variations	
3.5.5.2 Skeletal	Skeletal malformations and variations	
3.5.5.3 Soft tissue	Visceral malformations and variations	
	4 RESULTS AND DISCUSSION	
4.2 Maternal toxic Effects	Pregnancy Rate – high and approximately equivalent across groups (96.0-100.0%). No dams aborted, delivered early, or were removed from the study. Maternal toxicity: 18 dams out of 25 at 100 µg/kg/day died or were sacrificed moribund on gd 12(one), 13 (eight), 14 (eight), gd 16 (one). Clinical signs were limited to animals dosed at 100 µg/kg/day. Signs included external bleeding around eartag; pale eyes, ears, paws and tail; bleeding from vagina; prone position; laboured, slow or shallow breathing; chromodacryorrhoe and pilo-erection. They exhibited the following signs at necropsy: blood in vagina and amniotic sacs, blood mixed with ingesta in gastro-intestinal tract, pale organs including ovaries, spleen, kidneys, liver, adrenals, lungs, and multiple red foci on lungs. All other females survived and were pregnant. There were no apparent treatment-related clinical signs of toxicity at the other doses. Clinical weight loss (defined as >= 5g over a weight period) was observed in one dam each 50.0 and 100.0 µg/kg/day on gd 15. At the scheduled necropsy, there were no treatment-related findings. All pregnant dams had one or more live foetuses at scheduled sacrifice except for one at 50 µg/kg/day with a fully resorbed litter. Maternal body weights and weight gains were equivalent across all groups for all time points or intervals. Maternal weight gain for the pre-treatment period, gd 0-6, exhibited a significant dose-related downward trend, with the value at 100 µg/kg/day significantly reduced compared to the control value. The dams at 100 µg/kg/day or at any other dose never exhibited significantly reduced body weights or weight gains during or after the treatment, so the effect on gd 0-6 is considered biologically irrelevant. Maternal gravid uterine weight and absolute and relative liver weights were statistically and biologically equivalent across all groups. Maternal food consumption exhibited no treatment-related changes. A slight dose-related ($p < 0.05$) upward trend was present for food consumption for gd 18-20, unaccompanied by any significant pairwise comparisons to the control group.	
4.3 Teratogenic /	There were no treatment-related effects on any gestational	

Section A 6.08.1-01 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rats	
embryotoxic effects	parameters, including pre- or post-implantation loss, number of foetuses per litter, foetal sex ratio, or foetal body weight per litter. There were no treatment-related changes in the incidence of individual or pooled external, visceral, skeletal, or total malformations or variations. There were significant ($p< 0.05$) dose-related upward trends for percent foetuses (all foetuses or males and females separately) with malformations per litter and for percent litters with visceral malformations, but no significant pairwise comparisons for any parameter to the control group. There was only one foetus in the study with all of the observed external malformations at 25 µg/kg/day. There were four foetuses with skeletal malformations, one each at 0.0 and 12.5 µg/kg/day and two foetuses at 25 µg/kg/day. A total of 84 foetuses in 39 litters across all groups exhibited visceral malformations, all but one exhibited bilateral hydroureter (ureter greatly distended along its length from the renal pelvis to the urinary bladder)– the most common visceral malformation in the historical control data for this rat strain. Foetal visceral and skeletal variations were equally distributed across all groups and included enlarged lateral ventricles and distended ureters for visceral variations and extra (fourteenth) rib, short (thirteenth) rib, wavy ribs, reduced ossification in thoracic and caudal centra, and incomplete ossification of nasals, sacral centra, pubis and ischium for skeletal variations.	
4.4 Other effects	There were no significant effects of treatment on any gestational parameters, including number of ovarian corpora lutea; total number of uterine implantation sites; pre- or post-implantation loss; number of live foetuses per litter, sex ratio or foetal body weight per litter, when calculated as all foetuses, or males or females separately.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The present study was designed to evaluate the potential of Chlorophacinone (2-((4-chlorophenyl) phenyl acetyl) 1H-indane-1,3(2H)-dione) to produce maternal and developmental toxicity (including teratogenicity) when administered by gavage during major organogenesis in CD Sprague-Dawley rats. Timed pregnant rats were exposed to Chlorophacinone dissolved in corn oil and administered by gavage once daily, on gestational days 6 through 15 at doses 0.0, 12.5, 25.0, 50.0, 100.0 µg/kg/day, equivalent to 0.0, 6.25, 1112.5, 25.0, 50.0 µg/ml corn oil, at dosing volume of 2 ml/kg. 25 sperm-positive females were allocated to each group. Clinical observations were recorded daily, except during the dosing period when they were made twice daily. Maternal	

Section A 6.08.1-01 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rats	
	body weights were taken on gd 0,6,9,12,15,18,and 20. Feed consumption was measured for the intervals gd 0-6, 6-9, 9-12, 12-15, 15-18, 18-20. At scheduled sacrifice on gd 20 (approximately one and a half days before expected parturition), the dams were evaluated for body, liver and gravid uterine weight. Ovarian corpora lutea were counted and the status of uterine implantation sites (resorptions, dead foetuses, live foetuses) was recorded. All foetuses were dissected from the uterus, counted, weighed, sexed, and examined for external abnormalities. Approximately one-half of the live foetuses in each litter were examined for visceral malformations and variations. Control group consisted of animals that received the vehicle and historical control data set. The study was conducted according to the FIFRA testing guidelines (EPA 83-3, 1988) and EC Method B.13.	
5.3 Results and discussion	Profound maternal toxicity, including 72 % mortality (18 dams out of 25) and observations pre- and post-mortem consistent with the anticoagulation mechanism of action of the test article (external bleeding, pale extremities, pale organs, blood in gastrointestinal tract and amniotic sacs of the uterus) were observed only at the highest dose level tested – 100 µg/kg/day. There were no effects of treatment on maternal body weights, or food consumption at any dose. Pregnancy rate was high and equivalent across groups. Only one dam at 50 µg/kg/day had a fully resorbed litter; all remaining dams had live litters at scheduled sacrifice. The numbers of litters and foetuses examined were 25 (406), 24 (373), 25 (410), 24 (395), and 7 (110), at 0.0, 12.5, 25.0, 50.0, 100.0 µg/kg/day, respectively. There were no treatment-related effects on any gestational parameters, including pre- or post-implantation loss, number of foetuses per litter, foetal sex ratio, or foetal body weight per litter. There were no treatment-related statistically significant changes in the incidence of individual or pooled external, visceral, skeletal, or total malformations or variations. There were significant ($p < 0.05$) dose-related upward trends for percent foetuses (all foetuses or males and females separately) with malformations per litter and for percent litters with visceral malformations, but no significant pairwise comparisons for any parameter to the control group. The apparent increases in foetal (visceral) malformations were due to the incidences of bilateral hydroureter, the most common foetal visceral malformation observed in control rat foetuses. The incidence at 100 µg/kg/day was approximately comparable to that in the most recent study in the performing laboratory's historical control data set, and the apparent increase was observed only at a dose that	

Section A 6.08.1-01 **Annex Point IIA VI.6.8.1**		**Teratogenicity study** Developmental toxicity study in rats	
		resulted in profound maternal mortality. Chlorophacinone administered orally by gavage during major organogenesis in Sprague-Dawley rats resulted in no indication of developmental toxicity including teratogenicity. The NOAEL for maternal toxicity was 50.0 µg/kg/day and the NOAEL for developmental toxicity was greater than 100.0 µg/kg/day in rats under the conditions of this study.	
5.4	**Conclusion**		
5.4.1	LO(A)EL maternal toxic effects	100 µg/kg/day	
5.4.2	NO(A)EL maternal toxic effects	50.0 µg/kg/day	
5.4.3	LO(A)EL embryotoxic / teratogenic effects	>100.0 µg/kg/day	
5.4.4	NO(A)EL embryotoxic / teratogenic effects	100.0 µg/kg/day	
5.4.5	Reliability	1	
5.4.6	Deficiencies	No deficiencies were found in this well-conducted study.	

Section A 6.08.1-01 Annex Point IIA VI.6.8.1	**Teratogenicity study** Developmental toxicity study in rats	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

12 October 2005 (revised 23 December 2005)

Materials and Methods

Applicant version is adopted summarised as follows:

Summary: Chlorophacinone was tested to produce maternal and developmental toxicity (including teratogenicity) when administered by gavage during major organogenesis in CD Sprague-Dawley rats.

Timed pregnant rats were exposed to Chlorophacinone dissolved in corn oil and administered by gavage once daily, on gestational days 6 through 15 at doses 0, 12.5, 25, 50, 100 µg/kg/day.

The numbers of litters and foetuses examined were 25 (406), 24 (373), 25 (410), 24 (395), and 7 (110), at 0, 12.5, 25, 50, 100 µg/kg/day, respectively.

The highest dose caused high mortality (18 of 25) but in 7 surviving dams foetuses were posible to evaluate for developmental toxicity.

The study was conducted according to the FIFRA testing guidelines (EPA 83-3, 1988) and EC Method B.13.

Results and discussion

Applicant version is adopted with some summary remarks:

Maternal effects:

No alterations were observed in pregnancy rate (96-100%) in all groups. No dams aborted, delivered early, or were removed from the study.
Mortality: 18/25 at 100 µg/kg/day died or were sacrificed moribund on gd 12 (1), 13 (8), 14 (8), 16 (1).
Clinical signs were limited to animals dosed at 100 µg/kg/day. Signs included external bleeding; pale eyes, ears, paws and tail; bleeding from vagina; prone position; laboured, slow or shallow breathing; chromodacryorrhoe and pilo-erection. They exhibited the following signs at necropsy: blood in vagina and amniotic sacs, blood mixed with ingesta in gastro-intestinal tract, pale organs including ovaries, spleen, kidneys, liver, adrenals, lungs, and multiple red foci on lungs. All other females survived and were pregnant.
There were no apparent treatment-related clinical signs of toxicity at the other doses. At the scheduled necropsy, there were no treatment-related findings.

All pregnant dams had one or more live foetuses at scheduled sacrifice except for one at 50 µg/kg/day with a fully resorbed litter.
Maternal body weights and weight gains were equivalent across all groups for all time points or intervals.
Maternal gravid uterine weight and absolute and relative liver weights were statistically and biologically equivalent across all groups.

Maternal food consumption exhibited no treatment-related changes.

For **maternal toxicity a NOAEL of 50 µg/kg bw/day** was adopted on the basis of mortality at higher dose (LOAEL 100 µg/kg bw/day).

Developmental effects

Chlorophacinone administered orally during major organogenesis (gestational days 6 through 15) gave no indication of developmental toxicity including teratogenicity at the highest doses of 100 µg/kg/day which are causing high maternal mortality (18 of 25, 72%) with enough surviving dams (7 of 25, 28%) for evaluation of embryotoxicity and teratogenicity.
No developmental effects were noted at any dose. So, NOAEL for developmental toxicity was considered the highest tested dose with about 20 % dams surviving. In the first study in rat, the highest dose of 100 µg/kg bw per day caused 72% mortality (18 out of 25) without any significant observed effect in foetus of the surviving dams. In the second study in rats, 100 % mortality was observed at 75 µg/kg bw/day and at 25 µg/kg bw/day, a high mortality (13 of 16) was also

Section A 6.08.1-01 Annex Point IIA VI.6.8.1	Teratogenicity study Developmental toxicity study in rats	
	observed but no significant effect were detected in the foetus of the surviving dams. So it is concluded that no developmental effect was observed at the highest maternal tolerable doses. Strictly NOAEL for developmental toxicity cannot be established. For a practical point of view for later assessments, a **NOAEL for developmental toxicity in rats of 100 µg/kg bw/day is adopted.**	
Conclusion	Applicant version is adopted: For **maternal toxicity a NOAEL of 50 µg/kg bw/day** was adopted on the basis of mortality at higher dose (LOAEL 100 µg/kg bw/day). Clinical signs of toxicity and necropsy pathology demonstrated that mortalities were due to internal haemorrhage related with the anticoagulant properties of the substance. No developmental effects were noted at any dose including at the highest maternal tolerable doses. Therefore, NOAEL for developmental toxicity was considered the highest tested dose with about 20 % dams surviving. Strictly NOAEL for developmental toxicity cannot be established. For a practical point of view for later assessments, a **NOAEL for developmental toxicity of 100 µg/kg bw/day is adopted.**	
Reliability	1	
Acceptability	Accepted	
Remarks		

Table A 6.8.1-1: Table for teratogenic effects

Maternal effects

Parameter	control data		12.5 µg/kg/day	25.0 µg/kg/day	50.0 µg/kg/day	100.0 µg/kg/day	dose-response + / -
	historical	study					
Number of dams examined	29	25	25	25	25	25	
Clinical findings during application of test substance	Not reported			Urine-reddish color	Alopecia limbs	Bleeding around eartag; vaginal bleeding; lethargy; pale extremities; dyspnea; chromodacryorrhea; hunched; piloerection	+
Mortality of dams	0%	0%	0%	0%	0%	72 % **(18 out of 25)**	+
Abortions	0%	0	0	0	0	0	-
Body weight gain *day 0-6, day 6-15, day 15-20*	0-20 day: 164.1 g	0-6 day 45.5g; 6-15 day 52.7g; 15-20 day 77.2g	0-6 day 46g; 6-15 day 52.4g; 15-20 day 73.1g	0-6 day 42.3g; 6-15 day 56.0g; 15-20 day 81.8g	0-6 day 41.2g; 6-15 day 56.2g; 15-20 78.3g	0-6 day- 38.8g; 6-15 day 54.2g; 15-20 day 78.8g	-
Food consumption	Not reported	Mean 75.8 g/kg/day +/- 0.8	Mean 76.2 g/kg/day +/- 1.0	Mean 78.4 g/kg/day +/- 1.2	Mean 77.7 g/kg/day +/- 0.9	Mean 77.0 g/kg/day +/- 2.0	-
Pregnancies	Not reported	25/25	24/25	25/25	25/25	25/25	-
Necropsy findings in dams dead before end of test	Not reported	Normal	Normal	Normal	Normal	Blood in vagina and amniotic sac; dark ingesta in small intestine; blood and food in stomach; blood on face; pale organs; red foci on all lobes in lungs	+

Table A 6.8.1-2: Table for teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Parameter	control data		12.5 µg/kg	25.0 µg/kg	50.0 µg/kg	100.0 µg/kg	dose-response + / -
	historical	study					
Corpora lutea *state total/number of dams*	16.97	16.68/25	16.38/24	16.88/25	16.16/25	16.14/7	-
Implantations *state total/number of dams*	15.86 per litter/29	16.72/25	16.21/24	16.88/25	16.08/25	16.00/7	-
Resorptions *state total/number of dams*	0.59 litter/29	0.48/25	0.67/24	0.48/25	0.28/25	0.29/7	-
Total number of foetuses	15.28 live/ litter	16.24	15.54	16.40	16.46	15.71	-
Total number of litters	29	25	24	25	24	7	+
Foetuses / litter	15.28	16.24	15.54	16.40	16.46	15.71	-
Live foetuses / litter	15.28	16.24/25	15.54/24	16.40/25	16.46/25	15.71/7	-
Dead foetuses / litter *state ratio*	3.56%/ litter	0	0	0	0	0	-
Foetus weight (mean) *[g]*	3.642g	3.579	3.541	3.631	3.604	3.658	_
Foetal sex ratio *[state ratio m/f]*	57/43%	8.28/7.96	7.92/7.63	8.28/8.12	8.38/8.08	7.14/8.57	
percent males	57	51.0	51.0	50.5	50.9	45.4	

Table A 6.8.1-3: Table for teratogenic effects

Examination of the foetuses

Parameter	control data		12.5 µg/kg	25.0 µg/kg	50.0 µg/kg	100.0 µg/kg	dose-response + / -
	historical	study					
Number of foetuses examined		406	373	410	395	110	
Number of foetuses examined viscerally		205	186	206	196	55	
Number of foetuses examined skeletally		201	187	204	199	55	
External malformations [%]	0	0.00	0.00	0.24	0.00	0.00	-
External variations [%]	Not reported	0.00	0.00	0.24	0.00	0.00	-
Skeletal malformations [%]	0%	0.50	0.53	0.98	0.00	0.00	-
Skeletal variants [%]	Not reported	9.45	11.23	8.82	12.56	1.82	-
Visceral malformations [%]	21.6% foetuses	2.93	6.45	12.62	13.78	23.64	+
Variants visceral [%]	Not reported	72.82	77.42	66.50	70.40	100.0	-

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**		**Teratogenicity study** Developmental toxicity study in rabbits	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxx XX., Xxxx XX., Xxxxx XX., (XXXx): Developmental toxicity Evaluation of Chlorophacinone Administered by Gavage to New Zealand White Rabbits. Unpublished report No:XXXXXXXXX. Final report date (January 9, XXXX). XXXXXXXXXXXXXXXXXXXXXXXXXXXXX, XXXXXXXXXXXXXXX, XX (Dates of experimental work September XXXX – April XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	FIFRA testing guidelines (EPA 83-3, 1988). In accordance with EC Method B.31.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No GLP deviations were noted.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone (2-((4-chlorophenyl) phenyl acetyl) 1H-indane-1,3(2H)-dione) CAS # 3691-35-8	
3.2.1	Lot/Batch number	Lot # XXXXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Yellow odourless powder	
3.2.2.2	Purity	XXXXX%	
3.2.2.3	Stability	Not specified	
3.3	**Test Animals**		
3.3.1	Species	Rabbits	
3.3.2	Strain	New Zealand White	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Females – nulliparous	
3.3.5	Age/weight at study initiation	Five months, 2859 to 3989 g on gestational day (gd) 0	
3.3.6	Number of animals per group	16	
3.3.7	Control animals	Yes	
3.3.8	Mating period	Naturally mated by the supplier prior to shipment to the laboratory.	
3.4	**Administration/**	Oral – gavage	

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**		**Teratogenicity study** Developmental toxicity study in rabbits			
	Exposure				
3.4.1	Duration of exposure	13 consecutive days			
		Rabbit	day 7 to 19 incl.	post mating	
3.4.2	Postexposure period	11 days			
3.4.3	Type	Gavage			
3.4.4	Concentration	Gavage at doses of 0.0, 5.0, 10.0, 25.0, 75.0 µg/kg/day			
3.4.5	Vehicle	Corn oil			
3.4.6	Concentration in vehicle	0.0, 2.5, 5.0, 12.5, 37.7 µg/ml			
3.4.7	Total volume applied	2 ml/kg			
3.4.8	Controls	Vehicle – corn oil			
3.5	**Examinations**				
3.5.1	Body weight	Maternal weight –on gd 0, 3, 7, 9, 12, 15, 19, 21, 24, 27, 30			
3.5.2	Food consumption	Maternal food consumption recorded over intervals gd 3-7, 7-9, 9-12, 12-15, 15-19, 19-21, 21-24, 24-27 and 27 to 30.			
3.5.3	Clinical signs	Yes – once daily on gestational day (gd) 0-6 – prior to dosing, and on gd 20-30 after dosing period. Twice daily – at dosing and 1 hour after dosing throughout the dosing period (gd 7-19).			
3.5.4	Examination of uterine content	Gravid uterine weight, ovarian corpora lutea counted and uterine contents determined (number of implantation sites, resorptions, dead foetuses, live foetuses)			
3.5.5	Examination of foetuses				

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rabbits	
3.5.5.1 General	Number of fetuses per litter, Foetal Weight, Sex and Sex Ratio, Number of dead fetuses, Number of live fetuses, External malformations and variations	
3.5.5.2 Skeletal	Skeletal malformations and variations	
3.5.5.3 Soft tissue	Visceral malformations and variations	
	4 RESULTS AND DISCUSSION	
4.2 Maternal toxic Effects	Pregnancy Rate – high and approximately equivalent across groups (93.3-100.0%). One doe at 5.0 µg/kg/day delivered early on gd 29, and one doe at 25.0 µg/kg/day aborted on gd 23; both were removed from the study. Mortality: All 16 does at 75 µg/kg/day died or were sacrificed moribund (100 %) on gd 12(one), 15 (five), 16 (three), 17 (three), 18 (two), 21 (one), 23 (one). At 25 µg/kg/day, 13 out of 16 does died (81%): one on gd 17, one on gd 18, three on gd 19, five on gd 20, and three on gd 21. All other females at lower doses survived and were pregnant. All pregnant does had one or more live fetuses at scheduled sacrifice. Maternal body weights and weight gains were equivalent across all groups for all timepoints or intervals. Maternal weight gain for gd 12-15, exhibited a significant dose-related downward trend, with no significant pairwise comparisons to the control group. Maternal gravid uterine weights and absolute and relative liver weights were statistically and biologically equivalent across all groups. Treatment-related clinical observations were limited to does at 25.0 and 75.0 µg/kg/day prior to death. They included: external bleeding around mouth, ears, and the urogenital system, pale eyes, ears, and lips/gums, lethargy, and blood in pan beneath cage. There were no treatment-related clinical signs of toxicity at 5.0 and 10.0 µg/kg/day. Clinical weight loss (defined as >= 150g over a weight period) was observed at 0.0 (3 does), 5.0 (2), 10.0 (1), and 25.0 (4) µg/kg/day on gd 9; at 75.0 µg/kg/day (1) on gd 15; at 10.0 µg/kg/day (1) on day 17; at 0.0 µg/kg/day (2) and 10.0 µg/kg/day (2) on gd 19; at 0.0 µg/kg/day (1) on gd 21; at 10.0 µg/kg/day (1) on gd 24; and at 0.0 µg/kg/day (2), 5.0 µg/kg/day (100 and 10.0 µg/kg/day (1) on gd 30 prior to scheduled sacrifice. Does which died or were sacrificed moribund at 25 or 75 µg/kg/day exhibited the following signs at necropsy: blood in neck and over thoracic cavity, blood in vagina, uterus and amniotic sacs, blood mixed with ingesta in gastro-intestinal tract, pale organs including ovaries, spleen, kidneys, liver, and multiple red foci on intestines, appendix and lungs. At scheduled necropsy, there were no treatment-	

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rabbits	
	related findings in surviving does. Maternal food consumption exhibited no treatment-related changes, except for a significant reduction at 75.0 µg/kg/day (prior to demise of most of the does) for gd 12-15. The numbers of litters and fetuses evaluated were 16 (135), 14 (115), 16 (125), 2 (16) at 0.0, 5.0, 10.0, 25.0 µg/kg/day; no does survived to scheduled sacrifice at 75.0 µg/kg/day.	
4.3 Teratogenic / embryotoxic effects	There were no significant effects of treatment on any gestational parameters, including number of ovarian corpora lutea, total number of uterine implantation sites, pre- or post-implantation losses, number of live fetuses per litter, sex ratio or fetal body weight per litter, when calculated as all fetuses, or males or females. There were no treatment related changes in the incidence of individual or pooled external, visceral, skeletal or total malformations or variations. External malformations were observed in two fetuses, one at 0.0 µg/kg/day (agnathia and aglossia) and one at 10.0 µg/kg/day (exophthalmia). Visceral malformations were observed in three fetuses - one at 0.0 µg/kg/day (small lung lobes) and two in different litters at 5.0 µg/kg/day (one with abnormal development of cerebral hemispheres and ectopic tissue below the skull, and one with mild hydrocephaly). One fetus at 0.0 µg/kg/day exhibited a skeletal malformation, fused sternebrae. There were no fetal external variations observed. Fetal visceral and skeletal variations were equally distributed across groups and included enlarged lateral ventricles of the cerebrum, variations in papillary muscles of the heart, and size variations in gall bladder for visceral variations, and predominantly extra (13^{th}) rib, and extra sternebral ossification site, floating extra rib and bipartite center in thoracic centrum as skeletal variations.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The present study was designed to evaluate the potential of Chlorophacinone (2-((4-chlorophenyl) phenyl acetyl) 1H-indane-1,3(2H)-dione) to produce maternal and developmental toxicity (including teratogenicity) when administered by gavage during major organogenesis in New Zealand White Rabbits. Timed pregnant rabbits were exposed to test article, dissolved in corn oil and administered by gavage once daily, on gestational days 7 through 19 at doses 0.0, 5.0, 10.0, 25.0, 75.0 µg/kg/day, equivalent to 0.0, 2.5, 5.0, 12.5, 37.7 µg/ml corn oil, at dosing volume of 2 ml/kg. There were 16 females per group. Clinical observations were taken daily, except during the dosing period when they were made twice daily. Maternal body weights were taken on gd	

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rabbits	
	0, 3,7,9,12,15,19,21,24,27,30. Feed consumption was measured for the intervals gd 3-7, 7-9, 9-12, 12-15, 15-19, 19-21, 21-24, 24-27, 27-30. At scheduled sacrifice on gd 30, the does were evaluated for body, liver and gravid uterine weight. Ovarian corpora lutea were counted and the status of uterine implantation sites (resorptions, dead fetuses, live fetuses) was recorded. All fetuses were dissected from the uterus, counted, weighed, and examined for external abnormalities. All live fetuses in each litter were examined for visceral malformations and variations and sexed internally.	
5.3 Results and discussion	Profound maternal toxicity, including 81% mortality (13 out of 16) at 25.0 µg/kg/day and 100 %mortality (16 out of 16) at 75.0 µg/kg/day. Observations pre- and post-mortem consistent with the anticoagulation mechanism of action of the test article (external bleeding, pale extremities, pale organs, blood in gastrointestinal tract and amniotic sacs of the uterus) were observed at the two highest dose levels tested – 25.0 µg/kg/day and 75.0 µg/kg/day. There were no effects of treatment on maternal body weights, or food consumption at any dose, except for significantly reduced feed consumption at 75.0 µg/kg/day for gd 12-15 prior to demise of the does. Pregnancy was high and equivalent across groups (only one female was not pregnant in the entire study). All does had live litters at scheduled sacrifice. The numbers of litters and fetuses evaluated were 16 (135), 14 (115), 16 (125), 2 (16) at 0.0, 5.0, 10.0, 25.0 µg/kg/day; no does survived to scheduled sacrifice at 75.0 µg/kg/day. There were no treatment-related effects on any gestational parameters, including pre- or post-implantation loss, number of fetuses per litter, fetal sex ratio, or fetal body weight per litter. There were no treatment-related statistically significant changes in the incidence or severity of individual or pooled external, visceral (including cranio-facial), skeletal or total malformations or variations. In a range-finding study in pregnant rabbits various aspects of coagulation function, prothrombin time and activated partial thromboplastin time were measured. There were no effects on PT or APTT at doses of 1.0, 2.0 or 5.0 µg/kg/day but increased levels at 10 µg/kg/day. These findings were consistent with the known action of the test material and the lack of response at doses of 10 µg/kg/day or less in the teratogenic study. Chlorophacinone administered orally by gavage during major organogenesis in New Zealand White Rabbits resulted in no indication of developmental toxicity including teratogenicity. The NOAEL for maternal toxicity was 10.0 µg/kg/day and the NOAEL for developmental toxicity was	

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rabbits	
	at least 25.0 µg/kg/day in rabbits under the conditions of this study.	
5.4 Conclusion	Chlorophacinone administered orally by gavage during major organogenesis in New Zealand White Rabbits resulted in no indication of developmental toxicity including teratogenicity. The NOAEL for maternal toxicity was 10.0 µg/kg/day and the NOAEL for developmental toxicity was at least 25.0 µg/kg/day in rabbits under the conditions of this study.	
5.4.1 LO(A)EL maternal toxic effects	25.0 µg/kg/day	
5.4.2 NO(A)EL maternal toxic effects	10.0 µg/kg/day	
5.4.3 LO(A)EL embryotoxic / teratogenic effects	>25.0 µg/kg/day	
5.4.4 NO(A)EL embryotoxic / teratogenic effects	25.0 µg/kg/day	
5.4.5 Reliability	1	
5.4.6 Deficiencies	No deficiencies were found in this well-conducted study.	

Section A 6.08.1-02 Annex Point IIA VI.6.8.1	**Teratogenicity study** Developmental toxicity study in rabbits	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

October 2005

Materials and Methods

Applicant version is adopted summarised as follows:
Chlorophacinone was tested to produce maternal and developmental toxicity (including teratogenicity) when administered by gavage during major organogenesis in New Zealand White Rabbits. Timed pregnant rabbits were exposed to test substance, dissolved in corn oil and administered by gavage once daily, on gestational days 7 through 19 at doses 0, 5, 10, 25, 75 µg/kg/day.

The numbers of litters and fetuses evaluated were 16 (135), 14 (115), 16 (125), 2 (16) at 0, 5, 10, 25 µg/kg/day; no does survived to scheduled sacrifice at 75 µg/kg/day; high mortality ocurred at 25 µg/kg/day (13 of 16) but at least the foetuses of 2 does were possible to evaluate for embryotoxicity and teratogenicity.

Results and discussion

Applicant Version is adopted summarised as follows:

Maternal toxicity

Pregnancy rate was high and equivalent across groups (93.3-100.0%) with no dose related changes.

Mortality, clinical and pathology: At the highest dose (75 µg/kg/day), all 16 does died or were sacrificed moribund (100 %). At 25 µg/kg/day, 13 of 16 does died (81%). All other females at lower doses survived and were pregnant and had one or more live fetuses at scheduled sacrifice. Clinical observation included: external bleeding around mouth, ears, and the urogenital system, pale eyes, ears, and lips/gums, lethargy, and blood in pan beneath cage. Signs at necropsy included: blood in neck and over thoracic cavity, blood in vagina, uterus and amniotic sacs, blood mixed with ingesta in gastro-intestinal tract, pale organs including ovaries, spleen, kidneys, liver, and multiple red foci on intestines, appendix and lungs.

Clinical and pathology of surviving does: Treatment-related clinical observations were limited to does at 75 and 25 µg/kg/day prior to death. There were no treatment-related clinical signs of toxicity at 10 and 5 µg/kg/day. At scheduled necropsy, there were no treatment-related findings in surviving does.

Maternal body weights and weight gains were equivalent across all groups for all timepoints or intervals with a significant dose-related downward trend, with no significant pairwise comparisons to the control group.

Organ weight: Maternal gravid uterine weights and liver weights were statistically and biologically equivalent across all groups.

Maternal food consumption exhibited no treatment-related changes, except for a significant reduction at 75.0 µg/kg/day, prior to death.

NOAEL for maternal toxicity: **A value of 50 µg/kg bw/day** was adopted on the basis of mortality at higher dose**.** Clinical signs of toxicity and necropsy pathology demonstrated that mortalities were due to internal haemorrhage related with the anticoagulant properties of the substance.

Developmental effects

Chlorophacinone administered orally in rabbits during major organogenesis (gestational days 7 through 19) gave no indication of developmental toxicity including teratogenicity at the highest doses evaluated of 25 µg/kg/day which are causing high maternal mortality (13 of 16, 81%) with surviving does (3 of 16) for evaluation of embryotoxicity and teratogenicity.

There were no significant effects of treatment on any gestational parameters, including number of ovarian corpora lutea, total number of uterine implantation sites, pre- or post-implantation losses, number of live fetuses per litter, sex ratio or fetal body weight per litter, when calculated as all fetuses, or males or females. There were no treatment related changes in the incidence of individual or pooled external, visceral, skeletal or total malformations or variations.

There were no fetal external variations observed. Fetal visceral and skeletal variations were equally distributed across groups.

Section A 6.08.1-02 **Annex Point IIA VI.6.8.1**	**Teratogenicity study** Developmental toxicity study in rabbits	
Conclusion	No developmental effects were noted at any dose. So, NOAEL for developmental toxicity was considered the highest tested dose with about 20 % does surviving. At 75 µg/kg bw/day, 100 % mortality was observed, and at 25 µg/kg bw/day, a high mortality (13 of 16) was also observed but no significant effect were detected in the foetus of the surviving dams. So it is concluded that no developmental effect was observed including at the highest dose with surviving does. Strictly, NOAEL for developmental toxicity cannot be established. For a practical point of view for later assessments, a **NOAEL in rabbit for developmental toxicity of 25 µg/kg bw/day is adopted**. Chlorophacinone administered orally by gavage during major organogenesis (gd 7 to 19) in New Zealand White Rabbits resulted in no indication of developmental toxicity including teratogenicity. For **maternal toxicity a NOAEL of 50 µg/kg bw/day** was adopted on the basis of mortality at higher dose**.** Clinical signs of toxicity and necropsy pathology demonstrated that mortalities were due to internal haemorrhage related with the anticoagulant properties of the substance. No developmental effects were noted at any dose. So, NOAEL for developmental toxicity was considered the highest tested dose with about 20 % does surviving. At 75 µg/kg bw/day, 100 % mortality was observed, and at 25 µg/kg bw/day, a high mortality (13 of 16) was also observed but no significant effect were detected in the foetus of the surviving dams. So it is concluded that no developmental effect was observed including at the highest dose with surviving does. Strictly, NOAEL for developmental toxicity cannot be established. For a practical point of view for later assessments, a **NOAEL in rabbit for developmental toxicity of 25 µg/kg bw/day is adopted**.	
Reliability	1	
Acceptability	Accepted	
Remarks		

Table A 6.8.1-1: Table for teratogenic effects

Maternal effects

Parameter	control data		5.0 µg/kg/day	10.0 µg/kg/day	25.0 µg/kg/day	75.0 µg/kg/day	dose-response + / -
	Historical	study					
Number of dams examined	50	16	16	16	16	16	
Clinical findings during application of test substance	Not reported	Few faeces in pan, loose stools, pulling hair	Teeth clipped, few faeces in pan, alopecia neck, abdomen, pulling hair	Diarrhea, few faeces in pan, hair in pan, pulling hair	Blood in pan, in mouth and on fur, blood coming from nose, few faeces in pan, pale eyes, hair in pan,	Blood in pan, pale ears, gums, eyes, lethargy, blood in ears, mouth, and perioral, few, small or no faeces; pale ears, eyes, lips; unsteady gait; red tinged urine	+
Mortality of dams *state %*	0%	0%	0%	0%	**81% (13 of 16)**	**100% (16 of 16)**	+
Abortions	0	0	1/16 – early delivery on gd 29	0	1/16 aborted on gd 23	0	-
Body weight gain *day 7-19, day19-30, day 3-30*	Days 0-30; 658g	7-19 day 81g; 19-30 day 193g; 3-30 day 526g;	7-19 day 165g; 19-30 day 168g; 3-30 day 599g;	7-19 day 153g; 19-30 day 199g; 3-30 day 589 g;	7-19 day 110g; 19-30 day 189g; 3-30 day 472g;	7-19 day 191g	-
Food consumption Gd 3-30	Not reported	Mean 158.9 g/day +/- 7.0	Mean 169.2 g/day +/- 10.2	Mean 165.6 g/day +/- 8.9	Mean 151.2 g/day +/- 33.9	-	-
Pregnancies on gd 30 *pregnancy rate or %*	Not reported	16/16	14/16	16/16	2/16	-	-
Necropsy findings in dams dead before end of test	Not reported	Normal	Normal	Normal	Pale liver, spleen, kidneys; diffuse haemorrhaging in lungs; uterus-blood in all amniotic sacs; blood periorally, around urogenital area, and nostrils; subcutaneous haemorrhages, advanced autolysis; dark ingesta in intestines	Pale liver, spleen, kidneys ; blood around mouth, urogenital area, nostrils; pale eyes; diffuse hemorrhaging in esophagus, lungs, pericardium, pancreas, thoracic cavity; uterus-blood in all amniotic sacs and vagina; advanced autolysis; deep subcutaneous haemorrhaging in the area of neck and abdomen	+

Table A 6.8.1-2: Table for teratogenic effects

Litter response (Caesarean section data)

Parameter	control data		5.0 µg/kg/day	10.0 µg/kg/day	25.0 µg/kg/day	dose-response + / -
	historical	study				
Corpora lutea per doe	8.90/50	10.00/16	9.21/14	9.38/16	9.00/2	-
Implantations	7.96/50	8.75/16	8.71/14	8.00/16	8.00/2	-
Resorptions per litter	0.44/50	0.25/16	0.36/14	0.06/16	0.00/2	-
total number of fetuses	-	135	115	125	16	-
total number of litters	50	16	14	16	2	-
fetuses / litter	7.67/49	8.50/16	8.35/14	7.94/16	8.00/2	-
live fetuses / litter	7.67/49	8.44/16	8.21/14	7.81/16	8.00/2	-
dead fetuses / litter	0.00/50	0.06/16	0.14/14	0.13/16	00.0/2	-
fetus weight (mean) [g]	53.30	48.01	49.71	50.00	50.55	-
Fetal sex ratio [m/f ratio] Percent males	3.80/3.88 49.5	4.13/4.31 47.25	4.36/3.86 52.46	4.00/3.81 52.50	3.00/5.00 36.51	-

Table A 6.8.1-3: Table for teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Parameter	control data		5.0 µg/kg/day	10.0 µg/kg/day	25.0 µg/kg/day	dose-response + / -
	historical	study				
External malformations [%]	0.27	0.74	0.00	0.80	0.00	-
External variations [%]	0.27	0.00	0.00	0.00	0.00	-
Skeletal malformations [%]	1.86	0.74	0.00	0.00	0.00	-
Skeletal variants [%]	36.44	43.0	57.4	45.6	**100.0**	-
Visceral malformations [%]	0.53	0.74	1.74	0.00	0.00	-
Variants visceral[%]	9.04	14.1	14.8	13.6	18.8	-

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	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [x]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	

Detailed justification:

Waiver for multigeneration study in rodents on Chlorophacinone.

The following is a series of rationales to waiver the requirement to perform a multigeneration study on the anticoagulant rodenticide active substance Chlorophacinone under the Biocidal Products Directive 98/8/EEC.

1 INTRODUCTION.

The Biocidal Products Directive (98/8/EEC 'the Directive) requires a multigeneration study in rodents as part of the suite of toxicology tests in order to assess the possible adverse consequences to reproduction of long term exposure over several generations to the biocidal active substance Chlorophacinone.

It is a unique feature of the rodenticides that the test species used in the multigeneration study is also the target species. This gives rise to several questions: Is it relevant to consider the possible use of rodent reproduction studies to predict possible effects of rodenticides in humans, and is it scientifically feasible? Can the data be derived using other species? Given that at least one rodenticide molecule has been used for over forty years in human medicine, are there data in the human that are more relevant than animal data would be? Are there other data that demonstrate the potential, or lack of potential, adverse reproductive properties of active substances used as rodenticides?

The Directive states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". A more detailed waiver concept is given in the TNsG on data requirements.

The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*".

The TNsG recommendations were further refined in an Addendum to the TNsG entitled Refined waiving concept for rodenticides (TMII03-item9a-CA-Jun03-Doc9-TNsG.doc). These include:

The study is technically not possible to perform,

Use of other data,

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Data evaluated with regard to agricultural use
Read-across from data on related substances
Evaluation of acceptable human data,
The study is not scientifically necessary
The choice of species is not appropriate
The study is not necessary owing to limited exposure and toxicity profile.
The Notifier has prepared a scientific justification based on this guidance to waive the requirement for these studies.
Before the waiving arguments are given, it will be useful to review the way the coagulation system works in mammals and the mechanism by which the anticoagulant rodenticides function.

2 FUNCTION

Anticoagulant rodenticides such as Chlorophacinone function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver.
Blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin to the insoluble fibrous protein fibrin, which binds platelets and blood cells to form a solid mass referred to as a blood clot, sealing the site of the haemorrhage and preventing further blood loss. Fibrinogen is present in the blood, but thrombin is not. Thrombin factor IIa in the scheme below) is formed at the site of injury from prothrombin (factor II), which is present in the blood. Conversion of prothrombin to thrombin occurs via the coagulation cascade, in which the blood clotting factors are employed. Without these blood factors clotting cannot take place, and the haemorrhage will not be controlled by clot formation. If the blood vessel is large and/or serves a vital organ, the haemorrhage will be fatal. The synthesis of a number of blood coagulation factors (factors II [prothrombin], VII [proconvertin] IX [Christmas factor], X [Stuart-Prower factor] and the coagulation inhibiting proteins C and S) is dependent upon vitamin K, which acts as a co-enzyme.

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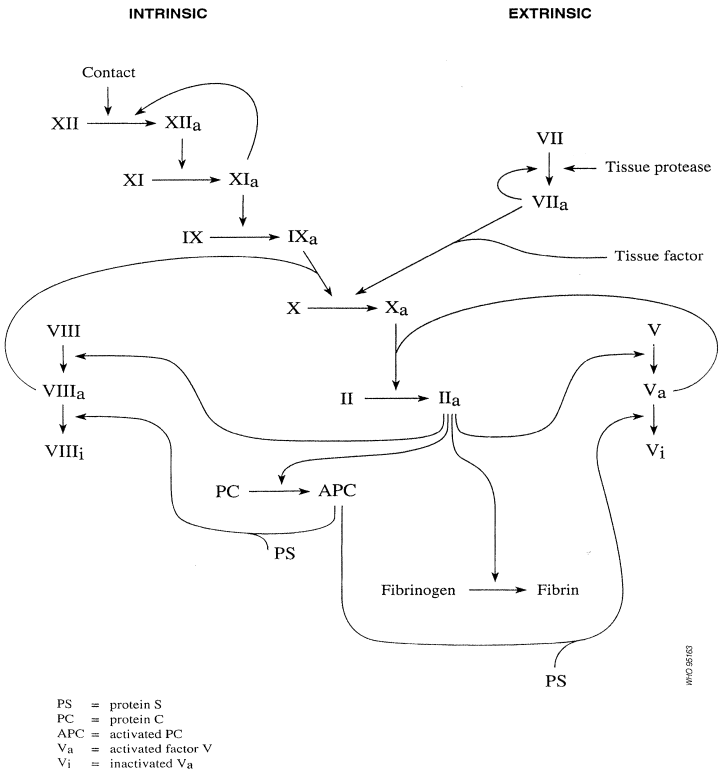
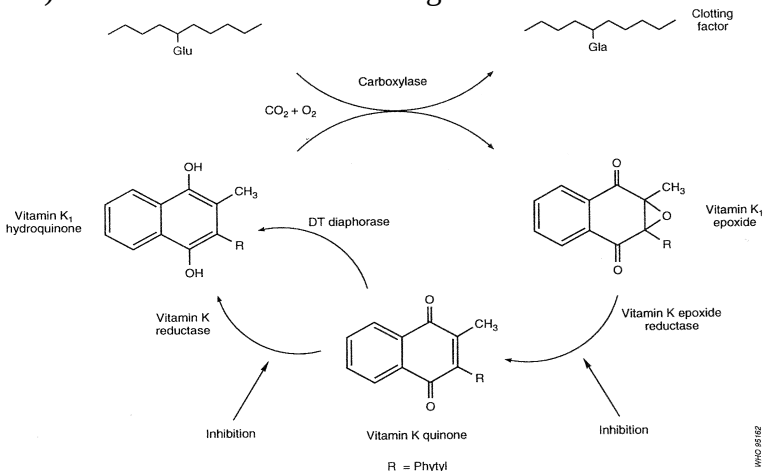
Annex Point 6.8.2 2 generation studies



Vitamin K hydroquinone is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide provides the energy required for the carboxylation reaction where glutamate (Glu) in the precursor is converted to γ-carboxyglutamate (Gla) to make the activated clotting factor.



The anticoagulant rodenticide active substances such as Chlorophacinone work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place.
The action is cumulative, increasing levels of the anticoagulant leading to increased clotting times, such that in the event of a significant haemorrhage, death occurs. The amount of vitamin K in the body is finite, and progressive blocking of the regeneration of vitamin K will lead to an increasing probability of a fatal haemorrhage. In general

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terms, progressive intake of anticoagulants results in death. The active substances are highly toxic and bioaccumulative. The oral LD_{50} of Chlorophacinone is 6.26 mg/kg. Rodenticide baits generally contain 50 ppm Chlorophacinone and are fatal after one to three meals.

3 TECHNICAL FEASIBILITY

3.2 Study design

Multigeneration studies seek to determine the consequences of medium-term exposure to the active substance by the daily dietary administration to rats for 70 days prior to pairing for mating, through pregnancy, parturition and lactation, to a parental generation, and similar administration to a filial (F_1) generation, through to weaning of the F_2 offspring. They are typically dosed via the dietary route, employing three increasing dose levels to groups of 25 rats of each sex, in comparison to a similar group of untreated animals (the control group).

The study consists of a pre-mating period, 10 weeks, for males and at least two oestrous cycles for the females (although in most cases both sexes are treated for 10 weeks prior to mating). The animals are paired for mating (1M:1F within groups) for up to three weeks and the females are allowed to litter naturally. Numbers of live young are counted, and at four days post partum the litter may be culled to a standard size (typically 4M and 4F). Offspring are reared to weaning (21 days post partum), and typically one male and one female are selected from each litter to make up a filial (F_1) generation. The F_1 generation animals are reared on the same test diet concentrations as their parents, typically for 70 days, before being paired for mating (avoiding brother/sister pairing). The females are allowed to litter and rear their young to weaning in the same manner as was the parent generation, and the study terminates at the weaning of the F_2 offspring. Each adult generation is exposed to the test diets for at least 113 days. In the case of equivocal findings, the animals may be mated a second time, further extending the duration of the study.

3.3 Haemorrhagic events in the reproductive cycle

It is possible to conduct short-term animal studies provided the accumulated dose never reaches lethal levels, but as the LD_{50} of these molecules is low, the level for low lethality (e.g. LD_{10}) can be anticipated to be lower, such that the amount administered daily during the 100+ days of each generation in a multigeneration study would be low, but not as low as in a carcinogenicity study. In a 90 day study with Difethialone, there were deaths at around 90 days in the highest dose group of 8 µg/kg/day.

As stated above, the progressive accumulation of the

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anticoagulant rodenticides leads to an increased probability of death by haemorrhage. It is important to emphasise that increasing the probability of haemorrhage increases the probability of death. Animals in a risk-free situation would live longer than animals at risk of haemorrhage. An example of this can be seen in a study conducted with Difethialone: in an acute cat study, there was one death. Post mortem revealed lethal haemorrhage at the site of a tapeworm attachment in the gut. Other cats without tapeworms survived at the same dosage. Thus it is the probability of haemorrhage that increases the probability of death.

There are several events in the reproductive cycle that are associated with incidental or inevitable haemorrhage. Mating may cause haemorrhage, as the rats often fight and may bite each other during courtship. Ovulation causes minor haemorrhage. The change over in placental nutrition during days 12 - 14 of pregnancy causes significant haemorrhage (visible as blood in the vaginal smear – in the Chlorophacinone rat teratology study, rats were noted as bleeding from the vagina at 100 µg/kg/dayat around day 14 of pregnancy), and parturition is always associated with major haemorrhage in the mother. The new-born pup is also susceptible to haemorrhage from the umbilicus (although this is closed by muscular action) and during play with siblings in the post partum period. While it is possible to devise a dose that does not lead to a lethal accumulation during the pre-mating period, the long depuration time, and the risks of haemorrhage associated with normal pregnancy, especially at parturition, mean that it is not possible to administer anticoagulants prior to mating without the anticoagulant still having an effect during pregnancy and at parturition. Even lower dose levels down to 5 µg/kg/day would carry risk of lethal haemorrhage.

3.4 Choice of species

Rodents are used in safety testing because they are small (easy to handle and house), readily available (large numbers can be bred in captivity), and they have a relatively short life span (studies are of shorter duration than with longer-lived species). In the case of rodenticides, designed to kill the wild form of the test species at low doses, reproduction testing of the target species is inherently difficult because of the increased risk of death by haemorrhage, outlined in 3.2 above. It is logical to see if there are alternative species, suitable for reproduction tests that are less sensitive to these active substances. However, comparison of LD_{50} values in other mammals shows that for each active substance the range of tolerance between species is generally one order of magnitude, and all are very low in absolute terms, so there is

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nothing to be gained from considering other species. (See Table 6.8.2-1).
There are also practical difficulties with reproduction testing in non-rodent species. It would not be possible to bring large group sizes of dogs or cats into breeding season at the same time, such that studies would need to be performed on a series of individual pairs of animals, and data collated after several years. The relatively long maturation times of these larger animals renders a true multigeneration study impossible to perform. Guinea pigs show delayed implantation and small litter size, and are therefore not feasible for this type of study.

3.5 Dose-setting and the Maximum Tolerated Dose

The test substance is incorporated in the diet at levels intended to demonstrate toxicity at the highest dose level, without adversely affecting adult survival over the length of the study (toxicity typically manifest as reduced body weight gain, but occasionally a more subtle indicator such as altered enzyme levels, changes in function of an organ that can be demonstrated by organ weight analysis or microscopic change at cellular level may be used).
The intention is to administer sufficient test material such that the animal has to respond to the chemical burden i.e. it is placed under toxic stress. The implication is that if the animal does not respond to the stress by showing adverse reproductive effects, then the chemical is considered unlikely to show adverse reproductive effects in man at lower doses not inducing overt toxic stress. Secondly, if the animal is not stressed sufficiently to show a toxic response, it has not been stressed sufficiently to demonstrate the potential to cause adverse reproductive effects.

3.6 Route of Administration of the Test Substance

Dietary admixture is the traditional method for multigeneration studies. However, the low concentrations required would mean that the diets could not be formulated accurately. An alternative is administration orally, by gavage. Experience with 90 day studies shows that oral administration is feasible over this duration, although levels of 8 μg/kg/day were associated with deaths after approximately 90 days.

3.7 Antidotal treatment

Studies are presented in the dossier which administer vitamin K as an ‘antidote’. These studies variously show that it is possible to use vitamin K in the treatment of low single doses of anticoagulants.
For Chlorophacinone, rats were given approximately 5 mg/kg bw/day for 24, 48 or 72 hours, via the diet, and vitamin K administered for 14 days. All rats given chlorophacinone for 24 hours survived, and 3/5 rats given

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Chlorophacinone for 48 hours survived but all rats treated for 72 hours died (reference A 6.10-01).
The anticoagulant active substances are highly lipophilic. They have been shown to accumulate in the liver. The inhibition of the regeneration of vitamin K occurs by blocking, i.e. competitive binding of the active substance and the vitamin K reductase enxyme (see above) to form a lipophilic complex, which will accumulate in the liver in the same manner as the active substance. Long term co-administration of vitamin K as an antidote, would result in the accumulation in the liver of the lipophilic complex; not the active substance. As there would be no free active substance present the test would not be valid.

3.8 Absence of reproductive risk

The anticoagulant action is the sole pharmacological action of the materials. The mode of action has been described in detail. It is difficult to demonstrate that this is the sole mode of action, as administration is acutely lethal, but it is supported by the available short-term toxicology data. The short-term studies (up to 90-days duration) in rats and dogs have shown no adverse effects on the reproductive organs (macroscopic condition, organ weight analysis and histology). The absence of effects on the reproductive organs indicate that a direct effect on reproduction and fertility is unlikely.

4 USE OF OTHER DATA

4.2 Data evaluated with regard to agricultural use

Chlorophacinone is registered for agricultural uses. All of the available data are presented in the BDP dossier: no other data have been derived specifically to defend agricultural uses.

4.3 Long-term human data

There is long term experience in humans with warfarin, widely used in anti-clotting therapy in humans for over forty years, with no association with adverse effects on fertility. Warfarin was the first of the anti-vitamin K rodenticides. The anticoagulant rodenticides fall into two categories: inandones, such as chlorophacinone, and hydroxycoumarins such as warfarin, bromadialone and difethialone.

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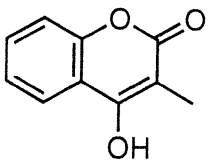
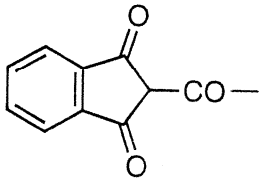
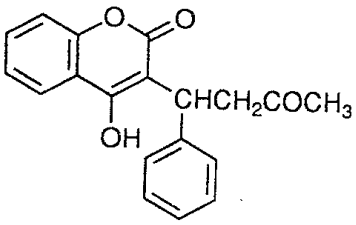
- hydroxycoumarins:



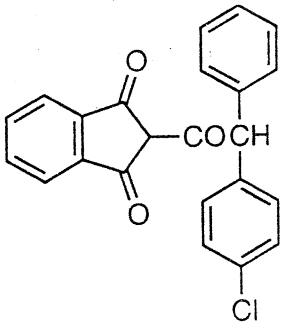
- indandiones:



The molecules all have significant structural similarity to the forms of vitamin K shown in Section 2 above. It can be seen that this structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K. The major differences in the active substances lie in the 'tail', which has varying degrees of lipophilicity. In general, the longer, and more lipophilic the 'tail' the longer the half-life, and more potent the active substance.



Warfarin



Chlorophacinone
Difethialone
Bromadialone

It has been established that the molecules are structurally similar, and all have the same mode of action. It is therefore appropriate to use information in humans in one molecule,

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warfarin, to support the risk assessment of Chlorophacinone. This 'bridging' is an acceptable strategy under the TNG Risk assessment for human health (Section 3.2.2.5 '(Quantitative) structure-activity relationships ((Q)SARs)'). Warfarin is the most frequently prescribed oral anticoagulant human drug. It is the eleventh most frequently prescribed drug in the USA (EU figures not available), with annual sales of $500 million. It is used in stroke prevention, in treatment of vascular heart disease and deep vein thrombosis. For stroke and heart disease, including patients with prosthetic heart valves, duration is 'lifelong' i.e. the patient takes the drug for the rest of their life. (Horton, J., Bushwick, B.M., Warfarin therapy: Evolving strategies in anticoagulation. American Family Physician, February 1, 1999). Doses employed in humans are typically 3 – 9 mg/person/day (dose equivalent to 0.05 – 0.15 mg/kg/day for a 60 kg human [British National Formulary, www.bnf.org]), with most doses being in the 4 – 6 mg/person/day range (Horton op cit). Treatment is associated with increased risk of bleeding episodes, but long-term use in humans over forty years has not been associated with any adverse effects on fertility. The sole long-term effect is bone protein depletion in female humans after 10-12 years of continuous use (WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995).

While the traditional use of warfarin has been associated with heart and blood disorders in the elderly, there is a significant cohort of patients, both male and female, of reproductive age with conditions such as mitral valve replacement or deep vein thrombosis (DVT). There are no indications of any adverse effects on fertility (i.e. mating performance) of either sex undergoing treatment with AVKs The absence of adverse effects in thousands of humans following four decades of long term warfarin therapy is considered sufficient evidence that warfarin does not adversely affect fertility in the human. Therefore a study in rats would not add to the sum of knowledge on the subject.

4.4 Exposure

The predominant use of anticoagulant rodenticides is at bait points, (varying in design for given situations to provide on a case-by-case basis for protection from enviromental factors such as sunlight or moisture, to prevent access to or interference by non-target animals/children/humans or to incorporate more formal physical obstruction e.g, enclosed boxes designed to be 'tamper-proof'), protected such that members of the general public cannot easily gain access to the baits within. This minimises the chances of secondary exposure, and reduces risk.

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	Where sale to the general public is permitted, block baits (and some pelleted and grain baits) are sold in plastic (LDPE) sachets, such that the user is not directly exposed to the bait. In theory, exposure could occur when partly used baits are cleared up. In this case, exposure should again be minimal, because the user should wear protective equipment (rubber gloves) to guard against rodent-born disease, such as leptospirosis and hepatitis. Amateur use is intermittent, typically occurring at a maximum of three times a year. This does not constitute long term exposure. In terms of long-term risk, manufacturers regularly monitor the health of personnel, including regular assessment of clotting times. This immediately provides a warning if exposure is occurring, and allow for both vitamin K administration (if necessary to remedy the individual condition) and implementation of measures to prevent further exposure. Pest control operators are advised to wear protective clothing, not only because of the inherent acute toxicity of the active substances, but principally because the wild rodents themselves are significant disease vectors. **5 CONCLUSION** In conclusion, a waiver for a multigeneration study on anticoagulant rodenticides is scientifically justified, based on the absence of effects on fertility following long term administration in humans. This is supported by short-term studies in animals, where there were no adverse findings in reproductive tissues. A waiver of the studies is further supported by the practical difficulties of performing a study. For the Biocidal Products Directive 98/8/EEC, a waiver for the requirement to submit a multigeneration study under Annex IIA, Section 6.8 for anticoagulants is requested.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	The applicant justifies non-submission by presenting some common argument to that for non submission of long term toxicity and carcinogenicity, as well as some specific argument related with the reproduction but in the same line of reasoning: (a) Problem in technical feasibility due to hemorrhagic event in the reproductive cycle in the rat and no feasibility of other experimental species for reproduction test. (b) Absent of reproduction risk as anticoagulant action is the sole pharmacological action (c) Supported by data of other hydroxycoumarins and indandione anticoagulants and history of human exposure with them. The TNG of data requirement indicate: "If, in exceptional circumstances, it is

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	claimed that such testing is unnecessary, this claim must be fully justified". The Addenda TNG for refining waiving for rodenticides made more flexible criteria for waiving due to the difficulties due to that "rodenticides designed to kill the wild form of the recommended test species, reproduction or long-term testing of the target species may be inherently difficult". There some weaknesses of the arguments: • The low toxicity argued in human is based with data with other chemical with order of magnitude of different acute toxicity in the rat. • Short term toxicity can not easily demonstrate that other mode of action might be relevant for low dose in long term toxicity and carcinogenicity. In spite of these weaknesses, globally there are strong reasons supporting the waiving due to the difficulties to do multigeneration reproduction studies and in favour of avoid to do more unnecessary animal experiments.
Conclusion	Justification of non-submission may be provisionally accepted provisionally to be reconsidered after the detail evaluation of other related data which are used for the justification.
Remarks	

Table A 6.8.2-1: Comparison of acute median lethal doses for various rodenticides in seven mammalian species

Rodenticide	Acute oral (LD_{50} mg/kg) in species*:						
	Rat	Guinea-pig	Rabbit	Dog	Cat	Sheep	Pig
Brodifacoum	0.26	2.78	0.29	0.25-3.56	~25	>25	0.5-2
Bromadiolone	>0.56-<0.84	2.8	1.0	10+	>25+	-	3
Chlorophacinone	6.26						
Difenacoum	1.8	50	2	~50	100	100	80-100
Difethialone	0.56	-	0.75	11.8@	>16@	-	2-3@
Diphacinone	3.0	-	35	3-7.5	14.7	-	150
Flocoumafen	0.46	>10	0.7	0.075-0.25	>10	>5	~60
Warfarin	58.0	-	800	20-50	6-40	-	1-5

* After WHO/IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva 1995)
Bromadiolone rat data: LiphaTech (unpublished 1987)

+ MTD

@ LiphaTech data

Section A 6.09-01 **Annex Point IIIA VI.1**		**Delayed neurotoxicity** Pharmacological investigations in rats, mice and guinea pigs	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx, XX. and Xxxxxxxxx, X. (XXXX) LM 2219 Pharmacological approach. XXXXXXXXXXXXX XXXXXXXXX. No report number available. Report dated 10 January XXXX (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	No. Report consists of a number of screening studies for which no guidelines are available.	
2.3	**GLP**	No. Studies were screening investigations conducted at a centre of excellence for rodenticide research but without GLP accreditation.	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	LM 2219. (difethialone) the test material is in the same class as chlorophacinone, acting as an anticoagulanr rodenticide. The studies provide information on the neurotoxicity of Vitamin K antagonists in general and so are applicable to chlorophacinone.	
3.2.1	Lot/Batch number	XXXXXXXX, analysis no XXXXX	
3.2.2	Specification	As given in section 2 for LM 2219.	
3.2.2.1	Description	No description of test material provided in study report	
3.2.2.2	Purity	XXXXX%	
3.2.2.3	Stability	Not provided in study report	
3.3	**Presentation of work**	The study consisted of a number of screening investigations in several species. Details of the animals are given below. Details of administration and observations will be provided separately for each test method, together with a method reference number. The applicant's method summary will simply list the method reference. A toxicological investigation was completed prior to beginning the pharmacological tests. A single dose of difethialone, 200 mg/kg bw, was administered by gavage as a suspension in 10% acacia to a group of 10 male Swiss mice, in weight range 20 to 22g. The animals were examined one and 24 hours after dosing. Since no signs of toxicity, changes in behaviour or mortality were observed, and the dose represented double the highest	

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		dose to be used in the pharmacology studies, the highest dose for pharmacologic examinations, 100 mg/kg bw, was considered to be harmless.	
3.4	**Test Animals**		
3.4.1	Species	1) Rats 2) Mice 3) Guinea pigs	
3.4.2	Strain	1) Sprague-Dawley OFA IFFA CREDO or Wistar Cesal 2) Swiss OF1 IFFA CREDO 3) Dunkin hartley albinos IFFA CReDO	
3.4.3	Source	1) IFFA CREDO, St, Germain sur l'Arbresle or CESAL, Montmedy Farm 2) IFFA CREDO 3)IFFA CREDO	
3.4.4	Sex	As detailed in specific methods	
3.4.5	Age/weight at study initiation	As detailed in specific methods	
3.4.6	Number of animals per group	As detailed in specific methods	
3.4.7	Control animals	As detailed in specific methods	
3.5	**Administration**		
3.5.1	Method 3.1.1.1	<u>Antianginal activity: electrocardiogram in the curarized mouse</u> Swiss mice, 18 to 22g, were fasted for 24 hours prior to receiving an injection of 10 mg/kg gallamine triiodoethylate in apyrogenic saline in a dose volume of 10 mL/kg bw. This blocks respiration immediately and irreversibly. ECG recordings were made to record the cardiac survival time of mice placed into respiratory arrest. Animals given a treatment liable to reduce cardiac oxygen consumption have a longer cardiac survival time. An effective dose (ED 100) that increases the cardiac survival by 100% can be calculated. The test material, 100 mg/kg bw, was administered once by gavage as a suspension in 10% acacia and delivered in a volume of 20 mL/kg bw. The test material was administered one hour prior to dosing with the curarizing agent. 60 mg/kg bw diltiazem was used as the control.	
3.5.2	Method 3.1.1.2	<u>Antianginal:anticalcium: rat duodenum in vitro</u> The concentration of anticalcium drug that inhibits calcium chloride-induced spasm can be determined in a test for anticalcium activity performed on rat duodenal tissue, in vitro, in a calcium free, depolarizing nutrient medium. A 2 cm fragment of duodenum is rapidly removed from a killed and exsanguinated rat and placed in a cuvet containing calcium-free Tyrode's solution maintained at a temperature of 37-38°C.	

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	One end of the duodenum fragment is attached to the floor of the cuvet and the other end attached to an isotonic strain gauge. The completed mount is then left to stand for an hour during which period it is rinsed approximately 10 times. A record of the background fragment movement is made for a few seconds then a calcium chloride solution (1 mg/0.5 mL distilled water) is added. The tissue is allowed to react over a period of 1.5 minutes and then rinsed and allowed to stand for 5 minutes. The procedure is repeated until two identical spasms are recorded. Then process was repeated except that the test material was injected (0.5mL) 30 seconds after addition of the calcium chloride and reactions recorded for the following minute. Difethialone was tested at 2.5 mg/L. Nifedipine, 2.5 µg/L served as the positive control.	
3.5.3 Method 3.1.2.1	<u>Alpha-blocking activity: adrenaline-induced mortality</u> Swiss mice weighing 18 to 22g, were given an intraperitoneal injection (10 mL/kg bw) of a solution of adrenaline in apyrogenic distilled water. The adrenaline dose resulting in 90% mortality within 90 minutes of injection was established. Alpha-blockers protect animals against adrenaline–induced mortality. The test material was administered by gavage, as a suspension in 10% acacia, in a dose volume of 20 mL/kg bw, one prior to the adrenaline injection. Difethialone was administered on a geometric scale with three doses 1, 5 and 25 mg/kg bw. Prazosine, 0.5 mg/kg bw was the positive control. The effective dose (ED_{50}) resulting in 50% inhibition of mortality compared with controls can then be calculated.	
3.5.4 Method 3.1.2.2	Antihypertensive activity: arterial blood pressure in genetically hypertensive rats (SHR) Arterial blood pressure is measured by an indirect route on conscious rats using oscillometric methods. Genetically hypertensive rats (SHR rats of the Okamoto strain, at least 12 weeks old) were placed in a quiet room, warmed in a hot box to 37°C for 15 minutes and then removed. An occlusive cuff was placed around the upper end of the tail and a sensor, linked to an electrosphygmograph, placed downstream of the cuff. Recordings of systolic blood pressure (SBP) are made when the cuff is deflated and oscillations reappear. The rats were acclimatised to the procedure prior to initiating the test. On the first day, reference SBP values are recorded before any treatment has occurred. One week later SBP is recorded three hours after dosing with the test material. The test material, difethialone, was administered as a solution in 10% acacia by gavage to achieve a dose level of 100 mg/kg	

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	bw. Two measurements were made for each rat. The positive control was alphamethyldopa, administered by gavage at a dose level of 150 mg/kg bw. Mean SBP values do not normally vary significantly between recording occasions unless affected by treatment. A minimum efficient dose, defined as the lowest dose producing a significant difference between pre- and post-treatment SBP values, can be determined from this assay.	
3.5.5 Method 3.1.3.1	Antiarrhythmic activity: chloroform-induced arrhythmias, according to Lawson's method Female Swiss mice, weighing 18 to 22g, were placed in a chloroform-saturated atmosphere and the electrocardiogram viewed on an oscilloscope as soon as respiration had stopped. The presence or absence of ventricular fibrillation was checked and scored as 10 – fibrillation present; 5 – fibrillation equivocal or 0 – normal. The test material was administered by intraperitoneal injection as a suspension in 3% acacia in a dose volume of 10 mL/kg bw to achieve a dose level of 100 mg/kg bw. The positive control was disopyramide, 60 mg/kg bw, administered by intraperitoneal injection. The effective dose (ED_{50}) was the test material concentration providing 50% protection in comparison with control.	
3.5.6 Method 3.2.1.1	<u>Central sedative activity: tube test, according to Boissier et al</u> The assay investigates drug effects on muscle tone and equilibrium function. Swiss mice of either sex, weighing 18 to 22g, were introduced headfirst into a glass tube. The internal diameter of the tube was 25 mm for mice weighing 18 to 20g and 28 mm for mice weighing 20 to 22g. A mark was placed at 20 cm from the 'head' end of the tube. When the mouse reached the 'head' end, the tube was set in an upright position. When the tube is inverted the mice endeavour to climb backwards up the tube. The test is positive if the animal climbs past the 20 cm mark within 30 seconds. Mice are preselected before initiating dosing – only those mice that can normally complete a positive test are included in the main study. The animals are dosed with test material, a suspension in 10% acacia, administered in a dose volume of 20 mL/kg bw by gavage, one hour before entering the tube. Doses of 1, 10 and 100 mg/kg bw were administered. The positive control was 10 mg/kg bw diazepam. The effective dose (ED_{50}) is defined as the dose concentration that inhibits by 50% the climbing ability of test mice in comparison with controls.	

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3.5.7 Method 3.2.1.2	Central sedative activity: rotating rod test (Rota rod) according to Boissier The assay measures how long mice are able to maintain their balance on an axle rotating at low speed as an assessment of equilibrium reflexes. Swiss mice of either sex, weighing 20 to 25g, are placed onto a 3 cm diameter wooden rod that is rotated by a motor at 6 rpm. The test material, a suspension in 10% acacia, was administered by gavage in a dose volume of 20 mL/kg bw. Doses of 1, 10 and 100 mg/kg bw were administered. The positive control was 5 mg/kg bw diazepam. One hour after dose administration the animal is placed on the rota rod and observed for a minute. Any mouse falling of the rod will be replaced on the rod only once. The assay is scored as 2 = mouse has no falls; 1 = mouse has one fall; 0 = mouse falls off twice. The effective dose (ED_{50}) is that which causes a drop in 50% scores for treated animals in comparison with controls.	
3.5.8 Method 3.2.1.3	Central sedative activity: escape test, according to Boissier et al. Male Swiss mice, weighing 18 to 22g, are placed into a parallelopipedic case without a lid. There is a board lined with fine netting that leads in and out of the case. The obliquely placed access/egress board was marked 2 cm from the top. The mice were maintained in a quiet, well-lit room and observed for 5 minutes to determine the number of times an escape was made. Escape was defined as crossing the access/egress board mark. The test material, a suspension in 10% acacia was administered by gavage in a dose volume of 20 mL/kg bw, at dose levels of 1, 10 or 100 mg/kg bw. The animals were dosed one hour before placing into the test arena. The effective dose (ED_{50}) was defined as that reducing the number of escapes by 50% in comparison with controls.	
3.5.9 Method 3.2.2.1	Anticonvulsant activity: pentylentetrazole-induced convulsions Subcutaneous injection of pentylentetrazole causes convulsions characterised by generally lethal clonic/tonic seizures. Administration of an anticonvulsant before dosing with pentylentetrazole will increase survival rates. Swiss mice weighing 18 to 22g were injected subcutaneously with 100 mg/kg pentylentetrazole in apyrogenic distilled water in a dose volume of 10 mL/kg bw. Deaths were recorded three hours later. The test material, a suspension in acacia, as administered by gavage at dose levels of 1, 10 or 100 mg/kg bw in a volume of 20 mL/kg one hour before the s.c. injection of pentylentetrazole. Phenobarbital, 20 mg/kg bw, was the positive control.	

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	The effective dose (ED_{50}) defined as the dose inhibiting mortality by 50%,	
3.5.10 Method 3.2.2.2	<u>Anticonvulsant activity: ocular electroshock: supramaximal convulsions</u> Transcranial stimulation is achieved by means of a corneal electrode applied to each eye in presence of 0.9% NaCl. The generator conditions are set as follows: output: 30 mA frequency: 100 Hz width: 1ms shock duration: 300ms. Electrical stimulation in this manner results in tonic seizure with hyperextension of the hind legs. Administration of an anticonvulsant before transcranial stimulation inhibits the occurrence of seizures. Seizures are scored on a three point scale – 0 = absence; 1 = clonic seizure; 2 = tonic seizure. The animals should have one seizure without dying to complete the test. Swiss mice, weighing 18 to 22g, were dosed by gavage with a suspension of LM2219 in acacia in a dose volume of 20 mL/kg, one hour before transcranial stimulation. The dose levels were 1, 10 or 100 mg/kg bw. Phenobarbital, 20 mg/kg bw, was the positive control. The effective dose (ED_{50}) is defined as the dose inhibiting scores by 50% in comparison with controls.	
3.5.11 Method 3.2.2.3	Anticonvulsant activity: strychnine-induced convulsions, according to Azoulay Subcutaneous injection of strychnine causes lethal convulsions. A dose of 1.25 mg/kg bw has been established as causing approximately 90% mortality in mice. Administration of an anticonvulsant prior to the strychnine injection will increase the survival rate. Chlormezanone, dosed at 100 mg/kg bw, was the positive control. Swiss mice, weighing between 18 and 22g, were fasted for 24 hours and then administered a dose of difethialone, 100 mg/kg bw, as a suspension in acacia (dose volume 20 mL/kg bw) by gavage. An hour later they were subcutaneously injected with 10 mL/kg bw strychnine in apyrogenic distilled water. The effective dose (ED_{50}) is defined as the dose inhibiting mortality by 50% in comparison with controls.	
3.5.12 Method 3.2.3.1	<u>Antidepressant activity: inhibition of reserpine-induced ptosis, according to Rubin et al</u> Intraperitoneal injection of reserpine results in palpebral ptosis, graded as: 0 = normal eye 1 = palpebra cover ¼ of eye surface 2 = palpebra cover ½ of eye surface 3 = palpebra cover ¾ of eye surface	

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	4 = eye is fully closed. Swiss mice of either sex, weighing 18 to 22g, were injected with 5 mg/kg reserpine in 0.1% acetic acid in a dose volume of 10 mL/kg bw and eyes examined one and a half hours later for ptosis effects. The treatment group was similarly dosed with reserpine but this was followed immediately by gavage administration of a 20 mL/kg bw suspension of difethialone in 10% acacia at a dose level of 50 mg/kg bw. The positive control was imipramine at a dose level of 20 mg/kg bw. The effective dose (ED_{50}) is defined as the dose inhibiting ptosis occurrence by 50% in comparison with controls.	
3.5.13 Method 3.2.3.2	Antidepressant activity: potentiation of the effects of 5-hydroxytryptophan, according to Pugsley and Lippman Intraperitoneal injection of 5-hydroxytryptophan results in serotonin accumulation in the brain causing stereotypic movements in the mouse. Drugs preventing re-uptake of serotonin promote accumulation in the brain and thereby potentiate these stereotypies. Female Swiss mice, weighing between 18 and 22g, were injected i.p. with 300 mg/kg bw 5-hydroxytryptophan in 3% acacia solution in a dose volume of 10 mL/kg bw. Thirty minutes later the animals were observed for stereotypic responses graded as 0 or 1 for absence or presence of hindleg extension, tremor, excitement or tossing of the head. The test animals were dosed by one of two routes, intraperitoneal injection of 25 mg difethialone/kg bw 10 mL/kg bw, 30 minutes before dosing with 5-hydroxytryptophan or by gavage (20 mL/kg bw) one hour before dosing with 5-hydroxytryptophan. Difethialone was administered as a suspension in acacia. The positive control was imipramine dosed at 15 mg/kg bw i.p. and the negative control was desipramine, dosed at 15 mg/kg bw i.p. The effective dose (ED_{50}) is defined as the dose reducing the number of scores of "4" by 50% in comparison with controls.	
3.5.14 Method 3.2.3.3	<u>Antidepressant activity: test of reserpine-induced akinesia, according to Bourin et al</u> Reserpine-induced akinesia can be reversed by direct or indirect acting dopaminergic agents. Female Swiss mice, weighing 18 to 22g, were given an intraperitoneal injection of reserpine, 2.5 mg/kg bw in 0.1% acetic acid in distilled water in a dose volume of 10 mL/kg bw. The mice were observed 4.5 hours later using the following grading system: 0 = mouse able to move over a distance exceeding its body length 1 = small movements; unable to move over a distance	

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	exceeding its body length 2 = akinesia, complete absence of movement. The test material was administered at a dose level of 50 mg/kg bw, as a suspension in acacia in a dose volume of 10 mL/kg bw, by intraperitoneal injection approximately 4 hours after injection of reserpine. Apomorphine, dissolved in apyrogenic saline and administered s.c. at a dose level of 0.4 mg/kg bw, in a dose volume of 10 mL/kg, was given 30 minutes before assessment of reactions. The effective dose (ED_{50}) is defined as the dose reducing the scores by 50% in comparison with controls.	
3.5.15 Method 3.2.3.4	<u>Antidepressant activity: MAOI activity, tryptamine test</u> Intraperitoneal injection of tryptamine causes characteristic stereotypic behaviour in mice. These effects can be potentiated by MAO inhibitors. Female Swiss mice, weighing 18 to 22g, were given an intraperitoneal injection of tryptamine, 100 mg/kg bw in apyrogenic distilled water in a dose volume of 10 mL/kg bw. This is a threshold dose established not to cause stereotypies. Thirty minutes after dosing the animals were observed for stereotypic responses graded as 0 or 1 for absence or presence of hindleg extension, tremor, excitement or lateral movements of the head. Difethialone was administered by gavage as a suspension in acacia at a dose volume of 20 mL/kg. The dose, 1, 10 or 100 mg/kg bw, was given either one or six hours prior to administering tryptamine. Tranylcypromine, 2.2 mg/kg bw, dosed by gavage was the positive control. The effective dose (ED_{50}) is defined as the dose reducing the number of scores of "4" by 50% in comparison with controls.	
3.5.16 Methods 3.3.1; 3.3.2 and 3.3.3	<u>Spasmolytic activity in vitro, by the method of Magnus</u> Atropinic or papaverinic activity can be tested using the rat duodenum and antihistaminic H1 activity can be tested by same methods using the guinea pig ileum. A standard formulation of Tyrode's solution is prepared (for the antihistaminic test 500 µg atropine/L is added to limit uncontrolled contractions. Contractions were induced by acetylcholine hydrochloride in distilled water, 250 µg/L or histamine dihydrochloride in distilled water, 25 µg/L. After killing and exsanguination, the rat or guinea pig has a 2 cm long piece of duodenum or ileum rapidly excised and fixed in oxygenated Tyrode's solution. The excised duodenum was maintained at 37-38°C and the ileum at 34-36°C. One end of the excised tissue was attached to the base of a cuvet and the other end to an isotonic strain gauge. The tissue was then allowed to stand for 30 minutes during which time it was rinsed 4-5 times with Tyrode's solution.	

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	The contractions produced by injection of 0.5 mL of spasmogenic agents, left in place for 90 seconds, were recorded. To observe antihistaminic effects, difethialone was added preventatively, prior to injection of the spasmogenic agent, and left in place for 30 seconds before rinsing. To observe atropinic or papaverinic effects, difethialone was added curatively, after injection of the spasmogenic agent, and left in place for 30 seconds before rinsing. Relaxant tests were conducted after testing contractants. The excised tissues were rinsed and unused for 5 minutes after each contraction. For atropinic activity, difethialone was dosed at 50 mg/L and atropine sulphate was the control, dosed at 7.5 µg/L, in distilled water. For papaverinic activity, difethialone was dosed at 5 mg/L and papaverine was the control, dosed at 3.75 µg/L, in distilled water. For antihistaminic activity, difethialone was dosed at 5 mg/L and thiazinamium was the control, dosed at 5 µg/L, in distilled water. The concentration of difethialone reducing contractant spasm by 50% was determined.	
3.5.17 Methods 3.4.1.1	<u>Analgesic activity: test of acetic acid-induced abdominal writhing according to Koster et al</u> Injection of 0.4% acetic acid by intraperitoneal injection to Swiss female mice (weighing 18 to 22g) at a dose volume of 30 mL/kg bw causes abdominal writhing. The writhing episodes are counted for 5 minutes from 10 minutes after injection. Difethialone, at dose levels of 1, 10 or 100 mg/kg bw, was administered by gavage, in a dose volume of 20 mL/kg bw as a suspension in acacia, one hour before the schedule observation time. The positive control was 200 mg/kg bw aspirin The ED_{50} was calculated as the dose concentration reducing the number of writhing bouts by 50% in comparison with controls.	
3.5.18 Methods 3.4.2.1	<u>Anti-inflammatory activity: carrageenan-induced plantar oedema, according to Winter et al</u> Difethialone was administered by gavage, in a dose volume of 10 mL/kg, as a suspension in 10% acacia solution. Female Wistar rats, in weight range 100 to 200g, were used for the test. One hour after difethialone administration, the hind leg was experimentally inflamed by plantar subcutaneous injection of 0.05 mL of 1% carrageenan in apyrogenic isotonic saline. The paw volume was measured by plethysmography before and 3 hours after injection of the phlogogenic agent. Oedema reaches maximum volume	

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	in control rats at approximately 3 hours post-injection. Difethialone was administered at a dose level of 100 mg/kg bw. The positive control was 75 mg/kg bw phenylbutazone. The volume of oedema generated for each animal was calculated and the difethialone dose inhibiting oedema formation by 30% was determined in comparison with controls.	
3.5.19 Methods 3.4.3.1	<u>Gastric antacid activity: mouse stomach pH</u> Initially the pH of the mouse stomach is measured. Gastric pH increases in presence of materials liable to reduce acid secretions. Swiss mice, 18 to 22 g are used. The animals are fasted for 24 hours prior to dosing and housed on grids to prevent them eating sawdust bedding or faeces. One hour before scheduled termination, by cervical dislocation, the mice are dosed by intraperitoneal injection of the test material at a dose level of 100 mg/kg bw in a dose volume of 10 ml/kg bw. Difethialone was prepared in a 3% acacia solution. The positive control was 80 mg/kg bw cimetidine. The mouse was opened along the median ventral line and the stomach opened in situ along the greater curvature. The pH of the gastric fluid was measured and a second pH measurement was obtained from the gastric wall near the pylorus if considered necessary. The minimum active dose was determined as the lowest dose effecting a significant difference in pH value in comparison with controls (Student's t test).	
3.6 Examinations	See methodologies detailed above in Section 3.4.1 to 3.4.19	
	4 RESULTS AND DISCUSSION	
4.2 Body Weight	Bodyweights not recorded in these screening tests	
4.3 Clinical signs of toxicity	Clinical signs were not checked in these tests for pharmacological activity. The dose levels were set below the lethal threshold based on a toxicology screening study. Any effects observed in the screening tests are recorded in the appropriate table of results.	
4.4 Other	All pharmacological activities investigated in this study are described in the relevant tables. See Tables 6.9-01 to 6.9-21.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	See relevant methodologies detailed above in Section 3.4.1 to 3.4.19.	
5.3 Results and discussion	The tabulated results for each individual test within the study are presented in Tables 6.9-01 to 6.9-19. Difethialone showed no antianginal activity *in vivo* or *in vitro*. Difethialone showed no antihypertensive activity.	

Section A 6.09-01 **Annex Point IIIA VI.1**	**Delayed neurotoxicity** Pharmacological investigations in rats, mice and guinea pigs	
	Difethialone showed no sedative activity. Difethialone showed no anticonvulsant activity in the various tests conducted. Difethialone showed no antidepressant activity. Difethialone showed no antispasmodic activity in a variety of *in vitro* tests. Difethialone showed no analgesic, anti-inflammatory or gastric antiacid activity in various tests designed to investigate these endpoints.	
5.4 Conclusion	Difethialone was investigated, in various screening tests, for potential pharmacological activity other than its known anticoagulant properties. At non-lethal doses the product showed no pharmacological activity in these tests.	
5.4.1 Reliability	2	
5.4.2 Deficiencies	No. The work was intended to be used for screening purposes and provides useful additional information on methods of action not exhibited by the rodenticide under investigation.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2007	
Materials and Methods	*Applicant version is adopted*	
Results and discussion	As no specific study has been performed for neurotoxicity of Chlorophacinone, studies with difethialone and other chemicals are presented by the Notifier. However only the data for difethialone is considered of interest due to its structural similarity and mode of action as antivitamin K. Data with other drugs are only considered as negative or positive controls. The tabulated results for each individual test within the study are presented in Tables 6.9-01 to 6.9-19. Difethialone showed no antianginal, antihypertensive, sedative, anticonvulsant, antidepressant, antispasmodic analgesic, anti-inflammatory or gastric antiacid activity in various tests designed to investigate these endpoints.	
Conclusion	Difethialone was investigated, in various screening tests, for potential pharmacological activity other than its known anticoagulant properties. At non-lethal doses the product showed no pharmacological activity in these tests. Due to thestructural similarities, no pharmacological activity may be deduced for cjhlorophacinone. .	
Reliability	*3*	
Acceptability	*Not accepted for risk assessment*	
Remarks	The observation is scientifically valid but not useful for risk assessment purpose.	

Table A 6.9-1: Table for activity on cardiovascular system

Antianginal activity – electrocardiogram of curarized mouse

Test material	Dose (mg/kg)	No. of animals	Mean cardiac survival (minutes)	Percentage increase over control
Control	0	7	8.43	--

Diltiazem	60	8	13.13	55.75
Difethialone	100	8	7.13	0

P< 0.05 (student's t test)
Difethialone had no effect on cardiac survival

Table A 6.9-2: Table for activity on cardiovascular system

Antianginal activity – anticalcium activity

Test material	Dose (µg/kg)	Rat number	$CaCl_2$ spasm height (cm)	Percentage inhibition
Control Difethialone	0 2500	1	16 14	-- 12.5
Control Nifedipine	 2.5	1	15.6 5	-- 67.95
Control Difethialone	0 2500	2	21.5 21	-- 2.33
Control Nifedipine	0 2.5	2	20.5 1.3	-- 93.66

Difethialone showed no anticalcium activity *in vitro*

Table A 6.9-3: Table for antihypertensive activity

Antihypertensive activity – noradrenaline induced mortality

Test material	Dose (mg/kg)	Number of animals	Number of deaths	Percentage inhibition
Control	0	10	9	--
Prazosine	0.5	10	2**	77.77
Difethialone	1 5 25	10 10 10	6 9 8	33.33 0 11.11

** p<0.01 (Fisher's test)
Difethialone showed no alpha-blocking activity

Table A 6.9-4: Table for antihypertensive activity

Antihypertensive activity – arterial blood pressure

Test material	Dose (mg/kg)	Number of animals	Mean arterial blood pressure before treatment (Hg cm)	Mean arterial blood pressure after treatment (Hg cm)
Control	0	9	24.00	22.72
Alphamethyldopa	150	9	23.06	18.39***
Difethialone	100	10	24.15	22.70

*** $p<0.001$

Difethialone did not modify arterial blood pressure

Table A 6.9-5: Table for antiarrhythmic activity

Antiarrhythmic activity – chloroform induced arrhythmia

Test material	Dose (mg/kg)	Number of animals	Mean scores	Percentage inhibition
Control	0	10	9.50	--
Disopyramide	60	10	0.50***	94.74
Difethialone	100	10	8.50	10.53

*** $p<0.001$ (Mann and Witney U test)

Difethialone does not protect animals from chloroform induced arrhythmias

Table A 6.9-6: Table for central sedative activity

Central sedative activity – tube test

Test material	Dose (mg/kg)	Number of animals	Mean number of mice having climbed up	Percentage inhibition
Control	0	10	8	--
Diazepam	10	10	2*	75.00
Difethialone	1	10	7	12.50
	10	10	8	0
	100	10	8	0

* $p<0.05$ (Fisher's test)

Difethialone exhibits no activity in the tube test

Table A 6.9-7: Table for central sedative activity

Central sedative activity – Rota rod

Test material	Dose (mg/kg)	Number of animals	Mean scores	Percentage inhibition
Control	0	10	1.90	--
Diazepam	5	10	0.30**	84.21
Difethialone	1	10	1.90	0
	10	10	1.90	0
	100	10	2.00	0

** $p<0.01$ (Mann and Witney U test)

Difethialone exhibits no activity in the rotarod test

Table A 6.9-8: Table for central sedative activity

Central sedative activity – Escape test

Test material	Dose (mg/kg)	Number of animals	Mean number of escapees	Percentage inhibition
Control	0	8	7.88	--
Difethialone	1	8	5.75	27.03
	10	8	6.00	23.86
	100	8	6.13	2.21

Difethialone did not alter the number of mouse escapees

Table A 6.9-9: Table for anticonvulsant activity

Anticonvulsant activity – pentylentetrazole induced convulsions

Test material	Dose (mg/kg)	Number of animals	Number of deaths	Percentage inhibition
Control	0	10	10	--
Phenobarbital	25	10	0***	100
Difethialone	1	10	10	0
	10	10	10	0
	100	10	9	10

*** p< 0.001 (Fisher's test)

Difethialone did not alter the number of deaths following induced convulsions

Table A 6.9-10: Table for anticonvulsant activity

Anticonvulsant activity – ocular electroshock

Test material	Dose (mg/kg)	Number of animals	Mean scores	Percentage inhibition
Control	0	8	2.00	--
Phenobarbital	20	8	0.75**	62.50
Difethialone	1	8	2.00	0
	10	8	1.88	6
	100	8	2.00	0

*** p< 0.01 (Mann and Witney U test)

Table A 6.9-11: Table for anticonvulsant activity

Anticonvulsant activity – strychnine-induced convulsions

Test material	Dose (mg/kg)	Number of animals	Number of deaths	Percentage inhibition
Control	0	10	8	--
Chlormezanone	100	10	3*	62.50
Difethialone	100	10	7	12.50

** p< 0.05 (Fisher's test)

Table A 6.9-12: Table for antidepressant activity

Antidepressant activity – reserpine-induced ptosis

Test material	Dose	Number	Mean scores	Percentage

	(mg/kg)	of animals		inhibition
Control	0	5	2.40	--
Imipramine	20	5	0**	100
Difethialone	50	5	1.20	50

** p< 0.01 (Mann and Witney U test)
Difethialone did not show a significant reduction in the reserpine-induced ptosis in the mouse.

Table A 6.9-13: Table for antidepressant activity

Antidepressant activity – potentiation of 5-hydroxytryptophan effects

Test material	Dose (mg/kg)	Number of animals	Mean scores
Control	0	10	0.60
Imipramine	15	10	3.56***
Desipramine	15	10	0.70
Difethialone	25	10	0.80

*** p< 0.001 (Student's t test)
Difethialone did not potentiate the effects of 5-hydroxytryptophan and does not interfere with axonal re-uptake of serotonin or serotonin release. Difethialone has no serotonin-like activity or monoamine oxidase inhibitor (MAOI) activity.

Table A 6.9-14: Table for antidepressant activity

Antidepressant activity – reserpine-induced akinesia

Test material	Dose (mg/kg)	Number of animals	Mean scores	Percentage inhibition
Control	0	8	2.00	--
Apomorphine	0.4	8	0**	100
Difethialone	50	8	1.88	6

** p< 0.01 (Mann and Witney U test)
Apomorphine administered subcutaneously; control and difethialone given by oral gavage
Difethialone showed no dopaminergic activity

Table A 6.9-15: Table for antidepressant activity

Antidepressant activity – monoamineoxidase inhibiting activity – tryptamine test at 1 or 6 hours

Test material	Dose (mg/kg)	Number of animals	Mean scores at 1 hour	Mean scores at 6 hours
Control	0	10	0	0
Tranylcypromine	2.2	10	3.60	2.90
Difethialone	1	10	0	0
	10	10	0	0
	100	10	0	0

*** p< 0.001 (Mann and Witney U test)

Difethialone did not show any MAOI activity.

Table A 6.9-16: Table for antispasmodic activity

Antispasmodic activity – atropinic activity *in vitro* – rat duodenum

Test material	Dose (µg/L)	Rat number	Acetylcholine spasm height (cm)	Percentage inhibition
Control	0	1	8.5	--
Difethialone	50,000		8.3	2.35
Control	0		8.5	--
Atropine	7.5		2.1	75.29
Control	0	2	15.5	--
Difethialone	50,000		13.8	10.97
Control	0		11.2	--
Atropine	7.5		2.4	78.57

Difethialone showed no atropinic activity *in vitro*

Table A 6.9-17: Table for antispasmodic activity

Antispasmodic activity – papaverinic activity *in vitro* – rat duodenum

Test material	Dose (µg/L)	Rat number	Acetylcholine spasm height (cm)	Percentage inhibition
Control	0	1	17.3	--
Difethialone	5		15.0	13.29
Control	0		18.0	--
Papaverine	3.75		7.5	58.33
Control	0	2	13.3	--
Difethialone	5		13.1	1.50
Control	0		11.5	--
Papaverine	3.75		4.5	60.87

Difethialone showed no papaverinic activity *in vitro*

Table A 6.9-18: Table for antispasmodic activity

Antispasmodic activity – antihistaminic activity *in vitro* – guinea pig ileum

Test material	Dose (µg/L)	Guinea pig	Histamine spasm height (cm)	Percentage inhibition

		number		
Control	0	1	9	--
Difethialone	5000		14	0
Control	0		15.5	--
Thiazinamium	5		6	61.29
Control	0	2	14	--
Difethialone	5000		13	7.14
Control	0		14	--
Thiazinamium	5		0	100

Difethialone showed no antihistaminic H1 activity *in vitro*

Table A 6.9-19: Table for analgesic activity

Antidepressant activity – reserpine-induced akinesia

Test material	Dose (mg/kg)	Number of animals	Mean scores	Percentage inhibition
Control	0	10	7.90	--
Aspirin	200	10	2.40**	69.62
Difethialone	1	10	10.10	0
	10	10	14.70*	0
	100	10	11.70	0

* $p < 0.05$ (Student's t test)

** $p < 0.01$ (Student's t test)

Difethialone showed no analgesic activity and did not protect animals from pain induced by injection of acetic acid

Table A 6.9-20: Table for anti-inflammatory activity

Anti-inflammatory activity – carrageenan-induced oedema

Test material	Dose (mg/kg)	Number of animals	Mean oedema volume	Percentage inhibition
Control	0	10	15.20	--
Phenylbutazone	75	10	11.20*	26.32
Difethialone	100	7	17.29	0

* $p < 0.05$ (Student's t test)

Difethialone showed no anti-inflammatory activity

Table A 6.9-21: Table for gastric antiacid activity

Anti-ulcerous activity – mouse stomach pH

Test material	Dose (mg/kg)	Number of animals	Mean stomach pH
Control	0	6	1.70
Cimetidine	80	6	4.32**
Difethialone	100	6	1.58

** $p < 0.01$ (Student's t test)

Difethialone showed no gastric antiacid activity

Section A 6.10-01 **Annex Point IIA VI.6.10**		**Subchronic oral toxicity** Antidotal treatment study in rats	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxxxxxxx XX., (XXXX): Antidotal Treatment Study Following Oral Exposure to Chlorophacinone in Rats. Unpublished report No: XXXXXX (July 5, XXX). XXXXXXXXXXXXXXXXXXXXX. (Dates of experimental work December 10, XXX – March 1, XXXX).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	No guideline is available for this study. Report states compliance with EPA 86-1.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Referred to in report as Chlorophacinone 0.005 % pelleted end-use product (CPN)(certified)	
3.2.1	Lot/Batch number	Lot # XXXX	
3.2.2	Specification	Not specified	
3.2.2.1	Description	Green-colored pellets	
3.2.2.2	Purity	0.0051 %	
3.2.2.3	Stability	Not stated in report	
3.3	**Test Animals**		
3.3.1	Species	Rat	
3.3.2	Strain	Crl:CD BR	
3.3.3	Source	XXXXXXXXXXXXXXXXXXXXXXX, USA	
3.3.4	Sex	Males	
3.3.5	Age/weight at study initiation	Approximately 10 weeks. 291.0 – 379.0g	
3.3.6	Number of animals per group	10	
3.3.7	Control animals	Yes	
3.4	**Administration/ Exposure**	Oral administration of Chlorophacinone-baited diets, followed by administration of vitamin K_1 as an antidote.	
3.4.1	Duration of treatment	Males selected for dosing with chlorophacinone since they have been shown to be slightly more sensitive to coumarin derived anticoagulant rodenticides. The duration of treatment with chlorophacinone varied.	

Section A 6.10-01 **Annex Point IIA VI.6.10**	**Subchronic oral toxicity** Antidotal treatment study in rats	
	The control and high dose group were dosed over 72 hours and the high dose group received 5.03 mg chlorophacinone/kg bw/day; the low dose group had a single dose at 5.28 mg chlorophacinone/kg bw/day and the intermediate group had two doses over 48 hours, receiving 4.73 mg chlorophacinone/kg bw/day. 1 to 2 hours after last treatment, each rat was given a subcutaneous injection of Vitamin K1 and for the following 13 days the antidote was administered orally.	
3.4.2 Frequency of exposure	Daily for 1, 2 or 3 days	
3.4.3 Postexposure period	The animals were observed for 8 to 10 days after completion of antidotal treatment.	
3.4.4 <u>Oral</u>		
3.4.4.1 Type	Three groups of 10 males were offered chlorophacinone (0.005 % pelleted end use product) for 24, 48, 72 hours respectively, as their sole dietary source of food. The mean amount of the chlorophacinone consumed on a mg/kg body weight/day basis was 5.28, 4.73, and 5.03 respectively. At the end of each of the exposure periods, basal diet replaced the chlorophacinone diet. 1-2 hours after the exposure period the first five animals in each group were given a single subcutaneous injection of Vitamin K_1 at a dose of 5 mg/kg body weight. The animals received Vitamin K_1 at a dose of 5 mg/kg body weight/day by oral gavage for the following 13 days. The remaining 5 animals in each group received no antidotal treatment. Control animals followed the same antidotal treatment schedule as the 72-hour-exposed animals. 8-10 days after discontinuing the antidotal treatment, all surviving animals were sacrificed.	
3.4.4.2 Concentration	Chlorophacinone - 5.28, 4.73, and 5.03 mg/kg body weight/day Vitamin K_1 - 5 mg/kg body weight administered as an aqueous colloidal solution	
3.4.4.3 Vehicle	Chlorophacinone was administered in a known weight of diet – ranging from 488 to 544g which was available ad libitum for 24, 48 or 72 hours. Vitamin K_1 was administered as an aqueous colloidal solution	
3.4.4.4 Controls	Received basal diet. All animals were fasted for 18 hours before initial presentation of chlorophacinone baited diet	
3.5 Examinations		
3.5.1 Observations		

Section A 6.10-01 **Annex Point IIA VI.6.10**	**Subchronic oral toxicity** Antidotal treatment study in rats	
3.5.1.1 Clinical signs	Cageside observations performed hourly for the first 8 hours after initial presentation of the baited diet and at hourly intervals through working day for the next 6 days. For remainder of study the animals were checked three times each day. A thorough physical examination was completed weekly.	
3.5.1.2 Mortality	Mortality and moribundity assessed twice daily.	
3.5.2 Body weight	Body weight recorded in weeks –2 and -1 prior to treatment and at weekly intervals during the study.	
3.5.3 Food consumption	1 week prior to treatment, over the entire period of chlorophacinone-baited diet administration, at the end of the first week of study, and at weekly intervals thereafter	
3.5.4 Haematology	Blood samples for prothrombin time collected 2 weeks prior to treatment from all animals and at study termination for all survivors. Samples collected from orbital sinus. Analysed using Coag-A-Mate X2 with maximum time set to 50 seconds	
3.6 Sacrifice and pathology		
3.6.1 Organ Weights	No	
3.6.2 Gross and histopathology	Gross pathology all dose groups including decedents and animals surviving to scheduled termination. Necropsy examination involved external body surface, all orifices, cranial cavity and external surfaces of brain, thoracic, abdominal and pelvic cavities and their viscera, nasal cavity and paranasal sinuses, cervical tissues and organs, the carcass and checking for any signs of haemorrhage. A full EC compliant list of tissues was preserved for possible histopathology although no histological examination was actually conducted.	
3.6.3 Statistics	Bodyweight, food consumption and prothrombin time data were analysed using appropriate methods	
	4 RESULTS AND DISCUSSION	
4.2 Observations		
4.2.1 Clinical signs	Compound-related findings began to appear on study Day 1: dark-red, swollen digit(s); sanguineous discharge and/or red crust on nose, paws, and skin/fur; red/black urogenital discharge; pale body; soft feces; compound in feces; localized swelling; head tilt; rough haircoat; labored respiration; wheezing; limited use of hindlimbs; languid appearance; prostrate appearance; tremors; and cold to touch. In the 24- and 48-hour-exposure groups, the compound related findings in the surviving animals had subsided by Day 4; this was the fourth and the third day of antidotal treatment for the respective groups. No treatment-related findings were seen after day 4 due to	

Section A 6.10-01 **Annex Point IIA VI.6.10**		**Subchronic oral toxicity** Antidotal treatment study in rats	
		the death of the animals in the 72-hour-exposure group. For animals surviving to the end of the first week there were no treatment-related clinical signs evident during the weekly clinical assessment.	
4.2.2	Mortality	There were no deaths during the period of exposure to chlorophacinone i.e. during the first 72 hours of study (days 0, 1 or 2). The first deaths occurred on Day 3. All animals exposed to chlorophacinone-baited diet and not treated with Vitamin K_1 antidote died within 4-5 days. Group 1 (Basal diet) – no mortality observed. Group 2 (24 hour exposure to chlorophacinone) - the five antidote-treated animals exposed to chlorophacinone-baited diet survived to the scheduled sacrifice. The five non-antidote-treated animals died before day 4. Group 3 (48 hour exposure to chlorophacinone) – three antidote-treated animals survived to the scheduled sacrifice. Two antidote-treated animals and five non-antidote-treated animals did not survive past the fourth day. Group 4 (72 hour exposure to chlorophacinone) – None of the antidote treated or non-antidote treated animals survived to the scheduled sacrifice.	
4.3	**Body weight gain**	There were slight decreases in the mean body weight change of the 24- and 48-hour-exposure groups compared to controls for study Days 0-7. There were slight increases in the mean body weight change of the 24- and 48-hour-exposure groups compared to controls for study Days 7-14 and Days 14-21 for the 48-hour-exposure group only. Over the entire study, Days 0-21, the mean body weight change between the groups was comparable. Chlorophacinone treatment for 24, 48 or 72 hours followed by antidotal treatment had no clear effect on bodyweight or weight gain among surviving rats	
4.4	**Food consumption and compound intake**	The average amount of chlorophacinone-baited diet per day was similar or slightly increased compared to the basal diet consumed by the control group. For the remainder of the first week, when only basal diet was offered, the amount of food consumed in the 24- and 48-hour-exposure groups was slightly less than in the controls. Thereafter, the mean food consumption values between the groups were similar.	
4.5	**Blood analysis**		
4.5.1	Haematology	Prothrombin time: Data did not reveal any changes that were considered to be of potential biological importance. The mean prothrombin time values for the chlorophacinone-treated groups were similar to the control values at the end of the study.	
4.6	**Sacrifice and pathology**		
4.6.1	Gross and histopathology	Treatment-related findings noted at necropsy of the animals found dead or sacrificed in moribund condition included:	

Section A 6.10-01 **Annex Point IIA VI.6.10**	**Subchronic oral toxicity** Antidotal treatment study in rats	
	Dark tissues or dark areas in a tissue; enlarged, distended, or swollen tissues; fluid in cavities; and gelatinous areas. These findings were observed in the thymus; abdominal, thoracic and cranial cavities; kidney; urinary bladder; paws; prostate; skeletal muscle; stomach; brain; epididymis; testis; subcutaneous tissues; and/or lymph nodes of the 24, 48 and 72 hour exposure groups. No compound related findings were noted at the scheduled sacrifice.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	Chlorophacinone is an anticoagulant rodenticide. The study was designed to determine the effectiveness of vitamin K_1 as an antidote for chlorophacinone-induced toxicity. Three groups of 10 males were offered chlorophacinone (0.005 % pelleted end use product) for 24, 48, 72 hours respectively, as their sole dietary source of food. The mean amount of the chlorophacinone consumed on a mg/kg body weight/day basis was 5.28, 4.73, and 5.03 respectively. At the end of each of the exposure periods, basal diet replaced the chlorophacinone diet. 1-2 hours after the exposure period the first five animals in each group were given a single subcutaneous injection of Vitamin K_1 at a dose of 5 mg/kg body weight. The animals received Vitamin K_1 at a dose of 5 mg/kg body weight/day by oral gavage for the following 13 days. The remaining 5 animals in each group received no antidotal treatment. Control animals followed the same antidotal treatment schedule as the 72-hour-exposed animals. 8-10 days after discontinuing the antidotal treatment, all surviving animals were sacrificed. The parameters evaluated during the study were mortality, clinical /cageside observations, body weight, food consumption, prothrombin time. Gross necropsy was performed on the animals found dead or sacrificed in moribund condition, and on the animals sacrificed at the end of the study.	
5.3 Results and discussion	Chlorophacinone-baited diets produced significant evidence of toxicity suggestive of exposure to an anticoagulant rodenticide including death after 24, 48 and 72 hours of exposure. The cageside findings were similar to those expected (various signs of hemorrhage) for an anticoagulant rodenticide. The mean amount of chlorophacinone consumed over the 24, 48 or 72 hour periods (5.28, 4.73 and 5.03 mg/kg bw/day) was equivalent to approximately 1.5 x the oral LD_{50} value for male rats and represented significant over exposure. All animals in each chlorophacinone-exposure group, that did not receive any Vitamin K_1, died. All of the animals fed	

Section A 6.10-01 **Annex Point IIA VI.6.10**	**Subchronic oral toxicity** Antidotal treatment study in rats	
	for 24 hours and subsequently administered Vitamin K_1 survived to the scheduled sacrifice. Three of five animals fed chlorophacinone for 48 hours and subsequently administered Vitamin K_1 survived to the scheduled sacrifice. None of the animals fed for 72 hours survived to the scheduled sacrifice. Chlorophacinone related cageside findings were resolved in all animals in the 24-hour exposure group by the fourth dose of Vitamin K_1. Chlorophacinone related cageside findings were resolved in all animals in the 48-hour exposure group by the third dose of Vitamin K_1. Slight decreases in body weight were seen in the chlorophacinone-exposed animals that survived to the end of the first week of study. These changes were attributed to the general decline in health status after exposure to chlorophacinone-baited diet. Body weight gain improved during the second and the third weeks of study in the surviving antidote-treated animals. These changes were attributed to the general improvement in health status after Vitamin K_1 treatment was initiated. At the end of the study, prothrombin time values in the chlorophacinone-treated groups were similar to control group values. Necropsy of the animals found dead or sacrificed in moribund condition revealed dark tissues or dark areas in a tissue; enlarged, distended, or swollen tissues; fluid in cavities; and gelatinous areas. The findings were observed in the thymus; abdominal, thoracic, and cranial cavities; kidney; urinary bladder; paws; prostate; skeletal muscle; stomach; brain; epididymis; testis; subcutaneous tissues; and/or lymph nodes. There were no treatment-related findings were noted at the scheduled sacrifice.	
5.4 Conclusion	Chlorophacinone-baited diets produced significant evidence of toxicity suggestive of exposure to an anticoagulant rodenticide including death after 24, 48 and 72 hours of exposure. The cageside findings were similar to those expected (various signs of hemorrhage) for an anticoagulant rodenticide. All animals in each respective chlorophacinone-exposure group that did not receive any Vitamin K_1 died. All of the animals fed for 24 hours and subsequently administered Vitamin K_1 survived to the scheduled sacrifice. Chlorophacinone related cageside findings were resolved in all animals in the 48-hour exposure group by the third dose of Vitamin K_1. Vitamin K_1 was an effective antidotal treatment for animals exposed to an anticoagulant rodenticide at significant overexposure (circa 1.5 fold the acute LD_{50}) for 24 hours. Antidotal effectiveness reduced with longer periods of exposure to chlorophacinone.	

Section A 6.10-01 **Annex Point IIA VI.6.10**	**Subchronic oral toxicity** Antidotal treatment study in rats	
5.4.1 LO(A)EL	Not applicable	
5.4.2 NO(A)EL	Not applicable	
5.4.3 Reliability	1	
5.4.4 Deficiencies	None	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date October 2005

Materials and Methods

Results and discussion

Adopted applicant version.
Summary:
Chlorophacinone-baited diets produced significant evidence of toxicity suggestive of exposure to an anticoagulant rodenticide including death after 24, 48 and 72 hours of exposure.
Findings were similar to those expected (various signs of hemorrhage) for an anticoagulant rodenticide.
All animals in each respective chlorophacinone-exposure group that did not receive any Vitamin K1 died.
All of the animals fed for 24 hours and subsequently administered Vitamin K1 survived to the scheduled sacrifice.
Chlorophacinone related cage side findings were resolved in all animals in the 48-hour exposure group by the third dose of Vitamin K1.

Conclusion

Vitamin K_1 was an effective antidotal treatment for animals exposed to an anticoagulant rodenticide at significant overexposure (circa 1.5 fold the acute LD_{50}) for 24 hours.

Antidotal effectiveness reduced with longer periods of exposure to chlorophacinone.

Reliability 1

Acceptability Accepted

Remarks

Table A 6.10-1: Results of antidotal treatment

Exposure group	Number of dead / number of investigated		Time of death (range)	Observations
	Vitamin K_1	Non-Vitamin K_1		
Basal diet	0/5	0/5		No pathology findings observed in both groups
24 hours	0/5	5/5	Day 3-4	Day 0: Hour 1 - slight sanguineous discharge from nose, wheezing; Hour 2 - chromodacryorrhea; Hour 7 – Sore(s on skin/fur); Day 1 - slight sanguineous discharge from nose, compound in feces Day 2 - red crust on right front paw Day 3-4 – pale body, cold to touch, limited activity of hind limbs. The compound related findings in the surviving animals subsided by Day 4; the fourth day of the antidotal treatment.
48 hours	2/5	5/5	Day 3-4	Day 1 - dark red swollen digit, soft feces, compound in feces; Day 2 - red crust on right front paw, slight sanguineous discharge from nose, pale body, dark red swollen areas; Day 3-4 – pale body, limited activity of hind limbs, languid, prostrate, tremors The compound related findings in the survived animals subsided by Day 4; the third day of the antidotal treatment.
72 hours	5/5	5/5	Day 3-4	Day 2 – Hour 2 – dark red swollen digit, compound in feces, Hour 5 - slight sanguineous discharge from nose; Day 3-4 - dark red swollen digit; pale body, head tilt, languid, prostrate, tremors, localised swelling axillary/maxillary. None survived to the end of the study.

Table A 6.10-2: Mean bodyweights

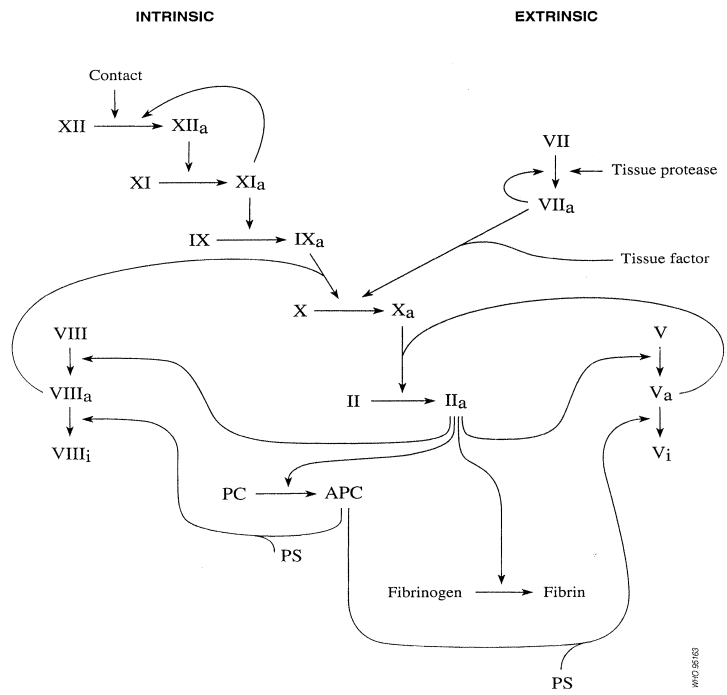
Bodyweight interval	Bodyweight (g) and weight gains			
	Group 1 control	Group 2 24 hr exposure	Group 3 48 hr exposure	Group 4 72 hr exposure
Day 0	335.3	332.1	341.4	331.9
Day 7	390.2 (54.9)	377.0 (49.4)	380.3 (44.7)	--
Day 14	427.1 (36.9)	420.2 (43.2)	424.3 (44.0)	--
Day 21	457.5 (30.4)	449.4 (29.2)	462.3 (38.0)	--
Gain for Day 0-21	(122.2)	(121.8)	(126.7)	--
Mean weight gains shown in parentheses				

Table A 6.10-3: Pre and post treatment prothrombin times (seconds)

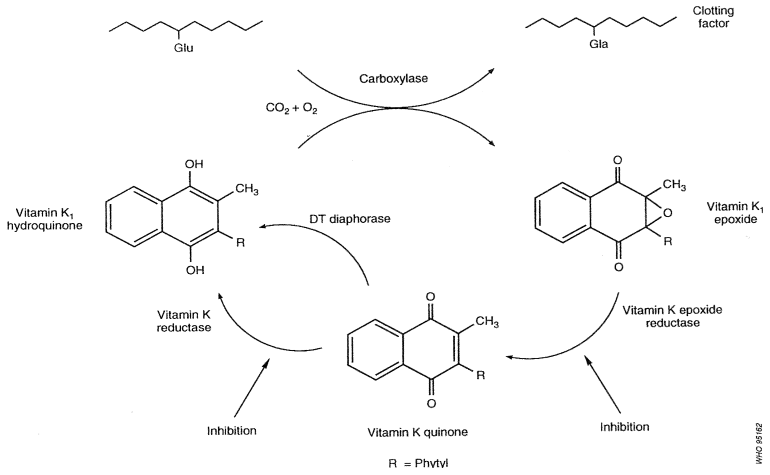
Group	Week	
	-2 pre-treatment	3
Group 1 control	14.4	14.9
Group 2 24 hr exposure	14.0	15.1
Group 3 48 hr exposure	14.6	14.6
Group 4 72 hr exposure	14.1	--

Section A 6.10-02 Annex Point IIA, 6.10	Mechanistic studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	In the absence of a specifc study investigating the mode of action of chlorophacinone, a summary of the findings on mechanisms of action of anticoagulant rodenticides as a family (including coumarin and indandione derivatives), presented in WHO IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva, 1995 ISBN 92 4 157175 6) is summarised below. Anticoagulant rodenticides such as Chlorophacinone function by inhibiting the ability of the blood to clot at the site of a haemorrhage, by blocking the regeneration of vitamin K in the liver. Death is due to haemorrhage. Blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin (factor IIa in the scheme below) to the insoluble fibrous protein fibrin, which binds platelets and blood cells to form a solid mass referred to as a blood clot, sealing the site of the haemorrhage and preventing further blood loss. Fibrinogen is present in the blood, but thrombin is not. Thrombin is formed at the site of injury from prothrombin (factor II) which is present in the blood. Conversion of prothrombin to thrombin occurs via the coagulation cascade, in which the blood clotting factors are employed. Without these blood factors clotting cannot take place, and the haemorrhage will not be controlled by clot formation. If the blood vessel is large and/or serves a vital organ, the haemorrhage will be fatal. The synthesis of a number of blood coagulation factors (factors II [prothrombin], VII [proconvertin] IX [Christmas factor], X [Stuart-Prower factor] and the coagulation inhibiting proteins C and S) is dependent upon vitamin K, which acts as a co-enzyme.	

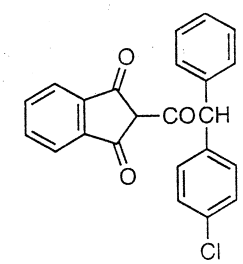
Section A 6.10-02 Mechanistic studies
Annex Point IIA, 6.10



Vitamin K hydroquinone is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide provides the energy required for the carboxylation reaction where glutamate (Glu) in the precursor is converted to γ-carboxyglutamate (Gla) to make the activated clotting factor.



The anticoagulant rodenticide active substances such as Chlorophacinone work by blocking the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place.
The structure of Chlorophacinone is:

Section A 6.10-02 Annex Point IIA, 6.10	**Mechanistic studies**
	 The molecule has significant structural similarity to the forms of vitamin K shown above. It can be seen that this structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate vitamin K. The amount of vitamin K in the body is finite, and progressive blocking of the regeneration of vitamin K will lead to an increasing probability of a fatal haemorrhage. In general terms, progressive intake of anticoagulants results in death.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	In the absence of specific studies, the Notifier report the mechanisms of action of related rodenticides
Conclusion	Accepted justification
Remarks	

Section A 6.11-01 Annex Point IIA, 6.11	Studies on other routes of administration	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Chlorophacinone has been tested in a variety of animal models by various routes of exposure (typically oral, dermal or inhalation) identified as the most common routes of human exposure. The well-defined mode of action indicates that anti-coagulant rodenticides are rapidly absorbed from the gastro-intestinal tract and easily absorbed through skin (maximum plasma concentrations attained within a few hours), following which they are rapidly accumulated in the liver and then excreted mainly as unchanged parent molecule or following hydroxylation. The activity of the molecules in blocking the epoxide cycle within the liver is unaffected by the route of initial administration and the resultant pharmacologic effect – haemorrhage due to loss of clotting factors is similarly unaffected by the exposure route. The time required to reduce plasma prothrombin to critical levels is measured in days (2-3 days before the typical heamorrhagic response is observed) and this will not be affected by the initial route of exposure. Systemic exposure involving parenteral administration is unlikely to alter the maximum plasma concentration and therefore unlikely to affect induction of a haemorrhagic syndrome. Use of warfarin for the treatment of thromboembolic disease is typically by oral administration but maintenance doses can be administered intravenously. Over 40 years of human exposure to this treatment regimen, using doses considerably higher than would arise in human acidental exposure scenarios, have not indicated any enhanced risk from parenteral exposure. The physical nature of the active molecule precludes intravenous or other parenteral human accidental exposure. In a review of accidental human exposure (WHO IPCS monograph 175) all cases of human poisoning by coumarin or indandione anticoagulant rodenticides have involved ingestion of significant quantities of rodenticide. Since the mode of action is well-characterised and the antidotal treatment is highly specific, further tests to investigate systemic toxicity following parenteral exposure are considered inappropriate and may be in contravention of the guiding principles relating to animal testing laid out in the Technical Notes for Guidance and in Directive 86/609/EC.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

Section A 6.11-01 Annex Point IIA, 6.11	Studies on other routes of administration
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	Aplicant made comments about datail studies
Conclusion	Accepted justification
Remarks	

Section A 6.12.1-01 **Annex Point IIA VI.6.9.1**		**Human case report (medical surveillance data)** Surveys of manufacturing plant personnel	
		1 REFERENCE	**Official use only**
1.1	**Reference**	XXXXXXXXXXX, XXXX, personal letter	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.2	**Substance**	Two laboratories concerned with research and development, manufacture and packaging of anticoagulant rodenticides. No specific products are mentioned but the principles of medical supervision refer to three rodenticide active ingredients – difethialone, bromadiolone and chlorophacinone and it can be assumed that the exposure being supervised relates to these materials or products containing these materials. The test substance is as specified in section 2.	
3.3	**Persons exposed**		
3.3.1	Sex	Not specified	
3.3.2	Age/weight	Not specified	
3.3.3	Known Diseases	Not specified	
3.3.4	Number of persons	Not specified	
3.3.5	Other information	The letter refers to “several persons” exposed to anticoagulant rodenticides during the manufacturing and packaging processes but gives no further detail.	
3.4	**Exposure**	Not specified	
3.4.1	Reason of exposure	Occupational/ accidental During manufacturing or packaging of active or products. Three categories of possible exposure identified: research chemists: poorly defined risk; production chemists: often exposed to pure active and concentrates, but exposure effectively eliminated by equipment engineering; bait manufacturers: manipulating low concentrations in dilute products, but exposure effectively eliminated by equipment engineering.	
3.4.2	Frequency of exposure	Not specified	
3.4.3	Overall time period of exposure	Not specified	
3.4.4	Duration of single exposure	Not specified	
3.4.5	Exposure concentration/dose	Not specified	

Section A 6.12.1-01 **Annex Point IIA VI.6.9.1**	**Human case report (medical surveillance data)** Surveys of manufacturing plant personnel	
3.5 Examinations	Staff monitored twice a year. Special attention given to control of prothrombin rate which is recorded one or two times annually or after any possible direct exposure to the active substance or products.	

Section A 6.12.1-01 **Annex Point IIA VI.6.9.1**	**Human case report (medical surveillance data)** Surveys of manufacturing plant personnel	
3.6 Treatment	Advice to physicians was issued jointly by Syngenta Crop Protection AG; Sorex Ltd; Lipha SA, BASF and Bayer Crop Science in relation to treatment of anticoagulant rodenticide poisoning as an aid to recognition of intoxication and dissemination of an agreed effective treatment regimen: Since rodenticides may be mixed with other chemicals, it is important to establish the product involved in poisoning incident. Second generation anticoagulant rodenticides, like chlorophacinone, act by interfering with prothrombin synthesis, disrupting clotting mechanisms and increasing tendency to haemorrhage. Since these products have longer body half-lives than the first generation rodenticides, bleeding can be prolonged and it may be necessary to prolong antidotal treatment for weeks rather than days. Typically signs of poisoning do not develop immediately but after less severe cases there is an increased tendency to bleed and effects including bruising, bleeding from nose or gums, blood in stools or urine and excesive bleeding from minor cuts/abrasions. More severe cases may involve massive (internal) haemorrhage, acute abdominal pain, shock or coma. Changes in prothrombin time are a reliable indicator of intoxication with anticoagulant and may be detected as early as 12-18 hours after ingestion of toxin and prior to onset of clinical signs. Daily monitoring of prothrombin time is recommended. Increased times will be reversed rapidly by administration of an effective antidote. Where antidote is required, Vitamin K_1 (Phytomenadione) should be administered intravenously followed by an extended period of daily oral dosing. Other analogues of Vitamin K are ineffective. Intramuscular administration should be avoided to reduce risk of inducing intramuscular haemorrhage. 10 - 20 mg of Vitamin K_1 (or 0.25 mg/kg for children) should be injected by slow intravenous infusion/injection (1.0 mg/minute). Check prothrombin times at 3 – 6 hourly intervals, repeat injection if no improvement evident. If it proves necessary to transfuse a patient, the use of plasma expander should be avoided (e.g dextran). Once prothrombin times have stabilised oral Vitamin K_1 treatment at 10 mg four times a day should be continued, checking prothrombin times daily. When prothrombin time has been normal for three days patient can be discharged with twice daily treatment of 10 mg b.i.d. oral Vitamin K_1. Oral supportive treatment may need to be continued for up to several months.	

Section A 6.12.1-01 **Annex Point IIA VI.6.9.1**	**Human case report (medical surveillance data)** Surveys of manufacturing plant personnel	
3.7 Remarks	The physician reponsible for medical supervision of the two sites involved in rodenticide production states that from 1987 he has seen no disease due to anticoagulant rodenticide. Reference is made to an isolated incident of intoxication (during medical supervision of a previous post-holder) due to a person biting his nails but no further information is provided for this anecdotal case.	
	4 RESULTS	
4.2 Clinical Signs	No information available	
4.3 Results of examinations	No information available	
4.4 Effectivity of medical treatment	No information available	
4.5 Outcome	No information available	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	In a brief written communication from the medical supervisor of two sites involved in manufacture, production and packaging of anticoagulant rodenticides, the doctor describes the medical supervision of staff at risk of exposure. Primary medical care is based on monitoring of staff prothrombin rates biannually.	
5.3 Results and discussion	No available monitoring results.	
5.4 Conclusion	The physician reponsible for medical supervision of the two sites involved in rodenticide production states that from 1987 to 1999 he has seen no disease due to anticoagulant rodenticide.	
	Evaluation by Competent Authorities	

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	October 2004
Materials and Methods	Applican version is accepted with some additional remarks: In a brief hand written communication from the medical supervisor of two sites involved in manufacture, production and packaging of anticoagulant rodenticides, the doctor describes the medical supervision of staff at risk of exposure. Primary medical care is based on monitoring of staff prothrombin rates biannually. Special attention given to control of prothrombin rate which is recorded one or two times annually or after any possible direct exposure to the active substance or products.
Results and discussion	No detail is available of prothrombin monitoring results.
Conclusion	Applican version is accepted with some additional remarks. The physician responsible for medical supervision of the two sites involved in rodenticide production states that from 1987 to 1999 have seen no disease due to anticoagulant rodenticide.

Section A 6.12.1-01 **Annex Point IIA VI.6.9.1**	**Human case report (medical surveillance data)** Surveys of manufacturing plant personnel	
Remarks		

Section A 6.12.2-01 Annex Point IIA, 6.9	**Human case report** Clinical cases, poisoning and other incidents	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The medical supervisor for the two Lipha sites responsible for the production of anticoagulant rodenticides (active substance and products containing the active) and packaging the products, confirmed that no cases of human poisoning have been reported among the exposed staff. **The International Programme on Chemical Safety (IPCS)** under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organisation published a Monograph "Environmental Health Criteria 175" Anticoagulant Rodenticides. This document includes a review of effects on humans of the long-acting anticoagulants (second generation anticoagulants including hydroxycoumarin and indandione compounds). This sets out a number of clinical cases and the outcome following accidental or unintentional poisoning and suicide attempts in acute or subacute exposure. In the majority of cases, severe or less severe haemorrhage and associated increased prothrombin times responded well to Vitamin K_1 antidotal treatment where this was provided initially by parenteral injection and followed by long term oral administration until prothrombin times were stabilised. Incidents of human exposures to rodenticides are **reported to poison control centres** in countries where such facilities exist. In 1988, for example, the American Association of Poison Control Centers (AAPCC) received accounts of **10,626 cases of human exposures to rodenticides**. These incidents represented 17% of reported exposures involving pesticides and 0.8% of the total number of cases reported in the AAPCC system. The rodenticide incidents included 4190 cases involving "anticoagulants" (principally warfarin) and 5133 involving "long-acting anticoagulants" (second-generation anticoagulants plus the indandione compounds). More than 95% of the rodenticide cases were classified as "accidental". Most of the remainder were classified as "intentional" and included attempted suicides. Of the 10,540 rodenticide incidents for which the ages of victims were reported, 9406 (89%) involved children under 6 years of age (Litovitz et al., 1994). Victims in nearly 32% of the rodenticide exposure incidents reported to the AAPCC in 1988 were treated in health care facilities. However, the medical outcome "none" was reported in more than 93% of the 5708 incidents for which information regarding	

Section A 6.12.2-01 Annex Point IIA, 6.9	**Human case report** Clinical cases, poisoning and other incidents

outcomes was reported. The remaining 380 cases included 333 with "minor" medical effects, 41 with "moderate" effects, 4 with "major" effects, and two deaths (Litovitz et al., 1994). In 1993, the Swedish Poison Information Centre received 338 enquiries concerning exposures to anticoagulant rodenticides. This number represented 0.6% of all enquiries to the centre and 37% of the enquiries concerning pesticides. Of the anticoagulant rodenticide enquiries, 202 pertained to warfarin and 136 to "superwarfarin" compounds (Persson, 1994).

Human exposure to second-generation and indandione anticoagulants produces symptoms consistent with anticoagulation effects (e.g., haematomas, haematemesis, haematuria, easy bruisability). Treatment of cases of exposure, particularly of substantial and repeated exposure, may require vitamin K_1 therapy and monitoring of prothrombin times for periods of many months (Rauch et al., 1994). Suicide and/or unintentional poisonings with anticoagulant rodenticides have occurred in many countries. Thus, Ungvary (1994) reported 70 cases, mostly involving children, that occurred in Hungary between 1988 and 1993.

Warfarin is widely used as a therapeutic and preventive agent in the treatment of thromboembolic disease. Patients have been maintained for years on this treatment with control of the prothrombin level, which should be kept between 10 and 30% of normal. **Diphacinone** has also been used as a drug because of its long-lasting action (the half-life in humans is 15-20 days). It ceased to be listed in the American Medical Association Drug Evaluations, (AMA, 1980) because of its structural relation to phenindion, which had been reported to have adverse effects.

Acute poisoning

Typical features of poisoning result from increased bleeding tendency and include: minor poisoning: coagulation disturbance detected only by laboratory analyses;
moderate poisoning:
coagulation disturbance resulting in haematomata, haematuria, blood in faeces or excessive bleeding from minor cuts or abrasions, gum bleeding;
severe poisoning:
retroperitoneal haemorrhage, severe gastrointestinal bleeding, cerebrovascular accidents, massive haemorrhage (internal bleeding) resulting in shock. If anaemia or liver disease is present then the above features may be more severe and persistent and the poisoning may be more difficult to control (Anonymous, 1988).

The onset of the signs of poisoning may not be evident until a few days after ingestion.

Poisoning incidents - Cases of human poisoning with

Section A 6.12.2-01 Annex Point IIA, 6.9	**Human case report** Clinical cases, poisoning and other incidents

"**superwarfarins**" **were reviewed by Katona & Wason (1989)**.
Fourteen members of a family in the Republic of Korea were poisoned by eating **warfarin**-containing maize meal. The first symptoms appeared 7-10 days after the beginning of exposure and were followed by massive bruises or haematomata on the buttocks in all cases (Lange & Terveer, 1954).
Pribilla (1966) reported a total dose of about **1000 mg of warfarin** to be fatal after 13 days of consumption.
Out of a total of 741 infants, **177 died after the use of warfarin-contaminated talc in Viet Nam**. The concentrations of **warfarin** in the powder varied from **1.7 to 6.5%** (Martin-Bouyer et al., 1983).
A 73-year-old woman suffered from recurrent episodes of hypoprothrombinaemia. Clotting tests and further investigation showed that this was due to a **warfarin** rodenticide intentionally mixed in the woman's cough syrup by her daughter-in-law. As the patient had as many as seven relapses, it was possible to compare different types of therapy. Menadione had no effect (Nilsson, 1957).
Several suicidal attempts with chlorophacinone have been reported. **Murdoch (1983)** reported a case of ingestion of 625 mg **chlorophacinone** (250 ml of a 0.25% concentrate formulation) by a 37-year-old woman. The prolonged anticoagulant action of chlorophacinone persisted for at least 45 days even though treatment was given. It was found that menadiol, the synthetic analogue of vitamin K_1, was ineffective. The natural form, phytomenadione, was effective only when given at high dosage (20 mg daily) 30 days after the ingestion of chlorophacinone.
In a case reported by **Dusein et al. (1984)**, the amount of ingested **chlorophacinone** was unknown. After adequate therapy, the prothrombin level became normal within 4 weeks.
Vogel et al. (1988) reported the case of an 18-year-old woman hospitalized 3 days after **ingesting approximately 100 mg chlorophacinone.** Under high-dose vitamin K_1 therapy (160 mg) the prothrombin time was normalized, but it increased again following withdrawal of vitamin K_1. After prolonged vitamin K_1 administration, the prothrombin time finally became normal after 7 weeks.
Brodifacoum poisoning has occurred in South Sumatra, Indonesia. Some of the villagers used a 0.005% brodifacoum rice grain bait as a food source even though they knew it was poisonous and unfit for human consumption. They attempted to remove the rodenticide by repeated washing, rinsing and cooking before eating the rice. Because of the delay in the appearance of poisoning symptoms it appeared that they had

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been successful, thus encouraging further attempts to purify the rice baits. As a result, deaths occurred before appropriate remedial treatment could be initiated (Anonymous, 1985).
Jones et al. (1984) reported the first case of human **brodifacoum** poisoning in a 17-year-old boy who attempted suicide by ingesting approximately 7.5 mg (0.12 mg/kg) of **brodifacoum** in Canada. He was initially seen with gross haematuria, followed by epistaxis and gum bleeding. The prothrombin time and the activated partial thromboplastin time were notably prolonged. He was treated for 56 days with either parenteral or oral vitamin K_1 and either fresh or stored plasma until coagulation values remained normal and stable.
Lipton & Klass (1984) reported a similar case in a 31-year-old mentally disturbed woman who ingested over a 2-day period approximately thirty 50-g packages of Talon-G (approximately 75 mg of **brodifacoum**). Prothrombin time and activated partial thromboplastin time were considerably prolonged (respectively 6-fold and 4-fold above normal values). After 4 days of therapy with high doses of vitamin K_1 (up to 125 mg/day), partial correction in the prothrombin time occurred. Vitamin K_1 therapy continued with interruptions for 8 months until normal prothrombin time levels were found.
Chong et al. (1986) reported a case of suicidal poisoning after ingestion of 10 mg **brodifacoum** (as 0.05% Klerat). The coagulation test became normal after large doses and prolonged use of vitamin K_1 over 6 months.
A case of intentional ingestion of **brodifacoum** (200 g of Talon G, 0.005% brodifacoum) was reported by Hoffman et al. (1988). A profound decrease in the levels of factors II, VII, IX and X, lasting 43 days after ingestion, was observed. Treatment with subcutaneous vitamin K_1 in doses up to 100 mg per day was effective.
Weitzel et al. (1990) described three patients with severe bleeding disorders due to deficiency of the vitamin K-dependent blood clotting proteins after ingestion of an anticoagulant. Although the patients denied any ingestion, **brodifacoum** was detected in their serum at concentrations of 7.6 nmol/litre, 270.7 nmol/litre and 2759 nmol/litre, respectively. The anticoagulant effect was found to persist long after **brodifacoum** was no longer detectable in the serum. A half-life of approximately **16-36 days** was determined for brodifacoum in the plasma.
Kruse & Carlson (1992) reported the case of a 25-year-old man who attempted suicide by consuming a **brodifacoum** rodenticide. He developed a severe coagulopathy that was treated with vitamin K_1 and fresh frozen plasma and he was discharged from hospital with oral phytomenadione. Fifteen

Section A 6.12.2-01 Annex Point IIA, 6.9	**Human case report** Clinical cases, poisoning and other incidents

weeks later the man presented again with a history of further brodifacoum ingestion. He suddenly became comatose and computer tomography revealed a subarachnoid haemorrhage that led to brain death 24 h later.

Wallace et al. (1990) described the clinical course of a patient poisoned with **brodifacoum** in a suicide attempt. He developed microhaematuria and melaena. His clotting factors were depressed and were poorly responsive to vitamin K treatment.

Barlow et al. (1982) reported a case of attempted suicide with 25 mg of **difenacoum** (500 g of rat bait) followed several months later by 1800 g of rat bait. The patient was treated with vitamin K_1 (phytomenadione) for 48 and 42 days, respectively, until the pharmacological effect of difenacoum ceased.

Nighoghossian et al. (1990) reported an unusual coagulopathy after accidental exposure to a **diphenacoum** rodenticide. A 59-year-old man developed subacute tetraparesis following severe sudden neck pain, which on clinical examination was shown to be due to a subdural cervical haematoma. Prothrombin complex activity was low and diphenacoum was present in the plasma. Specific medical management led to a complete recovery.

Greeff et al. (1987) reported accidental **bromadiolone** poisoning in two children, resulting in prolonged anticoagulation. Descarboxyprothrombin levels were increased in both cases by 27% and 29.9%, respectively (normal, non-detectable level). The first child rapidly recovered after treatment with high-dose intravenous factor IX-prothrombin complex and vitamin K_1. The clotting profile became normal on the third day after admission. The second child gave a poor response to 10 mg intravenous vitamin K_1 and the dose was increased to 20 mg.

<u>Controlled human studies</u>

Single oral doses of 60, 70, 80 or 120 mg **warfarin** decreased the prothrombin concentrations in volunteers to zero by the third day. After the administration of 50 mg vitamin K_1, the prothrombin concentrations returned by the sixth day to 60, 70, 55 and 63%, respectively, of the normal value (Anonymous, 1965).

When a single oral dose of 20 mg chlorophacinone was given to three volunteers, the lowest prothrombin times were 35, 34 and 38% of the pretreatment value on days 2, 4 and 2, respectively. Eight days after administration without any treatment the values were 80, 100 and 90%, respectively (**Anonymous, 1965**).

<u>Effects of short- and long-term exposure</u>

Two cases of occupational exposure to **brodifacoum** and **difenacoum** were reported by Park et al. (1986). The

Section A 6.12.2-01 Annex Point IIA, 6.9	**Human case report** Clinical cases, poisoning and other incidents
References	exposure was of a chronic nature (2 and 4 years, respectively). Plasma analysis in the first patient revealed the presence of both difenacoum and brodifacoum in the range of 30-50 µg/litre. In both patients unexpectedly high concentrations of vitamin K_1 2,3-epoxide were found in the presence of normal clotting factor activities and antigen levels suggesting the presence of coumarin anticoagulants in the liver. A case of poisoning in a 23-year-old man resulting from prolonged skin contact during the process of preparing and distributing **warfarin** baits has been reported (Fristedt & Sterner, 1965). Epidemiological studies During a production run preparing ready-to-use **flocoumafen** bait (0.005% in baits) in a formulation plant, the effect of the rodenticide on blood coagulation factors was monitored in 12 subjects, using the classical prothrombin time test, a modified prothrombin time technique and measurement of prothrombin (factor II) concentration in blood. No adverse health effects were observed in any subject involved in formulation operations. No changes were observed in any of the three tests that could be ascribed to absorption of **flocoumafen** into the body (Tuinman & Van Sittert, 1986). Litovitz TL, Clark LR, & Soloway RA (1994) 1993 Annual report of the American Association of Poison Control Centers, Toxic Exposure Surveillance System. Am J Emerg Med, 12: 546-584. Persson H (1994) [Annual report 1993.] Stockholm, Swedish Poison Information Centre, 24 pp (in Swedish). Rauch AE, Weininger R, Pasquale D, Burkart PT, Dunn HG, Weissman C, & Rydzak E (1994) **Superwarfarin** poisoning: A significant public health problem. J Community Health, 19: 55-65. Ungvary G (1994) Anticoagulant poisoning incidents in Hungary. Budapest, National Institute of Occupational Health (Unpublished report). Katona B & Wason S (1989) **Superwarfarin** poisoning. J Emerg Med, 7: 627-631. Lange PF & Terveer J (1954) **Warfarin** poisoning. US Armed Forces Med J, 5: 872-877. Pribilla O (1966) Murder caused by **warfarin**. Arch Toxicol, 21: 235-249. Martin-Bouyer G, Linh PD, Tuan LC, Barin C, Khahn MB, Hoa DQ, Tourneau J, & Guerbois H (1983) Epidemic of haemorrhagic disease in Vietnamese infants caused by **warfarin**-contaminated talc. Lancet, 1: 230-232. Nilsson IM (1957) Recurrent hypoprothrombinaemia due to poisoning with **dicumarol**-containing rat-killer. Acta

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Vogel JJ, de Moerloose Ph, Bouvier CA, Gaspoz J, & Riant P (1988) Anticoagulation prolongée lors d'une intoxication à la **chlorphacinone**. Schweiz Med Wochenschr, 118: 1915-1917.
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Barlow AM, Gay AL, & Park BK (1982) **Difenacoum** (Neosorexa) poisoning. Br Med J, 285: 541.
Nighoghossian N, Ruel JH, French P, Froment JC, & Trouillas P (1990) Hématome sous-dural cervico-dorsal par intoxication aux raticides **coumariniques**. Rev Neurol (Paris), 146(3): 221-223.
Greeff MC, Mashile O, & MacDougall LG (1987) "Superwarfarin" (**bromadiolone**) poisoning in two children resulting in prolonged anticoagulation. Lancet, 1: 1269.
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Section A 6.12.2-01 **Annex Point IIA, 6.9**	**Human case report** Clinical cases, poisoning and other incidents
	Clin Pharmacol, 21: 289-294. Fristedt B & Sterner N (1965) **Warfarin** intoxication from percutaneous absorption. Arch Environ Health, 11: 205-208. Tuinman CP & Van Sittert NJ (1985) Biomedical monitoring of personnel in Sorex Ltd (Widnes, UK) involved in a formulation run with the rodenticide **WL 108366**. The Hague, Shell Internationale Petroleum Maatschappij B.V. (Report No. HSE 85.006).
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2004

Evaluation of applicant's justification	Applicant presents a justification of no submission of data BUT actually made a review of the public available information. However applicant does not made a quantitative evaluation of dose level and effect severity in order to get an analysis and conclusions useful for risk assessment based in human data. Most data commented by applicant seems to be withdrawn from the monograph published by WHO-IPCS: *"Environmental Health Criteria 175" Anticoagulant Rodenticides" (1995) A review of more recent publications have not been submitted by the Applicant"*. **Applicant had to be done a more updated review of existing published data.** American Association of Poison Control Centers (AAPCC) received accounts of **10,626 cases of human exposures to rodenticides**. The rodenticide incidents included 4190 cases involving "anticoagulants" (principally warfarin) and 5133 involving "long-acting anticoagulants" (second-generation anticoagulants plus the indandione compounds). More than 95% of the rodenticide cases were classified as "accidental". However only very limited data is publically available with specific medical reports, doses ingested and description of severity of effects and response by therapeutic protocols applied. In the Applicant review, information about human data is presented on the main families of anticoagulant rodenticides: • First generation hydroxycoumarins (warfarin) • Second generations hydroxycoumurins (brodifacoum, bromadiolone, difenacoum, diphethialone, flocoumafen) • Indandiones (chlorophacinone, diphacinone) Data are reported of human poisoning with: • Warfarin (4 references) • Chlorophacinone (3 papers) • Brodifacoum (8 references) • Diphenacoum (2 references) • Bromadiolone (1 reference) Cases of controlled human studies are reported for: • Warfarin • Chlorophacinone Cases of occupational short term and long term exposure are reported for: • Brodifacoum • Difenacoum • Warfarin • Flocoumafen (epidemiological study in formulation plant) Many cases of human poisoning are described with warfarin (a first generation hydroxycoumarin) and with some of the second generation coumarins also known as superwarfarins or "long acting anticoagulants" as brodifacoum, bromodiolone and diphenacoum. Chlorophacinone is an indandione anticoagulant, a diferent family of chemicals, although acting also by the mechanism of interfering the synthesis of Vitamin K1 and causing similar effect than the second generation hydroxycoumarin. Although it is of general interest to consider other related anticoagulant rodenticides, specially those more related to chlophacionone (indandiones), in order to use human data for evaluation the most important and useful data are dose of chlorophacinone. Only limited data of poisoning with Chlorophacinone are presented by aplicant. They are related with several suicidal attempts with chlorophacinone which have been reported: Murdoch DA (1983) Prolonged anticoagulation in chlorphacinone poisoning. Lancet, 1: 355-356. Dusein P, Manigand G, & Taillandier J (1984) Hypoprothrombinémie sévère et prolongée après intoxication par chlorphacinone. Presse Méd, 13(30): 1845. Vogel JJ, de Moerloose Ph, Bouvier CA, Gaspoz J, & Riant P (1988)

Anticoagulation prolongée lors d'une intoxication à la chlorphacinone. Schweiz Med Wochenschr, 118: 1915-1917.
Anonymous (1965) Technical report on chlorophacinone. Lyon, France, Lipha S.A.

Murdoch (1983) reported a case of ingestion of 625 mg chlorophacinone (250 ml of a 0.25% concentrate formulation) by a 37-year-old woman. The prolonged anticoagulant action of chlorophacinone persisted for at least 45 days even though treatment was given. It was found that menadiol, the synthetic analogue of vitamin K1, was ineffective. The natural form, phytomenadione, was effective only when given at high dosage (20 mg daily) 30 days after the ingestion of chlorophacinone.

In a case reported by **Dusein et al. (1984)**, the amount of ingested chlorophacinone was unknown. After adequate therapy, the prothrombin level became normal within 4 weeks.

Vogel et al. (1988) reported the case of an 18-year-old woman hospitalized 3 days after ingesting approximately 100 mg chlorophacinone. Under high-dose vitamin K1 therapy (160 mg) the prothrombin time was normalized, but it increased again following withdrawal of vitamin K1. After prolonged vitamin K1 administration, the prothrombin time finally became normal after 7 weeks.

Controlled human studies has been described with warfarin and chlorophacinone When a single oral dose of 20 mg chlorophacinone was given to three volunteers, the lowest prothrombin times were 35, 34 and 38% of the pretreatment value on days 2, 4 and 2, respectively. Eight days after administration without any treatment the values were 80, 100 and 90%, respectively (Anonymous, 1965).

Plasma chlorophacinone determinations were performed in three cases of intoxication. The risk of bleeding was minimal when the plasma level was below 1 mg/litre (Burcuoa et al., 1989).

Some limited cases of intoxications of non-target wild vertebrade animals has been described with second generation anticoagulant in field observations. Primary intoxication with poisonings of pheasants and partridges by chlorophacinone used against Microtus arvalis have been reported (Giban, 1974). Studies of secondary toxicity have been also described. Barn owls (Tyto alba) were fed rats poisoned with diphacinone, chlorophacinone, coumafuryl, difenacoum, bromadiolone or brodifacoum. Five out of six owls died of haemorrhaging after feeding on rats killed with brodifacoum after 8 to 11 days. Sublethal haemorrhaging, but no mortality, occurred in owls fed rats killed with difenacoum. One owl died following 10 days of treatment with bromadiolone-poisoned rats, while five showed no symptoms. No abnormalities were observed in two owls fed rats killed with diphacinone, coumafuryl or chlorophacinone. Owls that died behaved normally until 24 h or less before death, when they became lethargic and stopped eating (Mendenhall & Pank, 1980).

Radvanyi et al. (1988) fed American kestrels (Falco sparverius) on meadow voles that had been maintained on 2% chlorophacinone. Voles consumed approximately 53 mg of 2% chlorophacinone (1.14 mg a.i.) before dying within 6 days. No kestrels fed poisoned mice, for up to 21 consecutive days, died. Haematomas were observed on the pectoral muscles, lungs, liver and heart of exposed birds.

Radvanyi A, Weaver P, Massari C, Bid D, & Broughton E (1988) Effects of chlorophacinone on captive kestrels. Bull Environ Contam Toxicol, 41: 441-448.

Giban J (1974) Use of chlorophacinone in the struggle against the common vole *(Microtus arvalis Pallas)* and against the musk rat *(Ondatra zibethicus).* In: Proceedings of the 6th Vertebrate Pest Conference, Sacramento, California. Davis, California, University of California, pp 263-271.

Burucoa Ch, Mura P, Robert R, Boinot C, Bouquet S, & Piriou A (1989) Chlorophacinone intoxication: A biological and toxicological study. Clin Toxicol, 27: 79-89.

Conclusion	Human data of acute poisoning and short and long occupational exposure, as well as case of intoxication o wild animal for direct or secondary intoxication represent data that has low usefulness for quantitative risk assessment but they can confirm the hazard of causing health concern due to their anticoagulant effects. Doses from 20 mg/person have been proved to cause clear anticoagulant alteration demonstrated by alteration of prothrombine time. Doses of 100 mg or higher have caused severe signs. The effect of anticoagulant, including chlorophacinone persint during weeks. Data of other anticoagulant rodenticides srtructurally derived from coumarin as warfarin, brodifacoum, bromodiolone and diphenacoum are useful to understand anticoagulant mechanism and clinical consequence but data with them cannot directly extrapolated to understand toxicokinetic and toxicodynamic of Chlorophacinone and cannot be used to extrapolate quantitative evaluation for risk assessment of chlorophacinone.
Remarks	Those published papers described in summary in this justification have been asked to the Applicant and were received by the Reporteur and their content has been revised and checked to be in agreement with the description in Applicant report.

Section A 6.12.3-01 **Annex Point IIA, 6.9**	**Human case report (health records)** Industrial health records	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The physician responsible for two sites involved in conception, manufacture and packaging of anticoagulant rodenticides including difethialone has provided information relating to the medical supervision of staff involved in these activities over a fifteen-year period. During that period there were no described cases of human accidental exposure to or poisoning by chlorophacinone. The medical care of staff working at the manufacturing plants (staff most at risk of accidental exposure to high levels of difethialone) involved two annual medical visits to monitor prothrombin times. French national legislation requires identification, evaluation and declaration of professional risks. Three categories of at risk workers were identified - research chemists: poorly defined risk; production chemists: often exposed to pure active and concentrates, but exposure effectively eliminated by equipment engineering; bait manufacturers: manipulating low concentrations in dilute products, but exposure effectively eliminated by equipment engineering. All three groups are monitored using the prothrombin test. Full details of health records, beyond confirmation by the site physician of no effects, were not available. Specific personal records are covered by confidentiality agreements and would not normally be available for public scrutiny.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2004	

Section A 6.12.3-01 **Annex Point IIA, 6.9**	**Human case report (health records)** Industrial health records
Evaluation of applicant's justification	The data described in this justification for non-submission of data probably had to be described in the dossier but it is OK if described here. Indicated that physician in manufacturing diphethialone and chlorophacinone did not reported cases of human accident and that staff were monitored using prothrombin test. It is justified that full report are not available because of confidentiality agreement they are not available for "public scrutiny". Notification if data in anonymous statistical style is most probably not affecting personal individual confidentiality while the quantitative data of small subclinical alteration in prothrombin test would be very valuable data for risk assessment.
Conclusion	Accepted justification.
Remarks	Notifier was asked to give some more detail of the worker monitoring and data of prothrombine time were supplied but it seems that these data are not available.

Section A 6.12.4-01 **Annex Point IIA, 6.9**	**Epidemiological study** General population epidemiological studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	The general population is not exposed to the molecule and epidemiology studies are not required. The active molecule and concentrates are available only in areas of rodenticide production which are under strict procedural and engineering controls to minimise exposure. Exposure of the general populus to rodenticide products is unlikely. The mode of action of the active substance results in immediate obvious ill-health (see 12.2-02 for case histories of individuals exposed) such that exposure is identified rapidly. Any general exposure of a population would be immediately obvious. There is no need for specific epidemiological investigations on the general population. Anticoagulant rodenticides are vitamin K antagonists. The main site of their action is the liver, where several of the blood coagulation precursors undergo vitamin-K-dependent post-translation processing before they are converted into the respective procoagulant zymogens. The point of action appears to be the inhibition of K_1 epoxide reductase. Anticoagulant rodenticides are easily absorbed from the gastrointestinal tract, and may also be absorbed through the skin and respiratory system. After oral administration, the major route of elimination in various species is through the faeces. The metabolic degradation of warfarin and indandiones (including chlorophacinone) in rats mainly involves hydroxylation. However, the second-generation anticoagulants are largely eliminated as unchanged compounds. The low urinary excretion precludes isolation of metabolites from the urine. The liver is the main organ for accumulation and storage of rodenticide anticoagulants. Accumulation also occurs in the fat. One epidemiological study with a second generation rodenticide in a production facility is available: During a production run preparing ready-to-use flocoumafen bait (0.005% in baits) in a formulation plant, the effect of the rodenticide on blood coagulation factors was monitored in 12 subjects, using the classical prothrombin time test, a modified prothrombin time technique and measurement of prothrombin (factor II) concentration in blood. No adverse health effects were observed in any subject involved in formulation operations. No changes were observed in any of the three tests that could be ascribed to absorption of flocoumafen into the body (Tuinman & Van Sittert, 1986).	
Reference	Tuinman CP & Van Sittert NJ (1985) Biomedical monitoring of personnel in Sorex Ltd (Widnes, UK)	

Section A 6.12.4-01 **Annex Point IIA, 6.9**	**Epidemiological study** General population epidemiological studies
	involved in a formulation run with the rodenticide WL 108366. The Hague, Shell Internationale Petroleum Maatschappij B.V. (Report No. HSE 85.006).
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*

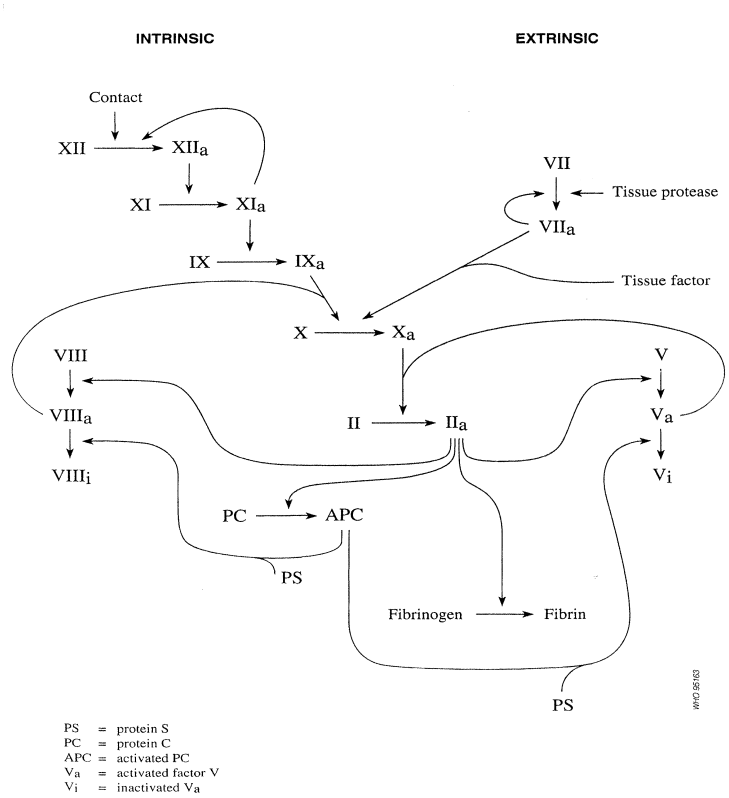
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	The data described in this justification for non-submission of data probably had to be described in the dossier but it is OK if described here. No submission of data is justified because "The general population is not exposed and epidemiology is not required". It is also argued that exposure to general population to rodenticides products is unlikely. However it is showed an epidemiological study with another second generation rodenticides (flocoumafen) in a formulation plant and no effect were observed in any of the applied test related with anticoagulant effect. **SEE COMMENTS IN SECTION 6.12.2 (Human cases)**
Conclusion	Accepted justification.
Remarks	Notifier was asked to supply the full version of the cited reports and publications and this additional information has been supplied from the Applicant to the Raporteur.

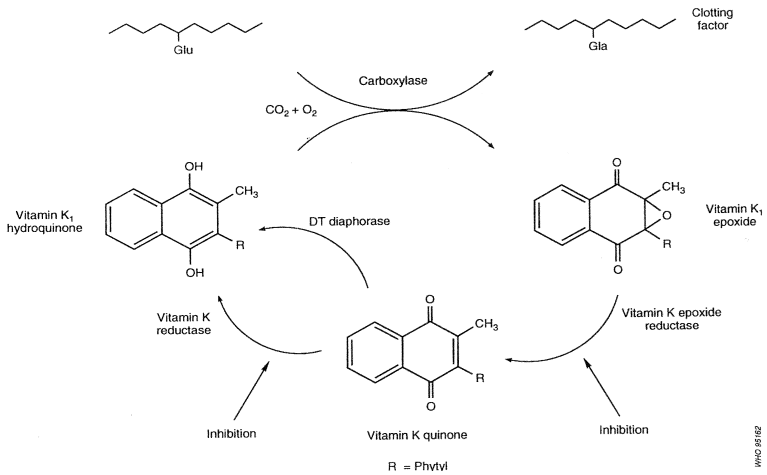
Section A 6.12.5-01 Annex Point IIA, 6.9	**Human case report** Diagnosis of poisoning	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Chlorophacinone is a second-generation anti-coagulant rodenticide. The anti-coagulants are a closely-related group of active substances, which share the same mode of action in mammals. They have been in use for over four decades, both as rodenticides and as human pharmaceuticals (in treatment of clotting disorders, and in cases of atrial valve replacement). Many poisoning incidents (both intentional and unintentional) have been reported for the group of active substances. A few cases of intoxications from occupational exposure to anticoagulants have also occurred. The information is generally available from several sources, and no single report is submitted with this dossier. This 'justification' consists of a summary of human poisoning information. Symptoms of acute intoxication by anticoagulant rodenticides range from increased bleeding tendency in minor or moderate poisoning to massive haemorrhage in more severe cases. The signs of poisoning develop with a delay of one to several days after absorption. The plasma prothrombin concentration is one guide to the severity of intoxication. This is a more sensitive indication than overall tests such as prothrombin time. In repeated occupational exposure, direct measurement of either trace amounts of circulating descarboxyprothrombin or circulating vitamin K 2,3-epoxide may provide a more sensitive assessment. Treatment of anticoagulant poisoning is graded according to the severity of intoxication. Specific pharmacological treatment consists of parenteral administration of vitamin K_1 with, in serious cases, co-administration of blood components. Measurement of prothrombin time helps to determine the effectiveness and required duration of treatment. The following provides background information on the mechanism of action of the anti-coagulant rodenticides. Vitamin K is a collective name for a number of related compounds, which all may function as co-enzymes for the enzyme gamma-glutamate carboxylase. They all contain the functional naphthoquinone ring structure, but differ in their aliphatic side chains. Vitamin K_1 (phytomenadione) contains a side chain composed of four isoprenoid residues, one of which is unsaturated. The vitamin K_2 compounds (menaquinones) have side chains which vary from 1 to 13 isoprenoid residues, all of which are unsaturated. They are	

Section A 6.12.5-01 Annex Point IIA, 6.9	**Human case report** Diagnosis of poisoning
	generally referred to as MK-n, where n is the number of isoprenoid residues. Vitamin K_3 (menadione) has no side chain, but upon ingestion it is converted into MK-4 by a liver enzyme. The two products commercially available for human use are K_1 and MK-4. Both are equally active, but for some reason K_1 is almost exclusively used in Europe and North America, whereas MK-4 (also known as menatetrenone) is used in Asia, notably Japan. K_3 is not used any more for humans because of its adverse side effect, haemolysis, but is frequently added to animal food. Both 4-hydroxycoumarin derivatives and indandiones are antagonists of vitamin K. Their use as rodenticides is based on the inhibition of the vitamin K-dependent step in the synthesis of a number of blood coagulation factors. The vitamin K-dependent proteins involved in the coagulation cascade are the procoagulant factors II (prothrombin), VII (proconvertin), IX (Christmas factor) and X (Stuart-Prower factor), and the coagulation-inhibiting proteins C and S. All these proteins are synthesized in the liver. Before they are released into the circulation the various precursor proteins undergo substantial (intracellular) post-translational modification. Vitamin K functions as a co-enzyme in one of these modifications, namely the carboxylation at well-defined positions of 10-12 glutamate residues into gamma-carboxyglutamate (Gla). The presence of these Gla residues is essential for the procoagulant activity of the various coagulations factors. Vitamin K hydroquinone (KH2) is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide (KO) provides the energy required for the carboxylation reaction. The epoxide is then recycled in two reduction steps mediated by the enzyme KO reductase. The latter enzyme is the target enzyme for coumarin anticoagulants. Their blocking of the KO reductase leads to a rapid exhaustion of the supply of KH_2, and thus to an effective prevention of the formation of Gla residues. This leads to an accumulation of non-carboxylated coagulation factor precursors in the liver. In some cases these precursors are processed further without being carboxylated, and (depending on the species) may appear in the circulation. At that stage the under-carboxylated proteins are designated as descarboxy coagulation factors (Stenflo et al., 1974; Nelsestuen et al., 1974). Normal coagulation factors circulate in the form of zymogens, which can only participate in the coagulation cascade after being activated by limited proteolytic degradation. Descarboxy coagulation factors have no procoagulant activity (i.e. they cannot be activated) and neither they can be converted into the active zymogens by vitamin K action. Whereas in anticoagulated

Section A 6.12.5-01 Annex Point IIA, 6.9	**Human case report** Diagnosis of poisoning

humans high levels of circulating descarboxy coagulation factors are detectable, these levels are negligible in warfarin-treated rats and mice. Reviews by Vermeer (1990) and Furie & Furie (1990) give further details.





Vitamin K hydroquinone is the active co-enzyme, and its oxidation to vitamin K 2,3-epoxide provides the energy required for the carboxylation reaction where glutamate (Glu) in the precursor is converted to γ-carboxyglutamate (Gla) to make the activated clotting factor.
The anticoagulant rodenticide active substances such as chlorophacinone work by blocking the regeneration of

Section A 6.12.5-01 Annex Point IIA, 6.9	**Human case report** Diagnosis of poisoning
References	vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place. The description of clinical symptoms of rodenticide poisoning, defined loosely as a haemorrhagic syndrome, can be found in study summaries presented in section 6 for acute and subacute exposure. IPCS Environmental Health Criteria 175. Anticoagulant RodenticidesMonograph., World Health Organisation 1995 Stenflo J, Fernlund P, Egan W, & Roepstorff P (1974) Vitamin K dependent modifications of glutamic acid residues in prothrombin. Proc Natl Acad Sci (USA), 71(7): 2730-2733. Nelsestuen GL, Zytkovicz TH, & Howard JB (1974) The mode of action of vitamin K: Identification of gamma-carboxyglutamic acid as a component of prothrombin. J Biol Chem, 249: 6347-6350. Vermeer C (1990) gamma-Carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. Biochem J, 266: 625-636. Furie B & Furie BC (1990) Molecular basis of vitamin K-dependent gamma-carboxylation. Blood, 75: 1753-1762.
Undertaking of intended data submission [ ]	

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	The data described in this justification for non-submission of data probably had to be described in the main dossier form but it is OK if described here a Justification. It is not well understood, why not to include such public available information in the dossier. Information is based in public data and on the known mechanism of toxicity.
Conclusion	Accepted justification
Remarks	Notifier was asked to supply the cited references and it has been done.

Section A 6.12.6-01 **Annex Point IIA, 6.9**	**Human case report** (sensitisation/allergenicity observations)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The delayed contact hypersensitivity studies (III-A 6.1.5) indicated that the active molecule in anticoagulant rodenticides is not a potential sensitiser. This is consistent with the reported human exposure cases where sensitisation is not included in the case study as a point of concern. The medical supervisor responsible for two production, manufacturing and packaging plants involved in rodenticide production reports no diseases among the work population and this must include the lack of any allergic or sensitisation responses. Coumarin is well known as being non-sensitising. The potential for the molecule to undergo the Michael reaction involving addition of nucleophiles across α,β-unsaturated carbonyl compounds (a factor involved in the reactivity of a large number of sensitisers) is present but the weight of evidence indicates coumarin and the cinnamate esters do not add nucleophilic groups to proteins. It is postulated that the lactone ring system in coumarin is stabilised through conjugation of the double bond with the aromatic ring. (Kimber, I and Maurer, T. Toxicology of Contact Hypersensitivity.) This theoretical lack of sensitising potential is substantiated by the results of the animal studies. The issue of allergenicity is more pertinent in terms of antidotal treatment. Intravenous injection of Vitamin K_1 can induce anaphylactic shock and it is important that only slow injection is used intravenously or, in cases of concern, the injected dose should be administered by another parenteral route e.g. subcutaneously or as an intramuscular depot.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2004	

Evaluation of applicant's justification	The data described in this justification for non-submission of data probably had to be described in the dossier but it is OK if described here. No submission is justified by: Medical supervisor of two production plant neither did nor reported cases. Coumarin is well known as being non-sensitising. (NOTE: However Chlorophacinone is not a coumarin but a diandione). It is supported by results in animal studies. The antidotal treatment with Vit K1
Conclusion	Accepted justification
Remarks	Data described in summary in this justification have been asked to the Notifier and were received by the Raporteur.

Section A 6.12.8-01 Annex Point IIA, 6.9	Human case report (prognosis following poisoning)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	The laboratory control of orally administered coumarin derivatives has been carried out using the classical one-stage prothrombin time test (Quick, 1935) or modified techniques such as "Thrombotest" (Owren, 1959). However, these tests have been designed for clinical monitoring of circulating clotting factors during anticoagulant therapy. The monitoring of occupational exposure to rodenticides requires the prothrombin time test to be of sufficient sensitivity to measure changes in the normal range (Tuinman & Van Sittert, 1985). Repeated occupational exposure to low levels of anticoagulant rodenticides could gradually deplete vitamin-K-dependent coagulation factors in the blood. To detect unwanted exposure of humans in an early state, a careful screening of those at risk is recommended. The question is which screening method is the most suitable to monitor low levels of rodenticide ingestion. Prothrombin time and related tests are "overall" clotting tests, which were developed for monitoring patients under deep anticoagulation. These tests are easy to perform and do not require complicated equipment, but they are relatively insensitive when used for monitoring milder anticoagulation states (Tuinman & Van Sittert, 1985; Ross et al., 1992; Travis et al., 1993). If possible, specific and more sensitive tests should be used. The most sensitive test, applicable over a wide range of anticoagulation states, is the direct detection of descarboxy-prothrombin using a monoclonal antibody specifically recognizing the descarboxy form of prothrombin (Widdershoven et al., 1987). Another marker for monitoring poor vitamin K status at an early stage is descarboxy-osteocalcin (Knapen et al., 1993), but the commercial test kits presently available need to be substantially improved and simplified before they can be recommended for this purpose in routine laboratories. Another method was suggested by Park et al. (1986), who repeatedly injected 10 mg of vitamin K_1 into factory workers who had been exposed to brodifacoum. The authors observed that 2-4 h after injection the circulating KO/K ratios were significantly elevated even 18 months after the prothrombin times had returned to normal values. This suggests that very low liver concentrations of brodifacoum can be detected from the altered KO/K ratio rather than from tests based on blood coagulation parameters. Because this method requires vitamin K administration shortly before	

Section A 6.12.8-01 Annex Point IIA, 6.9	Human case report (prognosis following poisoning)	
	blood sampling, it is only applicable in cases of anticoagulant poisoning, and not for the routine control of plant workers. Methods for the direct detection of coumarin anticoagulants in plasma and serum have been reported, all of which are based on the extraction of plasma and pre-purification of the sample, followed by HPLC analysis with fluorescence detection (Hunter, 1983; Murphy et al., 1989; Felice & Murphy, 1989; Felice et al., 1991; O'Bryan & Constable, 1991). However, such facilities will not be available in most routine laboratories. Moreover, the blood sampling should be performed within a reasonably short period after ingestion of the coumarins, because these drugs are rapidly cleared by the liver. This places severe restrictions on the applicability of these techniques, particularly for the second-generation anticoagulants. Plasma chlorophacinone determinations were performed in three cases of intoxication. The risk of bleeding was minimal when the plasma level was below 1 mg/litre (Burcuoa et al., 1989). All suspected poisoned patients should receive medical attention immediately. Rapid determination of prothrombin time and search for evidence of bleeding is essential and may have to be maintained for several weeks. Gastric lavage or induction of emesis are indicated in all cases of superwarfarin rodenticide ingestion if it was recent and the amount is possibly lethal or uncertain. Repeated administration of activated charcoal is useful. Cathartics could also be administered Vitamin K_1 is the specific antidote of choice. Depending on whether the poisoning is due to first or second generation anti-coagulants, the dosage may differ as well as the duration of treatment. Dosage is dependent on coagulation parameters, mainly prothrombin time. If the patient is bleeding severely, 25 mg of vitamin K_1 (phytomenadione) should be given by slow intravenous injection. Prothrombin time should be checked at 3-hourly intervals in severe cases and after 8-10 h in less severe cases. If no improvement occurs, vitamin K_1 injection should be repeated. Doses of up to 125-200 mg/day have been given without adverse effects (Lipton & Klass, 1984; Sheen et al., 1994). In moderate to minor cases of poisoning, vitamin K_1 may be given in lower doses. After initial parenteral vitamin K_1 administration, oral treatment can be continued for a prolonged period of time. Oral treatment can also be sufficient in minor cases. The major difference between first generation active rodenticides such as warfarin and second-generation rodenticides is that the latter can cause increased bleeding for a longer period of time than warfarin, as they have a much longer half-life in the body. Therefore vitamin	

Section A 6.12.8-01 Annex Point IIA, 6.9	Human case report (prognosis following poisoning)
	K_1 should be given for months rather than weeks. It is also prudent to monitor prothrombin time for some time after cessation of this treatment to ensure that there is no regression. In warfarin-resistant individuals, 10 times the normal dose of warfarin is required to achieve a reduction in the plasma prothrombin level. However, these individuals also respond more strongly to the effect of vitamin K (O'Reilly et al., 1963; O'Reilly et al., 1964). Patient should be kept in hospital until the prothrombin time has remained normal for 3 days. It is suggested that oral treatment with 10 mg vitamin K_1 twice daily may be necessary for up to 60 days with close monitoring of prothrombin time. According to Hoffman et al. (1988), factor analysis allows for a detailed evaluation of the course of toxicity, and the response to therapy. Monitoring the prothrombin time alone could offer a false sense of confidence and delay effective treatment. The addition of bittering agents to anticoagulant rodenticides is aimed at discouraging human consumption and thereby avoiding accidental exposure. The bittering agent used in chlorophacinone based baits is bitrex (denatomium benzoate (see III-B 6.5-01), the bitterest substance known to man. The addition of bitrex renders chlorophacinone based baits inedible to man, thus minimising potential for use in intentional poisoning and significantly reducing the risk of accidental ingestion. The prognosis following anticoagulant rodenticide ingestion or exposure is for delayed onset of clinical signs indicative of haemorrhagic events that will rapidly lead to death. The time course for increasing severity of toxic signs is often paralleled by rising prothrombin times. However if Vitamin K_1 antidotal therapy is administered before onset of massive haemorrhagic trauma and generally before prothrombin rate reaches near zero, then recovery is swift and complete. Due to the long-acting nature of these compounds and accumulation in the liver, initial parenteral Vitamin K_1 treatment must be followed by a sustained regimen of oral antidote administration until prothrombin times remain at basal levels. The human case studies presented in III-A 6.12.2-01 indicate the effectiveness of antidotal therapy.
References	Quick AJ (1935) The prothrombin in haemophilia and in obstructive jaundice. J Biol Chem, 109: 73. Owren PA (1959) Thrombotest: A new method for controlling anticoagulant therapy. Lancet, 2: 754-758. Tuinman CP & Van Sittert NJ (1985) Biomedical monitoring of personnel in Sorex Ltd (Widnes, UK) involved in a formulation run with the rodenticide WL 108366. The Hague, Shell Internationale Petroleum

Section A 6.12.8-01 Annex Point IIA, 6.9	Human case report (prognosis following poisoning)	
	Maatschappij B.V. (Report No. HSE 85.006). Ross GS, Zacharski LR, Robert D, & Rabin DL (1992) An acquired hemorrhagic disorder from long-acting rodenticide ingestion. Arch Intern Med, 152: 410-412. Travis SF, Warfield W, Greenbaum BH, Mobkisher M, & Siegel JE (1993) Spontaneous hemorrhage associated with accidental brodifacoum poisoning in a child. J Pediatr, 122: 982-984. Widdershoven J, van Munster P, De Abreu R, Bosman H, van Lith Th, van der Putten-van Meyel M, Motohara K, & Matsuda I (1987) Four methods compared for measuring des-carboxy-prothrombin (PIVKA-II). Clin Chem, 33(11): 2074-2078. Knapen MHJ, Jie K-SG, Hamulyák K, & Vermeer C (1993) Vitamin K-induced changes in markers for osteoblast activity and urinary calcium loss. Calcif Tissue Int, 53: 81-85. Park BK, Choonara IA, Haynes BP, Breckenridge AM, Malia RG, & Preston FE (1986) Abnormal vitamin K metabolism in the presence of normal clotting factor activity in factory workers exposed to 4-hydroxycoumarins. Br J Clin Pharmacol, 21: 289-294. Hunter K (1983) Determination of coumarin anticoagulant rodenticide residues in animal tissues by high-performance liquid chromatography: Fluorescence detection using post-column techniques. J Chromatogr, 270: 267-276. Murphy MJ, Ray AC, & Bailey EM (1989) A high performance liquid chromatography method for the detection of Brodifacoum in serum. Vet Hum Toxicol, 31(3): 228-231. Felice LJ & Murphy MH (1989) The determination of the anticoagulant rodenticide brodifacoum in blood serum by liquid chromatography with fluorescence detection. J Anal Toxicol, 13: 229-231. Felice LJ, Chalermchaikit T, & Murphy MJ (1991) Multicomponent determination of 4-hydroxycoumarin anticoagulant rodenticides in blood serum by liquid chromatography with fluorescence detection. J Anal Toxicol, 15: 126-129. O'Bryan SM & Constable DJC (1991) Quantification of brodifacoum in plasma and liver tissue by HPLC. J Anal Toxicol, 15: 144-147. Burucoa Ch, Mura P, Robert R, Boinot C, Bouquet S, & Piriou A (1989) Chlorophacinone intoxication: A biological and toxicological study. Clin Toxicol, 27: 79-89. Lipton RA & Klass EM (1984) Human ingestion of a 'superwafarin' rodenticide resulting in a prolonged anticoagulant effect. J Am Med Assoc, 252: 3004-3005. Sheen SR, Spiller HA, & Grossman D (1994) Symptomatic	

Section A 6.12.8-01 Annex Point IIA, 6.9	Human case report (prognosis following poisoning)	
	brodifacoum ingestion requiring high-dose phytonadione therapy. Vet Hum Toxicol, 36(3): 216-217. O'Reilly RA, Aggeler PM, & Leong LS (1963) Studies on the coumarin anticoagulant drugs: The pharmacodynamics of warfarin in man. J Clin Invest, 4: 1542-1551. O'Reilly RA, Aggeler PM, Haag MS, Leong LS, & Kropatkin ML (1964) Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs. N Engl J Med, 271: 809-815. Hoffman RS, Smilkstein MJ, & Goldfrank LR (1988) Evaluation of coagulation factor abnormalities in long-acting anticoagulant overdose. Clin Toxicol, 26(3/4): 233-248.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004
Evaluation of applicant's justification	The data described in this justification for non-submission of data probably had to be described and included in the Dossier but it is OK if described here. Information is based in public data, mainly from the experience of other anticoagulant rodenticides. **SEE COMMENT IN 12.2-01 (Human cases)**
Conclusion	Accepted justification
Remarks	The Notifier was asked to supply the cited references and additional information has been supplied.

Section A 6.13-01 Annex Point IIA, 6.13	Toxic effects on livestock and pets	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Data relating to various species and by various routes (see below) have been presented in the full toxicity evaluation of chlorophacinone. Review of the available information indicates there are no ethical grounds (that would not contravene the requirements of Directive 86/609/EC which militates against unnecessary testing using animals) for performing further studies on animals (either livestock or more particularly pet species) to elucidate the mode of toxic action for chlorophacinone. The highly specific pharmacological activity for indandione-type anticoagulant rodenticides has been discussed extensively in III-A 6.5 and III-A 6.8.2. The acute, subacute and long-term effects of this rodenticide on the target species has been discussed in relation to tests on laboratory rodents. The local tolerance of rabbits to chlorophacinone exposure is presented in section A 6.1.4-01. Antidotal therapy for intoxicated animals is presented in section A 6.10-01. Acute toxicity to dogs is presented in section A 6.1.1-02. A comparison of rodenticide potencies and their effects is also presented in section III-A 6.7-01. Given the types of exposure investigated and the well-known nature of the mode of action for this test substance class it is not considered appropriate to conduct further animal tests. Further information in relation to pet/livestock exposure was collated by various manufacturers in an industry – wide approach to rodenticide safety. Advice to veterinarians was issued jointly by Zeneca Public Health, Sorex Ltd; Rhone Poulenc, Lipha SA, Bayer and American Cyanamid Company in relation to treatment of anticoagulant rodenticide poisoning as an aid to recognition of intoxication and dissemination of an agreed effective treatment regimen. Since rodenticides may be mixed with other chemicals, it is important to establish the product involved in poisoning incident. Second generation anticoagulant rodenticides, like difethialone, act by interfering with prothrombin synthesis, disrupting clotting mechanisms and increasing tendency to haemorrhage. Since these products have longer body half-lives than the first generation rodenticides, bleeding can be prolonged and it may be necessary to prolong antidotal treatment for weeks rather than days. Animals may typically be exposed to rodenticides by one of two routes -	

Section A 6.13-01 Annex Point IIA, 6.13	Toxic effects on livestock and pets	
	consumption of anticoagulant-based rodent bait or secondary poisoning due to consumption of poisoned rodents. Clinical signs are unlikely to develop within the first 24 hours following poisoning and may not appear for several days but then develop rapidly, becoming more severe and normally progressing to death by haemorrhage if untreated. Typical signs resulting from increased tendency to haemorrhage may include nasal/oral bleeding and propensity for bruising, blood in faeces or urine, excessive bleeding from minor cuts or abrasions, laboured breathing, pale mouth and cold gums, anorexia andgeneral weakness, haematomas or subcutaneous swelling. In more severe cases shock, coma and/or massive haemorrhage (usually internal) may be observed. A reliable diagnostic aid is measurement of prothrombin time – anticoagulant activity increases clotting time and successful treatment can be monitored by observing prothrombin time rapidly return to normal levels. Vitamin K_1 (Phytomenadione) is the only effective antidote for all cases of indandione-type anticoagulant poisoning (other Vitamin K analogues are ineffective). General treatment should involve induction of vomiting if animal is presented within approximately 6 hours of suspected poisoning. Collect blood sample for prothrombin time using smallest feasible needle to avoid induction of venepuncture haemorrhage. Administer parenteral injection of 2 to 5 mg/kg Vitamin K_1. Subcutaneous or intramuscular injection be be required ince some preparations of Vitamin K_1 can cause anaphylaxis if injected too rapidly by intravenous route. The prothrombin time will normally fall rapidly following initial injection of antidote. The animal will then require long term supportive care involving daily administration of Vitamin K_1 (2 to 5 mg/kg/day) orally for 3-4 weeks even after symptoms have regressed. Prothrombin times should be monitored and treatment extended if times become elevated.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004

Evaluation of applicant's justification	The TNG in Chapter 3 point 6.13 explicitly state that these data may be specifically relevant for type 14 (rodenticides). This data may be relevant e.g. for product types **14**, 15 and 23 (ingestion of baits). An expert judgement is required to decide whether any studies are needed (see Chapter 1.2, point 4). So the Notifier cannot justify that data is not relevant. In the first paragraph of the same point in the TNG is stated that"an estimation on toxic effects and exposure … is required". It is also indicated that only exceptionally toxicity testing in livestock and pets is required. So Notifier cannot justify submission of data under the argument of not doing unnecessary animal experiment as no new animal experiment is required but only "an estimation". So Notifier had to present such "estimation" in the dossier. Notifier made here a discussion of the data of toxicity in different species emphasising that the mode of action of toxicity is well know and the possible type of effects that may be observed. This is useful information but should be notice that it had to be presented in the dossier. It is mentioned that data of exposure to pets/livestock has been reported by manufacturers about rodenticides safety. However no specific quantitative estimation of potential exposure is indicated and no "quantitative" estimation of toxicity to pets/livestock is made.
Conclusion	It is accepted that no new testing experiment should be done to evaluate toxicity to pets/livestock and that the type of effect predicted on the bases of the toxicological-pharmacological mode of action is acceptable as well as the conclusion that vitamin K1 is the only effective antidote as observed in the tested animals. However Notifier was asked to supply with quantitative estimation of potential exposure and toxicity on pets/livestock, and if existing, data of reported intoxications.
Remarks	Additional comments about quantitative assessment was received from the Notifier

Section A 6.14-01 Annex Point IIA, 6.14	Other tests related to exposure of humans	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Chlorophacinone has been demonstrated to produce two hydroxylated degradation products, **neither of which is pharmacologically or toxicologically active.** The defined mode of action for Chlorophacinone is specific to the parent molecule's ability to disrupt the epoxide cycle by inhibition of Vitamin K reductase or Vitamin K epoxide reductase. In cases of accidental exposure the therapeutic response is well characterised. Since the rodenticide class of materials (including coumarin and indandione type derivatives) includes warfarin, used as a pharmaceutic therapy, extensive human data are available for indicating possible side-effects or biological effects of long term low level exposure to anti-coagulant rodenticides. Although no cases of embryopathy have been reported arising from use of first generation anti-coagulants as rodenticides, some developmental effects have been identified for warfarin used as a therapeutic agent and administered during pregnancy. However, chlorophacinone is not used in this manner and is only available for possible human exposure if bait containing very low concentrations of rodenticide is interfered with during rodenticide usage. In all cases of possible human exposure to anticoagulant rodenticides, the use of simple diagnostic tests, such as the one stage Quick test or Owren thrombotest, can be used to determine the patients prothrombin times or levels of prothrombin (factor II) in plasma and to allow the correct administration of Vitamin K_1. Given the extensive database on human exposure to anticoagulant rodenticides, it is not considered appropriate to conduct further animal tests to further elucidate the well-characterised response.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	

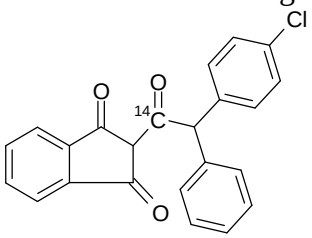
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2004

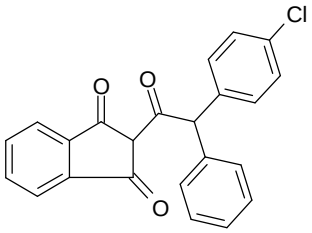
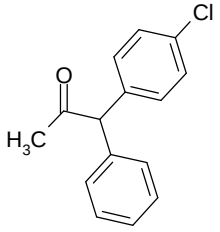
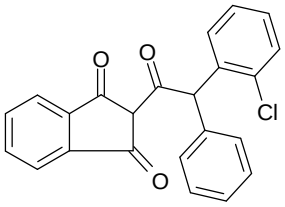
Evaluation of applicant's justification	Applicant affirms that "Chlorophacinone has been demonstrated to produce two hydroxylated degradation products, **neither of which is pharmacologically or toxicologically active".** **However in any place of the dossier studies have been identified demonstrated if the hydroxylated metabolites are or not active for the anticoagulant property causing the main end point of toxicity of Chlorophacinone.**
Conclusion	Accepted justification
Remarks	

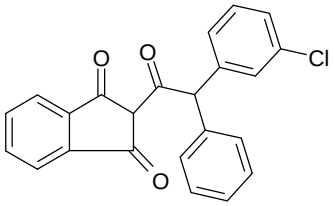
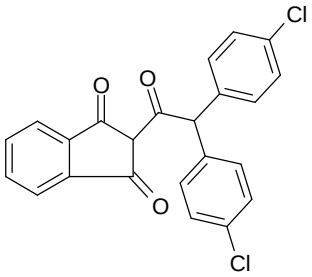
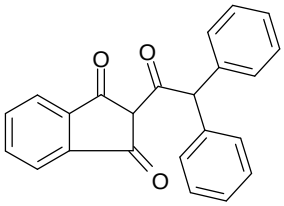
Section A 6.15.1-01 Annex Point IIA, 6.15.1	Identification of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated foods or feedingstuffs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [X]**	
Detailed justification:	Formulated products containing the active substance Chlorophacinone are not used for direct application to foods or feedingstuffs or to surfaces and areas where foods or feedingstuffs are prepared or stored. Formulated products containing the active substance are only used in the vicinity surrounding the areas used for storage of foods and feedingstuffs for protection and general hygiene purposes. Furthermore, the use of rodenticides in these areas is regulated by National food safety and food hygiene laws. Chlorophacinone is not volatile and the use patterns of the formulated products are such that incidental contamination of foods and feedingstuffs or surfaces used for storage and preparation is not possible. It is therefore considered that additional animal studies for determining the identity and concentration of residues in food and feedingstuffs are not necessary. However, the CEFIC Rodenticide Working Group (RWG), of which Liphatech is a member in good standing, is investigating the development of a multiresidue analytical method for the anticoagulant rodenticides defended by the RWG members. While the use patterns of the anticoagulants preclude incidental contamination of food and feedingstuffs, RWG members recognise the importance of having a multiresidue method available in case of accidental or deliberate contamination of food or feedingstuffs. As TNsG for 98/8 do not give guidance on food matrices, RWG intend developing methods for the food types described under Directive 91/414 (i.e. a cereal; high water content food: high fat content food and a high acid content food). The methods will be presented when they are available.	
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities
EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2004
Evaluation of applicant's justification	No data is identified in the dossier to clarify if residue and residual metabolites or degradated compounds are or not active as anticoagulant.
Conclusion	It is necessary the final report to deal with a multi-residue analytical method for the determination of residues in foods or feedstuff.
Remarks	

Section A 7.1.1.1.1-01 **Annex Point IIA VII.7.6.2.1**		**Hydrolysis as a function of pH and identification of breakdown products**	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxx, X., XXX, ^{14}C-chlorophacinone: Hydrolysis at three different pH values. XXXXX., laboratory report no. XXXXX, 10 December XXXX (unpublished). Section no.: A 7.1.1.1.1-01.	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. The study was performed to OECD guideline 111 and US EPA guideline OPPTS 835.2110.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No. The study was conducted to the recommended guidelines (EC method C.7, OECD 111).	
		3 MATERIALS AND METHODS	
3.2	**Test material (radiolabelled)**	As given in section 2. Acetyl-^{14}C-chlorophacinone. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione. chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	Batch no. XXXXX (a second batch of the test material was supplied after the pre-test for use on the main test, batch no. XXXXXXX).	
3.2.2	Specification	Specific activity 2257 MBq/mmol, 5.990 MBq/mg (2118 MBq/mmol, 5.620 MBq/mg for batch no. XXXXXXX.	
3.2.3	Purity	RCP (radiochemical purity) 97.2% by TLC, 97.3% by HPLC (batch no. XXXXXXX.4% by TLC).	
3.2.4	Further relevant properties	Position of radiolabel given below: 	

Section A 7.1.1.1.1-01 **Annex Point IIA VII.7.6.2.1**		**Hydrolysis as a function of pH and identification of breakdown products**	
3.3	**Test material (non-radiolabelled)**	As given in section 2. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione. chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.3.1	Lot/Batch number	XXXX.	
3.3.2	Specification	No further details.	
3.3.3	Purity	XXXXX%.	
3.3.4	Further relevant properties	Structure below: 	
3.4	**Reference material (PCPP)**	PCPP (CAS): 1-(4-chlorophenyl) 1-phenyl-propanone-2.	
3.4.1	Lot/Batch number	Lot no. M3153.	
3.4.2	Specification	No further details.	
3.4.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.4.4	Further relevant properties	Structure below: 	
3.5	**Reference material (LM 828)**	LM 828 (CAS): 2-((2-(2-chlorophenyl)-1-oxo-2-phenyl) ethyl-1H-indene-1-3-(2H)-dione.	
3.5.1	Lot/Batch number	Lot no. ANA 178.	
3.5.2	Specification	No further details.	
3.5.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.5.4	Further relevant properties	Structure below: 	
3.6	**Reference material (LM**	LM 3257 (CAS): 2-((2-(3-chlorophenyl)-1-oxo-2-phenyl) ethyl-1H-indene-1-3-(2H)-dione.	

Section A 7.1.1.1.1-01 Annex Point IIA VII.7.6.2.1		Hydrolysis as a function of pH and identification of breakdown products	
	3257)		
3.6.1	Lot/Batch number	Lot no. ANA 179.	
3.6.2	Specification	No further details.	
3.6.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.6.4	Further relevant properties	Structure below: 	
3.7	**Reference material (LM 106)**	LM 106 (CAS): 2-((2-2-di(4-chlorophenyl)-1-oxo) ethyl-1H-indene-1-3-(2H)-dione.	
3.7.1	Lot/Batch number	Lot no. ANA 106.	
3.7.2	Specification	No further details.	
3.7.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.7.4	Further relevant properties	Structure below: 	
3.8	**Reference material (LM 3256)**	LM 3756 (CAS): 2-(((2-2-diphenyl)-1-oxo) ethyl)-1H-indene-1-3-(2H)-dione.	
3.8.1	Lot/Batch number	Lot no. JB 4286.	
3.8.2	Specification	No further details.	
3.8.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.8.4	Further relevant properties	Structure below: 	
3.9	**Testing procedure**	The hydrolytic behaviour of chlorophacinone was investigated in sterile aqueous buffer (pH values 4, 7 and 9) at a concentration of *ca* 0.5 mg/L and temperature of 50°C. An additional investigation was carried out at pH 4 only at temperatures of 60 and 70°C.	

Section A 7.1.1.1.1-01 **Annex Point IIA VII.7.6.2.1**	**Hydrolysis as a function of pH and identification of breakdown products**	
3.9.1 Test system	Acidic, neutral and alkaline buffer solutions were prepared using distilled, deionised water as described in Table A 7.1.1.1.1-1. All buffer solutions were sterilised by autoclaving (30 mins, 120°C) and adjusted to final pH by addition of either 0.1M sodium hydroxide or 0.1M hydrochloric acid.	
3.9.2 Pre-test, 50°C	The treatment and incubation of the test solutions is summarised in Table A 7.1.1.1.1-2. chlorophacinone was prepared in the test samples at a concentration of *ca* 0.5 mg/L. Individual aliquots of treated buffer solutions (10 mL pH 4, 20 mL pH 7 and 9) in tightly closed sterile glass vessels were incubated at *ca* 50°C. To avoid photolytic effects vessels were covered in foil and incubation was performed in the dark in a thermo-regulated water bath under agitation. The pre-test at pH 4 was repeated due to significant adsorption to glassware, only the results from the repeat samples are given.	
3.9.2.1 pH, duration of the test, no. of replicates,	See Table A 7.1.1.1.1-2.	
3.9.2.2 Sampling	Samples were taken for analysis at 0, 2.4 hours and 1, 5 days. At each sampling interval, samples were submitted to ultrasonication (15 mins) to release material adsorbed to the glassware. The level of radioactivity in the buffer solutions was quantified by LSC. Sub-samples were taken for analysis as described in Section 3.9.4. The pH of the buffer solutions was checked at each sampling occasion.	
3.9.3 Main test, 60 and 70°C	The main test was conducted only at pH 4 and was carried out at temperatures of 60 and 70°C. The main test was conducted in a similar manner to the pre-test.	
3.9.3.1 pH, duration of the test, no. of replicates,	See Table A 7.1.1.1.1-4.	
3.9.3.2 Sampling	At 60°C, samples were taken for analysis at 0 and 4 days. At 70°C, samples were taken for analysis at 0, 1 and 4 days. At both temperatures, after 4 days sampling was stopped as less than 10% degradation of chlorophacinone was observed. Buffer samples were partitioned (x 2) with dichloromethane. All processing steps were carried out under red light. Extracts were quantified by LSC. Samples containing significant radioactivity were analysed by TLC using the conditions specified in Section 3.9.4. Confirmatory analysis was carried out on selected samples by HPLC. The pH of the buffer solutions was checked at each sampling occasion.	
3.9.4 Analytical methods	Analysis by HPLC was conducted using a reverse phase gradient system (see Table A 7.1.1.1.1-3 for details).	

Section A 7.1.1.1.1-01 Annex Point IIA VII.7.6.2.1		Hydrolysis as a function of pH and identification of breakdown products	
		Analysis conducted by TLC was carried out using silica plates (0.25 mm). TLC plates were developed in either SS1 acetone/ diethylamine (9:1 v/v) or SS2 ethylacetate/ diethylamine/ methanol (23/2/1 v/v/v). Non radiolabelled reference standards were visualised using UV light (254 nm). Radioactive regions were quantified using a linear analyser. The RCP determinations were conducted using both HPLC and TLC.	
		4 RESULTS	
4.2	**Recovery of applied radioactivity, pre-test, 50°C**	The recovery of applied radioactivity from the buffer solutions in the pre-test is summarised in Table A 7.1.1.1.1-5. The amount of applied radioactivity recovered was 93.9, 102.1 and 100.3% after 5 days, indicating a complete mass balance. Consequently, any evolved volatile components were not significant. The pH of the test solutions was maintained throughout the study.	
4.3	**Profile of components, pre-test, 50°C**	The level of chlorophacinone observed in the sterile aqueous buffer solutions is summarised in Table A 7.1.1.1.1-6. At a temperature of 50°C, < 5% degradation was observed after 5 days at pH values of 7 and 9. At pH 4 the amount of chlorophacinone remaining after 5 days was 58.4%.	
4.4	**Recovery of applied radioactivity, main-test, 60 and 70°C**	The recovery of applied radioactivity from the buffer solutions in the main-test is summarised in Table A 7.1.1.1.1-7. The amount of applied radioactivity recovered was 100.1 and 98.9% after 4 days, indicating a complete mass balance. Consequently, any evolved volatile components were not significant. The pH of the test solutions was maintained throughout the study.	
4.5	**Profile of components, main-test, 60 and 70°C**	The level of chlorophacinone observed in the sterile aqueous buffer solutions of the main study is summarised in Table A 7.1.1.1.1-8. At temperatures of 60 and 70°C, insignificant degradation of chlorophacinone was observed at pH 4. The study was not conducted at temperatures of 60 and 70°C at pH values of 7 and 9 insufficient degradation was observed at lower temperatures.	
4.6	**Hydrolysis rate constant (k_h)**	Chlorophacinone was stable to hydrolysis at pH values of 7 and 9. In buffer solutions at pH 4 significant degradation of chlorophacinone was observed, however, at higher temperatures (i.e. 60 and 70°C) no significant degradation was observed. Consequently, the degradation of	

Section A 7.1.1.1.1-01 **Annex Point IIA VII.7.6.2.1**	**Hydrolysis as a function of pH and identification of breakdown products**	
	chlorophacinone observed in buffer solutions at pH 4 at a temperature of 50°C was considered to be due to some surface catalysed reaction. The degradation observed could also have been due to the ultrasonication employed to release glass adsorbed radioactivity in these samples (note this procedure was not conducted at elevated temperature). Overall, it is considered that the degradation observed at pH 4 was not due to hydrolysis.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The hydrolytic behaviour of chlorophacinone was investigated in sterile aqueous buffer (pH value 4, 7 and 9) at a temperature of 50°C. An additional investigation was carried out at temperatures of 60 and 70°C at pH 4 only. The GLP study was conducted to OECD Guideline 111 in 2003.	

5.3 Results and discussion	The recovery of the applied radioactivity ranged from 93.9 to 104.6% throughout the investigation. The pH of the buffer solutions was maintained throughout the duration of the study. Although some degradation of chlorophacinone was observed in buffer solutions at pH 4 at a temperature of 50°C, no significant degradation was observed at higher temperatures, it was concluded that the degradation observed was anomalous and not due to hydrolysis.	
5.4 Conclusion	Chlorophacinone is stable to hydrolysis with an estimated half-life of > 1 year at all environmentally relevant pH values. No significant degradation products were formed. The hydrolytic degradation of chlorophacinone is not considered to be a significant process in the environment.	
5.4.1 Reliability	1.	
5.4.2 Deficiencies	None.	

	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2007	
Materials and Methods	OECDm 111 and US EPA guideline OPPTS 835.2110.	
Results and discussion		
Conclusion	Based on this result, it is concluded with no need for further testing in accordance with the OECD guideline that chlorophacinone is stable in water at pH~4, 7 and 9 up to 70ºC, with a half-life greater than or equal to one year.	
Reliability	1.	
Acceptability	Acceptable	
Remarks	In the pre-test which was conducted at 50ºC, M2 appeared above 10%, 30.9%. Due to the results of the test at 60 and 70ºC, where all the metabolites were below 10%, M2 is considered of no relevance.	

Table A 7.1.1.1.1-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
4	0.01M Citrate buffer	Citric acid (0.357 g), sodium chloride (0.078 g) and sodium hydroxide (0.083 g) dissolved in water (1 L).
7	0.01M Phosphate buffer	Potassium dihydrogen phosphate (0.105 g) and disodium phosphate dihydrate (0.159 g) dissolved in water (1 L).
9	0.01M Borate buffer	Dipotassium hydrogen phosphate (0.072 g) and disodium tetraborate decahydrate dissolved in water (1 L).

Table A 7.1.1.1.1-2: Description of test system for pre-test at 50°C

Criteria	Details
Purity of water	Deionised water, further purified using a purification unit (ELGA water purifier) to produce ultra pure water.
Preparation of test medium	Test substance (*ca* 93.3, 104.8 or 111.6 µg), dissolved in acetonitrile (320, 400 or 400 µL) was diluted with buffer solution (160, 200 or 200 mL final volume) for pH stocks 4, 7 and 9, respectively.
Sub-sample size	10 mL (pH 4), 20 mL (pH 7 and 9).
Test concentrations (mg a.i./L)	0.583, 0.524 and 0.558 for pH's 4, 7 and 9.
Temperature (°C)	50 ± 1°C.
Controls	Not applicable.
Identity and concentration of co-solvent	Acetonitrile 0.2% v/v.
Replicates	Eight replicates for each pH value (intended for duplicate samples at four sampling intervals).
Sampling intervals	0, 2.4 hours and 1, 5 days.

Table A 7.1.1.1.1-3: Description of other equipment used

Glassware	The bulk treated buffer solution was prepared in measuring cylinders. The individual sub-samples (10 mL or 20 mL) were incubated in tightly closed sterile glass vessels.
Other equipment	HPLC equipment: Pump (Merck-Hitachi L-6200 or L-7100), autosampler (Merck-Hitachi AS-2000 and L-7200), UV detector (Merck-Hitachi L-4000 and L-7400) and ^{14}C detector (Packard flow scintillation analyser 500TR). TLC equipment: Automatic TLC-Linear analyser (Tracemaster 40) with data processing system (Berthold CHROMA ver 7.25).

Table A 7.1.1.1.1-4: Description of test system for main test at 60 and 70°C

Criteria	**Details**
Purity of water	Deionised water, further purified using a purification unit (ELGA water purifier) to produce ultra pure water.
Preparation of test medium	Test substance (*ca* 116.7 µg), dissolved in acetonitrile (1300 µL) was diluted with buffer solution (200 mL final volume) for pH 4 stock.
Sub-sample size	10 mL (pH 4).
Test concentrations (mg a.i./L)	0.593.
Temperature (°C)	59.6 ± 0.1°C and 69.2 ± 0.1°C.
Controls	Not applicable.
Identity and concentration of co-solvent	Acetonitrile 0.65% v/v.
Replicates	Eight replicates for each pH value (intended for duplicate samples at six sampling intervals).
Sampling intervals	60°C : 0 and 4 days. 70°C : 0, 1 and 4 days.

Table A 7.1.1.1.1-5: Recovery of applied radioactivity from pre-test samples at 50°C

Incubation time (days)	**Recovery of applied radioactivity (% AR)**		
	pH 4[1]	**pH 7[2]**	**pH 9[3]**
0	100.0	100.0	100.0
2.4 hours	97.3	101.2	99.7
1	94.9	104.6	100.8
5	93.9	102.1	100.3

Values are means of duplicate samples (nominal concentration *ca* 0.5 mg/L).

Table A 7.1.1.1.1-6: Profile of radioactivity from pre-test samples at 50°C

Sample times (days)	Buffer components (% AR)			
	chlorophacinone	Met 1	Met 2	Total
pH 4				
0	90.9	4.0	5.3	100.2
2.4 hours	89.1	2.7	5.4	97.2
1	75.4	2.5	16.9	94.8
5	58.4	4.5	30.9	93.8
pH 7				
0	94.0	3.8	2.9	100.7
2.4 hours	96.4	2.0	2.7	101.1
1	99.4	3.1	2.1	104.6
5	96.1	4.5	1.5	102.1
pH 9				
0	95.9	2.4	2.2	100.5
2.4 hours	94.4	3.0	2.3	99.7
1	96.9	1.9	1.9	100.7
5	96.8	3.5	n.d	100.3

n.d – not detected.
Values are means of duplicate samples.

Table A 7.1.1.1.1-7: Recovery of applied radioactivity from main-test samples at 60 and 70°C

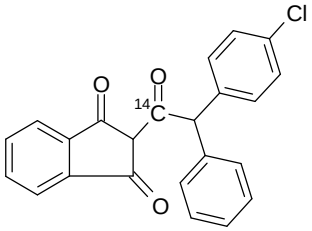
Incubation time (days)	Recovery of applied radioactivity (% AR)					
	pH 4, 60°C			pH 4, 70°C		
	Organic	Aqueous	Total	Organic	Aqueous	Total
0	99.2	0.8	100.0	99.2	0.8	100.0
1	--	--	--	93.4	0.2	93.6
4	99.7	0.4	100.1	98.0	0.9	98.9

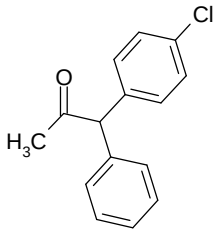
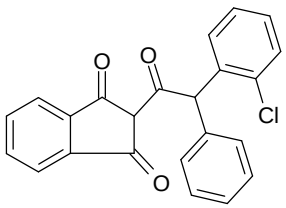
Values are means of duplicate samples (nominal concentration *ca* 0.5 mg/L).

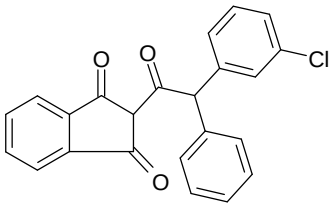
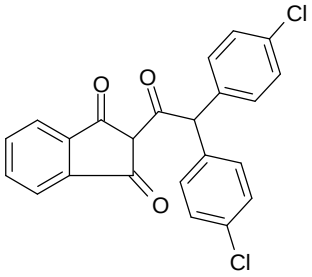
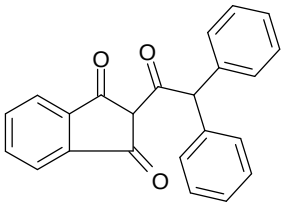
Table A 7.1.1.1.1-8: Profile of radioactivity from main test samples at 60 and 70°C

Sample times (days)	Buffer components (% AR)				
	chlorophacinone	Met 1	Met 2	Origin	Total
pH 4 60°C					
0	98.0	n.d	0.5	0.7	98.5
4	94.7	n.d	1.7	3.3	96.4
pH 4 70°C					
0	98.0	n.d	0.5	0.7	98.5
1	97.4	1.3	1.3	0.3	100.0
4	88.8	4.5	4.2	0.5	97.5

n.d – not detected.

Section A 7.1.1.1.2-01 Annex Point IIA VII.7.6.2.2		Phototransformation in water including identity of transformation products	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Xxxxx, X (XXX), ^{14}C-chlorophacinone: Aqueous Photolysis Under Laboratory Conditions. XXXXX, Laboratory Report No. XXXXX, 04 March XXXX (unpublished). Section No.: A 7.1.1.1.2-01.	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	LiphaTech S.A.S.	
1.2.2	Companies with letter of access	None.	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. Commission Directive 95/36/EC of 14 July 1995 amending Council Directive 91/414/EEC; Annex II: 2.9.2 and 7.2.1.2 Photochemical degradation, OECD Guideline for Testing of Chemicals, draft document, August 2000 and EPA OPPTS 835.2210.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No. Study performed to recommended guideline.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. Acetyl-^{14}C-chlorophacinone. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione. chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	XXXXXXX	
3.2.2	Specification	Specific activity: 2118 MBq/mmol, 5.62 MBq/mg.	
3.2.3	Purity	RCP (radiochemical purity): XXX% by HPLC.	
3.2.4	Radiolabelling	Position of radiolabel given below: 	
3.2.5	Further relevant properties	None.	

Section A 7.1.1.1.2-01 Annex Point IIA VII.7.6.2.2		Phototransformation in water including identity of transformation products	
3.3	**Test material (non-radiolabelled)**	As given in section 2. Chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione. chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.3.1	Lot/Batch number	XXXX	
3.3.2	Specification	No further details.	
3.3.3	Purity	XXXX%	
3.3.4	Further relevant properties	Not applicable.	
3.4	**Reference material (PCPP)**	PCPP (CAS): 1-(4-chlorophenyl) 1-phenyl-propanone-2.	
3.4.1	Lot/Batch number	Lot no. M3153.	
3.4.2	Specification	No further details.	
3.4.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.4.4	Further relevant properties	Structure below:  	
3.5	**Reference material (LM 828)**	LM 828 (CAS): 2-((2-(2-chlorophenyl)-1-oxo-2-phenyl) ethyl-1H-indene-1-3-(2H)-dione.	
3.5.1	Lot/Batch number	Lot no. ANA 178.	
3.5.2	Specification	No further details.	
3.5.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.5.4	Further relevant properties	Structure below:  	
3.6	**Reference material (LM 3257)**	LM 3257 (CAS): 2-((2-(3-chlorophenyl)-1-oxo-2-phenyl) ethyl-1H-indene-1-3-(2H)-dione.	
3.6.1	Lot/Batch number	Lot no. ANA 179.	
3.6.2	Specification	No further details.	

Section A 7.1.1.1.2-01 Annex Point IIA VII.7.6.2.2		Phototransformation in water including identity of transformation products	
3.6.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.6.4	Further relevant properties	Structure below: 	
3.7	**Reference material (LM 106)**	LM 106 (CAS): 2-((2-2-di(4-chlorophenyl)-1-oxo) ethyl-1H-indene-1-3-(2H)-dione.	
3.7.1	Lot/Batch number	Lot no. ANA 106.	
3.7.2	Specification	No further details.	
3.7.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.7.4	Further relevant properties	Structure below: 	
3.8	**Reference material (LM 3256)**	LM 3756 (CAS): 2-(((2-2-diphenyl)-1-oxo) ethyl)-1H-indene-1-3-(2H)-dione.	
3.8.1	Lot/Batch number	Lot no. JB 4286.	
3.8.2	Specification	No further details.	
3.8.3	Purity	> 98%, (non radiolabelled reference standard used for qualitative analysis only).	
3.8.4	Further relevant properties	Structure below: 	
3.9	**Testing procedure**	The rate of photolysis of chlorophacinone in pH 7 aqueous buffer solution and natural pond water was investigated using simulated sunlight (Hanau Suntest).	
3.9.1	Test system	pH 7 buffer (0.01M): Prepared with potassium dihydrogen phosphate (0.107 g) and di-sodiumhydrogenphosphate dihydrate (0.203 g) in de-ionised water. Buffer pH adjusted with hydrochloric acid if necessary and sterilised by autoclave before use.	

Section A 7.1.1.1.2-01 **Annex Point IIA VII.7.6.2.2**		**Phototransformation in water including identity of transformation products**	
		Natural pond water: Sampled from a site at Fröschweiher, Möhlin AG, Switzerland on 18 December 2002. Sterilised by gamma irradiation before use. The treatment and incubation of the test solutions is summarised in Table A 7.1.1.1.2-1. Further details of the test system and equipment used are provided in Table A 7.1.1.1.2-2.	
3.9.2	Properties of light source	Simulated sunlight (Hanau Suntest CPS), see Table A 7.1.1.1.2-2.	
3.9.3	Determination of irradiance	The intensity of light was measured with a LI-1800 spectrophotometer (Li-Cor Inc./USA) before and at the end of irradiation.	
3.9.4	Temperature	The temperature of the test solutions in the vessels was kept constant at 25.0 ± 0.1°C by means of a refrigerated circulating cooler.	
3.9.5	pH	Buffer : pH 7 Pond water: pH 8.1 (pre sterilisation), 8.4 (post sterilisation). Measurements were taken at the beginning and end of the exposure period, see Table A 7.1.1.1.2-3, to confirm that pH was maintained throughout the study.	
3.9.6	Duration of the test	The definitive phase of the study was conducted over 13 days.	
3.9.7	Number of replicates	Duplicate exposed and single dark control samples at each sampling interval.	
3.9.8	Sampling	Irradiated: 0, 4 hours, 1, 3, 4, 7, and 13 days. Dark control: 1, 3 and 13 days. At each sampling interval the level of radioactivity in solution (including a rinse of the test vessel with acetonitrile) was quantified by LSC and analysed directly by HPLC. Volatile traps were sampled and exchanged with fresh reagent at each sampling interval. Sunlight measurements and temperatures were recorded at each sampling interval.	
3.9.9	Analytical methods	Chromatographic analysis (RCP and test solutions) was performed using HPLC with a reversed phase (acetonitrile/0.1% trifluoro acetic acid) gradient system.	
3.9	**Transformation products**		
3.9.1	Method of analysis for transformation products	The levels of chlorophacinone and corresponding degradation products were monitored using HPLC as described in Section 3.9.9.	
		4 RESULTS	
4.2	**Screening test**	A preliminary test was performed using the same methodologies as described above. The test was only used as a range finding exercise and to check the suitability of the analytical methods and consequently the results have not	

Section A 7.1.1.1.2-01 Annex Point IIA VII.7.6.2.2		Phototransformation in water including identity of transformation products	
		been summarised.	
4.3	**Actinometer data**	A chemical actinometer was not used for this study.	
4.4	**Photolysis data**		
4.4.1	Recovery of applied radioactivity, mass balance	The recovery of applied radioactivity from the exposed samples and dark controls is summarised in Table A 7.1.1.1.2-3. The amount of applied radioactivity (AR) recovered from the buffer and pond water exposed samples ranged from 79.7 to 104.9% (overall average 90.9%) and 76.9 to 108.6% (overall average 88.9%), respectively and a complete mass balance was generally achieved. Low recoveries were attributed to incomplete collection of CO_2. Losses were incurred during LSC measurement of radioactivity in buffer or pond water solutions. Samples collected at the 13 day interval were acidified and the radioactivity re-trapped prior to measurement by LSC. Improved recovery (92.2% of applied) was observed for the pH 7 buffer samples using this alternative method. In the dark controls, recoveries were greater than or equal to 97.7% AR in all samples indicating a complete mass balance. Measurements of the pH of the buffer solutions and pond water at the beginning and end of the incubation period indicated that the pH of the solutions was maintained over the test period. Microbiology tests performed at the end of the incubation period confirmed that sterility of the samples was maintained.	
4.4.2	Concentration values	The percent AR recovered as chlorophacinone and degradation products in aqueous buffer and pond water solutions exposed to artificial sunlight and dark controls, at each sampling interval, is summarised in Table A 7.1.1.1.2-4. Analysis of samples was performed by HPLC.	
4.4.3	Photolysis rate constant, k^c_p	The photolysis of chlorophacinone under artificial sunlight was rapid in both buffer solution and pond water, with 41.5 and 22.1% AR, respectively remaining as chlorophacinone after 1 day. The calculated DT_{50} and DT_{90} values are presented graphically in Figures A 7.1.1.1.2-1 and A 7.1.1.1.2-2 and the results are summarised in Table A 7.1.1.1.2-5. The best fit DT_{50} values for the photolysis of chlorophacinone in sterile buffer solution and sterile pond water were determined to be 0.78 and 0.45 days, respectively. The buffer solution DT_{50} (0.78 days) following continuous “Suntest” irradiation corresponded to 2.2 days	

Section A 7.1.1.1.2-01 Annex Point IIA VII.7.6.2.2		Phototransformation in water including identity of transformation products	
		natural summer sunlight at latitude 50°N and to 2.1 days at latitude 30-40°N, based on standard calculations. The pond water DT_{50} (0.45 days) following continuous "Suntest" irradiation corresponded to 1.3 days natural summer sunlight at latitude 50°N and to 1.2 days at latitude 30-40°N, based on standard calculations.	
4.4.4	Kinetic order	The photolysis of chlorophacinone, under artificial sunlight, gave a good correlation to pseudo first order kinetics (R^2 values were ≥ 0.99).	
4.4.5	Reaction quantum yield (ϕ^c_E)	The sunlight reaction quantum yield (ϕ^c_E) of the test substance was not determined.	
4.5	**Specification of the transformation products**	Photolysis of chlorophacinone in aqueous sterile buffer solution and sterile pond water led primarily to the formation of carbon dioxide, which reached levels of 85.8 and 69.1% AR, respectively after 13 days. Three unidentified photolysis product (M1, M2 and M3) were observed in the buffer solution and pond water samples. Levels of M2 and M3 were not significant (> 10% AR). In pond water, M1 reached a level of 23.4% AR after 4 days, declining thereafter to < 10% AR at 13 days. In buffer solution, M2 was a minor component observed at only 0.8% AR.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The rate of photolysis of chlorophacinone in aqueous solution was investigated under artificial sunlight in sterile pH7 buffer and in sterile pond water. The GLP study was conducted to the OECD Guideline for Testing of Chemicals, draft document, August 2000 and EPA OPPTS 835.2210 guideline, in 2004.	
5.3	**Results and discussion**	The amount of applied radioactivity (AR) recovered from the buffer and pond water exposed samples ranged from 79.7 to 104.9% (overall average 90.9%) and 76.9 to 108.6% (overall average 88.9%), respectively. A satisfactory mass balance was achieved with low recoveries attributable to incomplete collection of carbon dioxide. Photolysis of chlorophacinone in aqueous sterile buffer solution and sterile pond water led primarily to the formation of carbon dioxide, which reached levels of 85.8 and 69.1% AR, respectively after 13 days. Three unidentified photolysis products; M1, M2 and M3 were also observed in buffer and pond water samples reaching maximum levels of 23.4, 4.4 and 8.8% AR, respectively. Levels of each compound were declining at the final sampling interval (13 days). The pH and sterility of the test solutions was maintained throughout the incubation period. Photolysis of chlorophacinone under artificial sunlight was rapid in buffer solution and pond water. Photolysis gave a	

Section A 7.1.1.1.2-01 **Annex Point IIA VII.7.6.2.2**	**Phototransformation in water including identity of transformation products**	
	good correlation to pseudo first order kinetics.	
5.3.1 k^c_p	Rate constants were 0.88712 and 1.52564 $days^{-1}$ for the buffer and pond water samples, respectively.	
5.3.2 ϕ^c_E	The quantum yield was not determined.	
5.3.3 $t_{1/2E}$	The rate of photochemical degradation of chlorophacinone was determined in aqueous systems with simulated sunlight and the DT_{50} values ranged from 0.78 days in buffer solution to 0.45 days in pond water. The buffer solution DT_{50} (0.78 days) following continuous "Suntest" irradiation corresponded to 2.2 days natural summer sunlight at latitude 50°N and to 2.1 days at latitude 30-40°N, based on standard calculations. The pond water DT_{50} (0.45 days) following continuous "Suntest" irradiation corresponded to 1.3 days natural summer sunlight at latitude 50°N and to 1.2 days at latitude 30-40°N, based on standard calculations.	
5.4 Conclusion	Photolysis of chlorophacinone in aqueous solution is rapid.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	Yes. A calculation of quantum yield was not performed. In addition, the study made no attempt to identify the photolysis components formed in significant quantities (i.e. > 10% AR). As the study is conducted for classification purposes only (i.e. actual use of the biocidal products will not result in exposure to aquatic systems) the identity of the photolysis components is not considered relevant.	**x**
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	Commission Directive 95/36/EC of 14 July 1995 amending Council Directive 91/414/EEC; Annex II: 2.9.2 and 7.2.1.2 Photochemical degradation, OECD Guideline for Testing of Chemicals, draft document, August 2000 and EPA OPPTS 835.2210.	

Section A 7.1.1.1.2-01 Annex Point IIA VII.7.6.2.2	Phototransformation in water including identity of transformation products	
Results and discussion	From the results it can be concluded that chlorophacinone is rapidly degraded by direct sunlight in natural water bodies with half-lives of 0.78 (sterile aqueous buffer pH~7)and 0.45 days (sterile pond water pH~8.4) ranging from days at latitudes 30° N, 40° N or 50° N at 25ºC. These results demonstrate that ^{14}C-chlorophacinone will be rapidly degraded photochemically under natural conditions in the aquatic environment mainly to CO2 with a calculated half life of: The buffer solution DT_{50} (0.78 days) following continuous "Suntest" irradiation corresponded to 2.2 days natural summer sunlight at latitude 50°N and to 2.1 days at latitude 30-40°N, based on standard calculations. The pond water DT_{50} (0.45 days) following continuous "Suntest" irradiation corresponded to 1.3 days natural summer sunlight at latitude 50°N and to 1.2 days at latitude 30-40°N, based on standard calculations. Direct phototransformation in aqueous systems is considered to be a relevant process for the lifetime of when released into an aqueous environment.	
Conclusion	**5.3.2.** M1 reached a level of 23.4% AR after 4 days, declining thereafter to < 10% AR at 13 days; but since photolysis is a process which occurs mainly in the superficial layer of the water body this metabolite will not be further considered.	
Reliability	1	
Acceptability	Acceptable	
Remarks		

Table A 7.1.1.1.2-1: Description of test solution and controls

Criteria	Details
Purity of water	Deionised water used to prepare buffer samples. Pond water characteristics: Source: XXXXXX, XXXX, Switzerland Sampling date: December 18, 2002 pH: 8.1 (pre sterilisation), 8.4 (post sterilisation) DOC: 4.0 (pre sterilisation), 3.0 (post sterilisation) Suspended solids: 0.17 mg/L. Conductivity (µS at 20°C): 95.9 Redox potential (mV): 195 Oxygen content (mg/l): 5.5 Total residues after evaporation (mg/ml): 0.2
Preparation of test medium	Pre-test: Radioactive chlorophacinone was dissolved in acetonitrile (5 ml) to give a concentration of 82.7 µg/ml (determined by LSC). Aliquots of the solution (120 µl) were added to pH 7 buffer and pond water samples (15 ml). Main test: Radioactive chlorophacinone was dissolved in acetonitrile (6 ml) to give a concentration of 81.2 µg/ml (determined by LSC). Aliquots of the solution (150 µl) were added to pH 7 buffer and pond water samples (15 ml). Test solutions were contained in individual 25 ml vessels (inner diameter 2.1 cm, height 11.0 cm, exposed area 3.5 cm^2) constructed entirely of glass and covered with borosilicate glass lids.
Test concentrations (mg a.i./l)	Pre test: 0.66 µg/l Main test: 0.82 µg/l
Temperature (°C)	25.0 ± 0.1°C
Controls	Dark control samples were similarly prepared.
Identity and concentration of co-solvent	Acetonitrile (1% v/v).
Replicates	Duplicate exposed and single dark control at each sampling interval.

Table A 7.1.1.1.2-2: Description of test system

Criteria	Details
Glassware	Purpose built glass incubation tubes sealed with borosilicate glass lids.
Other equipment	Liquid scintillation counters: Packard TRI-CARB 2500TR, 2550TR, 2700TR, or 2900TR. HPLC system: Merck-Hitachi L-7000 series with Packard Flow 500TR ^{14}C-detector. Absorption spectra: Perkin Elmer UV/VIS Spectrophotometer Lambda 2
Method of sterilisation	Buffer solutions were sterilised by autoclave Pond water was sterilised by gamma irradiation. Glassware was sterilised by rinsing with ethanol/water (70:30; v/v).
Test apparatus	Individual aliquots (15 ml) of the test item in sterile pH 7 buffer and in sterile natural pond water were exposed to light in incubation tubes (25 ml, inner diameter 2.1 cm, height 11.0 cm, exposed area 3.5 cm^2) constructed entirely of glass and covered with borosilicate glass lids which absorb radiation below 290 nm, similar to the natural sunlight cut-off by ozone. The solutions were continuously irradiated through their borosilicate lids. Sterile filtered, humidified air was drawn through the incubation vessels over the solutions at about 10 ml/minute. Any radioactive carbon dioxide or organic volatiles in the purged air was captured in traps of ethylene glycol followed by 2N NaOH, respectively. The study was performed in a "Suntest CPS, Original Hanau" apparatus (Heraeus, Germany), equipped with a 1.8 kW xenon burner and an UV filter system Xenon Burner: 765 W/m^2. UV filtering (lambda < 800 nm) with controllable irradiance between 400 W/m^2 and 765 W/m^2 to a pre-set value. Filters: UV filter with a 290 nm cut-off to simulate natural sunlight. Exposure Area: Approximately 500 cm^2 The spectral energy distribution of the Xenon burner measured through the borosilicate glass lids was comparable to that of sunlight measured from 300 to 800 nm. The intensity of light was measured using a LI-1800 spectrophotometer (Li-Cor Inc./USA) before and at the end of irradiation.
Properties of simulated sunlight:	The spectral irradiance of the Suntest apparatus was measured at the start and end of the irradiation period, and compared with the

	spectral irradiance of sunlight. Summer sunlight at 50°N (Frankfurt/Basel, Switzerland) is about 96% that of latitude 30°N or 40°N. Additionally, it is assumed that the average daily radiation intensity from the sun is about 75% of the maximum intensity over a 12 h period, whereas the irradiation intensity in the Suntest was constant over time. The measured irradiance was related to the sunlight intensity of summer at latitude 50°N as follows: The integral of light intensity at 300 – 400 nm of the Xenon arc source was determined to be on average 44.1 W/m^2. The corresponding value of June 26, 2003 midday sunlight at the test facility (47.5°N, 7.8°E) was determined to be 43.2 W/m^2. The mean ratio of intensities (r) was calculated according to: r = 44.1/43.2 = 1.02 The experimental irradiation hours (h) were converted to midsummer sunlight days (d) by the equation: D = (h.r.F1.F2)/(0.75.12) Where: d = days summer sunlight h = hours of irradiation in the Suntest apparatus r = 1.02 F1 = 1.03 (correction for season (June 26, 2003, latitude 50N)) F2 = 0.96 (correction for season of latitude 50N to latitudes 30N-40N) 0.75 = Correction for diurnal variation of natural sunlight 12 = Conversion of hours into days

Table A 7.1.1.1.2-3: Mean recovery of applied radioactivity from sterile aqueous buffer and sterile natural pond water (main-test)

Incubation time (days)	Recovery of applied radioactivity (% applied)							
	pH 7 buffer[1]				Pond water[2]			
	Solution	CO_2	Other volatile	Total[3]	Solution	CO_2	Other volatile	Total[4]
Exposed								
0	102.5	n.app.	n.app.	102.6	101.8	n.app.	n.app.	101.9
0.17	101.6	1.0	n.app.	102.6	105.6	0.2	n.app.	105.9
1	70.8	15.3	<0.1	86.1	79.5	2.3	<0.1	81.8
3	23.5	57.0	<0.1	80.5	72.1	11.8	<0.1	83.9
4	17.4	65.4	<0.1	82.7	64.9	20.0	<0.1	84.9
7	7.1	82.7	<0.1	89.8	40.8	45.0	<0.1	85.8
13	6.4	85.8	<0.1	92.2	9.4	69.1	<0.1	78.6
Dark control								

0	101.2	n.app.	n.app.	101.2	101.7	n.app.	n.app.	101.7
1	100.8	<0.1	<0.1	100.9	98.6	<0.1	<0.1	98.6
3	100.2	0.2	<0.1	100.4	100.6	0.2	<0.1	100.8
13	97.4	0.4	<0.1	97.7	100.8	0.6	<0.1	101.5

n.app. = Not applicable.
[1] pH was 6.98 at the start of incubation and 6.91 at the end of incubation.
[2] pH was 8.50 at the start of incubation and 8.46 at the end of incubation.
[3] pH 7 buffer total recovery ranged from 79.7 to 104.9% (Overall mean = 90.9%).
[4] Pond water total recovery ranged from 76.9 to 108.6% (Overall mean = 88.9%).
Low recovery in samples was attributed to incomplete collection of CO_2. Losses were incurred during LSC measurement of radioactivity in buffer or pond water solution. For the 13 day sample only, the solutions were acidified and the radioactivity re-trapped prior to measurement by LSC.

Table A 7.1.1.1.2-4: Profile of radioactivity in aqueous buffer and sterile natural pond water (main-test)

Incubation time (days)	Mean recovery of applied radioactivity (% applied)											
	pH 7 buffer						Pond water					
	CPN	M1	M2	M3	CO_2[1]	Total	CPN	M1	M2	M3	CO_2[1]	Total
Exposed samples												
0	101.5	1.0	n.d.	n.d.	n.app	102.6	100.1	1.8	n.d.	n.d.	n.app	101.9
0.17	91.8	1.6	n.d.	2.5	6.7	102.6	72.0	19.1	n.d.	2.0	12.8	105.9
1	41.5	2.5	n.d.	8.8	33.2	86.1	22.1	16.4	4.4	3.4	35.5	81.8
3	6.7	5.1	n.d.	3.3	65.3	80.5	9.5	18.4	2.8	2.3	50.9	83.9
4	4.4	7.5	n.d.	0.5	70.3	82.7	2.0	23.4	4.1	n.d.	55.4	84.9
7	0.3	6.0	n.d.	0.1	83.5	89.8	n.d.	11.9	2.3	n.d.	71.7	85.8
13	n.d.	5.6	0.8	n.d.	85.8	92.2	n.d.	7.6	1.8	n.d.	69.1	78.6
Dark controls												
0	101.2	n.d.	n.d.	n.d.	n.d.	101.2	101.7	n.d.	n.d.	n.d.	n.d.	101.7
1	100.8	n.d.	n.d.	n.d.	0.1	100.9	91.2	7.4[2]	n.d.	n.d.	<0.1	98.6
3	100.2	n.d.	n.d.	n.d.	0.2	100.4	95.8	4.8[2]	n.d.	n.d.	0.2	100.8
13	92.8	4.6	n.d.	n.d.	0.4	97.7	89.9	10.9[2]	n.d.	n.d.	0.6	101.5

CPN = chlorophacinone.
n.app. = Not applicable.
n.d. = Not detected.
[1] Total CO_2 - Results include radioactivity dissolved in solution and collected in traps.
[2] Degradation attributed to instability during analysis, samples were processed prior to HPLC.

Figure A 7.1.1.1.2-1: DT_{50} and DT_{90} values for photolysis of chlorophacinone in sterile aqueous buffer (pH 7)

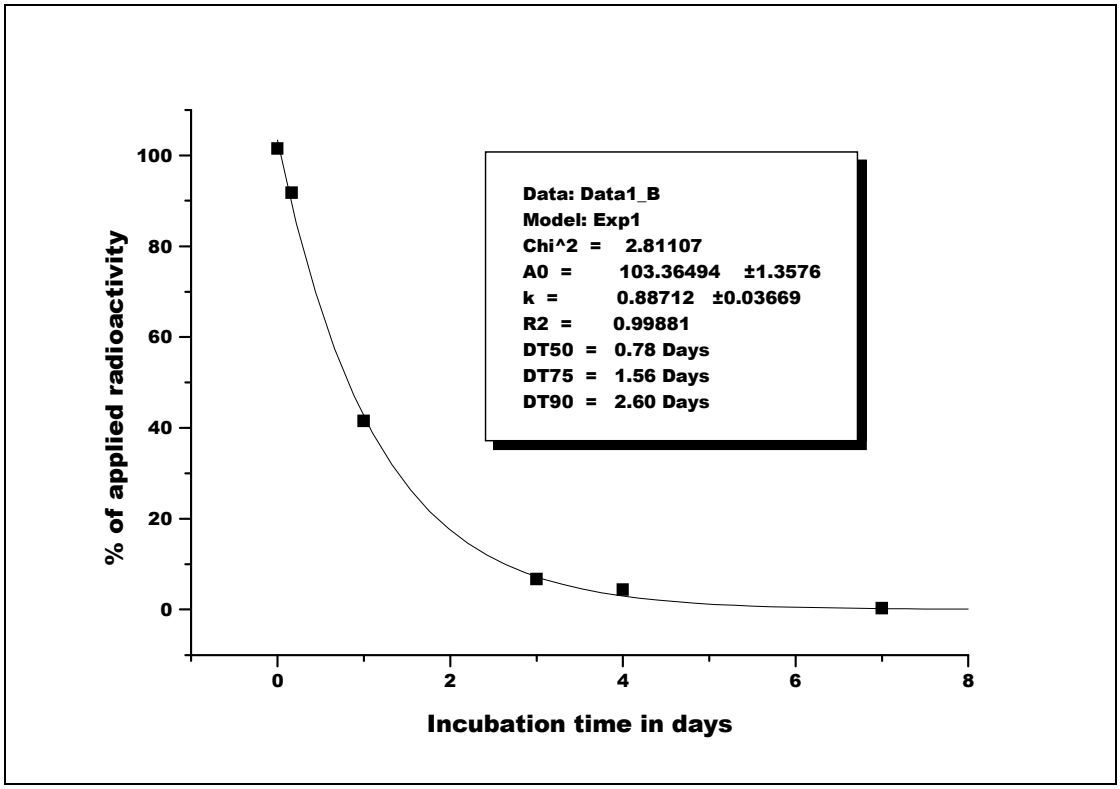


Figure A 7.1.1.1.2-2: DT_{50} and DT_{90} values for photolysis of chlorophacinone in sterile pond water

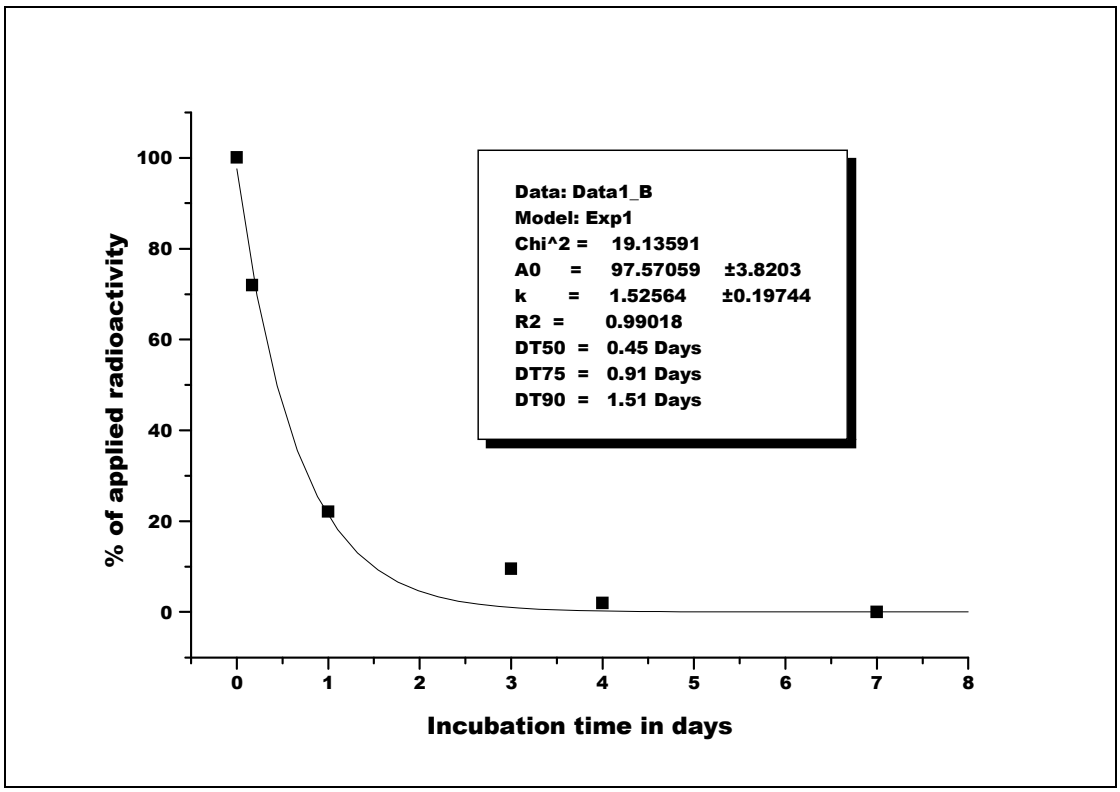
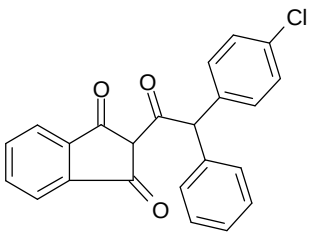


Table A 7.1.1.1.2-5: First order DT_{50} and DT_{90} values for the rate of photolysis of chlorophacinone in sterile aqueous buffer (pH7) and sterile pond water

Buffer	Data range (days)	$DT_{50(lab)}$ (days)	$DT_{90(lab)}$ (days)	Regression parameters		
				C_0 (% AR)	k ($days^{-1}$)	R^2
pH 7	0 to 13	0.78	2.6	103.36	0.88712	0.999
Pond water	0 to 13	0.45	1.5	97.57	1.52564	0.990

Section A 7.1.1.2.1-01 **Annex Point IIA VII.7.6.1.1**		**Biodegradability (ready)** Manometric respirometry test (OECD 301 F)	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxx, X., XXX, Ready biodegradability of chlorophacinone in a manometric respirometry test, XXX XXX., laboratory report no. XXXXXX, 14 January XXXX (unpublished). Section no. : A 7.1.1.2.1-01.	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. The study was performed to OECD guideline no. 301F.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No. The study was conducted to the recommended guideline (EC methods C.4 A to F or the corresponding OECD 301 A to F guidelines).	
		3 MATERIALS AND METHODS	
3.2	**Test material**	As given in section 2. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	XXXXXXX	
3.2.2	Specification	Expiry date 26 March 2005.	
3.2.3	Purity	XX.XX%.	
3.2.4	Further relevant properties	Structure below: 	
3.3	**Reference substance**	Sodium benzoate (Lot no. 403453/1). Purity 99.6%.	
3.3.1	Initial concentration of reference	*ca* 100 mg/L.	

Section A 7.1.1.2.1-01 **Annex Point IIA VII.7.6.1.1**		**Biodegradability (ready)** Manometric respirometry test (OECD 301 F)	
	substance		
3.4	**Testing procedure**	The ready biodegradability of chlorophacinone was investigated under aerobic conditions at a mean temperature of 22°C in the dark over a period of 28 days.	
3.4.1	Inoculum / test species	The test water consisted of purified water with added minerals as specified in Table A 7.1.1.2.1-1. The inoculum used was aerobic activated sewage sludge from a treatment plant (Füllinsdorf, Switzerland) treating predominantly domestic wastewater. The activated sewage sludge was washed twice with tap water by centrifugation and decanting. The level of suspended solids were determined by drying and the wet weight ratio calculated. The sewage sludge was diluted with test water to obtain a dry material concentration of 4 g/L. Prior to use, the sewage sludge was aerated at room temperature.	
3.4.2	Test conditions	The test material, where applicable, was added directly to the test vessels (500mL Erlenmeyer flasks) containing the diluted sewage sludge, the reference material dissolved in test water where applicable and test water (up to 250 mL volume). Dissolution was aided by ultrasonication (15 mins). The final concentration of the activated sludge was 30 mg dry material per L. Inoculum controls (prepared in duplicate) contained test water only. Procedural controls (prepared in duplicate) contained the reference material dissolved in the test water at a concentration of 100 mg/L. The abiotic control contained the test material dissolved in test water at a concentration of 100.0 mg/L, poisoned with mercury dichloride at a concentration of 10 mg/L. The toxic controls contained both the reference material (100 mg/L) and the test material (100.8 mg/L). The test item flasks (prepared in duplicate) contained only the test material dissolved in test water at a concentration of *ca* 100 mg/L. The test vessels were incubated in the dark at a temperature of 22°C for a period of 28 days. The pH of each individual test vessel was adjusted before addition of the activated sewage if necessary. The pH of the test vessels was measured at the end of the incubation period.	
3.4.3	Sampling	The oxygen consumption of each test vessel was monitored throughout the incubation period. The percentage biodegradation was calculated with reference to the theoretical oxygen demand (ThOD) calculated from chemical molecular formula. The ThOD of the reference and test materials were calculated to be 1.67 and 2.13 mg O_2/mg, respectively.	

Section A 7.1.1.2.1-01 **Annex Point IIA VII.7.6.1.1**		**Biodegradability (ready)** Manometric respirometry test (OECD 301 F)	
		4 RESULTS	
4.2	**Degradation of test substance**	The cumulative biochemical oxygen demand in the test vessels is summarised in Table A 7.1.1.2.1-2. The extent of biodegradation observed is summarised in Table A 7.1.1.2.1-3. The percentage of biodegradation of the test material was calculated based on the ThOD of 2.13 mg O_2/mg. No significant biodegradation of chlorophacinone was observed, consequently chlorophacinone can not be considered readily biodegradable under the conditions of the test. The percentage of biodegradation of the reference material was calculated based on the ThOD of 1.67 mg O_2/mg. In the procedural control, the reference material was biodegraded to the extent 85% after 14 and 28 days exposure, thus confirming the suitability of the inoculum and test conditions. The percentage of biodegradation observed in the toxic controls was calculated based on the ThOD of both the reference and test materials. The biodegradation of the reference material observed in the toxic control was 34% after 14 days. The test material did not have an inhibitory effect on the activated sewage sludge micro-organisms (> 25% difference of procedural controls). Measurements taken at the end of the incubation period, showed that the pH in the test vessels was maintained during the study.	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The ready biodegradability of chlorophacinone was investigated under aerobic conditions at a mean temperature of 22°C in the dark over a period of 28 days. The GLP study was conducted OECD guideline 301 F in 2003.	
5.3	**Results and discussion**	After 28 days, the extent of biodegradation of the test material was negligible. The results indicate that chlorophacinone can not be classified as readily biodegradable under the conditions of the test. The test material did not have an inhibitory effect on the sewage sludge microorganisms.	
5.4	**Conclusion**	chlorophacinone can not be classified as readily biodegradable under the conditions of the test.	
5.4.1	Reliability	1.	
5.4.2	Deficiencies	None.	

Section A 7.1.1.2.1-01 **Annex Point IIA VII.7.6.1.1**	**Biodegradability (ready)** Manometric respirometry test (OECD 301 F)	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	Chlorophacinone was investigated for its ready biodegradability in a manometric respirometry test over 28 days based on OECD 301 F (Ready Biodegradability. Manometric respirometry test.) by following its BOD.	
Results and discussion	**4.1.** For visual information it is recommended to include Figure 2: "Biodegradation of chlorophacinone and the reference item" graph from the study report.	
Conclusion	Chlorophacinone was found to be not ready biodegradable under the test conditions within 28 days.	
Reliability	1	
Acceptability	Acceptable	
Remarks	The percentage of biodegradation was calculated as the ratio BOD (mg O_2/mg a.s.) * 100/ThOD (mg O_2/mg a.s.)	

Table A 7.1.1.2.1-1: Composition of test water

Minerals	Amount of nutrient per Litre deionised water (mg)
KH_2PO_4	85
K_2HPO_4	217.5
$Na_2HPO_4.2H_2O$	334.0
NH_4Cl	5.0
$MgSO_4.7H_2O$	22.5
$CaCl_2$	36.4
$FeCl_3.6H_2O$	0.25

The pH of the final solution was adjusted from pH 7.8 to 7.4 by addition of diluted hydrochloric acid solution.
Analytical grade chemicals were used.

Table A 7.1.1.2.1-2: Cumulative biochemical oxygen demand in the test vessels

Sampling interval (days)	Cumulative biochemical oxygen demand, BOD (mg O_2/L)				
	Inoculum Control	Procedure Control	Abiotic Control	Toxic control [1]	Test material
0	0, 0	0, 0	0	0	0, 0
1	0, 0	5, 4	0	6	0, 1
2	0, 2	80, 78	0	73	1, 2
3	1, 3	98, 98	0	88	1, 3
4	2, 5	120, 121	0	115	2, 4
5	3, 7	130, 130	0	124	2, 4
6	4, 8	133, 133	0	126	3, 4
7	5, 9	138, 138	0	129	3, 5
8	6, 11	142, 142	0	132	3, 5
9	6, 12	145, 145	0	134	3, 5
10	7, 13	148, 148	0	136	4, 6
13	8, 16	153, 154	0	141	4, 7
14	9, 17	154, 155	0	143	4, 7
15	9, 17	155, 156	0	144	4, 8
16	9, 18	156, 157	0	145	4, 8
17	9, 19	156, 157	0	146	4, 8
18	10, 20	157, 159	0	147	4, 8
20	10, 22	158, 161,	0	149	4, 8
21	11, 22	158, 161	0	149	4, 8
22	11, 23	158, 162	0	150	4, 8
23	11, 24	158, 163	0	150	4, 8
24	11, 25	158, 163	0	150	4, 8
27	11, 27	158, 165	0	151	4, 8
28	12, 28	158, 166	0	152	4, 8

[1] Toxic control consisted of 100 mg/L reference material and 100.8 mg/L test material.

Table A 7.1.1.2.1-2: Extent of biodegradation observed

Sampling interval (days)	Percentage biodegradation		
	Procedure Control	Toxic control [1]	Test material
0	0, 0	0	0, 0
1	3, 2	2	0, 0
2	47, 46	19	0, 0
3	57, 57	23	0, 0
4	70, 70	29	-1, 0
5	75, 75	31	-1, 0
6	76, 76	31	-1, -1
7	78, 78	32	-2, -1
8	80, 80	32	-3, -2
9	81, 81	33	-3, -2
10	83, 83	33	-3, -2
13	84, 85	34	-4, -2
14	84, 85	34	-4, -3
15	85, 86	34	-4, -2
16	85, 86	34	-4, -3
17	85, 86	35	-5, -3
18	85, 86	35	-5, -3
20	85, 87	35	-6, -4
21	85, 87	35	-6, -4
22	84, 87	35	-6, -4
23	84, 87	35	-6, -4
24	84, 87	35	-7, -5
27	83, 87	35	-7, -5
28	83, 87	35	-7, -6

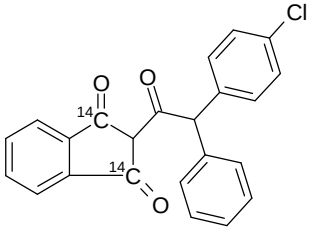
Negative values relate to less biodegradation than the control samples.

[1] Toxic control consisted of 100 mg/L reference material and 100.8 mg/L test material.

Section A 7.1.1.2.2-01 Annex Point IIA VII.7.6.1.2	Inherent biodegradability	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Based on the information obtained from the study described under Section A 7.1.1.2.1 (i.e. chlorophacinone is not readily biodegradable), and the further simulation test conducted under Section A 7.2.1, it is considered that chlorophacinone is not likely to be inherently biodegradable and therefore a test has not been performed.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification	The notifier assumes that the substance is not inherently biodegradable.	
Conclusion	Acceptable	
Remarks		

Section A 7.1.2.1.1-01 Annex Point IIIA XI.2.1	Biological sewage treatment, Aerobic biodegradation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Based on the information obtained from the study described under Section A 7.1.1.2.1 (i.e. chlorophacinone is not readily biodegradable), and the further simulation test conducted under Section A 7.2.1, it is considered that chlorophacinone is not likely to be biodegradable under the conditions of this test and therefore a test has not been performed.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification	The notifier assumes that chlorophacinone is not biodegradable under aerobic conditions in the biological sewage treatment.	
Conclusion	Acceptable	
Remarks		

Section A 7.1.2.1.2-01 Annex Point IIIA XII.2.1	Biological sewage treatment, Anaerobic biodegradation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Based on the information obtained from the study described under Section A 7.1.1.2.1 (i.e. chlorophacinone is not readily biodegradable), and the further simulation test conducted under Section A 7.2.1, it is considered that chlorophacinone is not likely to be biodegradable under the conditions of this test and therefore a test has not been performed.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification	The notifier assumes that chlorophacinone is not biodegradable under anaerobic conditions in the biological sewage treatment	
Conclusion	Acceptable	
Remarks		

Section A 7.1.3-01 Annex Point IIA7.7		Adsorption / desorption screening test	
		1 REFERENCE	Official use only
1.2	Reference	Xxxx, X XXXX, Adsorption / desorption of chlorophacinone in four soil types, XXXXXXXX., laboratory report no. XXX, 26 January XXX (unpublished). Section no.: A 7.1.3-01.	
1.3	Data protection	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	Guideline study	Yes. The study was performed to US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 163-1.	
2.3	GLP	Yes.	
2.4	Deviations	No. The study meets the requirements of the recommended guideline (recommended guidelines OECD 106- draft guideline for aerobic degradation, BBA or US EPA).	
		3 MATERIALS AND METHODS	
3.2	Test material (radiolabelled)	As given in section 2. Indan-^{14}C-chlorophacinone. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	Lot no. XXXXX.	
3.2.2	Specification	Specific activity 4.23 mCi/mmol. Chemical purity > XX%.	
3.2.3	Purity	RCP (radiochemical purity) > XXXX% by 2D TLC (determined prior to use on study) see Section 3.7.5.	
3.2.4	Further relevant properties	Position of radiolabel given below:  	
3.2.5	Method of analysis	RCP determined prior to use by TLC analysis, conditions specified in Section 3.7.5.	
3.3	Test material (non radiolabelled)	As given in section 2. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-	

Section A 7.1.3-01 Annex Point IIA7.7		Adsorption / desorption screening test	
		phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.3.1	Lot/Batch number	XXXXXXX.	
3.3.2	Specification	No further details.	
3.3.3	Purity	> XXX% (non-radiolabelled test material used for qualitative analysis only).	
3.3.4	Further relevant properties	None specified.	
3.4	**Degradation products**	Degradation products tested: Yes. The stability of the test material over the duration of the study was tested in the preliminary investigations and confirmed at the end of the study as described in Section 3.7.4, no significant degradation of chlorophacinone was observed.	
3.4.1	Method of analysis for degradation products	See Section 3.7.5.	
3.5	**Soil types**	Soils were obtained by Agrisearch Inc. and were air dried, sieved (2 mm) and stored at ambient room temperature prior to use on the study. The soils were characterised by A & L Great Lakes Laboratories Inc., the characterisation data for the soils is summarised in Table A 7.1.3-1. Four soil types were used (clay, sand, sandy clay loam and loam).	
3.6	**Testing procedure**	The sorption properties of chlorophacinone were investigated in four soils (of US origin) using the batch equilibrium technique.	
3.6.1	Test system	Tests were conducted in Teflon centrifuge tubes (50 mL), prior to use tubes were sterilised by autoclaving for 1 hour at 121°C and 15 psig.	
3.6.2	Test solution and Test conditions	Calcium acetate solution (0.01 M) was prepared by adding calcium acetate (1.76 g per l) to filtered, deionised, boiled, distilled water and stirring until dissolved. The solution was sterilised by filtration (0.2 µm). For the preliminary investigations, a stock solution of chlorophacinone in calcium acetate solution was prepared at a concentration of 3.0 µg/mL by adding the test material dissolved in acetonitrile. For the definitive study, a stock solution of the test material was prepared by adding chlorophacinone dissolved in acetonitrile (2310 µL) to 0.01M calcium acetate solution (826 mL). The working solutions were prepared at concentrations of 0.17, 0.34, 0.65, 1.24 and 2.56 µg/mL by diluting with further blank calcium acetate solution.	
3.7	**Test performance**	--	

Section A 7.1.3-01 Annex Point IIA7.7		Adsorption / desorption screening test	
3.7.1	Preliminary test	According to (a) "OECD 106": No. Preliminary tests were not conducted exactly according to the recommended guideline. Instead the soil to solution ratio, the required equilibration time, any losses to glassware and the stability and overall recovery of the test material over the duration of the study were investigated using the procedure described under Section 3.7.2.	
3.7.2	Screening test: Adsorption	According to (a)"OECD 106": Yes. Duplicate soil slurries were prepared using soil (1 g) and 0.01M calcium acetate solution (20 mL) containing chlorophacinone at a concentration of 3.0 µg/mL. The soil slurries were shaken (Eberbach shaker, 175 to 200 rpm) for a period of 48 hours. At intervals of 4, 8, 24 and 48 hours aliquots (100 µL) were removed and quantified by LSC after centrifugation.	
3.7.3	Screening test: Desorption	According to (a)"OECD 106": Not performed. A screening phase for the desorption step was not conducted, the equilibration time used for the desorption phase was selected as 24 hours to be consistent with the adsorption phase.	
3.7.4	Definitive study, Freundlich sorption isotherms	The study was conducted by preparing soil slurries containing 0.5 g soil and 40 mL 0.01M calcium acetate solution i.e. a soil to solution ratio of 1:80 w/v. The slurries were not pre-equilibrated. Solutions were prepared with radiolabelled chlorophacinone at actual concentrations of 0.17, 0.34, 0.65, 1.24 and 2.56 mg/L. Duplicate soil slurries were prepared for each soil at each concentration. The soil slurries were equilibrated with the test compound in the dark for a period of 24 hours at a temperature of *ca* 25°C using a mechanical shaker (Eberbach shaker, 175 to 200 rpm). After equilibration, the soil and aqueous layers were separated using centrifugation and the radioactivity in the aqueous layer quantified 'directly' by LSC. The radioactivity in the soil layer following the adsorption phase was quantified 'indirectly' by subtracting the amount in the aqueous layer, including the interstitial water, from the total applied radioactivity. One desorption step was performed by removing the entire aqueous layer and replenishing with an equal amount of fresh 0.01M calcium acetate solution. The soil slurries were shaken for a further 24 hour period prior to centrifugation, separation and quantification as before. Following the desorption step, the concentration of chlorophacinone in the soil layer was quantified combustion analysis. Additionally, sub-samples from the supernatant solutions from the adsorption and desorption phases were	

Section A 7.1.3-01 Annex Point IIA7.7		Adsorption / desorption screening test	
		chromatographically analysed by TLC to confirm the stability of the test compound over the duration of the study period.	
3.7.5	Chromatographic analysis	Chromatographic analysis of the supernatant solutions from the adsorption and desorption phases was conducted by TLC using silica plates (0.25 mm) developed in either methanol/ acetic acid (80/20, v/v), or acetone/ diethylamine (90/10 v/v). Non radiolabelled reference standards were visualised using UV light (254 nm). Radioactive regions were quantified using an Ambis Radioanalytical imaging system. The RCP determinations were similarly conducted using 2D TLC using both of the solvent systems given above in turn.	
		4 RESULTS	
4.2	**Preliminary test**	The investigations normally conducted in the preliminary study (i.e. soil to solution ratio, the required equilibration time, any losses to glassware and the stability and overall recovery of the test material over the duration of the study) were incorporated into the adsorption screening test, results are described in Section 4.3.	
4.3	**Screening test: Adsorption**	The amount of applied radioactivity recovered from the preliminary study ranged from 94.4 to 106.4% (average 101.8%), indicating a complete mass balance. The adsorption screening test indicated that equilibration of chlorophacinone in the soil slurries was achieved quickly and that after 24 hours only slight changes were observed in the concentrations detected in the aqueous layer. Therefore a period of 24 hours was selected as the equilibration period for the definitive study. Adsorption in the screening test was extensive. The results obtained are presented in Table A 7.1.3-2. The measured values for the soil distribution (partition) coefficient (K_D) after 24 hours ranged from 58 to 492 mL/g. Therefore in the definitive study a soil to solution ratio of 1:80 w/v was used. Some adsorption to the tubes was observed, however this was considered minimal in comparison to the adsorption of the test material to the soils when present.	
4.4	**Screening test: Desorption**	Not performed, equilibration period for desorption phase was set as the same as that for the adsorption phase (i.e. 24 hours).	
4.5	**Definitive study, Freundlich sorption isotherms**	Freundlich adsorption isotherms were determined for all soils over an actual concentration range of 0.17 to 2.56 µg/mL. The Freundlich sorption parameters determined for the adsorption and desorption phases of the study are summarised in Table A 7.1.3-3. The adsorption of chlorophacinone to soil gave a good correlation to the Freundlich equation (correlation 0.993 to 1.000).	

Section A 7.1.3-01 Annex Point IIA7.7		Adsorption / desorption screening test	
4.5.1	Adsorption parameters	The range of soil distribution (partition) coefficients for the adsorption and desorption phases of chlorophacinone in each soil over the concentrations used, i.e. K_D^{ads} and K_D^{des} was not determined in the study report. The amounts of chlorophacinone adsorbed to soil at the end of the adsorption phase ranged from 84.8 to 91.3% (average 87.5%), 36.6 to 51.4 (average 44.8%), 57.0 to 71.8 (average 62.6%) and 52.7 to 62.5 (average 57.0%) for the Mississippi clay, Maryland sand, Maryland sandy clay loam and California loam soils respectively. The Freundlich soil adsorption coefficient, K_F^{ads} was determined to be 80 to 1000 mL/g and the Freundlich exponent (1/n) 1.145 to 1.231. Freundlich soil adsorption coefficient normalised for organic carbon content, K_{OC}^{ads} was determined to be 15600 to 136000 mL/g, indicating chlorophacinone as 'non mobile' according to the SSLRC (Soil Survey and Land Research Council, UK) classification index. Adsorption to soil was strong for all soil types and was greatest (in terms of percentage adsorbed) for the Mississippi clay soil.	
4.5.2	Desorption parameters	The Freundlich soil desorption coefficient, K_F^{des} was determined to be 1.6×10^5 to 1.8×10^6 mL/g and the Freundlich exponent (1/n) 1.796 to 2.296. Freundlich soil desorption coefficient normalised for organic carbon content, K_{OC}^{des} was determined to be 14900 to 97000 mL/g. Generally, the desorption coefficients, although still significant, were slightly lower than the corresponding adsorption coefficients.	
4.5.3	Recovery over duration of study	The amounts of test material recovered at the end of the study (supernatant solutions from the adsorption and desorption phases plus the soil pellet extraction) ranged from 91.14 to 97.26% (average 95%).	
4.5.4	Stability over duration of study	Chromatographic analysis by TLC of the supernatant solutions from the adsorption and desorption phases of each soil type showed no significant degradation products (taken visually from details given in the report), indicating that the test material was stable over the duration of the study.	
4.6	**Degradation product(s)**	No significant degradation products were observed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The sorption properties of chlorophacinone were investigated in four soils (of US origin) using the batch equilibrium technique. The GLP study was conducted to US EPA Guidelines 163-1 in 1993.	

Section A 7.1.3-01 **Annex Point IIA7.7**	**Adsorption / desorption screening test**	
5.3 Results and discussion	Freundlich adsorption isotherms were determined for each soil with chlorophacinone over the nominal concentration range 0.17 to 2.56 µg/mL using a soil to solution ratio of 1:80 w/v (0.5 g soil dry weight to 40 mL solution) in the dark at a temperature of 25°C.	
5.3.1 Adsorbed a.s. [%]	The amount of test material adsorbed to the soils were > 85.19, > 36.63, > 57.00 and > 52.68% for the Mississippi clay, Maryland sand, Maryland sandy clay loam and California loam soils, respectively.	
5.3.2 Soil distribution (partition) coefficient, K_D	Not determined.	
5.3.3 Freundlich soil adsorption coefficient, K_F	For adsorption 80 to 1000 mL/g. For desorption 57 to 578 mL/g.	
5.3.4 Freundlich soil adsorption coefficient normalised for organic carbon content, K_{OC}	For adsorption 15,600 to 136,000 mL/g. For desorption 14,900 to 97,000 mL/g.	
5.3.5 Freundlich exponent, 1/n	For adsorption 1.145 to 1.231. For desorption 1.027 to 1.560.	
5.4 Conclusion	Chlorophacinone is rapidly and strongly sorbed to soil. The Freundlich soil sorption coefficient normalised for organic carbon content (K_{OC}) was 15,600 mL/g. This indicates chlorophacinone as 'non mobile' according to the SSLRC classification index. The Freundlich exponent (1/n) ranged from 1.145 to 1.231. Chlorophacinone, even if released indirectly to soil in small quantities, is not likely to move through the soil profile and is unlikely to reach groundwater in significant quantities.	
5.4.1 Reliability	2.	
5.4.2 Deficiencies	Yes. The study contained some deficiencies when compared to modern day standards. These deficiencies are discussed in more detail where appropriate under the relevant headings above and are not considered to have adversely affected the conclusions made.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	US EPA Guidelines 163-1 (1993).	
Results and discussion		

Section A 7.1.3-01 Annex Point IIA7.7	Adsorption / desorption screening test	
Conclusion	Chlorophacinone is strongly adsorbed to soil.	
Reliability	2	
Acceptability	Acceptable	
Remarks	Estimations of the K_{oc} based on the K_{ow} applying QSARs for soil and sediment would be several orders of magnitude lower than the experimental value retrieved in the adsorption/desorption screening test (K_{oc} from 136,000 to 15,600). The drastic difference reflects that other processes are involved apart from lipophilicity. As a conclusion, adsorption to soil does not depend only on the organic carbon content.	

Table A 7.1.3-1: Classification and physico-chemical properties of soils used as adsorbents

Parameter / Soil name	Soil 1	Soil 2	Soil 3	Soil 4
Source	XXXX	XXXX	XXXX	XXXX
Soil series	XXXX	XXXX	XXXX	XXXX
Textural classification, USDA	clay	sand	sandy clay loam	loam
Sand (%)	25	96	56	44
Silt (%)	33	1	21	47
Clay (%)	42	3	23	9
Organic matter (%)	4.8	0.1	2.0	0.8
Organic carbon (%) [1]	2.824	0.059	1.176	0.471
pH	5.9	6.2	7.0	6.7
Cation exchange capacity (MEQ/100 g)	24.3	1.1	6.85	4.3
Bulk density (g/mL)	1.22	1.44	1.34	1.57
Moisture content, (g/100 g soil) Field capacity (FC, 1/3 bar)	35.9	2.6	17.8	11.7

[1] Calculated as organic matter content ÷ 1.7 (as specified in the report).

Table A 7.1.3-2: Partition coefficient (K_D) against equilibration time

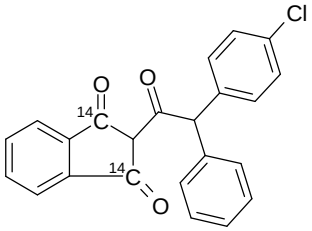
Parameter / Soil name	Estimated K_D value (mL/g)			
Equilibration shaking time (hours) / Soil	Mississippi clay	Maryland sand	Maryland sandy clay loam	California sandy loam
4	341	36	70	73
8	402	40	70	62
24	492	58	80	101
48	369	89	90	96

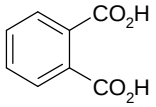
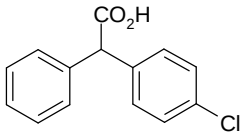
Table A 7.1.3-3: Soil adsorption/desorption parameters for chlorophacinone in four soils

Soil		Soil sorption parameters				
		K_D (mL/g)	K_F (mL/g)	1/n	Correlation	K_{OC} (mL/g)
Adsorption						
1	Mississippi, clay (pH 5.9, oc 2.824%)	n.a.	1000	1.231	0.993	35400
2	Maryland Sand (pH 6.2, oc 0.059%)	n.a.	80	1.170	0.994	136000
3	Maryland Sandy clay loam (pH 7.0, oc 1.176%)	n.a.	183	1.225	0.994	15600
4	California Sandy loam (pH 6.7, oc 0.471%)	n.a.	126	1.145	1.000 average 0.995	26900
Desorption						
1	Mississippi, clay (pH 5.9, oc 2.824%)	n.a.	578	1.089	0.992	20500
2	Maryland Sand (pH 6.2, oc 0.059%)	n.a.	57	1.560	0.983	97000
3	Maryland Sandy clay loam (pH 7.0, oc 1.176%)	n.a.	175	1.068	0.999	14900
4	California Sandy loam (pH 6.7, oc 0.471%)	n.a.	90	1.027	0.992 average 0.992	19100

n.a. – not analysed.

K_D – soil distribution (partition) coefficient.
K_F – Freundlich soil adsorption coefficient.
K_{OC} - Freundlich soil adsorption coefficient normalised for organic carbon content.
1/n – Freundlich exponent

Section A 7.2.1-01 **Annex Point IIIA VII.4, XII.1.1**		**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxx, X., XXXX, Aerobic soil metabolism of chlorophacinone, XXXXXXXX., laboratory report no. XX, 18 January XXX (unpublished). Section no. : A 7.2.1-01.	
1.3	**Data protection**	Yes	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. The study was performed to US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No. Apart from minor deviations, the study meets the requirements of the recommended guideline (recommended guidelines OCED - draft guideline for aerobic degradation, BBA or US EPA).	
		3 MATERIALS AND METHODS	
3.2	**Test material (radiolabelled)**	As given in section 2. Indan-^{14}C-chlorophacinone. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	Lot no. XXXXXX.	
3.2.2	Specification	Specific activity 4.23 mCi/mmol.	
3.2.3	Purity	RCP (radiochemical purity) > XX% by two TLC systems.	
3.2.4	Further relevant properties	Position of radiolabel given below: 	
3.2.5	Method of analysis	RCP determined prior to use by TLC analysis, conditions specified in Section 3.3.5.	

Section A 7.2.1-01 **Annex Point IIIA VII.4, XII.1.1**		**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
3.3	**Test material (non radiolabelled)**	As given in section 2. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.3.1	Lot/Batch number	Lot no XXXXXX.	
3.3.2	Specification	No further details.	
3.3.3	Purity	> XX%.	
3.3.4	Further relevant properties	Not applicable.	
3.4	**Reference material (phthalic acid)**	o-Phthalic acid (IUPAC): 1,2-Benzenedicarboxylic acid. o-Phthalic acid (CAS): o-Benzenedicarboxylic acid.	
3.4.1	Lot/Batch number	Lot no. not specified.	
3.4.2	Specification	No further details.	
3.4.3	Purity	XXX%, assigned (non radiolabelled reference standard used for qualitative analysis only).	
3.4.4	Further relevant properties	Structure below:  	
3.5	**Reference material (chlorophenyl-phenyl acetic acid)**	p-Chlorophenyl-phenyl acetic acid (IUPAC): Not available. p-Chlorophenyl-phenyl acetic acid (CAS): Not available.	
3.5.1	Lot/Batch number	Lot no JB3653.	
3.5.2	Specification	No further details.	
3.5.3	Purity	100%, assigned (non radiolabelled reference standard used for qualitative analysis only).	
3.5.4	Further relevant properties	Structure below:  	
3.6	**Test performance**	The route and rate of aerobic degradation of ^{14}C-chlorophacinone was investigated in one soil (sandy clay loam) of US origin in the dark under laboratory conditions at a temperature of 24 to 26°C and moisture content of 75% field capacity (1/3 bar moisture). To further investigate the effect of the experimental design on the recovery of evolved volatile components, the experiment was repeated using a fresh batch of soil sourced from the same location (textural classification on re-sampling was sandy loam).	

Section A 7.2.1-01 **Annex Point IIIA VII.4, XII.1.1**	**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
3.6.1 Test soils	The test soil was sampled from an agricultural field (US origin) and was sieved (2 mm) and stored (25°C) prior to use. The characterisation details of the soil samples are given in Table A 7.2.1-1. Prior to use the moisture content of the soil was adjusted to 75% field capacity (1/3 bar moisture). The microbial viability of the soils was determined at the start and end of the study.	
3.6.2 Treatment to soil samples	Soil samples (25 g dry weight) were treated with ^{14}C-chlorophacinone (*ca* 0.25 mg) dissolved in acetone (100 µL). The treatment rate (10.0 mg/kg dry weight) is equivalent to an application rate of 7500 g a.s./ha (assuming a soil density of 1.5 g/cm^3 and a mixing depth of 5 cm). The purity of the test material was confirmed before the treatment. Additional soil samples (sterilised by autoclaving) were similarly treated to be used as sterile samples.	
3.6.3 Incubation of soil samples	Following treatment, the moisture content of the soil samples was adjusted to 75% field capacity (1/3 bar moisture). Soil samples were incubated in foil covered Erlenmeyer flasks (250 mL) stoppered with polyurethane foam plugs at a temperature of *ca* 25°C. Soil moisture content was maintained periodically by addition of water. Separate soil samples were similarly treated and incubated, but attached to a series of trapping solutions and flushed daily with humidified air by vacuum to recover evolved volatile components. Duplicate soil samples were taken for analysis at intervals over a period of 182 days (0, 1, 3, 7, 14, 21, 30, 91 and 182 days). Additional sterile soil samples were taken for analysis after 30 and 182 days. Soil samples were extracted (x 3) with ethanol/ water (90/10 v/v) and (x 3) with acetone/ water (90/ 10 v/v) using ultrasonication and separated by centrifugation. Further reflux extractions were carried out, as necessary, using ethanol/ water (50/50 v/v), acetone/ water (50/50 v/v) acetone/ water/ phosphoric acid (70/30/1 v/v/v) and ultrasonication using ethanol/ 2N sodium kydroxide (1/1 v/v). The radioactivity content of the soil extracts and non-extracted soil residue (NER) were quantified using liquid scintillation counting (LSC) and combustion analysis, respectively. The levels of evolved volatile components was quantified by sampling the trapping solutions connected to separate flasks at regular intervals. Levels of carbon dioxide present were confirmed by barium carbonate precipitation.	

Section A 7.2.1-01 **Annex Point IIIA VII.4, XII.1.1**		**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
3.6.4	Chromatographic analysis	Routine chromatographic analysis of the soil extracts was performed using 2D-TLC using silica plates (0.25 mm) developed in methanol/ acetic acid (80/20 v/v) followed by acetone/ diethylamine (90/10 v/v). Non radiolabelled reference standards were visualised using UV light (254 nm). Radioactive regions were quantified using a plate scanner (Ambis radioanalytical imaging system). Confirmatory analysis on selected samples was conducted by HPLC using a reverse phase gradient system (Shimadzu LC-6A, SPD-6A UV detector and Ramona-5-LS radioactivity detector). Non radiolabelled test material was used as authentic reference standard.	
3.7	**Repeat test performance**	To further investigate the effect of the experimental design on the recovery of evolved volatile components, the experiment was repeated using a fresh batch of soil sourced from the same location.	
3.7.1	Test soils	The test soil for the repeat investigation was sampled from the same field as for the original study. The soil was treated as before, see Section 3.5.2. The characterisation details of the soil repeat soil sample is given in Table A 7.2.1-1. Although sourced from the same field, the repeat soil sample was classified texturally as sandy loam (as opposed to sandy clay loam). The sand content of the repeat soil sample was higher than the original batch (correspondingly the clay content was lower). The microbial viability of the repeat soil was determined at the start of the study and after 70 days.	
3.7.2	Treatment to soil samples	The repeat soil samples were treated in the same way as the original samples.	
3.7.3	Incubation of soil samples	The repeat soil samples were incubated in the same was as the original samples. For the repeated soil samples the arrangement with the trapping solutions was modified such that the volume was increased to improve trapping efficiency. Duplicate soil samples were taken for analysis at intervals over a period of 70 days (0, 14, 30, 45 and 70 days). Repeat soil samples were extracted using the same methods.	
3.7.4	Chromatographic analysis	Soil extracts from the repeat soil were chromatographically analysed using the same methods as described for the original soil samples, see Section 3.6.4.	
		4 RESULTS	
4.2	**Recovery and distribution**	Determinations of microbial biomass activity indicated that the soil was viable for the duration of the study. The recovery and distribution of applied radioactivity from the soil is summarised in Table A 7.2.1-2. The recovery of applied radioactivity from the individual original soil	

Section A 7.2.1-01 Annex Point IIIA VII.4, XII.1.1	Aerobic degradation in soil (initial study) 2 soil types, aerobic conditions	
	samples ranged from 72 to 101% AR (overall average 92%). The majority of the applied radioactivity was extractable from the soil and the levels observed steadily declined from 100% AR initially to 17% after 182 days. The amount of soil NER observed gradually increased to 11% AR after 182 days and was not considered significant. The level of volatile radioactivity recovered steadily increased to 50% AR after 182 days and was confirmed as carbon dioxide by barium carbonate precipitation. Following modification of the experimental design, a similar pattern was observed with the repeat soil. Recovery of applied radioactivity ranged from 95 to 107% AR (overall average 104%), indicating an improved mass balance. Consequently the low recovery of applied radioactivity from the original soil samples was attributed to incomplete recovery of carbon dioxide. If the shortfall in mass balance is assumed to have been due to incomplete recovery of carbon dioxide, the levels of carbon dioxide evolved could have potentially been as high as 65% AR after 182 days.	
4.3 Profile of components	The profile of components extracted from the original and repeat soil samples is summarised in Table A 7.2.1-3. Chromatographic analysis using 2D-TLC of the original soil samples indicated that chlorophacinone was steadily degraded in soil and comprised 100% AR initially, but declined to 56.8% after 30 days and 17.8% after 182 days. Degradation of chlorophacinone did not lead to the formation of any significant metabolites (i.e. > 10% AR). Chromatographic analysis of the repeat soil samples confirmed the profile of components, increasing the levels of carbon dioxide actually observed.	
4.4 Route of degradation	Based on the metabolites observed in the chromatographic profile of the soil extracts, as described in Section 4.3, a tentative degradation pathway is proposed in Figure A 7.2.1-01.	
4.4.1 Significant degradation products	Two distinct minor metabolites (i.e.< 10% AR) were observed in the original soil samples which were identified as o-phthalic acid and p-chlorophenyl-phenyl acetic acid which comprised maximum levels of 5.1 and 1.8% AR after 91 days. The metabolites were confirmed by co-chromatography with authentic reference standards. These metabolites were also observed in the repeat soil samples although at slightly higher levels due to the increased degradation of chlorophacinone observed on this occasion.	
4.5 Rate of degradation	In order to provide a consistent and modern approach to the degradation kinetics the DT_{50} and DT_{90} values have been recalculated by non-linear regression using the Solver	

Section A 7.2.1-01 **Annex Point IIIA VII.4, XII.1.1**	**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
	function in a Microsoft Excel spreadsheet to find the best fit between the observed experimental data and the first order rate equation, $C_T = C_0 \times \exp^{-kT}$. The line of best fit was determined by minimising the sum of the squares of the residuals between the actual data and the best fit line. This was achieved using the Solver function to change the values of C_0 and k and converge on a minimum value for the sum of the squares of the residuals. The rate constant, k, was then used to determine the DT_{50} (from LN(2)/k) and DT_{90} (from LN(10)/k) values. It is assumed that similar methods of calculating a first order DT_{50} value would provide equivalent results. The recalculation of the DT_{50} and DT_{90} values is presented graphically in Figures A 7.2.1-2 and 7.2.1-3 and summarised in Table A 7.2.1-4. The degradation of chlorophacinone, at a temperature of *ca* 25°C and moisture content of 75% 1/3 bar moisture content, give a good correlation to first-order kinetics (R^2 > 0.9). However, using the entire data set the DT_{90} value was visually underestimated as a biphasic degradation profile was observed and at later sampling intervals the degradation rate of chlorophacinone appeared to slow. Therefore the DT_{50} was estimated using the entire data set (0 to 182 days) and the DT_{90} value was estimated visually. The best fit first order DT_{50} value of chlorophacinone in soil was determined to be 47.3 days for a sandy clay loam soil. The corresponding DT_{90} value was > 200 days. To reflect an average EU outdoor temperature of 12°C the degradation rate has been converted using the Arrhenius equation with a default activation energy of 54.0 kJ/mol. Converted to a temperature of 12°C the DT_{50} value for Buckeystown sandy clay loam soil was 128 days. The degradation rate in the repeat soil samples was slightly quicker than that observed for the original soil samples. The DT_{50} and DT_{90} values were estimated using a similar procedure to that described above, see Table A 7.2.1-4. However as the repeat analysis was mainly carried out to improve the mass balance recovered, the degradation rate for these samples is not discussed further.	

Section A 7.2.1-01 **Annex Point IIIA VII.4, XII.1.1**	**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The route and rate of aerobic degradation of ^{14}C-chlorophacinone was investigated one soil (sandy clay loam) of US origin in the dark under laboratory conditions at a temperature of 24 to 26°C and moisture content of 75% field capacity (1/3 bar moisture). To further investigate the effect of the experimental design on the recovery of evolved volatile components, the experiment was repeated using a fresh batch of soil sourced from the same location (textural classification on re-sampling was sandy loam). The GLP study was conducted to the US EPA Guidelines (162-1) in 1994.	
5.3 Results and discussion	Recovery of applied radioactivity from the original soil samples ranged from 72 to 101% AR (average 92%). The recovery of the evolved volatile components was improved following modifications to the experimental design. The majority of the applied radioactivity was extractable from the soil at all sampling intervals. Significant levels of carbon dioxide were evolved and were potentially as high as 65% after 182 days. The level of soil NER observed did not exceed 11% AR. The level of chlorophacinone observed steadily declined with a biphasic degradation profile. A DT_{50} value of 128 days was determined for the original soil samples, at an equivalent temperature of 12°C. Degradation of chlorophacinone did not lead to the formation of any significant metabolites (i.e. > 10% AR). Several minor metabolites (i.e.< 10% AR) were observed.	
5.4 Conclusion	Chlorophacinone steadily degraded in soil under aerobic conditions, with an equivalent DT_{50} value of 128 days (12°C). Degradation of chlorophacinone did not lead to the formation of any significant metabolites (i.e. > 10% AR). Significant levels of carbon dioxide were evolved, up to 65% AR after 182 days.	
5.4.1 Reliability	2.	
5.4.2 Deficiencies	Yes. The study contained some deficiencies when compared to modern day standards. These deficiencies are discussed in more detail where appropriate under the relevant headings above and are not considered to have adversely affected the quality of the results.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A 7.2.1-01 Annex Point IIIA VII.4, XII.1.1	**Aerobic degradation in soil (initial study)** 2 soil types, aerobic conditions	
Date	September 2006	
Materials and Methods	The study was performed to US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1.	
Results and discussion	In soil under dark aerobic conditions in the laboratory (12°C extrapolated from 25°C), chlorophacinone is degraded steadily with an estimated DT_{50} value of 128 days. Degradation of chlorophacinone results predominantly in the formation of carbon dioxide (61.0% AR after *ca* 100 days) (mineralization). Metabolites (including o-phthalic acid and p-chlorophenyl acetic acid) do not exceed 10% AR at any sampling interval. Soil non-extractable residue (NER) comprises 9.0% AR after *ca* 100 days.	
Conclusion		
Reliability	2	
Acceptability	Acceptable	
Remarks	In OECD 307 it is recommended to use at least three additional soils in order to determine the rates of transformation.	

Table A 7.2.1-1: Classification and physico-chemical properties of soils used as adsorbents

Soil name		**Soil 1**	**Soil 2 (repeat study)**
Source		XXXXXX USA Sandy clay loam	XXXXXX USA Sandy loam
Sampling date		31 May 1991	31 August 1992
Soil order		Ultisol	Ultisol
Soil series		XXXX	XXXX
Soil horizon		A (0 to 15 cm)	A (0 to 15 cm)
Textural classification, USDA		Sandy clay loam	Sandy loam
Sand (%)		55.79	71.0
Silt (%)		21.41	22.3
Clay (%)		22.80	6.7
Organic matter (%)		2.03	1.7
Organic carbon (%) [1]		1.18	0.99
pH		7.0	7.2
Cation exchange capacity (MEQ/100 g)		6.85	8.7
Moisture content, (g/100 g soil)			
75% FMC at 1/3 bar		17.75	20.4
Bulk density, (g/cm^3)		1.34	1.23
Microbial biomass (CFU/g soil) [2]			
pre-study	PCA	> 3.0 x 10^8	> 3.0 x 10^8
	RBA	3.6 x 10^4	1.3 x 10^5
	ACT	1.1 x 10^7	6.3 x 10^7
	THIO	< 1.0 x 10^4	> 3.0 x 10^8
70 days	PCA	n.a	> 3.0 x 10^8
	RBA	n.a	3.8 x 10^5
	ACT	n.a	9.6 x 10^7
	THIO	n.a	> 3.0 x 10^8
182 days	PCA	> 3.0 x 10^8	n.a
	RBA	> 3.0 x 10^6	n.a
	ACT	1.0 x 10^8	n.a
	THIO	1.5 x 10^7	n.a
182 days (sterile)	PCA	< 10	n.a
	RBA	< 10	n.a
	ACT	< 10	n.a
	THIO	< 10	n.a

n.d – not determined.

[1] Calculated as organic matter content ÷ 1.724.

[2] CFU (colony forming unit)
Culture plates were incubated (72 hours, except 182 day plates which were incubated for 9 days) at a temperature of 25°C.
Culture types were: PCA (plate count agar, total bacteria), RBA (rose bengal agar, total fungi), ACT (actinomycete isolation agar, total actinomycetes) and THIO (thioglycollate agar, total anaerobes).

Table A 7.2.1-2: Recovery and distribution of radioactivity from aerobic soil samples

Sampling times (days)	Soil components (% AR)				Volatile (% AR)	Total [1] (% AR)
	Extract [2]	Reflux [3]	Soil NER	(sub-total)	carbon dioxide [4]	
Sandy clay loam						
0	100	n.p.	0	(100)	n.a.	100
1	97	n.p.	3	(100)	1	101
3	87	n.p.	4	(91)	4 (9)	95
7	80	3	5	(88)	8 (12)	96
14	73	3	6	(82)	13 (18)	95
21	67	7	4	(78)	16 (23)	93
30	54	8	5	(67)	21 (33)	88
91	22	9	9	(40)	36 (61)	75
182	17	8	11	(36)	50 (65)	85
Sandy loam						
0	97	n.p.	0	(97)	n.a.	97
14	42	21	9	(72)	34	106
30	21	25	10	(56)	50	105
45	16	23	9	(48)	57	105
70	10	23	10	(43)	64	106
Sterile (sandy clay loam)						
30	100	n.p.	3	(103)	n.p.	103
182	96	n.p.	6	(102)	n.p.	102

n.a. = not analysed

All values are means of duplicate samples.

[1] The recovery of applied radioactivity from the individual samples of the original soil ranged from 72 to 101% AR (overall average 92%). The recovery of applied radioactivity from the individual samples of the repeat soil ranged from 95 to 107% AR (overall average 104%).

[2] Soil extractions consisted of x 3 ethanol/ water (90/10 v/v) and x 3 with acetone/ water (90/ 10 v/v).

[3] Soil reflux extractions consisted of x 1 ethanol/ water (50/50 v/v) and, where necessary, x 1 acetone/ water (50/50 v/v) and x 1 acetone/ water / phosphoric acid (70/30/1 v/v/v). An additional extraction using ultrasonication using ethanol/ 2N sodium kydroxide (1/1 v/v) was also sometimes performed.

[4] Values in parentheses are maximum values of carbon dioxide potentially evolved, allowing for complete recovery.

Table A 7.2.1-3: Profile of radioactivity extracted from aerobic soil samples

Sample times (days)	Soil components (% AR)					
	Chloro-phacinone	Met 1	Met 2	Unknowns	Origin	Total
Sandy clay loam						
0	100.0	0.0	0.0	< 0.1	< 0.1	100.0
1	95.8	0.6	0.2	< 0.1	< 0.1	96.5
3	86.4	0.0	0.3	< 0.1	0.1	86.8
7	80.0	0.7	2.0	< 0.1	< 0.1	82.6
14	74.0	0.5	1.5	< 0.1	0.1	75.9
21	69.1	0.5	3.1	0.3	0.2	73.1
30	56.8	0.7	3.4	0.4	0.2	61.5
91	23.6	1.8	5.1	0.1	< 0.1	30.5
182	17.8	1.1	4.5	0.4	0.1	23.8
Sandy loam						
0	92.4	3.9	0.2	< 0.1	0.2	96.6
14	41.1	5.4	7.7	< 0.1	8.9	63.0
30	26.4	1.5	7.0	0.8	10.0	45.6
45	19.7	1.1	9.0	< 0.1	9.4	39.1
70	12.7	1.5	8.9	< 0.1	9.5	32.5
Sandy loam (sterile)						
30	99.0	0.3	0.3	< 0.1	< 0.1	99.5
182	94.1	1.0	0.2	< 0.1	0.2	95.5

n.d – not detected.
Met 1 - p-chlorophenyl-phenyl acetic acid.
Met 2 - o-phthalic acid.

Figure A 7.2.1-1: Postulated degradation pathway for chlorophacinone in aerobic soil

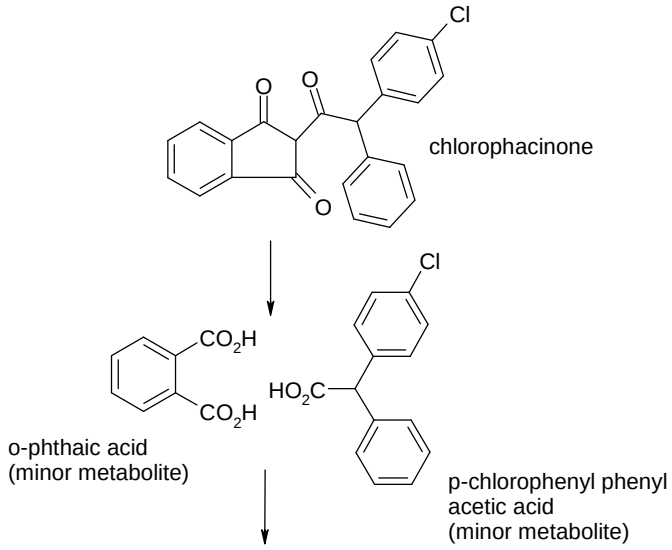


Figure A 7.2.1-2: Re-calculation of DT_{50} value for Buckeystown soil (Soil 1) using first-order kinetics

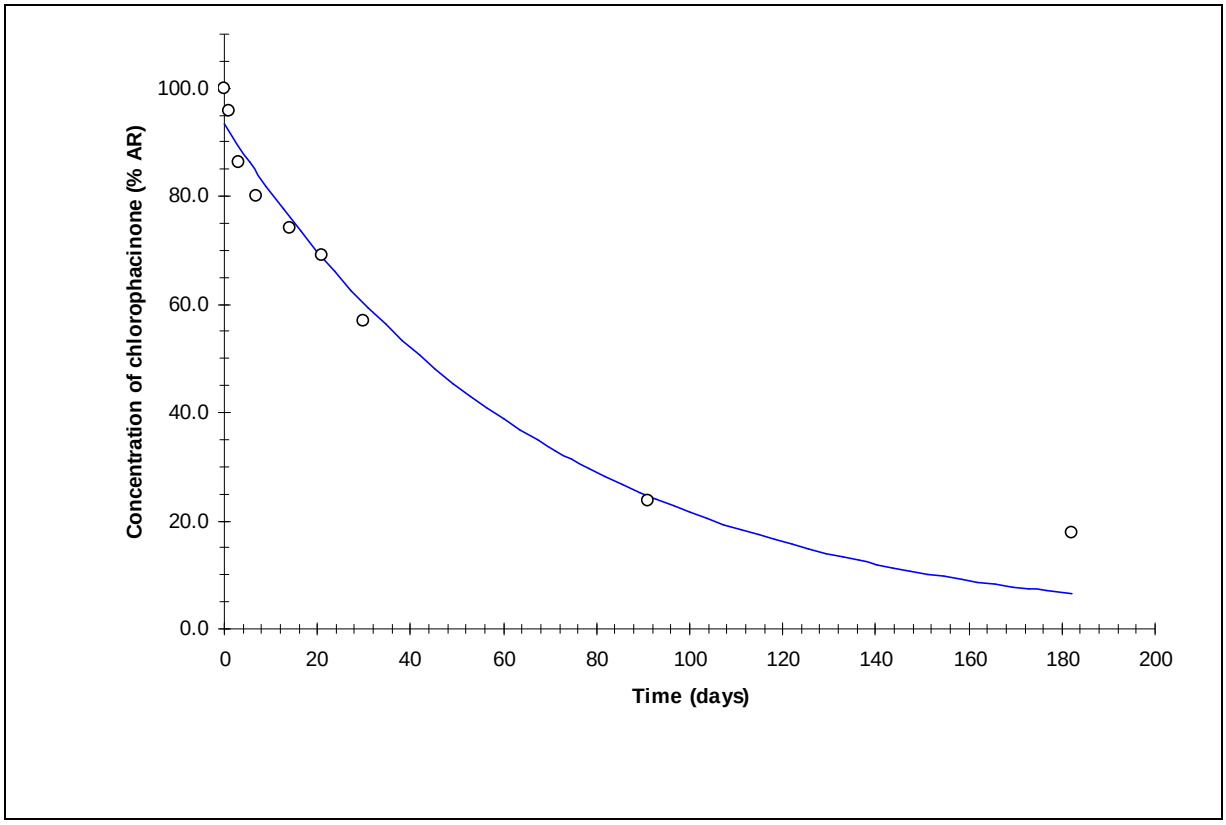


Figure A 7.2.1-3: Re-calculation of DT_{50} value for Buckeystown soil (Soil 2) using first-order kinetics

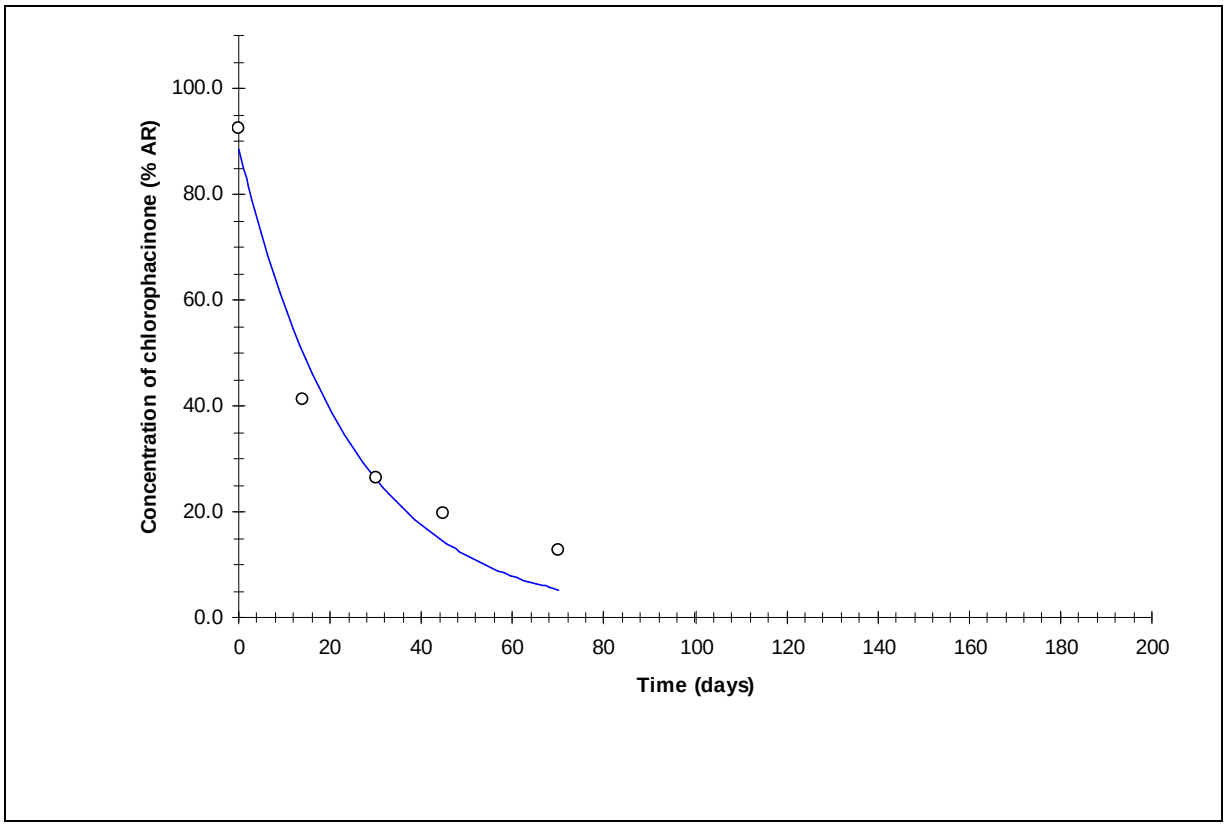


Figure A 7.2.1-3: Re-calculation of DT_{90} value for Buckeystown soil (Soil 2) using first-order kinetics

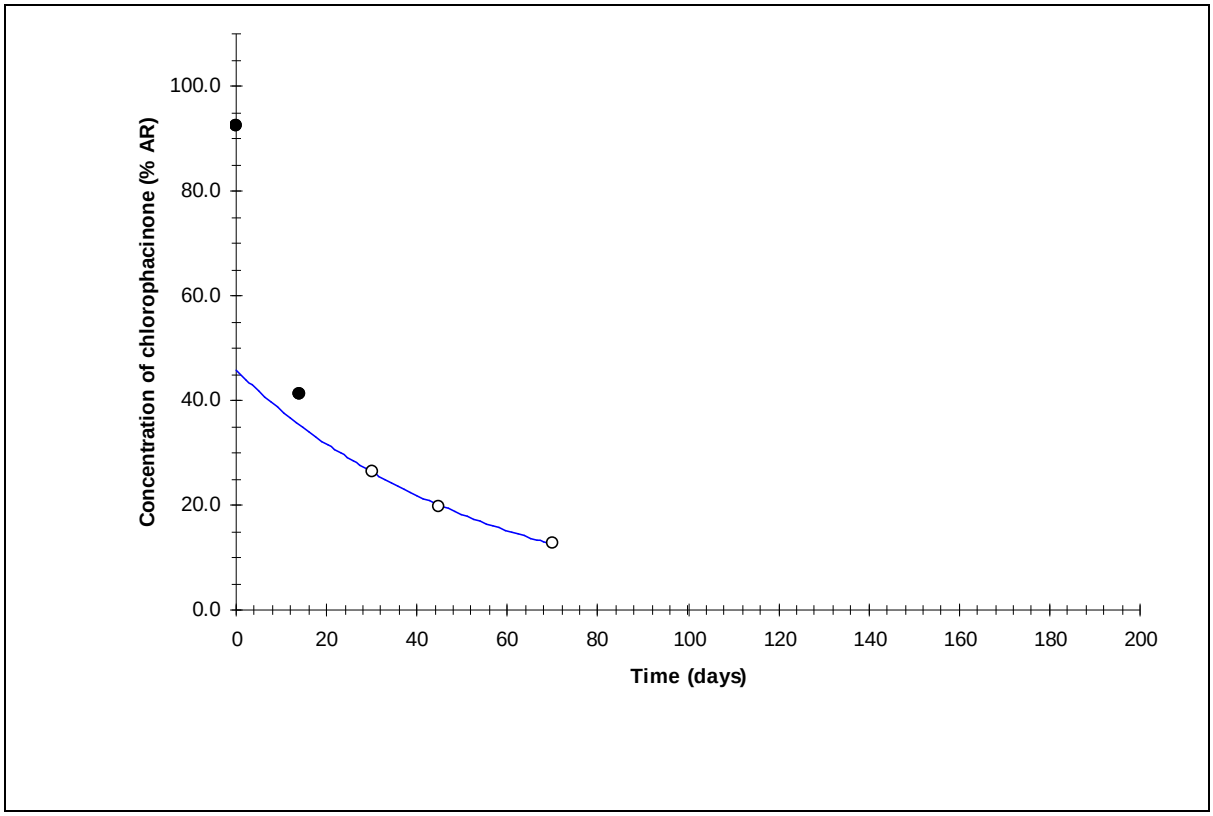


Table A 7.2.1-4: $DT_{50(lab)}$ and $DT_{90(lab)}$ values for the rate of aerobic degradation of chlorophacinone in soil

Soil type	Data range (days)	$DT_{50(lab)}$ (days)	$DT_{90(lab)}$ (days)	Regression parameters		
				C_0	k	R^2
Soil 1 (sandy clay loam)	0 to 182	47.3	> 200 [1]	93.508	0.01466	0.967
Soil 2 (sandy loam)	0 to 70	17.1	-	88.633	0.04063	0.955
	30 to 70	-	125 [1]	45.745	0.01846	0.999

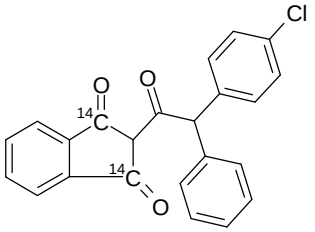
The soil was incubated at a temperature of 25°C and a moisture content of 75% 0.33 bar moisture.

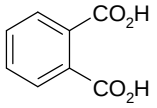
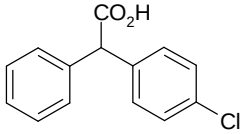
[1] DT_{50} (or DT_{90}) value was not demonstrated experimentally, result obtained by extrapolation.

Section A 7.2.2.1-01 Annex Point IIIA VII.4, XII.1.1, XII.1.4	Route and rate of degradation in three soils under appropriate conditions	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Although the DT_{50} value of chlorophacinone in soil, determined in the study described under Section A 7.2.1-01, is 128 days at a temperature of 12°C, the PEC/PNEC for soil is > 1. Further, as described under Section A 7.2.3.2, it is considered unlikely that chlorophacinone or any metabolite of chlorophacinone will move through the soil profile in significant quantities. Consequently, further laboratory soil degradation studies have not been performed.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification	Acceptable	
Conclusion	It is not considered necessary to perform this test.	
Remarks		

Section A 7.2.2.2-01 Annex Point IIIA XII.1.1, Annex VI, para. 85	Field soil dissipation and accumulation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The time taken for dissipation of 50% and 90% ($DT_{50field}$ and $DT_{90field}$) of the active substance under field conditions can be sufficiently estimated using the laboratory data described under Section A 7.2.1. Due to the low soil exposure of the active substance, the restricted usage conditions, accumulation in the field is not expected to be significant.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks		

Section A 7.2.2.3-01 Annex Point IIIA XII.1.4	Extent and nature of bound residues	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Under the laboratory study described under Section A 7.2.1, only minor levels of non extractable residues (NER) were observed (< 10% AR after 91 days). Therefore, due to the low soil exposure of the active substance, the restricted usage conditions, further investigations into the extent and nature of bound residues have not been performed.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks		

Section A 7.2.2.4-01 **Annex Point IIIA XII.1.1**		**Other soil degradation studies** Photo-degradation on a soil surface	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxx, X., XXX, Soil photolysis, XXXXXXXXXXXXXX laboratory report no. XXX, 10 August XXX (unpublished). Section no. : A 7.2.2.4-01.	
1.3	**Data protection**	Yes	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. The study was performed to US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No. No specific guideline is recommended for this study design. Apart from minor deviations, this study was conducted to generally accepted guidelines (SETAC, BBA or US EPA) for this study type.	
		3 MATERIALS AND METHODS	
3.2	**Test material (radiolabelled)**	As given in section 2. Chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl) indan-1, 3-dione. chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	Lot no. XXXXX.	
3.2.2	Specification	Specific activity 4.23 mCi/mmol.	
3.2.3	Purity	RCP (radiochemical purity) > XX% by 2D TLC systems.	
3.2.4	Further relevant properties	Position of radiolabel given below: 	
3.2.5	Method of analysis	RCP determined prior to use by TLC analysis, conditions specified in Section 3.5.6.	

Section A 7.2.2.4-01 **Annex Point IIIA XII.1.1**		**Other soil degradation studies** Photo-degradation on a soil surface	
3.3	**Test material (non radiolabelled)**	As given in section 2. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.3.1	Lot/Batch number	Lot no XXXXXX.	
3.3.2	Specification	No further details.	
3.3.3	Purity	> XX%.	
3.3.4	Further relevant properties	Not applicable.	
3.4	**Reference material (phthalic acid)**	o-Phthalic acid (IUPAC): 1,2-Benzenedicarboxylic acid. o-Phthalic acid (CAS): o-Benzenedicarboxylic acid.	
3.4.1	Lot/Batch number	Lot no. not specified.	
3.4.2	Specification	No further details.	
3.4.3	Purity	100%, assigned (non radiolabelled reference standard used for qualitative analysis only).	
3.4.4	Further relevant properties	Structure below:  	
3.5	**Reference material (chlorophenyl-phenyl acetic acid)**	p-Chlorophenyl-phenyl acetic acid (IUPAC): Not available. p-Chlorophenyl-phenyl acetic acid (CAS): Not available.	
3.5.1	Lot/Batch number	Lot no JB3653.	
3.5.2	Specification	No further details.	
3.5.3	Purity	100%, assigned (non radiolabelled reference standard used for qualitative analysis only).	
3.5.4	Further relevant properties	Structure below:  	
3.6	**Test performance**	The route and rate of photo-degradation of ^{14}C-chlorophacinone was investigated on a soil surface (sandy clay loam) exposed to an artificial light source.	
3.6.1	Test soils	The test soil was sampled from an agricultural field (of US origin) and was sieved (2 mm) and stored (25°C) under moist conditions prior to use. The characterisation details of the soil are given in Table A 7.2.2.4-1. The microbial viability of the soil was determined at the start of the study and after 30 days exposure. Sterile samples were prepared by autoclaving soil (2 g dry weight) directly in the glass vials for 1 hour on two consecutive working days at 15 psi and 121°C.	

Section A 7.2.2.4-01 **Annex Point IIIA XII.1.1**	**Other soil degradation studies** Photo-degradation on a soil surface	
3.6.2 Treatment to soil samples	Soil samples (2 g dry weight, depth 2 to 3 mm, prepared in quartz glass test tubes) at a moisture content of 75% 1/3 bar moisture content. Soil samples were treated with ^{14}C-chlorophacinone (*ca* 0.022 mg) dissolved in acetone (20 µL). The resulting treatment level corresponded to 10.8 mg/kg (the surface area of the glass vial was not specified). The purity of the test material was confirmed before the treatment.	
3.6.3 Incubation of soil samples	Following treatment, the quartz glass vials sealed with Teflon coated rubber stoppers. Sample tubes were placed in the test apparatus as described in Table A 7.2.2.4-2. Dark control soil samples were wrapped in foil and incubated in a laboratory incubator at 25°C.	
3.6.4 Properties of light source	An artificial light source was used, details are summarised in Table A 7.2.2.4-2.	
3.6.5 Sampling	Duplicate soil samples were taken for analysis at intervals over a period of 30 days (0, 1, 2, 3, 5, 9, 14, 21 and 30 days). Soil samples were extracted (x 3, 30 mins) with ethanol/ water (90/10 v/v) and (x 3, 30 mins) with acetone/ water (90/ 10 v/v) using ultrasonication and separated by centrifugation. Reflux extractions were performed using ethanol/ water (90/10 v/v, 2 hours). Further extractions were carried out, as necessary, by soxhlet using acetone/ water/ phosphoric acid (70/30/1 v/v/v, 1 hour) and ultrasonication using 6N sodium hydroxide (30 mins) at an elevated temperature (70°C). The radioactivity content of the soil extracts and non-extracted soil residue (NER) were quantified using LSC and combustion analysis respectively. At each sampling interval, the headspace from each sealed quartz glass test tube was sampled by syringe and passed through a series of trapping solutions (ethylene glycol plus 2 x potassium hydroxide) to attempt to recover evolved volatile components.	
3.6.6 Chromatographic analysis	Soil extracts were analysed chromatographically by 2D-TLC using silica plates (0.25 mm) developed in methanol/ acetic acid (80/20 v/v) followed by acetone/ diethylamine (90/10 v/v). Non radiolabelled reference standards were visualised using UV light (254 nm). Radioactive regions were quantified using a plate scanner (Ambis radioanalytical imaging system). Confirmatory analysis on selected samples was conducted by HPLC using a reverse phase gradient system (Shimadzu LC-6A, SPD-6A UV detector and Ramona-5-LS radioactivity detector). Non radiolabelled test material was used as authentic reference standard.	
	4 RESULTS	

Section A 7.2.2.4-01 Annex Point IIIA XII.1.1	Other soil degradation studies Photo-degradation on a soil surface	
4.2 Recovery and distribution	Determinations of microbial biomass activity indicated that the soil was viable for the duration of the study. The recovery and distribution of radioactivity from the soil samples is summarised in Table A 7.2.2.4-3. The recovery of applied radioactivity from the individual exposed soil samples ranged from 42.1 to 122.7% AR (average 88.6%) over the entire study period (i.e. 30 days). Over the period 0 to 5 days, the recovery of applied radioactivity from the individual samples ranged from 95.9 to 122.7% AR (average 107%). From 5 days and onwards the recovery of applied radioactivity declined from 97.9% to 49.5% AR, this decline is considered due to incomplete recovery of evolved volatile components (i.e. carbon dioxide) due to inadequacies in the experimental design (see trapping procedure described in Section 3.6.5. The recovery of applied radioactivity from the individual dark control soil samples ranged from 94.3 to 121.0% AR (average 106%), indicating a complete mass balance for these sample types. The majority of the applied radioactivity was extractable from the soil and the levels observed steadily declined from 97.8% AR initially to 44.1% after 30 days. The amount of soil NER observed was minimal and accounted for a maximum of 1.5% AR in the exposed samples. Evolved volatile components were potentially significant (*ca* 50%).	
4.3 Profile of components	The profile of components extracted from the soil samples is summarised in Table A 7.2.2.4-4. Chromatographic analysis using 2D-TLC indicated that chlorophacinone was quickly photo-degraded on a soil surface. Degradation of chlorophacinone led to the formation of 1 significant metabolite (i.e. > 10% AR), o-phthalic acid which was observed at a maximum level of 37.1% AR after 5 days. At least 3 other minor metabolites (i.e. < 10% AR) were observed. Significant amounts of carbon dioxide were potentially evolved.	
4.4 Route of degradation	Based on the metabolites observed in the chromatographic profile of the soil extracts, as described in Section 4.3, a tentative degradation pathway is proposed in Figure A 7.2.2.4-01.	
4.5 Rate of degradation	The rate of photo-degradation of chlorophacinone was recalculated using the procedure described in Section A 7.2.1-01. The recalculation of the DT_{50} and DT_{90} values is presented graphically in Figures A 7.2.2.4-2 and 7.2.2.4-3 and summarised in Table A 7.2.2.4-5. The photo-degradation of chlorophacinone, at a temperature of *ca* 25°C, gave a reasonable correlation to first-order kinetics, however using the whole data set i.e. 0 to 30 days it appeared visually that the DT_{90} value would be	

Section A 7.2.2.4-01 Annex Point IIIA XII.1.1	Other soil degradation studies Photo-degradation on a soil surface	
	underestimated. Therefore the DT_{50} and DT_{90} values were estimated by applying first-order kinetics to portions of the data sets. The DT_{50} value was determined using the data for 0 to 5 days. The DT_{90} values were determined using the data for 5 to 30 days. The DT_{50} and DT_{90} values for the degradation in the dark controls were determined using the entire data set. The best fit first order DT_{50} value of chlorophacinone in soil, corrected for the degradation observed in the dark controls was determined to be 4.1 days. The corresponding DT_{90} value was 32.1 days. To reflect an average EU outdoor temperature of 12°C the degradation rates have been converted using the Arrhenius equation with a default activation energy of 54.0 kJ/mol. Converted to a temperature of 12°C the DT_{50} and DT_{90} values for the photo-degradation of Buckeystown sandy clay loam soil are 11.1 and 86.8 days, respectively.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The route and rate of photo-degradation of ^{14}C-chlorophacinone was investigated on a soil surface (sandy clay loam) exposed to an artificial light source. The GLP study was conducted to the US EPA Guidelines 161-3 in 1992.	
5.3 Results and discussion	The recovery of applied radioactivity from the individual exposed soil samples ranged from 42.1 to 122.7% AR (average 88.6%) over the entire study period (i.e. 30 days). However, due to inadequacies in the experimental design, this was considered due to incomplete recovery of evolved carbon dioxide. The majority of the applied radioactivity was extractable from the soil at all sampling intervals. Significant levels of carbon dioxide were evolved, up to potentially *ca* 50%. The level of soil NER observed did not exceed 1.5% AR in the exposed samples. Photo-degradation of chlorophacinone on a sandy clay loam soil surface proceeded with a biphasic degradation profile. A DT_{50} value of 11.1 days was determined at an equivalent temperature of 12°C. Photo-degradation of chlorophacinone led to the formation of one significant (i.e. > 10% AR) metabolite, o-phthalic acid which was observed at a maximum level of 37.1% AR after 5 days. At least 3 other minor metabolites (i.e.< 10% AR) were observed.	
5.4 Conclusion	Chlorophacinone quickly photo-degraded on a soil surface when exposed to an artificial light source, with an equivalent DT_{50} value of 11.1 days (12°C). Degradation of chlorophacinone led to the formation of	

Section A 7.2.2.4-01 **Annex Point IIIA XII.1.1**	**Other soil degradation studies** Photo-degradation on a soil surface	
	significant amounts of the metabolite o-phthalic acid and carbon dioxide.	
5.4.1 Reliability	2.	
5.4.2 Deficiencies	Yes. The study contained some deficiencies when compared to modern day standards. These deficiencies are discussed in more detail where appropriate under the relevant headings above and are not considered to have adversely affected the quality of the results.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods	US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3.	
Results and discussion	The best fit first order DT_{50} value of chlorophacinone in soil at 25ºC, corrected for the degradation observed in the dark controls was determined to be 4.1 days. The corresponding DT_{90} value was 32.1 days. Photolysis of chlorophacinone on a soil surface proceeds rapidly with a DT_{50} of 11.1 days at an equivalent temperature of 12°C. Degradation of chlorophacinone results in the formation of a major metabolite o-phthalic acid (37.1% AR), carbon dioxide (potentially 50% AR) and three minor degradation products (< 10% AR).	
Conclusion		
Reliability	2	
Acceptability	Acceptable	
Remarks		

Table A 7.2.2.4-1: Classification and physico-chemical properties of soils used as adsorbents

Soil name	**Soil 1**
Source	XXXXXXXXX USA
Textural classification, USDA	Sandy clay loam
Sand (%)	55.79
Silt (%)	21.41
Clay (%)	22.80
Organic matter (%)	2.03
Organic carbon (%) [1]	1.18
pH	7.0
Cation exchange capacity (MEQ/100 g)	6.85
Moisture content, (g/100 g soil) 75% FMC at 1/3 bar	17.75
Bulk density, (g/cm^3)	1.34

Microbial biomass (CFU/g soil) [2]		
pre-study	PCA	> 3.0 x 10^8
	RBA	3.6 x 10^4
	ACT	1.1 x 10^7
	THIO	< 1.0 x 10^4

[1] Calculated as organic matter content ÷ 1.724.

[2] CFU (colony forming unit)
Culture plates were incubated (72 hours) at a temperature of 25°C.
Culture types were: PCA (plate count agar, total bacteria), RBA (rose bengal agar, total fungi), ACT (actinomycete isolation agar, total actinomycetes) and THIO (thioglycollate agar, total anaerobes).

Table A 7.2.2.4-2: Description of test system

Criteria	**Details**
Laboratory equipment	Soil samples in quartz glass test tubes were positioned horizontally in the test apparatus. Temperature was monitored using a thermocouple inside one of the sample tubes. Temperature of the sample tubes was regulated using chilled liquid coolant. A diagram is supplied in the study report.
Test apparatus	Heraeus Suntest unit, model CPS.
Properties of artificial light source:	Artificial light source used.
Nature of light source	Xenon lamp
Emission wavelength spectrum	290 to 800 nm.
Light intensity	During the exposure period the lamp intensity ranged from 3.7 4.3 x 10^5 W/cm^2 (lamp rated at 400 to 765 W/m^2). Natural sunlight on a clear sunny day at the test facility provided an intensity of 3.0 to 3.6 x 10^{-5} W/cm^2. Intensity of the light source was recorded using a full spectrum International Light Meter Model 1700 with SED 623 detector and a UVP Blak-Ray ultraviolet meter. Exposure cycle consisted of a 12 hour light/ dark exposure periods. Each exposure period of 12 hours was considered equivalent to 1 day sunlight exposure.
Filters	A UV filter was used to remove radiation below 290 nm.
Dark control samples:	Similar dark control samples were covered in foil and incubated separately in a laboratory incubator at the same temperature as the exposed samples.

Table A 7.2.2.4-3: Recovery and distribution of radioactivity from soil samples exposed to artificial light

Sampling times (days)	Soil components (% AR)				Volatile (% AR)	Total [1] (% AR)
	Extract	Reflux	Soil NER	(sub-total)	carbon dioxide [2]	
Exposed samples						
0	97.8	n.p.	2.5	(100.3)	n.a.	100.3
1	86.0	30.2	0.8	(116.9)	0.9	117.7
2	62.2	42.2	1.4	(105.8)	1.5	107.4
3	69.0	37.1	1.5	(107.6)	2.0	109.6
5	49.4	44.3	1.7	(95.3)	2.6	97.9
9	39.6	43.9	1.3	(84.7)	3.0 (12.3)	87.7
14	26.3	43.4	1.5	(71.1)	3.4 (25.6)	74.5
21	16.3	31.1	1.5	(48.8)	3.9 (46.8)	53.3
30	12.4	31.7	1.2	(45.2)	4.3 (50.5)	49.5
Dark controls						
0	97.8	n.p.	2.5	(100.3)	n.a.	100.3
1	96.6	n.p.	6.3	(102.9)	0.6	103.5
2	99.6	n.p.	7.4	(107.0)	1.1	108.1
3	94.1	15.7	3.0	(112.8)	1.4	114.2
5	92.8	8.9	1.9	(103.6)	1.8	105.4
9	88.3	16.3	14.4	(119.0)	2.0	121.0
14	86.0	11.1	2.9	(100.0)	2.3	102.3
21	82.5	18.8	2.7	(104.0)	2.5	106.5
30	75.8	12.6	3.0	(91.4)	2.9	94.3
Sterile						
30 (exposed	38.7	38.3	3.1	(80.1)	0.6	80.7
30 (dark control)	91.9	4.4	4.3	(100.6)	0.4	101.0

n.a. = not analysed

The values for the exposed samples are means of duplicate samples. The values for the dark controls are from single samples.

[1] For the exposed samples, the recovery of applied radioactivity from the individual samples ranged from 95.9 to 122.7% AR (average 107%) for the first 5 days and from 42.1 to 122.7% AR (average 88.6%) for 0 to 30 days overall. For the dark control samples the recovery of applied radioactivity from the individual samples ranged from 94.3 to 121.0% AR (average 106%) for 0 to 30 days overall.

[2] Values in parentheses are potential amounts allowing for incomplete recovery of carbon dioxide.

Table A 7.2.2.4-4: Profile of radioactivity extracted from aerobic soil samples

Sample times (days)	Soil components (% AR)						
	Chloro-phacinone	Met 1	Met 2	Unknowns		Origin	Total
				Unk 1	Unk 2		
Exposed samples							
0	97.4	n.d	0.4	n.d	n.d	n.d	97.8
1	84.1	2.8	22.4	3.5	1.2	0.1	114.1
2	63.3	3.0	31.4	1.2	2.8	n.d	101.5
3	62.3	4.1	31.1	4.2	1.9	n.d	103.4
5	41.0	3.5	37.1	6.6	2.6	n.d	90.7
9	38.1	3.0	31.4	4.2	2.0	n.d	78.7
14	25.8	2.1	33.3	1.8	2.2	0.1	65.1
21	15.1	1.9	21.2	1.6	2.4	0.2	42.3
30	13.1	2.3	20.8	1.6	1.6	n.d	39.3
Dark controls							
0 [2]	97.4	n.d	0.4	n.d	n.d	n.d	97.8
1	95.8	0.5	n.d	n.d	n.d	0.3	96.6
2	99.5	n.d	n.d	n.d	n.d	0.1	99.6
3	101.2	0.8	5.3	1.6	0.7	0.1	109.7
5	92.8	1.0	6.2	1.4	0.3	n.d	101.7
9	87.4	2.0	8.1	5.9	1.3	n.d	104.7
14	82.8	0.6	5.6	6.5	0.8	n.d	96.3
21	88.1	0.1	6.7	5.8	0.7	n.d	101.4
30	78.8	0.5	5.7	2.8	0.7	n.d	88.5
Sterile							
30 (exposed	39.8	2.5	27.6	1.2	3.8	n.d	74.8
30 (dark control)	87.6	n.d	n.d	6.3	n.d	1.0	94.9

n.d – not determined.
Met-1 p-Chlorophenyl-phenyl acetic acid.
Met-2 o-Phthalic acid.

Figure A 7.2.2.4-1: Postulated photo-degradation pathway for chlorophacinone on a soil surface

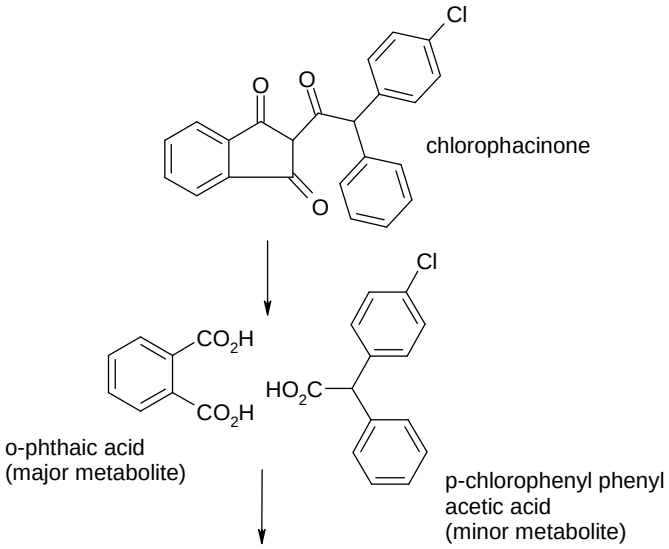


Figure A 7.2.2.4-2: Re-calculation of DT_{50} value for Buckeystown soil exposed to artificial light using first-order kinetics

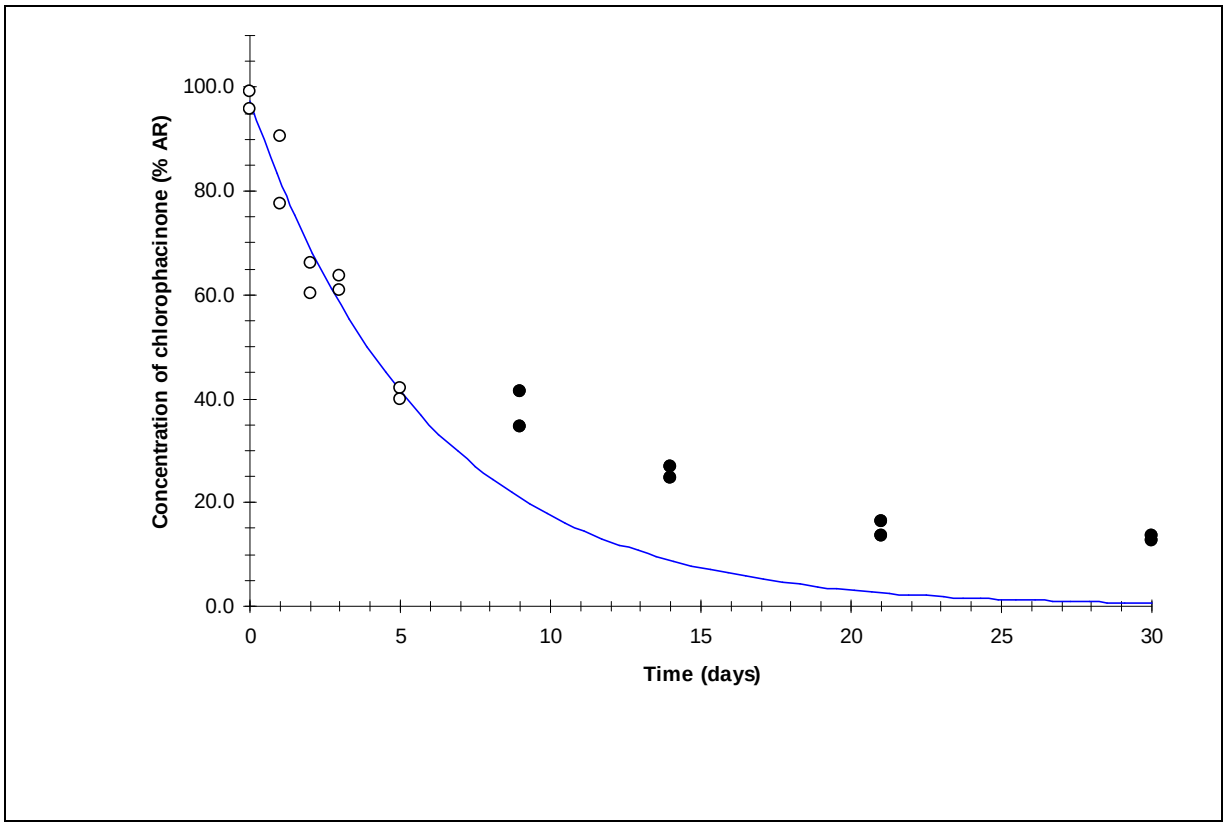


Figure A 7.2.2.4-3: Re-calculation of DT_{90} value for Buckeystown soil exposed to artificial light using first-order kinetics

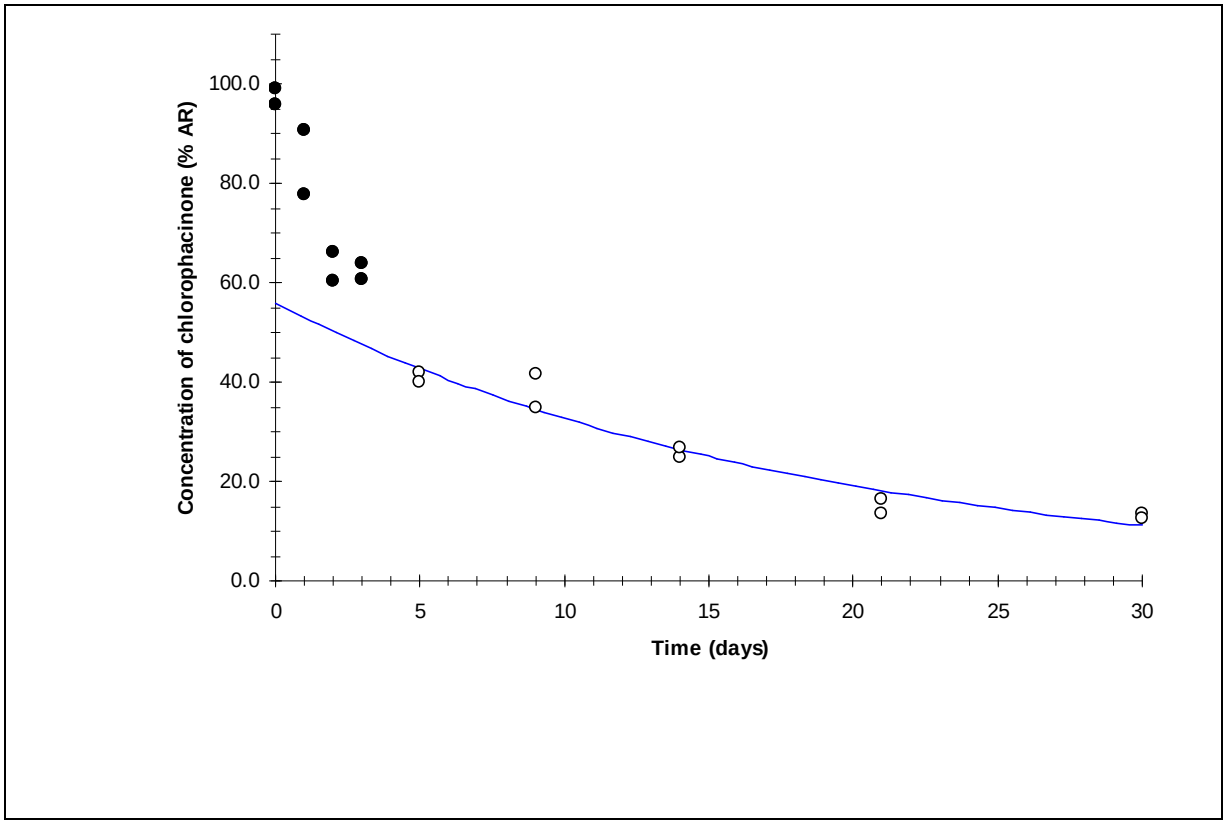


Figure A 7.2.2.4-4: Re-calculation of DT_{50} value for dark control Buckeystown soil using first-order kinetics

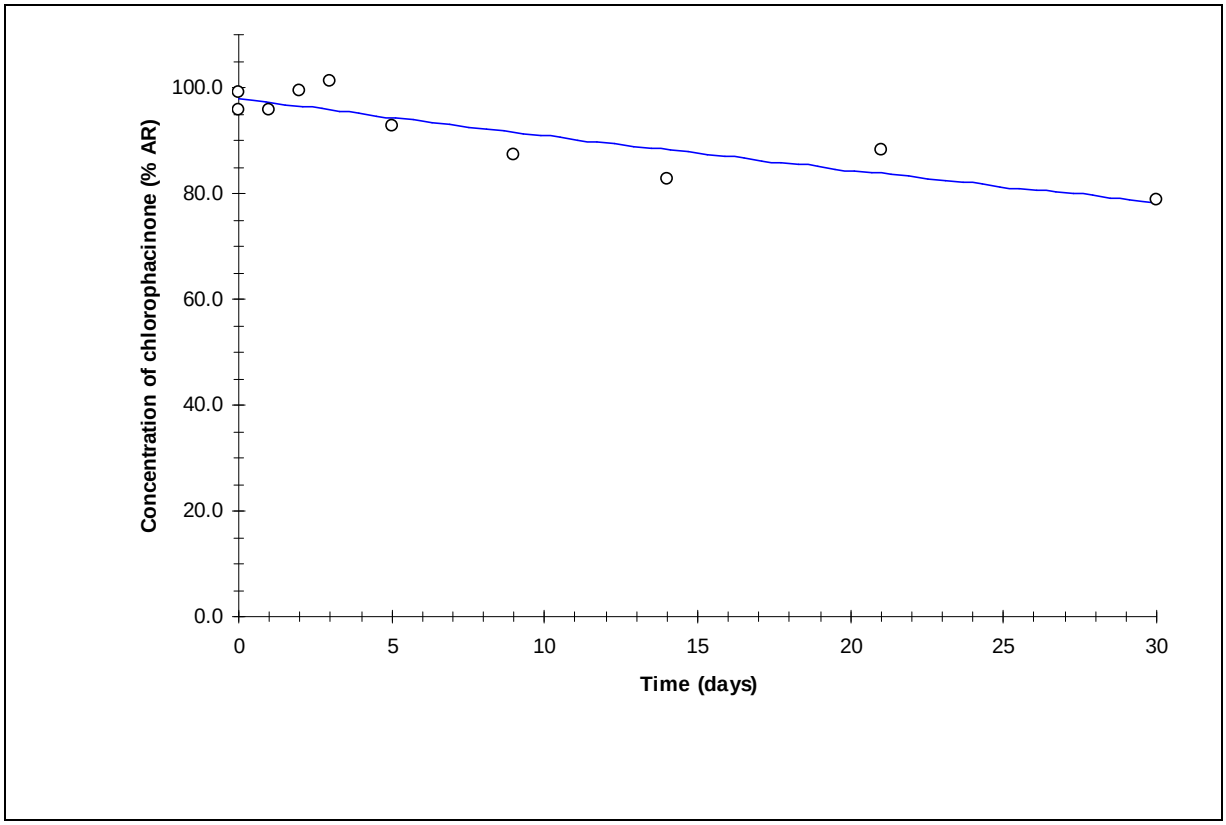


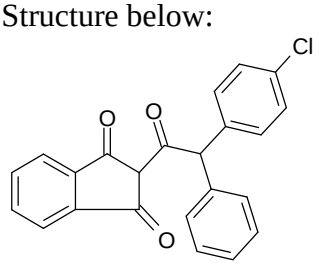
Table A 7.2.24-5: $DT_{50(lab)}$ and $DT_{90(lab)}$ values for the rate of photo-degradation of chlorophacinone on a soil surface

Soil type	Data range (days)	$DT_{50(lab)}$ (days)	$DT_{90(lab)}$ (days)	Regression parameters		
				C_0	k	R^2
Exposed	0 to 5	4.1	--	97.316	0.17120	0.943
	5 to 30	--	32.1	55.821	0.05358	0.932
Dark controls	0 to 30	93.4	310	97.873	0.00743	0.776
Photo-degradation in exposed samples corrected for dark controls	--	4.2	49.9	--	0.04615	--

The soil was incubated at a temperature of 23 to 25.4°C.

Section A 7.2.3.1-01 Annex Point IIIA XII.1.2	Adsorption / desorption (OECD 106) including metabolites	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	A separate adsorption / desorption study was not conducted as a complete investigation (including the determination of the Freundlich adsorption isotherms) was conducted in the study described under Section A 7.1.3-01. In the study described under Section A 7.2.1, degradation of chlorophacinone did not lead to the formation of any significant degradation products. Therefore, further adsorption/desorption studies on any soil metabolites are not required.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks		

Section A 7.2.3.2-01 Annex Point IIIA XII.1.3	Mobility in at least three soil types and where relevant mobility of metabolites and degradation products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Under the study described under Section A 7.1.3, the K_{oc} value for chlorophacinone in soil was ≥ 15600 mL/g. It is therefore considered that, even if present in soil, chlorophacinone would not be expected to leach through the soil profile in significant quantities. Furthermore, due to the low soil exposure of the active substance, the restricted usage conditions and the fact that degradation of chlorophacinone in soil does not lead to the formation of significant metabolites, it is considered unlikely that any metabolites of chlorophacinone would move through the soil profile in significant quantities.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks		

Section A 7.3.1-01 Annex Point IIIA VII.5		Phototransformation in air (estimation method)	
		1 REFERENCE	Official use only
1.2	Reference	Xxxx, XX., XXX, The estimation of photochemical oxidative degradation of chlorophacinone. XXXX, laboratory report no. XXXXXXXX, 21 January XXXX (unpublished). Section no. : A 7.3.1-01.	
1.3	Data protection	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	Guideline study	Not applicable. The calculation (QSAR estimation) was performed using the Atmospheric Oxidation Program v1.90 (AOPWIN).	
2.3	GLP	Not applicable (QSAR estimation).	
2.4	Deviations	No. The estimation was conducted using a widely accepted method (Atkins estimation based on structural relationships).	
		3 MATERIALS AND METHODS	
3.2	Test material	As given in section 2. chlorophacinone (IUPAC): 2-(2-(4-chlorophenyl)-2-phenylacetyl)indan-1, 3-dione chlorophacinone (CAS): 2-[(4-chlorophenyl)-phenylacetyl]-1*H*-indene-1, 3(2*H*)-dione.	
3.2.1	Lot/Batch number	Not applicable.	
3.2.2	Specification	Not applicable.	
3.2.3	Purity	Not applicable.	
3.2.4	Further relevant properties	Structure below:  Smiles notation : O=C3C(C(=O)c2c3cccc2)C(=O)C(c4ccc(cc4)CL)c1ccccc1.	
3.3	Reference substance	Not applicable.	

Section A 7.3.1-01 **Annex Point IIIA VII.5**	**Phototransformation in air (estimation method)**	
3.3.1 Initial concentration of reference substance	Not applicable.	
3.4 Testing procedure		
3.4.1 Calculation of half-lives	The photochemical oxidative degradation half-life of chlorophacinone in air was estimated using the Atmospheric Oxidation Program v1.90 (AOPWIN), which is based on the structural activity relationship (QSAR's) methods developed by Atkinson, R (1985 to 1996).	
	4 RESULTS	
4.2 Degradation of test substance	The half life and rate constant for the photochemical oxidative degradation of chlorophacinone in air via the hydroxyl reaction was estimated to be 14.3 hours and 9.00 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1}, respectively (based on 1.5 x 10^6 OH radicals per cm^3). Chlorophacinone does not have any olefinic or acetylenic bonds and therefore it is unlikely that there is a significant photochemical oxidative degradation of chlorophacinone in air via the ozone.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2 Materials and methods	The photochemical oxidative degradation half-life of chlorophacinone in air was estimated using the Atmospheric Oxidation Program v1.90 (AOPWIN), which is based on the structural activity relationship (QSAR's) methods developed by Atkinson, R (1985 to 1996).	
5.3 Results and discussion	The estimated half-life for the hydroxyl reaction in air is 14.3 hours. Furthermore, the vapour pressure of chlorophacinone as determined by OECD guideline no. 104 is 4.76 x 10^{-4} Pa (22.8°C) and Henry's law constant is 0.013725 $Pa.m^3.mol^{-1}$ (based on a water solubility of 13.0 mg/L). Therefore chlorophacinone is not expected to volatilise to air in significant quantities.	
5.4 Conclusion	Significant amounts of chlorophacinone are not likely to volatilise or persist in air.	
5.4.1 Reliability	1.	
5.4.2 Deficiencies	None.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods		
Results and discussion	The estimated half-life for the hydroxyl reaction in air is 14.3 hours.	

Section A 7.3.1-01 Annex Point IIIA VII.5	Phototransformation in air (estimation method)	
Conclusion	Furthermore, the vapour pressure of chlorophacinone as determined by OECD guideline no. 104 is 4.76 x 10^{-4} Pa (22.8°C) and Henry's law constant is 0.013725 $Pa.m^3.mol^{-1}$ (based on a water solubility of 13.0 mg/l). Therefore chlorophacinone is not expected to volatilise to air in significant quantities. In conclusion, significant amounts of chlorophacinone are not likely to volatilise or persist in air.	
Reliability	1	
Acceptability	Acceptable	
Remarks		

Section A7.4.1.1-01 Annex Point IIA VII.7.1		Acute toxicity to fish	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxxxx, XX. (XXXX). Chlorophacinone - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. XXXXXXXXXXXXXXXX laboratory report number XXXXXX, 18 May XXXX (unpublished).	
1.3	**Data protection**	Yes	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. US EPA FIFRA 72-1, comparable to OECD 203.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	None.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Chlorophacinone	
3.2.1	Lot/Batch number	XXXXXX	
3.2.2	Purity	XXX%	
3.2.3	Further relevant properties	Photolysis half-life of chlorophacinone in water is between 1 and 2 days, log octanol:water partition coefficient is 2.42 at pH 7, 23°C and solubility in water is 13 mg/l at 20°C.	**X**
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Stock solution prepared with 10 mg chlorophacinone/ml in acetone. Stock solution was then fed to a constant flow serial diluter where automated mixing with dilution water occurred prior to delivery to appropriate replicate test vessels.	
3.4	**Reference substance**	No.	
3.5	**Testing procedure**		
3.5.1	Dilution water	See Table A7.4.1.1-2.	
3.5.2	Test organisms	See Table A7.4.1.1-3.	**X**
3.5.3	Test system	See Table A7.4.1.1-4.	
3.5.4	Test conditions	See Table A7.4.1.1-5.	
3.5.5	Duration of the test	96 hours.	
3.5.6	Test parameter	Mortality and observations of toxicity.	
3.5.7	Sampling	Samples were taken from the control, low, mid and high	

Section A7.4.1.1-01 **Annex Point IIA VII.7.1**		**Acute toxicity to fish**	
		treatments for confirmatory analyses prior to test-start. Mid-water samples taken from each vessel at initiation and termination of the test.	
3.5.8	Monitoring of TS concentration	By HPLC.	
3.5.9	Statistics	LC_{50} by probit analysis.	
		4 RESULTS	
4.2	**Results test substance**		
4.2.1	Effect data (Mortality)	See Table A7.4.1.1-6.	
4.2.2	Other effects	See Table A7.4.1.1-6.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	None.	
4.4	**Test with reference substance**	Not performed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	Flow-through acute toxicity test with rainbow trout in accordance with OECD 203. Test media sampled at initiation and termination and analysed for chlorophacinone.	
5.3	**Results and discussion**		
5.3.1	LC_{50}	96-hour LC_{50} = 0.45 mg/l (95% confidence limits of 0.42 to 0.49 mg/l), based on mean measured concentrations.	**X**
5.4	**Conclusion**	See Table A7.4.1.1-9.	
5.4.1	Reliability	1	
5.4.2	Deficiencies	None.	
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		September 2006	

Section A7.4.1.1-01 Annex Point IIA VII.7.1	Acute toxicity to fish	
Materials and Methods	**3.1.3.** DT_{50} CPN photolysis = 2.2 days (buffer solution pH~7) and 1.3 (days pond water pH~8.4) natural summer sunlight at latitude 50°N, at 25ºC. US EPA FIFRA 72-1, comparable to OECD 203 (Fish, acute toxicity test). Twenty fish (Rainbow trout (*Oncorhynchus mykiss*)) (ten per replicate) were tested for 96 h under flow-through conditions. A preliminary test was conducted before in order to determine the toxically relevant range. All fish were fed a dry commercial pelleted food, *ad libitum*, daily except during the 48 h prior to and during the definitive test. No mortality ocurred in the fish test population during the two days prior to testing. **3.4.2.** Test organisms. Their age has not been reported.	
Results and discussion	Five nominal concentrations of the test material (1.0, 0.60, 0.36, 0.22 and 0.13 mg/l), a solvent control and a dilution water control. All the mean measured concentrations were above 80% of the nominal concetrations. The 96-hour LC_{50} value was 0.45 mg/l based on mean measured concentrations. $NOEC_{96\text{-}h}$ = 0.22 mg/l. **Comment: 5.2.1.** LC_0 = 0.22 mg/l and LC_{100} = 1.0 mg/l. 96 h. Mortality.	
Conclusion		
Reliability	1	
Acceptability	Acceptable	
Remarks	**Table A7.4.1.1-5**: Test conditions: Test solutions were not aerated.	

Table A7.4.1.1-1: Preparation of Test Substance solution for poorly soluble or volatile test substances

Criteria	Details
Vehicle	Acetone.
Concentration of vehicle	Maximum of 0.1 ml/l at highest chlorophacinone concentration.
Vehicle control performed	Yes, 0.1 ml acetone/l.

Table A7.4.1.1-2: Dilution water

Criteria	Details
Source	Well water.
Alkalinity	22 to 25 mg $CaCO_3$/l.
Hardness	25 to 27 mg $CaCO_3$/l.
pH	6.9 to 7.0
Conductivity	120 to 140 µmhos/cm.
Holding water different from dilution water	No.

Table A7.4.1.1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*).
Source	Commercial supplier in CA, USA.
Age/size	Mean wet weight 1.1 g, total length 36-54 mm.
Pretreatment	Holding period of at least 14 days under test conditions.
Feeding of animals during test	None. Feeding stopped 48 hours prior to test initiation.

Table A7.4.1.1-4: Test system

Criteria	Details
Test type	Flow-through.
Volume of test vessels	11 l, flow rate approximately 50 ml/min, giving *ca*. 6.5 volume turnovers/24 hours.
Volume/animal	1.1 l (0.15 g biomass/l).
Number of animals/vessel	10
Number of vessels/ concentration	2

Table A7.4.1.1-5: Test conditions

Criteria	Details
Test temperature	11 to 13°C
Dissolved oxygen	67 to 92% ASV
pH	6.7 to 7.2
Photoperiod	16 h daily

Table A7.4.1.1-6: Test substance concentrations

Nominal chlorophacinone concentration, mg/l	Measured concentration, mg/l					
	Initial (0 hour)		Final (96 hours)		Mean[1]	
Control	< LOD[2]	< LOD	< LOD	< LOD	< LOD	(-)
Acetone control	< LOD	< LOD	< LOD	< LOD	< LOD	(-)
0.13	0.19	0.10	0.096	0.098	0.12	(92)
0.22	0.26	0.26	0.17	0.17	0.21	(95)
0.36	0.44	0.45	0.32	0.33	0.39	(108)
0.60	0.67	0.62	0.56	0.42	0.57	(95)
1.0	1.1	1.0	0.82	0.85	0.94	(94)

[1] Based on original analytical data, not the rounded values presented for the 0 and 96 hour measurements. Values in brackets represent percentages of nominal concentrations;
[2] Below the limit of detection, 0.052 and 0.055 mg/l for 0 and 96 hour samples, respectively.

Table A7.4.1.1-7: Mortality data

Test Substance Concentration [mg/l]		Mean mortality (two replicates, each with 10 fish, per treatment)			
Nominal	**Mean measured**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	< LOD	0	0	0	0
Solvent control	< LOD	0	0	0	0
0.13	0.12	0	0	0	0
0.22	0.21	0	0	0[a]	0[a]
0.36	0.39	0[af]	5[agh]	10[a]	15[a]
0.60	0.57	5[abij]	60[ag]	85[ce]	95[d]
1.0	0.94	95[a]	100	100	100

[a] One or more survivors with darkened pigmentation;
[b] One or more survivors with darkened pigmentation and partial loss of equilibrium;
[c] One or more survivors with darkened pigmentation and complete loss of equilibrium;
[d] One or more survivors with darkened pigmentation and erratic swimming behaviour;
[e] One or more survivors lethargic with darkened pigmentation;
[f] One or more survivors with partial equilibrium loss;
[g] One or more survivors with complete equilibrium loss;
[h] One or more survivors swimming erratically;
[i] One or more survivors lethargic;
[j] One or more survivors lethargic with partial equilibrium loss.

Table A7.4.1.1-8: Effect data

Parameter	96 h [mg/l][1]	95 % c.l.
LC_{50}	0.45	0.42 to 0.49

[1] Based on mean measured concentrations.

Table A7.4.1.1-9: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fulfilled
Mortality of control animals <10%	**Yes**	-
Concentration of dissolved oxygen in all test vessels > 60% saturation	**Yes**	-
Concentration of test substance ≥80% of initial concentration during test	**Yes**	-

Section A7.4.1.1-02 Annex Point IIA VII.7.1		Acute toxicity to fish	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxxxx, XX (XXXX). Chlorophacinone - Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions. XXXXXXXXXXXXXXXX laboratory report number XXXXXX, 18 May XXXX (unpublished).	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. US EPA FIFRA 72-1, comparable to OECD 203.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	None.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Chlorophacinone	
3.2.1	Lot/Batch number	XXXXXX	
3.2.2	Purity	XXX%	
3.2.3	Further relevant properties	Photolysis half-life of chlorophacinone in water is between 1 and 2 days, log octanol:water partition coefficient is 2.42 at pH 7, 23°C and solubility in water is 13 mg/l at 20°C.	**X**
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Stock solution prepared with 2.5 mg chlorophacinone/ml in acetone. Stock solution was then fed to a constant flow serial diluter where automated mixing with dilution water occurred prior to delivery to appropriate replicate test vessels.	
3.4	**Reference substance**	No.	
3.5	**Testing procedure**		
3.5.1	Dilution water	See Table A7.4.1.1-11.	
3.5.2	Test organisms	See Table A7.4.1.1-12.	**X**
3.5.3	Test system	See Table A7.4.1.1-13.	
3.5.4	Test conditions	See Table A7.4.1.1-14.	
3.5.5	Duration of the test	96 hours.	
3.5.6	Test parameter	Mortality and observations of toxicity.	
3.5.7	Sampling	Samples were taken from the control, low, mid and high	

Section A7.4.1.1-02 Annex Point IIA VII.7.1		Acute toxicity to fish	
		treatments for confirmatory analyses prior to test-start. Mid-water samples taken from each vessel at initiation and termination of the test.	
3.5.8	Monitoring of TS concentration	By HPLC.	
3.5.9	Statistics	LC_{50} by probit analysis.	
		4 RESULTS	
4.2	**Results test substance**		
4.2.1	Effect data (Mortality)	See Table A7.4.1.1-15.	
4.2.2	Other effects	See Table A7.4.1.1-15.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	None.	
4.4	**Test with reference substance**	Not performed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	Flow-through acute toxicity test with bluegill sunfish in accordance with OECD 203. Test media sampled at initiation and termination and analysed for chlorophacinone.	
5.3	**Results and discussion**		
5.3.1	LC_{50}	96-hour LC_{50} = 0.71 mg/l (95% confidence limits of 0.63 to 0.83 mg/l), based on mean measured concentrations.	**X**
5.4	**Conclusion**	See Table A7.4.1.1-18.	
5.4.1	Reliability	1	
5.4.2	Deficiencies	The concentration of acetone in the solvent control and the maximum chlorophacinone treatment was 0.48 mg/l and therefore exceeded 0.1 ml/l. However, comparison of the solvent control data with those of the untreated control indicates there were no adverse consequences in this study.	
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		September 2006	
Materials and Methods		US EPA FIFRA 72-1, comparable to OECD 203 (Fish, acute toxicity test). Twenty fish (ten per replicate) Bluegill sunfish (*Lepomis macrochirus*) were tested for 96 h under flow-through conditions. A preliminary test was conducted before in order to determine the toxically relevant range. All fish were fed a dry commercial pelleted	

Section A7.4.1.1-02 Annex Point IIA VII.7.1	Acute toxicity to fish	
	food, *ad livitum*, daily except during the 48 h prior to, and during the definitive test. No mortality in the fish test population during the two days prior to testing. **3.1.3.** DT_{50} CPN photolysis = 2.2 days (buffer solution pH~7) and 1.3 (days pond water pH~8.4) natural summer sunlight at latitude 50°N, at 25ºC. **3.4.2.** Test organisms. The age of the organims was not reported.	
Results and discussion	Five nominal concentrations of the test material (1.2, 0.72, 0.43, 0.26 and 0.16 mg/l), a solvent control and a dilution water control. Mean measured concentrations (0.82, 0.52, 0.36, 0.24 and 0.11 mg/l) were ranged from 68-92 % of the nominal concetrations. The 96-hour LC_{50} value was 0.71 mg/l based on mean measured concentrations. **Comment: 5.2.1.** LC_0 (96 h) = 0.36 mg/l. 100% mortality was not reached.	
Conclusion		
Reliability	1	
Acceptability	Acceptable	
Remarks	Table A7.4.1.1-5: Test conditions: Test solutions were not aerated.	

Table A7.4.1.1-10: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Vehicle	Acetone.
Concentration of vehicle	0.48 ml/l maximum at highest chlorophacinone concentration.
Vehicle control performed	Yes, 0.48 ml acetone/l.

Table A7.4.1.1-11: Dilution water

Criteria	Details
Source	Well water.
Alkalinity	24 to 32 mg $CaCO_3$/l.
Hardness	25 to 32 mg $CaCO_3$/l.
pH	7.3 to 7.4
Conductivity	110 to 130 µmhos/cm.
Holding water different from dilution water	No.

Table A7.4.1.1-12: Test organisms

Criteria	Details
Species/strain	Bluegill sunfish (*Lepomis macrochirus*).
Source	Commercial supplier in CT, USA.
Age/size	Mean wet weight 0.53 g, total length 26-43 mm.
Pretreatment	Holding period of at least 14 days under test conditions.
Feeding of animals during test	None. Feeding stopped 48 hours prior to test initiation.

Table A7.4.1.1-13: Test system

Criteria	Details
Test type	Flow-through.
Volume of test vessels	11 l, flow rate approximately 50 ml/min, giving *ca*. 6.5 volume turnovers/24 hours.
Volume/animal	1.1 l (0.074 g of biomass per liter of flowing test solution per day)
Number of animals/vessel	10
Number of vessels/ concentration	2

Table A7.4.1.1-14: Test conditions

Criteria	Details
Test temperature	21 to 22°C
Dissolved oxygen	91 to 104% ASV.
pH	7.0 to 7.3
Photoperiod	16 h daily.

Table A7.4.1.1-15: Test substance concentrations

Nominal chlorophacinone concentration, mg/l	Measured concentration, mg/l					
	Initial (0 hour)		Final (96 hours)		Mean[1]	
Control	< LOD[2]	< LOD	< LOD	< LOD	< LOD	(-)
Acetone control	< LOD	< LOD	< LOD	< LOD	< LOD	(-)
0.16	0.12	0.13	0.098	0.075	0.11	(69)
0.26	0.26	0.26	0.23	0.22	0.24	(92)
0.43	0.40	0.43	0.28	0.31	0.36	(84)
0.72	0.59	0.57	0.48	0.44	0.52	(72)
1.2	0.87	0.78	0.83	0.81	0.82	(68)

[1] Based on original analytical data, not the rounded values presented for the 0 and 96 hour measurements. Values in brackets represent percentages of nominal concentrations;
[2] Below the limit of detection, 0.049 and 0.044 mg/l for 0 and 96 hour samples, respectively.

Table A7.4.1.1-16: Mortality data

Test Substance Concentration [mg/l]		Mean mortality (two replicates, each with 10 fish, per treatment)			
Nominal	**Mean measured**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control	< LOD	0	0	0	0
Solvent control	< LOD	0	0	0	0
0.16	0.11	0	0	0	0
0.26	0.24	0	0	0	0
0.43	0.36	0	0	0	0
0.72	0.52	0	0	0[b]	15[b]
1.2	0.82	0[ade]	0[ade]	35[bc]	70[bc]

[a] One or more survivors with darkened pigmentation;
[b] One or more survivors lethargic with darkened pigmentation;
[c] One or more survivors with partial equilibrium loss;
[d] One or more survivors swimming erratically;
[e] One or more survivors lethargic;

Table A7.4.1.1-17: Effect data

Parameter	96 h [mg/l][1]	95 % c.l.
LC_{50}	0.71	0.63 to 0.83

[1] Based on mean measured concentrations.

Table A7.4.1.1-18: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**Yes**	-
Concentration of dissolved oxygen in all test vessels > 60% saturation	**Yes**	-
Concentration of test substance ≥80% of initial concentration during test	**Yes**	-

Section A7.4.1.2-01 **Annex Point IIA VII.7.2**		**Acute toxicity to invertebrates** ***Daphnia magna***	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxx, XX. (1992). Chlorophacinone - Acute toxicity to daphnids (*Daphnia magna*) under flow-through conditions. xxxxxxxxxxxxxxxxxxxxxx., laboratory report number XXXXXX, 6 May XXXX (unpublished).	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. US EPA 72-2 comparable to OECD 202 (I) and EU C.2.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	None.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Chlorophacinone	
3.2.1	Lot/Batch number	XXXXXX	
3.2.2	Purity	XXX%	
3.2.3	Further relevant properties	Photolysis half-life of chlorophacinone in water is between 1 and 2 days, log octanol:water partition coefficient is 2.42 at pH 7, 23°C and solubility in water is 13 mg/l at 20°C.	**X**
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Stock solution prepared with 1.7 mg chlorophacinone/ml in acetone. Stock solution was then fed to a constant flow serial diluter where automated mixing with dilution water occurred prior to delivery to appropriate replicate test vessels.	
3.4	**Reference substance**	No.	
3.5	**Testing procedure**		
3.5.1	Dilution water	See Table A7.4.1.2-2.	
3.5.2	Test organisms	See Table A7.4.1.2-3.	
3.5.3	Test system	See Table A7.4.1.2-4.	
3.5.4	Test conditions	See Table A7.4.1.2-5.	
3.5.5	Duration of the test	48 hours.	
3.5.6	Test parameter	Immobilisation.	
3.5.7	Monitoring of TS	By HPLC.	

Section A7.4.1.2-01 **Annex Point IIA VII.7.2**		**Acute toxicity to invertebrates** ***Daphnia magna***	
	concentration		
3.5.8	Statistics	EC_{50} by probit analysis.	
		4 RESULTS	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	See Table A7.4.1.2-6.	
4.2.2	Effect data (Immobilisation)	See Table A7.4.1.2-7.	**X**
4.2.3	Other effects	See Table A7.4.1.2-7.	
4.3	**Results of controls**	See Table A7.4.1.2-7.	
4.4	**Test with reference substance**	Not performed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	An acute flow-through toxicity test with *D. magna* in general accordance with OCED 202 (I) and EC method C.2. Test media sampled at initiation and termination and analysed for chlorophacinone.	
5.3	**Results and discussion**		
5.3.1	EC_{50}	24-hour EC_{50}: > 820 µg/l ; 48-hour EC_{50}: 640 µg/l (with 95% confidence limits of 540 to 820 µg/l).	
5.4	**Conclusion**		
5.4.1	Reliability	1	
5.4.2	Deficiencies	None.	
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		September 2006	
Materials and Methods		US EPA 72-2 comparable to OECD 202 (I) (*Daphnia* sp., Acute Immobilisation Test and Reproduction Test) and EU C.2. Twenty invertebrates (*Daphnia magna*) (ten per replicate) were tested for 48 hours under flow-through conditions. A preliminary test was conducted before in order to determine the toxically relevant range. Daphnids were not fed during the 48-hour definitive exposure. **3.1.3.** DT_{50} CPN photolysis = 2.2 days (buffer solution pH~7) and 1.3 (days pond water pH~8.4) natural summer sunlight at latitude 50°N, at 25ºC.	

Section A7.4.1.2-01 Annex Point IIA VII.7.2	Acute toxicity to invertebrates *Daphnia magna*	
Results and discussion	**4.1.2.** Effect data. EC_0 (48 h) = 0.31 mg/l. 5 concentrations of the test material (nominal: 850, 510, 310, 180 and 110 µg/l), one solvent control and one dilution water control. All the mean measured concentrations were above 80% of the nominal concetrations. The 48-hour EC_{50} value was 0.64 mg/l.	
Conclusion	The tested substance chlorophacinone has a high toxicological effect on the invertebrate species *Daphnia magna*.	
Reliability	1	
Acceptability	Acceptable	
Remarks	**Table A7.4.1.2-8: Effect data:** [1] effect data based on mean measured (m) concentrations. EC_0 = 0.31 mg/l.	

Table A7.4.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Vehicle	Acetone
Concentration of vehicle	0.50 ml/l maximum at highest chlorophacinone concentration.
Vehicle control performed	Yes, 0.50 ml acetone/l

Table A7.4.1.2-2: Dilution water

Criteria	Details
Source	Fortified (hardened) well water (US EPA, 1975) and filtered to remove organic contaminants.
Alkalinity	110 – 120 mg $CaCO_3$/l
Hardness	170 – 180 mg $CaCO_3$/l
pH	8.0 - 8.1
Oxygen content	> 60% ASV
Conductivity	500 µmhos/cm
Holding water different from dilution water	No

Table A7.4.1.2-3: Test organisms

Criteria	Details
Source	Laboratory culture maintained at test facility.
Age	≤ 24 hours old at test start
Breeding method	Parthenogenic culture
Kind of food	Unicellular green algae and trout food suspension
Feeding frequency	Once daily
Pretreatment	None

Feeding of animals during test	None

Table A7.4.1.2-4: Test system

Criteria	Details
Renewal of test solution	Intermittent flow-through.
Volume of test vessels	1.4 l medium volume. Flow rate approximately 50 ml/cycle with *ca*. 167 cycles/24 hours.
Volume/animal	140 ml
Number of animals/vessel	10
Number of vessels/ concentration	2

Table A7.4.1.2-5: Test conditions

Criteria	Details
Test temperature	19 - 22°C
Dissolved oxygen	85 - 95% ASV
pH	8.0 - 8.2
Adjustment of pH	None
Quality/Intensity of irradiation	Sylvania Growlux and Cool White fluorescent lights at 38 to 52 footcandles
Photoperiod	16 hours daily

Table A7.4.1.1-6: Test substance concentrations

Nominal chlorophacinone concentration, µg/l	Measured concentration, µg/l					
	Initial (0 hour)		Final (48 hours)		Mean[1]	
Control	< LOD[2]	< LOD	< LOD	< LOD	< LOD	(-)
Acetone control	< LOD	< LOD	< LOD	< LOD	< LOD	(-)
110	100	110	95	99	100	(91)
180	170	170	160	160	160	(89)
310	280	280	280	280	280	(90)
510	500	490	490	500	500	(98)
850	830	810	830	810	820	(96)

[1] Based on original analytical data, not the rounded values presented for the 0 and 48 hour measurements. Values in brackets represent percentages of nominal concentrations;
[2] Below the limit of detection, 47 and 55 µg/l for 0 and 48 hour samples, respectively.

Table A7.4.1.2-7: Immobilisation data

Nominal Test-Substance Concentration [µg/l]		Mean Immobile *Daphnia* (%) (two replicates, each with 10 daphnids, per treatment)	
Nominal	**Mean measured**	**24 hours**	**48 hours**
Control	< lod	0	0
Solvent control	< lod	0	0
110	100	0	0
180	160	0	0
310	280	0[b]	0
510	500	0[b]	40[a]
850	820	45[a]	65[a]

lod: limit of detection, 47 and 55 µg/l for day 0 and 2 samples, respectively;
[a] all survivors lethargic;
[b] all survivors swimming erratically.

Table A7.4.1.2-8: Effect data

Endpoint	EC_{50}	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [µg/l]	> 820	-		
48 h [µg/l]	640	540 to 820		

Table A7.4.1.2-9: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	Fulfilled	Not fullfilled
Immobilisation of control animals <10%	**Yes**	**-**
Control animals not staying at the surface	**Not reported**	**-**
Concentration of dissolved oxygen in all test vessels >3 mg/l	**Yes**	**-**
Concentration of test substance ≥80% of initial concentration during test	**Yes**	**-**

Section A7.4.1.3-01 Annex Point IIA VII.7.3		Growth inhibition test on algae	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxx, X. (XXX). Toxicity of chlorophacinone to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test, XXXXX., laboratory report number XXXXX, 14 January XXX (unpublished).	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. OECD 201 and EU method C.3.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	None.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Chlorophacinone.	
3.2.1	Lot/Batch number	XXXXXX.	
3.2.2	Purity	XXXX% (w/w).	
3.2.3	Further relevant properties	Photolysis half-life of chlorophacinone in water is between 1 and 2 days, log octanol:water partition coefficient is 2.42 at pH 7, 23°C and solubility in water is 13 mg/l at 20°C.	**X**
3.2.4	Method of analysis	Not stated.	
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Primary stock solution prepared in N,N-dimethylformamide (DMF) (34.99 mg chlorophacinone/ml) and serially diluted with DMF to prepare a range of dosing solutions. Dosing solutions added to algal medium at a uniform rate of 50 µl/500 ml.	
3.4	**Reference substance**	No.	
3.5	**Testing procedure**		
3.5.1	Culture medium	Composition as prescribed by Guideline. $Na_2EDTA \cdot 2H_2O$ added at 0.1 mg/l of culture medium.	
3.5.2	Test organisms	See Table A7.4.1.3-2.	
3.5.3	Test system	See Table A7.4.1.3-3.	
3.5.4	Test conditions	See Table A7.4.1.3-4.	**X**
3.5.5	Duration of the test	72 hours.	

Section A7.4.1.3-01 Annex Point IIA VII.7.3		Growth inhibition test on algae	
3.5.6	Test parameter	Inhibition of culture growth, based on areas under the growth curves and average specific growth rates.	
3.5.7	Sampling	Algal counts: 0.2 – 1.0 ml from all flasks after 24, 48 and 72 hours, at least two measurements/sample with an electronic particle counter. Microscopic examination: control and nominal 1.6 mg/l treatments after 72 hours. Analysis: duplicate samples from each medium batch containing chlorophacinone and the solvent control immediately prior to inoculation and duplicate samples from stability batches of the same media incubated without algae under test conditions for 72-hours.	
3.5.8	Monitoring of TS concentration	By HPLC/UV-detection. See Table A7.4.1.3-6.	
3.5.9	Statistics	E_bC_{50} and E_rC_{50} values estimated by probit analysis. Dunnett's t-test used to determine significant differences from solvent control to locate NOE_bC and NOE_rC values.	
		4 RESULTS	
4.2	**Limit Test**	Not performed.	
4.3	**Results test substance**		
4.3.1	Initial concentrations of test substance	See Table A7.4.1.3-5.	
4.3.2	Actual concentrations of test substance	See Table A7.4.1.3-5.	
4.3.3	Cell concentration data	See Table A7.4.1.3-6.	
4.3.4	Effect data (cell multiplication inhibition)	72-hour E_bC_{50}: 1.7 mg/l. 72-hour E_rC_{50}: 2.2 mg/l (95% confidence limits: 0.7 – 9.1 mg/l). 72-hour NOE_bC: 0.72 mg/l. 72-hour NOE_rC: 0.72 mg/l.	
4.4	**Results of controls**	See Table A7.4.1.3-6.	
4.5	**Test with reference substance**	Not performed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	Algal growth inhibition test with *S. subspicatus* in accordance with OECD 201 and EU method C.3.	
5.3	**Results and discussion**		
5.3.1	NOEC	72-hour NOE_bC: 0.72 mg/l. 72-hour NOE_rC: 0.72 mg/l.	

Section A7.4.1.3-01 **Annex Point IIA VII.7.3**	**Growth inhibition test on algae**	
5.3.2 E_rC_{50}	72-hour E_rC_{50}: 2.2 mg/l (95% confidence limits: 0.7 – 9.1 mg/l).	
5.3.3 E_bC_{50}	72-hour E_bC_{50}: 1.7 mg/l.	
5.4 Conclusion	Validity criteria fulfilled.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	None.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods	OECD 201 (Alga, Growth Inhibition Test) and EU method C.3. The growth of the green algal species *Scenedesmus subspicatus* was investigated in a 72-hour static test. **3.1.3.** DT_{50} CPN photolysis = 2.2 days (buffer solution pH~7) and 1.3 (days pond water pH~8.4) natural summer sunlight at latitude 50°N, at 25ºC. **3.4.4.** Test conditions. Table 7.4.1.3-4 Please stablish whether there was aeration of dilution water.	
Results and discussion	The test concentrations were based on the results of a range-finding test without GLP. Five nominal concentrations 3.5, 1.6, 0.72, 0.35 and 0.16 mg/l in parallel with one control and a solvent control group. The measured concentrations varied in the range of 84 to 88% of the nominal values. The 72-hour E_bC_{50} value was 1.7 mg/l and the value of E_rC_{50} was 2.2 mg/l based on the nominal concentrations of the active substance.	
Conclusion		
Reliability	1	
Acceptability	Acceptable.	
Remarks	The new OECD algal inhibition Guideline (OECD 201, 2006) contains 3 validity criteria. One of these is a requirement for cell density to increase by a factor of at least ×16 in the control vessels in 72 hours. This was also stipulated in the original version of OECD 201, and the CPN study satisfies this requirement as indicated in the study report and the existing A7.4.1.3-01 summary. The revised guideline introduced 2 further criteria: 1. "The mean coefficient of variation for section-by-section growth rates .... must not exceed 35%". The mean coefficient of variation for the three 1-day sections in this study was 7.8%. This new validity criterion is satisfied. 2. "The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with .... *Desmodesmus subspicatus*". This study was performed with Scenedesmus subspicatus, now known as Desmodesmus subspicatus. The coefficient of variation for the control replicates over the entire study duration was 0.81%. This new validity criterion is also satisfied. *The algal study performed with chlorophacinone may therefore be considered valid according to all three criteria.*	

New tables included from Doc. IV-A 7.4.1.3-01.

Table 1: Algal cell densities during the test period of 72 hours

Nominal test item concentration (mg/L)	Flask No.	Density of algal cells (cell number x 10,000/mL) after					
		24 h		48 h		72 h	
Solvent control	1	2.6	2.4	7.1	7.3	32.9	32.8
	2	3.1	2.2	7.6	7.2	35.9	35.4
	3	4.6	2.8	7.8	7.5	38.7	38.8
	4	3.2	2.7	7.8	7.9	38.7	38.1
	5	2.5	2.0	7.2	6.7	39.6	38.3
	6	3.4	2.7	7.0	6.9	40.4	39.9
	m	2.85		7.33		37.46	
	s	0.51		0.37		2.70	
	n	6		6		6	
Control	1	2.2	2.0	8.2	7.3	40.0	41.6
	2	2.1	1.9	8.5	7.4	42.6	41.9
	3	2.3	2.2	6.7	7.3	41.2	39.9
	m	2.12		7.57		41.20	
	s	0.13		0.50		0.92	
	n	3		3		3	
0.16	1	2.1	1.9	6.7	7.1	32.5	33.0
	2	2.7	2.0	7.3	6.7	37.5	36.6
	3	2.3	2.0	6.3	6.4	32.9	33.5
	m	2.17		6.75		34.33	
	s	0.18		0.35		2.36	
	n	3		3		3	
0.35	1	2.9	2.0	7.8	7.2	24.7	23.4
	2	2.8	2.0	7.0	6.8	43.6	43.1
	3	1.9	1.8	7.9	7.5	46.5	46.0
	m	2.23		7.37		37.88	
	s	0.33		0.42		12.07	
	n	3		3		3	
0.72	1	3.1	1.9	8.1	8.1	44.6	45.2
	2	2.4	2.9	7.8	7.2	43.9	44.2
	3	2.9	1.9	7.6	6.7	39.1	38.9
	m	2.52		7.58		42.65	
	s	0.13		0.48		3.19	
	n	3		3		3	
1.6	1	1.7	1.4	7.2	6.6	14.8	13.9
	2	2.2	1.7	6.0	5.8	16.4	15.5
	3	2.6	1.7	7.2	5.9	15.0	15.6
	m	1.88		6.45		15.20	
	s	0.31		0.51		0.80	
	n	3		3		3	
3.5	1	1.5	1.3	2.4	2.1	1.6	1.5
	2	1.6	1.3	1.9	1.4	1.6	1.5
	3	1.5	1.1	1.3	1.4	1.9	1.7
	m	1.38		1.75		1.63	
	s	0.08		0.46		0.14	
	n	3		3		3	

m: mean value; s: standard deviation; n: number of flasks
At the start, 10,000 algal cells/mL were incubated.

Table 2: Areas under the growth curves (AUC) and percentage inhibition of AUC (I_{AUC}) during the test period

Nominal test item concentration (mg/L)	Areas under the growth curves (AUC) and % inhibition of AUC					
	0-24 h		0-48 h		0-72 h	
	AUC	I_{AUC} (%)	AUC	I_{AUC} (%)	AUC	I_{AUC} (%)
Solvent control	22	0.0	120	0.0	634	0.0
Control	13	39.6	106	12.3	667	-5.2
0.16	14	36.9	97	19.4	566	10.7
0.35	15	33.3	106	12.0	625	1.4
0.72	18	18.0	115	4.2	694	-9.5
1.6	11	52.3	87	28.1	322	49.1
3.5	5	79.3	18	84.9	35	94.5

AUC x 10,000
- % inhibition: increase in growth relative to that of solvent control

Table 3: Growth rates (r) and percentage inhibition of r (I_r) during the test period

Nominal test item concentration (mg/L)	Growth rate r and % inhibition of r					
	0-24 h		0-48 h		0-72 h	
	r (1/day)	I_r (%)	r (1/day)	I_r (%)	r (1/day)	I_r (%)
Solvent control	1.03	0.0	1.00	0.0	1.21	0.0
Control	0.75	27.6	1.01	-1.5	1.24	-2.7
0.16	0.77	25.5	0.95	4.2	1.18	2.4
0.35	0.80	23.1	1.00	-0.2	1.20	0.7
0.72	0.92	10.9	1.01	-1.7	1.25	-3.6
1.6	0.62	39.7	0.93	6.5	0.91	24.9
3.5	0.32	68.7	0.27	73.0	0.16	86.5

- % inhibition: increase in growth relative to that of solvent control

Table A7.4.1.3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Vehicle	N,N-dimethylformamide.
Concentration of vehicle	0.1 ml/l in all chlorophacinone treatments and the solvent control.
Vehicle control performed	Yes.

Table A7.4.1.3-2: Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus* CHODAT.
Strain	86.81 SAG (Universität Göttingen, Germany).
Laboratory culture	Yes.
Method of cultivation	Axenic laboratory culture.
Initial cell concentration	1.0×10^4 cells/ml (nominal).

Table A7.4.1.3-3: Test system

Criteria	Details
Volume of culture flasks	50 ml flasks containing 15 ml medium.
Culturing apparatus	Glass conical flasks each containing a magnetic stirrer bar, held in a temperature-controlled water bath.
Light quality	Fluorescent lighting (Philips TLD 36W/840), mean intensity: 8,500 lux.
Procedure for suspending algae	Continuous stirring.
Number of vessels/ concentration	3 for each chlorophacinone concentration and the untreated control, and 6 replicates for the solvent control.

Table A7.4.1.3-4: Test conditions

Criteria	Details
Test temperature	22 - 23°C
pH	7.9 to 8.0 at start to 8.1 - 8.6 at end
Light intensity	7,550 to 9,110 lux
Photoperiod	Continuous

Table A7.4.1.3-5: Analytical results

Nominal concentration of chlorophacinone [mg/l]	Measured concentration (mg/l)		Mean measured concentration (mg/l)	Mean measured concentration as % of nominal (%)
	0 h	72 h		
0.16	0.13	na	-	-

0.35	0.30	na	-	-
0.72	0.63	0.59	0.61	85
1.6	1.4	1.3	1.4	85
3.5	3.1	3.1	3.1	88

na: not analysed; below the 72-hour NOEC.

Table A7.4.1.3-6: Algal growth

Nominal Test Substance Concentration [mg/l]	Mean[a] algal cell density (× 10^4 cells/ml)			Mean areas under the growth curve		Mean growth rates (1/day)	
	24 h	48 h	72 h	0-72 h	% inhibition[b]	0-72 h	% inhibition[b]
Solvent control	2.85	7.33	37.46	634	-	1.21	-
Control	2.12	7.57	41.20	667	-5.2	1.24	-2.7
0.16	2.17	6.75	34.33	566	10.7	1.18	2.4
0.35	2.23	7.37	37.88	625	1.4	1.20	0.7
0.72	2.52	7.58	42.65	694	-9.5	1.25	-3.6
1.6[c]	1.88	6.45	15.20	322*	49.1	0.91*	24.9
3.5[d]	1.38	1.75	1.63	35*	94.5	0.16*	86.5

[a] means of duplicate measurements of samples taken from six (solvent control) or three (control and chlorophacinone) replicate flasks;
[b] percentage reduction in growth parameter relative to the solvent control value;
[c] microscopic examination after 72 h showed no apparent change in cell shape or size compared to the control;
[d] algal medium noticeably coloured by the test substance (yellow/pale yellow);
* significantly different from the solvent control ($p < 0.05$).

Table A7.4.1.3-7: Algal cell densities recorded in the untreated control (Initial inoculation density: 10,000 cells/mL, 3 replicate vessels, duplicate counts per replicate at each timepoint).

	0 h		24 h				48 h				72 h		
	cells/mL	logn	cells/mL			logn	cells/mL			logn	cells/mL		
	nom.		a	b	mean	of mean	a	b	mean	of mean	a	b	mean
rep. 1	10,000	9.21	22,000	20,000	21,000	9.95	82,000	73,000	78,000	11.26	400,000	416,000	408,000
rep. 2	10,000	9.21	21,000	19,000	20,000	9.90	85,000	74,000	80,000	11.29	426,000	419,000	423,000
rep. 3	10,000	9.21	23,000	22,000	23,000	10.04	67,000	73,000	70,000	11.16	412,000	399,000	406,000

Average specific growth rate, $\mu_{i-j} = (\ln X_j - \ln X_i)/(t_j - t_i)$

Table A7.4.1.3-8: Coefficient of variance for individual control replicates over the entire test duration (days 0-3)

	72 h ln X_j	0 h ln X_i	ln X_j - ln X_i	μ_{i-j}
rep. 1	12.92	9.21	3.71	1.24
rep. 2	12.96	9.21	3.75	1.25
rep. 3	12.91	9.21	3.70	1.23
mean				1.24
SD (σn - 1)				0.01
coeff var				**0.81%**

Table A7.4.1.3-9: Coefficients of variance for individual control replicates, section by section

(a) 0-24 h

	24 h ln Xj	0 h ln Xi	ln Xj - ln Xi	μi-j
rep. 1	9.95	9.21	0.74	0.74
rep. 2	9.90	9.21	0.69	0.69
rep. 3	10.04	9.21	0.83	0.83
mean				0.75
SD (σn - 1)				0.07
coeff var				**9.3%**

(b) 24-48 h

	48 h ln Xj	24 h ln Xi	ln Xj - ln Xi	µi-j
rep. 1	11.26	9.95	1.31	1.31
rep. 2	11.29	9.90	1.39	1.39
rep. 3	11.16	10.04	1.12	1.12
mean				1.27
SD (σn - 1)				0.14
coeff var				11.0%

(c) 48-72 h

	72 h ln Xj	48 h ln Xi	ln Xj - ln Xi	µi-j
rep. 1	12.92	11.26	1.66	1.66
rep. 2	12.96	11.29	1.67	1.67
rep. 3	12.91	11.16	1.75	1.75
mean				1.69
SD (σn - 1)				0.05
coeff var				3.0%

The mean coefficient of variation for all three 1-day sections was (9.3 + 11.0 + 3.0)/3 = 7.8%.

Table A7.4.1.3-10: Validity criteria for algal growth inhibition test according to revised OECD Guideline 201 (2006)

	Fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**	-
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 7% (*Desmodesmus subspicatus*).	**Yes**	-
The mean coefficient of variation for section-by-section growth rates did not exceed 35%.	**Yes**	-
Concentration of test substance ≥80% of initial concentration during test	**Yes**	-

Section A7.4.1.4-01 Annex Point IIA VII.7.4, Annex Point IIIA VII.3		Inhibition to microbial activity (aquatic)	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxx, X. (XXX). Toxicity of chlorophacinone to activated sludge in a respiration inhibition test. XXXXX., laboratory report number XXXXX, 14 January XXX (unpublished).	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. OECD 209 and EU method C.11.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	None.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Chlorophacinone.	
3.2.1	Lot/Batch number	XXXXXX.	
3.2.2	Purity	XX.XX% (w/w).	
3.2.3	Further relevant properties	Photolysis half-life of chlorophacinone in water is between 1 and 2 days, log octanol:water partition coefficient is 2.42 at pH 7, 23°C and solubility in water is 13 mg/l at 20°C.	**X**
3.3	**Reference substance**	3,5-dichlorophenol (Aldrich, Lot 02611ES).	
3.4	**Testing procedure**		
3.4.1	Culture medium	OECD synthetic sewage concentrate (16 g peptone, 11.0 g meat extract, 3.0 g urea, 0.7 g NaCl, 0.4 g $CaCl_2.2H_2O$, 0.2 g $MgSO_4.7H_2O$ and 2.8 g K_2HPO_4 per litre deionised water) used in the test at a dilution of 16:500 ml.	
3.4.2	Inoculum / test organism	See Table A7.4.1.4-2.	
3.4.3	Test system	See Table A7.4.1.4-3.	
3.4.4	Test conditions	See Table A7.4.1.4-4.	
3.4.5	Duration of the test	3 hour incubation.	
3.4.6	Test parameter	Respiration inhibition.	
3.4.7	Analytical parameter	Continuous dissolved oxygen measurements spanning approximately 15 minutes.	
3.4.8	Controls	Blank controls containing water, respiration substrate and inoculum, but without test or reference substance. There were no abiotic or vehicle	

Section A7.4.1.4-01 Annex Point IIA VII.7.4, Annex Point IIIA VII.3		Inhibition to microbial activity (aquatic)	
		controls (not appropriate, based on consideration of test substance properties and method of its introduction to the test system).	
3.4.9	Statistics	Probit analysis (chlorophacinone EC_{15} and 3,5-DCP EC_{50} plus 95% confidence limits).	
		4 RESULTS	
4.2	**Preliminary test**	Not performed.	
4.3	**Results test substance**		
4.3.1	Initial concentrations of test substance	10, 32, 100, 320 and 1,000 mg chlorophacinone/l (nominal).	
4.3.2	Concentration/ response curve	The inhibition of respiration for each treatment is presented in Table 7.4.1.4-05.	
4.3.3	Effect data	EC_{50} > 1000 mg/l NOEC (EC_{15}): 775 mg/l.	
4.3.4	Other observed effects	None.	
4.4	**Results of controls**	Respiration rates of start- and end-of-series blank controls were 1.480 and 1.484 mg O_2/l/min, respectively, and differed from one another by less than 15%.	
4.5	**Test with reference substance**	Performed.	
4.5.1	Concentrations	5, 16 and 50 mg 3,5-dichlorophenol/l.	
4.5.2	Results	EC_{50}: 12 mg/l (95% confidence limits: 11.2 to 12.8 mg/l).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	The effect of chlorophacinone on aerobic biological sewage treatment processes was assessed according to OECD Guideline 209 by determining inhibition of respiration of the micro-organisms present in activated sludge. Activated sludge was exposed over a period of three hours to concentrations of chlorophacinone. In addition, the reference substance 3,5-dichlorophenol (3,5-DCP) was tested.	
5.3	**Results and discussion**	Percentage respiration rate reductions in each of the chlorophacinone and reference treatments were based on nominal concentrations and were assessed in relation to the mean control rate. The two individual control rates met the 15% conformity requirement and the 3,5-DCP reference EC_{50} lay within the 5 to 30 mg/l range prescribed by the test guideline. The results are presented in Table 7.4.1.4-05. All concentrations of chlorophacinone were either near or above the aqueous solubility limit and undissolved test material was therefore observed in all preparations containing the test substance. The respiration inhibition, which remained below 20% and relatively unchanged between 320 and 1,000 mg/l, may have been limited by the solubility of the test substance under the conditions of the test.	
5.3.1	EC_{20}	Not calculated.	

Section A7.4.1.4-01 Annex Point IIA VII.7.4, Annex Point IIIA VII.3	Inhibition to microbial activity (aquatic)	
5.3.2 EC_{50}	> 1000 mg/l.	
5.3.3 EC_{80}	> 1000 mg/l.	
5.4 Conclusion	Both validity criteria were fulfilled.	
5.4.1 Reliability	1.	
5.4.2 Deficiencies	None.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods	OECD 209 (Activated Sludge, Respiration Inhibition Test) and EU method C.11.The inhibitory effect of the chlorophacinone on the respiration rate of aerobic wastewater microorganisms of activated sludge in a 3-hour respiration inhibition test. **3.1.3.** DT_{50} CPN photolysis = 2.2 days (buffer solution pH~7) and 1.3 (days pond water pH~8.4) natural summer sunlight at latitude 50°N, at 25ºC.	
Results and discussion	The following nominal concentrations of the active substance were tested: 10, 32, 100, 320 and 1000 mg/l. In addition, two controls and three different concentrations of the reference substance 3,5-dichlorophenol (5, 16 and 50 mg/l) were tested in parallel. The results of these treatments confirmed the suitability of the activated sludge. No adverse effects were detected below the water solubility limit of the substance.	
Conclusion	The maximum inhibition of respiration recorded was less than 20% at a much higher concentration than the water solubility limit what means that chlorophacinone does not appear to have significant negative effects for the microbial activity of the STP sludges.	
Reliability	1	
Acceptability	Acceptable	
Remarks		

Table A7.4.1.4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Chlorophacinone mixed with water, subjected to ultrasonication (15 min), followed by intense stirring (24 h) at room temperature and in darkness.
Vehicle	None.
Concentration of vehicle	Not appropriate.
Vehicle control performed	No.
Other procedures	None.

Table A7.4.1.4-2: Inoculum / test organism

Criteria	Details
Nature	Activated sludge.
Source	Sewage treatment works treating predominantly domestic sewage.
Sampling site	XXXX, Switzerland.
Preparation of inoculum for exposure	Sludge twice centrifuged and washed with tap water.
Pretreatment	No prior adaptation. Two day holding period prior to use, fed with synthetic sewage at 50 ml/l/day.

Table A7.4.1.4-3: Test system

Criteria	Details
Culturing apparatus	1 l glass flasks
Number of culture flasks/concentration	Two for blank control, one per test and reference concentration.
Aeration device	Compressed air, delivery device not reported.
Measuring equipment	Dissolved oxygen meter
Test performed in closed vessels due to significant volatility of TS	No. (Not appropriate, based on consideration of test substance properties).

Table A7.4.1.4-4: Test conditions

Treatment	Temperature (°C)		pH		Dissolved oxygen (mg/l)	
	Start	End	Start	End	Start	End
Control 1	19	19	7.0	7.9	8.8	8.0
Chlorophacinone (nominal mg/l):						
10	-	-	6.9	8.0	8.7	8.7
32	-	-	7.0	7.9	8.7	8.3
100	-	-	7.0	7.8	8.8	8.8
320	-	-	6.9	7.9	8.7	8.7
1,000	-	-	7.0	7.9	8.7	8.5
3,5-DCP (nominal mg/l):						
5	-	-	7.0	8.0	8.6	8.7
16	-	-	7.1	8.0	8.9	8.9
50	-	-	7.0	7.9	8.9	8.7
Control 2	-	-	7.0	7.9	8.7	8.3

- not measured.

Table A7.4.1.4-5: Test results

Treatment	Respiration rate (mg O_2/l/min)	% inhibition[1]
Control 1 Control 2	1.480 1.484	na na
Mean control	1.482	na
Chlorophacinone (nominal mg/l): 10 32 100 320 1,000	 1.430 1.417 1.410 1.238 1.283	 3.5 4.4 4.9 16.5 13.4
3,5-DCP (nominal mg/l): 5 16 50	 1.184 0.576 0.128	 20.1 61.1 91.3

[1] Percentage reduction in respiration rate relative to the mean control value;
na: not appropriate.

Section A7.4.2-01 **Annex Point IIIA VII.7.5**	**Bioconcentration**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [×]**	
Limited exposure [×]	**Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted*…". The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". **1. Predicted bioconcentration behaviour** An evaluation of the instrinsic potential for bioconcentration in aquatic organisms may be based on physical chemical properties such as the n-octanol/water partition coefficient (TGD v. 4.3.1, April, 2000). Two values of the Log_{10} n-octanol/water partition coefficient of chlorophacinone are available. A value of 1.93 is based on the shake flask method without control of pH in the medium and a value of 2.42 (pH 7) is based on the shake flask method with control of pH (Section A.3). Both values are less than 3.0 and thus indicate a relatively low propensity for bioconcentration in aquatic organisms (TGD on Risk Assessment, Part II, 2003). Furthermore, the extent of surface water contamination following the use of products containing chlorophacinine is expected to be very low and thus exposure to aquatic biota is limited. **2. Assessment of exposure** Chlorophacinone is incorporated at a concentration of 50 mg/kg into wax block and grain baits and is applied at 2000 mg/kg in tracking powder. Some applications or uses of the two baits are common to both formulations whilst those of the tracking-powder are product-specific. The various uses are outlined in Documents II-C1, II-C2 and II-C3. According to EUBEES 2, exposure of surface water bodies to chlorophacinone is not expected to arise following deployment in and around buildings and on waste dumps.	

Section A7.4.2-01 Annex Point IIIA VII.7.5	Bioconcentration
	Releases of chlorophacinone may occur *via* treated effluents discharged to receiving waters following use of wax blocks in sewers. As detailed in Section 2.3.1 of Document II-C1, according to a modification of the "SimpleTreat" model, and based on the assumption that no biodegradation occurs, some 99% of the influent load may remain in the aqueous phase and be discharged in the effluent. Peak in-sewer concentrations (9.7×10^{-5} mg total chlorophacinone/L, based on EUBEES 2 defaults) coincide with the first week of pulse-baiting campaigns and are assumed to be reduced by half over each of the following two weeks before falling back to a normal steady state level of 3.7×10^{-6} mg total chlorophacinone/L. The concentrations of chlorophacinone that enter treatment facilities are lower than the totals in sewers because residues contained in the bodies of rats and large block fragments is removed mechanically. On this basis, the steady-state concentration of chlorophacinone in the treated effluent prior to dilution in the receiving water is less than 3.7 ng/L. Following discharge, chlorophacinone would rapidly dissipate from the water column, based on its tendency to bind strongly to solids (Koc: ≥ 15,600 mL/g). Exposure of fish to chlorophacinone is consequently expected to be insignificant following the use of all chlorophacinone products, including wax blocks deployed in sewers. On the basis of a Log n-octanol/water partition coefficient of less than 3.0 and limited exposure in the aquatic compartment a bioconcentration study in fish is not necessary.
Undertaking of intended data submission [ ]	Not applicable.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2007
Evaluation of applicant's justification	
Conclusion	It is accepted that chlorophacinone has a low potential to bioconcentrate
Remarks	The BCF_{fish} was calculated from the log K_{ow} of 2.42; pH~7, 23ºC according to the TGD and resulted in BCF_{fish} of 22.75 l/kg.

Section A7.4.3.1-01 Annex Point IIIA XIII.1.3	Prolonged toxicity to an appropriate species of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [×]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted*…". The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of fish to chlorophacinone is therefore not expected to occur and a study of prolonged toxicity to fish is consequently unnecessary. Commissioning chronic exposure studies with fish in spite of the fact that no significant exposure of the aquatic compartment is anticipated is both unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data	
Remarks		

Section A7.4.3.1-01 Annex Point IIIA XIII.1.3	Prolonged toxicity to an appropriate species of fish
	COMMENTS FROM OTHER MEMBER STATE (specify)
Date	July 2007
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	The acute information provided is enough for the risk assessment in the aquatic compartment.
Remarks	

Section A7.4.3.2-01 Annex Point IIIA XIII.1.3	Effects on reproduction and growth rate on an appropriate species of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [×]	**Other justification [×]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted*…". The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (Larsen, 2003), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of fish to chlorophacinone is therefore not expected to occur and a study of effects on growth rate and reproduction of fish is consequently unnecessary. Commissioning chronic exposure studies with fish in spite of the fact that no significant exposure of the aquatic compartment is anticipated is both unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	The acute information provided is enough for the risk assessment in the aquatic compartment.	
Remarks		

Section A7.4.3.3.1-01 Annex Point IIIA XIII.1.3	Bioaccumulation in an appropriate species of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [×]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted*…". The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of fish to difethialone is therefore not expected to occur and a study of bioaccumulation in fish is consequently unnecessary. Commissioning bioaccumulation studies with fish in spite of the fact that no significant exposure of the aquatic compartment is anticipated is both unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	It is accepted that chlorophacinone has a low potential to bioaccumulate	
Remarks	Due to its low octanol-water partition coefficient (K_{OW}) of the substance bioaccumulation is not foreseen.	

Section A7.4.3.3.2-01 Annex Point IIIA XIII.1.3	Bioaccumulation in an appropriate invertebrate species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of aquatic invertebrates to chlorophacinone is therefore not expected to occur and a study of bioaccumulation in aquatic invertebrates is consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Give date of action	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	The acute information provided is enough for the risk assessment in the aquatic compartment.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE (specify)	
Date	July 2007	
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state	
Conclusion	It is accepted that chlorophacinone has a low potential to bioaccumulate	
Remarks		

Section A7.4.3.4-01 Annex Point IIIA XIII.1.3	Effects on reproduction and growth rate with an appropriate invertebrate species	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [×]	**Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of aquatic invertebrates to chlorophacinone is therefore not expected to occur and a study of effects on reproduction and growth rate with aquatic invertebrates is consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	The acute information provided is enough for the risk assessment in the aquatic compartment.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE (specify)	
Date	Give date of comments submitted	
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state	
Conclusion	Discuss if deviating from view of rapporteur member state	

Section A7.4.3.4-01 Annex Point IIIA XIII.1.3	Effects on reproduction and growth rate with an appropriate invertebrate species
Remarks	

Section A7.4.3.5.1-01 Annex Point IIIA XIII.1.3	Effects on sediment-dwelling organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of aquatic organisms to chlorophacinone is therefore not expected to occur and a study of effects on sediment-dwellers is consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	When no measured data are available, either for the determination of a PEC_{sed} or for the calculation of a $PNEC_{sed}$, no quantitative risk characterisation for sediment can be performed. In this situation, the assessment conducted for the aquatic compartment will also cover the sediment compartment for chemicals with a log K_{ow} up to 5, as in the present case (log K_{ow} = 2.42 pH~7, 23ºC).	
Remarks		

Section A7.4.3.5.2-01 Annex Point IIIA XIII.1.3	Aquatic plant toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the aquatic compartment to active substances contained in rodenticidal baits is considered to be negligible. Significant exposure of aquatic organisms to chlorophacinone is therefore not expected to occur and a study of effects on aquatic plants is consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	The existing database on the toxicity of chlorophacinone to aquatic organisms is considered sufficient, so that further testing of the effect on either other aquatic organisms or aquatic plants is not considered to be required.	
Remarks		

Section A7.5.1.1-01 Annex Point IIIA VII.7.4	Inhibition to microbiological activity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [×]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". Chlorophacinone is incorporated at a concentration of 50 mg/kg into two types of bait formulation: wax blocks and grains. Some applications are common to both formulations whilst others are product-specific. The various uses that result in transfer of chlorophacinone to the soil compartment, and the calculations made to estimate the corresponding concentrations of chlorophacinone in soil are outlined in Documents II-C1 and II-C2. Deployment of wax blocks in sewers and subsequent spreading of sludge on soil is not expected to result in significant quantities of chlorophacinone reaching open land because the bulk of the chlorophacinone load entering a waste-water treatment plant will remain associated with the aqueous phase. Possible impact on soil fertility following use of wax blocks in sewers is therefore not a matter for concern. Deployment of chlorophacinone baits around buildings is expected to cause soil contamination concentrated within 10 cm of bait stations, with a more diffuse distribution over the areas that carry target rodent traffic. The concentrated hot-spot and overall mean concentrations are estimated to be 0.1507 and 0.0036 mg chlorophacinone/kg soil. These values apply to strips of soil extending no more than 10 m from the baited edge of buildings and are therefore of limited relevance to wide-scale soil fertility. The same limited relevance applies to waste dumps and landfills, where the concentration of chlorophacinone in soil following deployment of wax blocks is estimated to be 0.007 mg/kg soil. **Conclusion** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the soil compartment to chlorophacinone contained in rodenticidal baits is feasible, with concentrations dependent on	

Section A7.5.1.1-01 Annex Point IIIA VII.7.4	Inhibition to microbiological activity
	deployment practices and product type. Exposure of the terrestrial compartment will be extremely localised and confined to small concentration 'hotspots' within a few cm of bait points located outdoors. The scope for widespread and significant exposure of the terrestrial compartment is therefore negligible and studies of the effects of chlorophacinone on terrestrial microbiological activity are consequently unnecessary.
Undertaking of intended data submission [ ]	Not applicable.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2007
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view
Conclusion	soil microorganisms test will not be requested taking into account that it is expected not to be the most sensitive species according to the test results in STPs.
Remarks	

Section A7.5.1.2-01 Annex Point IIIA XIII.3.2		Earthworm, acute toxicity test	
		1 REFERENCE	**Official use only**
1.2	**Reference**	XXXXX, XX. (XXXX). Chlorophacinone: acute toxicity (LC_{50}) to the earthworm (*Eisenia foetida*). XXXXXXXXXXXXXXXX., laboratory report number XXXXXXXXX, 16 June XXX (unpublished).	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	LiphaTech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes. OECD 207 (1984) and EU method Part C.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No.	
		3 METHOD	
3.2	**Test material**	Chlorophacinone	
3.2.1	Lot/Batch number	XXXXXX	
3.2.2	Purity	XXXX% (w/w)	
3.2.3	Further relevant properties	Photolysis half-life of chlorophacinone in water is between 1 and 2 days, log octanol:water partition coefficient is 2.42 at pH 7, 23°C and solubility in water is 13 mg/l at 20°C. Soil DT_{50}: 128 days at 12°C.	**X**
3.3	**Reference substance**	Chloroacetamide (separate study, November 1998).	
3.4	**Testing procedure**		
3.4.1	Preparation of the test substance	Two premixes (2,200 and 22,000 mg/kg) were prepared by mixing the test substance with sand. Appropriate quantities of the appropriate premix were mixed with additional sand to give a series of 100 g secondary mixtures.	
3.4.2	Application of the test substance	Secondary mixtures of chlorophacinone and sand were incorporated into bulk soil preparations prior to moisture adjustment and portioning into replicate test vessels.	
3.4.3	Test organisms	Adult *Eisenia foetida*, individual weights at start of test were 300 to 600 mg.	
3.4.4	Test system	Continuous exposure of six groups of 40 earthworms to five concentrations of the test substance in artificial substrate and one control treatment (substrate only) for 14 days. Each treatment consisted of four replicate test vessels (1 l glass containers with perforated covers). Earthworms not fed during the test. Nominal chlorophacinone concentrations were: 0 (control), 95, 171, 309, 556 and 1,000 mg/kg.	
3.4.5	Test conditions	See Table A7.5.1.2-3.	

Section A7.5.1.2-01 Annex Point IIIA XIII.3.2		Earthworm, acute toxicity test	
3.4.6	Test duration	14 days.	
3.4.7	Test parameter	Mortality, weight change and observations of toxicity.	
3.4.8	Examination	Earthworms retrieved from the soil after 7 and 14 days, washed, dried and batch-weighed (per replicate container). Final substrate moisture content was assessed by weighing bulk soil pooled according to treatment at the end of the test.	
3.4.9	Monitoring of test substance concentration	No.	
3.4.10	Statistics	None applied to chlorophacinone data.	
		4 RESULTS	
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	Nominal test substance concentrations were: 0 (control), 95, 171, 309, 556 and 1,000 mg chlorophacinone/kg dry weight artficial soil.	
4.2.2	Effect data (Mortality)	See Table A7.5.1.2-5.	
4.2.3	Other effects	Treatment-related weight losses (Table A7.5.1.2-6). Worms of the 556 and 1,000 mg/kg treatment groups were occasionally observed on the sides of the test vessels or on the surface of the soil during the course of the study.	
4.3	**Results of controls**		
4.3.1	Mortality	0%.	
4.3.2	Number/ percentage of earthworms showing adverse effects	Overall control group mean weight change was -1% relative to initial weights.	
4.3.3	Nature of adverse effects	Slight weight loss. See Table A7.5.1.2-6.	
4.4	**Test with reference substance**	Day 14 LC_{50}: 53.1 mg chloroacetamide/kg dry soil (95% confidence limits: 48.1 – 59.3 mg/kg).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	A 14-day acute toxicity test with *E. foetida* in accordance with OECD 207 and the EU part C test method. No deviations from the stated guidelines.	
5.3	**Results and discussion**		
5.3.1	LC_{50}	14-day LC_{50} > 1,000 mg chlorophacinone/kg dry weight artificial soil.	
5.3.2	Weight change	Mean weight decrease recorded between day 0 and day 14 in all treatment groups and showed a clear dose-response relationship. The maximum decrease relative to initial mean weight was -23% at 1,000 mg/kg.	

Section A7.5.1.2-01 Annex Point IIIA XIII.3.2	Earthworm, acute toxicity test	
5.4 Conclusion		
5.4.1 Reliability	1.	
5.4.2 Deficiencies	None.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods	OECD 207 (1984) (Earthworm, Acute Toxicity Tests) and EU method Part C. Groups of forty worms were allocated to soil containing 0, 95, 171, 309, 556 and 1000 mg chlorophacinone/l. Worms were observed for 14 days and counted on days 7 and 14. **3.1.3.** DT_{50} CPN photolysis = 2.2 days (buffer solution pH~7) and 1.3 (days pond water pH~8.4) natural summer sunlight at latitude 50°N, at 25ºC.	
Results and discussion	Weight loss accurred in all groups (1, 4, 5, 11, 19 and 23% weight loss). Under the conditions of this study, the LC_{50} value of the a.s. to the earthworm was > 1000 mg/kg dry artificial soil. NOEC (mortality) = 309 mg/kg dry artificial soil although weight loss was observed at lower concentrations (nominal concentrations).	
Conclusion	LC_0: not calculated LC_{50} > 1000 mg a.s./kg dry artificial soil. LC_{100} > 1000 mg a.s./kg dry artificial soil.	
Reliability	1	
Acceptability	Acceptable	
Remarks	10% organic matter content (OECD standard soil). **Table A7.5.1.2-7: Effect data**: NOEC (mortality) = 309 mg/kg dry artificial soil although weight loss was observed at lower concentrations.	

Table A7.5.1.2-1: Test organisms

Criteria	Details
Species/strain	*Eisenia foetida*
Source of the initial stock	XXXXXXXXXXXXXXX., UK
Culturing techniques	Maintained in the laboratory in artificial soil.
Age/weight	Adult *Eisenia foetida*, mean weight at start of test was 300 to 600 mg.
Pre-treatment	Acclimation in OECD artificial test substrate, duration not stated.

Table A7.5.1.2-2: Test system

Criteria	Details
Artificial soil test substrate	Industrial quartz sand: 70% (w/w) Kaolin clay: 20% (w/w) Sphagnum peat: 10% (w/w) Calcium carbonate sufficient to adjust pH to 5.7.
Test mixture	Nominal chlorophacinone concentrations were: 0 (control), 95, 171, 309, 556 and 1,000 mg/kg dry weight artficial soil.
Size, volume and material of test container	1 l glass containers
Amount of artificial soil (kg)/ container	739 g mean wet weight substrate.
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Artificial and continuous
Test performed in closed vessels due to significant volatility of test substrate	No, lids were perforated.

Table A7.5.1.2-3: Test conditions

Criteria	Details
Test temperature	20 to 22°C
Moisture content	See Table A.7.5.1.2-4
pH	See Table A.7.5.1.2-4
Adjustment of pH	Yes, with calcium carbonate to 5.7 at the start of the test.
Light intensity / photoperiod	Approximately 480 lux, continuous
Relevant degradation products	No major metabolites formed in an aerobic soil degradation study.

Table A7.5.1.2-4: Moisture content and pH during the test

Nominal concentration (mg/kg)	pH		Moisture content (% dry weight)	
	Day 0	Day 14	Day 0	Day 14
0, 95, 171, 309, 556, 1,000	not reported	not reported	34 - 35	31 - 32

Table A7.5.1.2-5: Mortality data

Chlorophacinone Concentration (nominal) [mg/kg artificial soil]	Mortality (%)	
	Day 7	Day 14
Control	0	0
95	0	0
171	0	0
309	0	0
556	2.5	5
1,000	5	15

Table A7.5.1.2-6: Weight-change data

Chlorophacinone Concentration (nominal) [mg/kg artificial soil]	Weight change (%) between day 0 and day 14
Control	-1
95	-4
171	-5
309	-11
556	-19
1,000	-23

Table A7.5.1.2-7: Effect data

Endpoint [14 d]	Nominal concentration (mg chlorophacinone/kg)
LC_{50}	> 1,000
NOEC (mortality)	309

Table A7.5.1.2-8: Validity criteria for acute earthworm test according to OECD 207

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	yes	-

Section A7.5.1.3-01 Annex Point IIIA XIII.1.3	Acute toxicity to (terrestrial) plants	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the soil compartment to active substances contained in rodenticidal baits is feasible, with concentrations dependent on deployment practices and product type. Exposure of the terrestrial compartment will be extremely localised and confined to small concentration 'hotspots' within a few cm of bait points located outdoors. The scope for widespread and significant exposure of the terrestrial compartment is therefore negligible and studies of the acute toxicity of chlorophacinone to plants are consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** July 2007	

Section A7.5.1.3-01 **Annex Point IIIA XIII.1.3**	**Acute toxicity to (terrestrial) plants**
Evaluation of applicant's justification	According to the TNsG document on "data requirements for active substances and biocidal products"; Chapter 3 "Additional data required for active substances and biocidal products"; Part A: "Additional data and guidance for active (chemical) substances". In Figure 3.2. "Testing strategy for terrestrial ecotoxicity studies" page 109, it is explained that when there is a direct exposure to soil 3 different soil studies should be requested, i.e. 7.5.1.1. Inhibition to microbial activity, 7.5.1.2. Acute toxicity to earthworms or other soil-non-target macro-organisms and 7.5.1.3. Acute toxicity to plants. The derivation of a $PNEC_{soil}$ from the $PNEC_{aquatic}$ based on the equilibrium partitioning method presents large uncertainties for the specific case of chlorophacinone due to the ecotoxicological profile of this molecule and the lack of knowledge related to the adsorption mechanism to soil particles, evidenced by the measured Koc values from the adsorption/desorption screening test (Doc. III-A 7.1.3-01). According to the TGD, the soil test set has to be required. "Calculation of PNEC using assessment factors" (TGD, part II Subchapter 3.6.2.2). "A dataset comprising of toxicity data for primary producers, consumers and decomposers is preferred". In this case, short-term toxicity test to soil microorganisms will not be requested taking into account that it is expected not to be the most sensitive species according to the test results in STP.
Conclusion	No test on plants has been requested according to TM's decision. RMS considers that uncertainty still remains.
Remarks	

Section A7.5.2.1-01 Annex Point IIIA XIII.1.3	Reproduction study with other soil non-target macro-organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted*…". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the soil compartment to active substances contained in rodenticidal baits is feasible, with concentrations dependent on deployment practices and product type. Exposure of the terrestrial compartment will be extremely localised and confined to small concentration 'hotspots' within a few cm of bait points located outdoors. The scope for widespread and significant exposure of the terrestrial compartment is therefore negligible and studies of the effects of chlorophacinone on the reproduction of soil non-target macro-organisms are consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	A proper risk assessment can be carried out with the information available.	
Remarks		

Section A7.5.2.1-01 Annex Point IIIA XIII.1.3	Reproduction study with other soil non-target macro-organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the soil compartment to active substances contained in rodenticidal baits is feasible, with concentrations dependent on deployment practices and product type. Exposure of the terrestrial compartment will be extremely localised and confined to small concentration 'hotspots' within a few cm of bait points located outdoors. The scope for widespread and significant exposure of the terrestrial compartment is therefore negligible and studies of the effects of chlorophacinone on the reproduction of soil non-target macro-organisms are consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	This test is not necessary to carry out the risk assessment in the soil compartment	
Remarks		

Section 7.5.3.1.1-01 Annex Point IIIA XIII.1.1		Acute oral toxicity on birds	
		1 REFERENCE	**Official use only**
1.2	**Reference**	Xxxxxx, XX. (XXX) Acute oral LD_{50} - bobwhite quail. Chlorophacinone XXXXXXXXXXXXXX. unnumbered laboratory report, 19 November XXXX (unpublished).	
1.3	**Data protection**	Yes.	
1.3.1	Data owner	Liphatech S.A.S.	
1.3.2	Companies with letter of access	None.	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	None cited. In-house method generally consistent with SETAC 1995.	
2.3	**GLP**	Yes.	
2.4	**Deviations**	No.	
		3 METHOD	
3.2	**Test material**	Chlorophacinone (termed 'ROZOL Technical' in the report).	
3.2.1	Lot/Batch number	Not stated.	
3.2.2	Purity	Not stated.	
3.2.3	Method of analysis in the diet	Not applicable.	
3.3	**Administration of the test substance**	Test substance mixed with corn oil and administered by intubation to the crop via stainless steel catheters.	
3.4	**Reference substance**	No.	
3.5	**Testing procedure**		
3.5.1	Test organisms	See Table A7.5.3.1.1-2.	
3.5.2	Test system	See Table A7.5.3.1.1-3.	
3.5.3	Duration of the test	14 days.	
3.5.4	Test parameter	Mortality and behaviour.	
3.5.5	Examination / Observation	See Table A7.5.3.1.1-3.	
3.5.6	Statistics	Determination of LD_{50} by probit analysis.	
		4 RESULT	
4.2	**Limit Test / Range finding test**	No	

Section 7.5.3.1.1-01 Annex Point IIIA XIII.1.1		Acute oral toxicity on birds	
4.3	**Results test substance**		
4.3.1	Applied concentrations	See Table A7.5.3.1.1-3.	
4.3.2	Effect data (Mortality)	See Table A7.5.3.1.1-5.	
4.3.3	Body weight	See Table A7.5.3.1.1-6.	
4.3.4	Feed consumption	See Table A7.5.3.1.1-6.	
4.3.5	Concentration / response curve	Not stated.	
4.3.6	Other effects	After 4 days first mortalities were recorded at 251 and 631 mg/kg bw and the first mortality at 398 mg/kg bw occurred on day 8. Toxic symptoms included lethargy, depression, indifferent response to external stimuli, wing droop, ruffled plumage, coordination loss and lower limb weakness. The final death occurred on day 10 at 398 mg/kg bw. No deaths or sub-lethal effects occurred in the control group or among birds dosed at 100 and 159 mg/kg bw.	
4.4	**Results of controls**		
4.4.1	Number/ percentage of animals showing adverse effects	None.	
4.5	**Test with reference substance**	Not performed.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.2	**Materials and methods**	An acute oral toxicity study with bobwhite quail (*Colinus virginianus*). Administration, by crop intubation of chlorophacinone in corn oil at doses of 100 to 631 mg/kg bw. The test duration was 14 days.	
5.3	**Results and discussion**		
5.3.1	LD_{50}	The 14-day LD_{50} of chlorophacinone to the bobwhite quail was 495 mg/kg bw (with 95% confidence limits of 383 to 641 mg/kg bw). Sub-lethal effects were observed from 251 mg/kg bw and included lethargy, depression, indifferent response to external stimuli, wing droop, ruffled plumage, coordination loss and lower limb weakness. Some symptoms persisted among survivors at 251 mg/kg bw and above up to the end of the study.	
5.4	**Conclusion**	Validity criterion of less than 10% mortality in the control treatment was achieved.	
5.4.1	Reliability	2.	

Section 7.5.3.1.1-01 Annex Point IIIA XIII.1.1	Acute oral toxicity on birds	
5.4.2 Deficiencies	No specification given for the test material. No macropathological examination of survivors or birds that died during the test.	X
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	Sixty adult birds into five treated groups and one control group of ten birds (Bobwhite quail (*Colinus virginianus*)) each (five males/five females) were exposed by oral intubation to five different concentrations (100, 159, 251, 398 and 631 mg/kg bw) of chlorophacinone technical (purity no stated) dispersed in corn oil.	
Results and discussion	LD_{50} = 495 mg/kg bw.	
Conclusion	**5.3.2. Deficiencies:** The test and recovery diets included sources of vitamin K that would have counteracted the effects of chlorofacinone.	
Reliability	3	
Acceptability	Not acceptable. The study is considered not acceptable since diets for feeding the birds included sources of vitamin K that could have counteracted the effects of the a.s. increasing the LC_{50} significantly. It is not necessary to repeat the test since another acute oral toxicity on birds study has been performed for the same species.	
Remarks		

Table A7.5.3.1.1-1: Method of administration of the test substance

Carrier / Vehicle	Details
Organic carrier	Corn oil.
Concentration of the carrier	Stock dispersions of 5 and 10 g chlorophacinone in corn oil, total volume 50 ml. Volume of appropriate dispersion adjusted according to treatment and body weight and to administer similar doses on volume:body weight basis. Control birds received corn oil only.
Function of the carrier / vehicle	To suspend the test substance.

Table A7.5.3.1.1-2: Test animals

Criteria	Details
Species/strain	*Colinus virginanus.*
Source	Laboratory breeding stock.
Age (in weeks), sex and initial body weight (bw)	Adults (>10 weeks), mean group weight at start of test was 190 to 205 g (males and females).
Amount of food	*Ad libitum*.
Age at time of first dosing	Adults.
Health condition / medication	Healthy.

Table A7.5.3.1.1-3: Test system

Criteria	Details
Test location	Indoors.
Holding pens	10 birds per pen, "Beacon" battery brooders, model no. B755, dimensions not reported.
Number of animals	60.
Number of animals per pen [cm²/bird]	10 [individual floor space allocation unknown].
Number of animals per dose	10 (5 males, 5 females).
Pre-treatment / acclimation	Pre-acclimation for two weeks. Temperature approximately 18 to 24°C, 14 hour photoperiod. Food and water available *ad libitum* apart from 16 hour fasting period prior to dosing.
Diet during test	Gamebird grower diet (Vitamin K included).
Dosage levels (of test substance)	0 (control), 100, 159, 251, 398 and 631 mg/kg bw.
Feed dosing method	Crop intubation.
Frequency, duration and method of animal monitoring after dosing	Body weight: 1, 3, 7 and 14 days. Food consumption: 1-7 and 8-14 days. Mortality and toxic symptoms: daily.

Table A7.5.3.1.1-4: Test conditions (housing)

Criteria	Details
Test temperature	Temperature 18.3 to 23.9°C.
Relative humidity	Not stated.
Photoperiod and lighting	14 hour photoperiod.

Table A7.5.3.1.1-5: Summary of mortality

Test substance dosage level [mg/kg bw]	Mortalities (out 10 total)
Control	0
100	0
159	0
251	1
398	3
631	7

Mortalities from day 4 onwards at 251 and 631 mg/kg bw and from day 8 at 398 mg/kg bw, last mortality on day 10.

Table A7.5.3.1.1-6: Summary of body weight and food consumption

Test substance dosage level [mg/kg bw]	Mean body weight (g/bird)[1]				Estimated mean food consumption (g/bird/day)	
	day 1	day 3	day 7	day 14	days 1-7	days 8-14
Control	190	203	210	213	18	21
100	194	203	211	212	18	21
159	197	207	208	211	16	19
251	204	186	213	213	17	22
398	205	206	214	210	19	17
631	199	207	208	215	14	27

[1] Males and females combined.
[2] Days in relation to dosing.

Section 7.5.3.1.1-02 **Annex Point IIIA XIII.1.1**	**Acute oral toxicity on birds**	
	6 REFERENCE	**Official use only**
1.1. Reference	Xxxxxx, XX. and Xxxxxxx, XX. (XXXX). Chlorophacinone: 30-day acute oral LD_{50} study in bobwhite quail (*Colinus virginianus*). XXXXXXXXXXXX., laboratory report number XXXXXX, 16 May XXXX (unpublished).	
1.2. Data protection	Yes.	
1.2.1. Data owner	Liphatech S.A.S.	
1.2.2. Companies with letter of access	None.	
1.2.3. Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.2. Guideline study	Yes. US EPA FIFRA 71-1, comparable to SETAC 1995.	
2.3. GLP	Yes.	
2.4. Deviations	No.	
	3. METHOD	
3.2. Test material	Chlorophacinone (termed Rozol Technical in the report).	
3.2.1. Lot/Batch number	XXXXXX	
3.2.2. Purity	XXX% (w/w).	
3.3. Administration of the test substance	Test substance mixed with corn oil and administered via oral gavage.	
3.4. Reference substance	No.	
3.5. Testing procedure		
3.5.1. Test organisms	See Table A7.5.3.1.1-8.	
3.5.2. Test system	See Table A7.5.3.1.1-9.	
3.5.3. Duration of the test	30 days.	
3.5.4. Test parameter	Mortality, behaviour and macropathology.	
3.5.5. Examination / Observation	See Table A7.5.3.1.1-9.	
3.5.6. Statistics	Determination of LD_{50} according to Litchfield and Wilcoxon.	
	4. RESULT	
4.2. Limit Test / Range finding test	Yes (two tests).	
4.2.1. Concentration	Range finding test 1: 1.0, 6.81 and 21.5 mg/kg bw; Range finding test 2: 215, 464 and 1,000 mg/kg bw.	

Section 7.5.3.1.1-02 Annex Point IIIA XIII.1.1	Acute oral toxicity on birds	
4.2.2. Number/ percentage of animals showing adverse effects	No deaths in range-finding test 1. Survivors only at 215 mg/kg bw in range-finding test 2.	
4.2.3. Nature of adverse effects	Mortality.	
4.3. Results test substance		
4.3.1. Applied concentrations	See Table A7.5.3.1.1-9.	
4.3.2. Effect data (Mortality)	See Table A7.5.3.1.1-11.	
4.3.3. Body weight	See Table A7.5.3.1.1-12.	
4.3.4. Feed consumption	See Table A7.5.3.1.1-12.	
4.3.5. Concentration / response curve	Not stated.	
4.3.6. Other effects	After 2 days first mortalities were recorded at 316 to 681 mg/kg bw. Toxic symptoms included lethargy, ruffled feathers, diarrhoea (sometimes containing blood), weakness, anorexia and bleeding from tail feathers. Final deaths occurred on day 5 at 316, 464 and 681 mg/kg bw. All surviving birds appeared normal from day 6 onwards. Post-mortem examination revealed haemorrhaging (intramuscular, subdermal, in body cavity) in 26 of the 28 birds that died during the test. One bird had a mottled liver with diffuse discolouration (at 464 mg/kg bw) and a white chalky substance surrounded the heart and upper hepatic lobes of one bird at 316 mg/kg bw. Post-mortem findings in birds sacrificed at study termination showed no abnormal tissue alterations in four birds selected from each of the control, 100 and 215 mg/kg bw groups or the two survivors from each of the 316 to 681 mg/kg bw treatments. Statistically significant bodyweight depression, relative to the control group, occurred at 316, 464 and 681 mg/kg bw, but was confined to day 3. Bodyweights of surviving birds were comparable to the control group at all subsequent measurements. Severe food avoidance was recorded for the 316, 464 and 681 mg/kg bw groups up to day 7, but from day 8 onwards, food consumption in all treatment groups was either equal to or greater than that of the control birds.	
4.4. Results of controls		
4.4.1. Number/ percentage of animals showing adverse effects	Diarrhoea noted on days 1 and 2, anorexia on day 3.	
4.5. Test with reference substance	Not performed.	

Section 7.5.3.1.1-02 **Annex Point IIIA XIII.1.1**	**Acute oral toxicity on birds**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.2. Materials and methods	An acute oral toxicity study with bobwhite quail (*Colinus virginianus*) in accordance with SETAC (1995). Administration, by oral gavage of chlorophacinone in corn oil at doses of 100 to 681 mg/kg bw. The test duration was 30 days.	
5.3. Results and discussion		
5.3.1. LD_{50}	The 30-day LD_{50} of chlorophacinone to the bobwhite quail was 257 mg/kg bw (with 95% confidence limits of 177 to 373 mg/kg bw).	
5.4. Conclusion	Validity criterion of less than 10% mortality in the control treatment was achieved.	
5.4.1. Reliability	1.	
5.4.2. Deficiencies	No.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods	US EPA FIFRA 71-1, comparable to SETAC 1995.	
Results and discussion	LD_{50} = 257 mg/kg bw.	
Conclusion		
Reliability	1	
Acceptability	Acceptable.	
Remarks		

Table A7.5.3.1.1-7: Method of administration of the test substance

Carrier / Vehicle	Details
Organic carrier	Yes, corn oil.
Concentration of the carrier	A premix of 5000 mg chlorophacinone in corn oil, total volume 100 ml. One ml of premix equal to 50 mg chlorophacinone. Total volume administered per bird was 3.10 ml (premix diluted as appropriate with additional corn oil, control birds received 3.10 ml corn oil only).
Function of the carrier / vehicle	To suspend the test substance.

Table A7.5.3.1.1-8: Test animals

Criteria	Details
Species/strain	*Colinus virginianus.*
Source	XXXXXXXXXXXXXXXXXXXXXXXXX, USA.
Age (in weeks), sex and initial body weight (bw)	Adults (27 weeks), group mean weights at start of test were 199 to 208 g (males and females).
Amount of food	*Ad libitum*.
Age at time of first dosing	Adults.
Health condition / medication	Healthy.

Table A7.5.3.1.1-9: Test system

Criteria	Details
Test location	Indoors.
Holding pens	Wire pens: 53.3 × 45.7 × 38.1 cm (10 birds per pen).
Number of animals	60.
Number of animals per pen [cm²/bird]	10 [244 cm²/bird].
Number of animals per dose	10 (5 males, 5 females).
Pre-treatment / acclimation	Quarantine and acclimation period of 45 days. Temperature approximately 18 to 21°C, humidity 49 to 71%, 8 hour photoperiod. Food and water available *ad libitum* apart from a 16-17 hour fasting period prior to dosing
Diet during test	Vitamin K deficient diet 'Teklad'.
Dosage levels (of test substance)	0 (control), 100, 215, 316, 464 and 681 mg/kg bw.
Feed dosing method	Oral gavage, 0 hours.
Frequency, duration and method of animal monitoring after dosing	Body weights: 3, 7, 14, 21 and 30 days. Food consumption: 1-3, 4-7, 8-14, 15-21 and 22-30 days. Mortality and toxic symptoms: daily.

Table A7.5.3.1.1-10: Test conditions (housing)

Criteria	Details
Test temperature	Temperature 18 to 21°C.
Relative humidity	Humidity 49 to 71%.
Photoperiod and lighting	8 hour photoperiod.

Table A7.5.3.1.1-11: Summary of mortality

Test substance dosage level [mg/kg bw]	Mortalities (out of 5 per sex, 10 total)		
	Males	Females	Total
Control	0	0	0
100	1	0	1
215	3	0	3
316	3	5	8
464	4	4	8
681	4	4	8

Mortalities from day 2 onwards at 316 mg/kg bw and above, and from day 4 at 100 and 215 mg/kg bw, last mortalities on day 5.

Table A7.5.3.1.1-12: Summary of body weight and food consumption

Test substance dosage level [mg/kg bw]	Mean body weight (g/bird)[1]						Estimated mean food consumption (g/bird/day)				
	day 1[2]	day 3	day 7	day 14	day 21	day 30	d 1-3	d 4-7	d 8-14	d 15-21	d 22-30
Control	200	197	198	201	200	203	7	14	14	12	13
100	204	196	200	207	202	207	8	14	16	13	15
215	202	194	199	207	200	202	8	15	17	16	13
316	199	178[3]	178	196	197	204	3	5	26	22	21
464	201	176[3]	197	204	203	208	2	10	21	20	17
681	208	175[3]	187	205	208	214	2	8	22	20	17

[1] Males and females combined.
[2] Days in relation to dosing.
[3] Significantly different to control at $p < 0.05$.

Section 7.5.3.1.2-01 Annex Point IIIA XIII.1.2	Short-term toxicity on birds	
	1. REFERENCE	**Official use only**
1.1. Reference	Xxxxxx, XX. and Xxxxx, XX. (XXXX). Chlorophacinone: 30-day acute dietary LC_{50} study in bobwhite quail. XXXXXXXXXXXXX, laboratory report number XXXXXXX, 16 May XXXX (unpublished).	
1.2. Data protection	Yes.	
1.2.1. Data owner	LiphaTech S.A.S.	
1.2.2. Companies with letter of access	None.	
1.2.3. Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1. Guideline study	Yes. US EPA FIFRA 71-2, comparable to OECD 205.	
2.2. GLP	Yes.	
2.3. Deviations	Report describes taking of samples of diets and despatch to sponsor for analysis. Analytical method and findings not reported.	
	3. METHOD	
3.1. Test material	Chlorophacinone (termed Rozol Technical in the report).	
3.1.1. Lot/Batch number	XXXXXXX	
3.1.2. Purity	XXX% (w/w).	
3.1.3. Method of analysis	UV absorption spectrometry	
3.2. Administration of the test substance	Suspended in corn-oil and incorporated into a pre-mix, subsequently used to prepare a range of diets at selected test concentrations.	
3.3. Reference substance	No.	
3.4. Testing procedure		
3.4.1. Test organisms	See Table A7.5.3.1.2-1.	
3.4.2. Test system	See Table A7.5.3.1.2-2.	
3.4.3. Test conditions	See Table A7.5.3.1.2-3.	
3.4.4. Duration of the test	30 days.	
3.4.5. Test parameter	Mortality, body weights, food consumption, sub-lethal effects (observations), gross pathology.	
3.4.6. Examination / Observation	See Table A7.5.3.1.2-2.	
3.4.7. Statistics	Details not reported.	
	4. RESULTS	

Section 7.5.3.1.2-01 **Annex Point IIIA XIII.1.2**	**Short-term toxicity on birds**	
4.1. Limit Test / Range finding test	Performed with dietary concentrations of 50, 100, 200, 400, 800 and 1600 mg/kg food.	
4.2. Results test substance		
4.2.1. Effect data (Mortality)	Table A7.5.3.1.2-4.	
4.2.2. Body weight	See Table A7.5.3.1.2-6.	
4.2.3. Food consumption	See Table A7.5.3.1.2-6.	
4.2.4. Other effects	Mortalities occurred in all treatments between 50 and 800 mg/kg food and were first observed on the second day of exposure. Signs of toxicity were noted among birds of all chlorophacinone treatments. Three birds of the 10 mg/kg food treatment group exhibited swollen feet on day 5 and blood-staining of the lower limbs was evident in one bird until day 8, after which the effects receded. Effects seen at all higher treatments included intra-muscular, subcutaneous and internal hamorrhaging, swollen and bloodstained legs and feet, reduced size, weakness and lethargy. Mean body weights of all treatment groups on days 5 and 30 were considered normal by comparison to the controls. During the exposure period, food consumption by birds given the treated diets was similar to the controls, except for the birds of the 200 and 400 mg/kg food treatments where consumption was lower. Food consumption by surviving birds generally matched that of the controls during the recovery period.	
4.3. Results of controls		
4.3.1. Number/ percentage of animals showing adverse effects	No treatment-related effects observed.	
4.4. Test with reference substance	Not performed.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1. Materials and methods	A short-term dietary toxicity test with bobwhite quail (*Colinus virginianus*) in accordance with OECD 205. Administration by dietary inclusion at nominal concentrations of 50 to 800 mg/kg food. The test duration was 30 days (five days on test diets, 25 days recovery on basal vitamin K deficient diet).	
5.2. Results and discussion	*Summarize relevant results; discuss relevant test material-specific properties (e.g. solubility, stability, adsorption behaviour, volatility).*	
5.2.1. LC_{50}	The 30-day LC_{50} of chlorophacinone to the bobwhite quail was 95 mg/kg food (with 95% confidence limits of 38 to 239 mg/kg food).	**X**
5.3. Conclusion	Mortality was less than 10% in the control treatment. Numbers of control groups, period on test diet and total test	

Section 7.5.3.1.2-01 Annex Point IIIA XIII.1.2	Short-term toxicity on birds	
	duration and observation frequency were in accordance with OECD 205.	
5.3.1. Reliability	2	
5.3.2. Deficiencies	No analytical confirmation of dietary concentrations.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods	US EPA FIFRA 71-2, comparable to OECD 205 (Avian Dietary Toxicity Test). 5 days fed with the test diet plus 25 days with basal diet. A corn oil suspension of the test material was incorporated into vitamin k deficient diet. Six groups of eleven-day-old bobwhite quail were fed diets containing 10, 50, 100, 200, 400 and 800 mg a.s. Four vehicle control groups (0 ppm a.i.) each received vitamin k deficient diet which had been mixed with corn oil. Water *ad libitum*.	
Results and discussion	The 30-day LC_{50} of chlorophacinone to the bobwhite quail was 95 mg/kg food (with 95% confidence limits of 38 to 239 mg/kg food). **5.2.1.** LC_{16} = 7 mg a.s/kg food and LC_{84} = 1,170 mg/kg food.	
Conclusion		
Reliability	2	
Acceptability	The test is acceptable, although minor deficiencies ocurred; which do not affect significantly the outcome of the test.	
Remarks	24 hour lighting (fluorescent lights). OECD 205 recommends a lighting period of 12-16 h/d. **Table A7.5.3.1.2-1: Test animals**: animal age range within the test: 11 to 41 days (from start to end of test. According to guideline OECD 205 the test temperature should be in the range of 30-32 ºC if the Bobwhite quail is between 8-14 days and 25-28 ºC in case they are above 14 days. Temperature should not be above 32ºC but in table Table A7.5.3.1.2-3 a range between 27.8-40ºC is stated.	

Table A7.5.3.1.2-1: Test animals

Criteria	Details
Species/strain	Bobwhite quail (*Colinus virginianus*).
Source	XXXXXXXXXXXXXXXXXXXX, USA).
Age (in weeks), sex and initial body weight (bw)	Birds hatched from eggs received from breeding farm and acclimated for 11 days before test initiation, not sexed, initial group mean body weights 21 – 22 g on day 0 of the test.
Age range within the test	11 to 41 days (from start to end of test).

Table A7.5.3.1.2-2: Test system

Criteria	Details
Test location	Held indoors.
Holding pens	Wire pens (45.7 cm × 61.0 cm × 45.7 cm).
Number of animals	100.
Number of animals per pen [cm²/bird]	10 [279].
Number of animals per dose	10.
Pre-treatment / acclimation	Pre-treatment in wire pens and fed basal diet.
Diet during test	Vitamin K deficient laboratory diet ('Teklad').
Dosage levels (of test substance)	0, 10, 50, 100, 200, 400 and 800 mg/kg food.
Dosing method	Dietary inclusion for first five days of test, then on basal diet only for remainder of test until day 30.
Frequency, duration and method of animal monitoring after dosing	Observations: daily; Gross pathology at termination and birds found dead during test; Body weights (days):1, 5 and 30. Food consumption (5 day periods): 5, 10, 15, 20, 25, 30.

Table A7.5.3.1.2-3: Test conditions (housing)

Criteria	Details
Test temperature	Range over entire test duration was 27.8 to 40.0°C.
Relative humidity	29 to 73%.
Photoperiod and lighting	24 hour lighting (fluorescent lights).

Table A7.5.3.1.2-4: Mortality data after test termination

Test substance nominal dosage level [mg/kg food]	Mortalities after test termination (out of 10 per treatment)		
	Number dead	Percent dead	Time of death (days)
Control (group 1)	0	0	-
Control (group 2)	0	0	-
Control (group 3)	0	0	-
Control (group 4)	0	0	-
10	0	0	-
50	4	40	2,4,4,5
100	8	80	2,3,3,4,4,4,4,4
200	8	80	2,2,2,2,3,3,4,5
400	9	90	2,2,3,3,4,4,4,5,5
800	8	80	4,4,4,4,7,8,8,9

Table A7.5.3.1.2-5: Validity criteria for short-term toxicity test according to OECD 205

	Fulfilled
Mortality of control animals < 10%	**Yes**
Test substance concentration > 80% of nominal concentration throughout the dosing period	**Not reported**
Lowest treatment level causing no compound-related mortality.	**Yes**
Lowest treatment level causing no other observable toxic effects	**No**

Table A7.5.3.1.2-6: Summary of body weight and food consumption

Test substance dosage level [mg/kg food]	Mean body weight (g/bird)				Estimated mean food consumption (g/bird/day)[1]					
	Day 1	Day 5	Day 30	Overall gain	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (I)	21	31	104	+83	7	5	7	10	11	13
Control (II)	22	28	102	+80	5	4	7	10	13	14
Control (III)	21	29	109	+88	9	5	8	11	13	14
Control (IV)	22	30	107	+85	8	6	7	10	11	12
Mean control	22	30	106	+84	7	5	7	10	12	13
10	21	30	110	+89	7	6	7	10	14	13
50	22	27	112	+90	5	5	8	13	14	19
100	21	34	115	+94	5	7	8	11	12	15
200	21	38	120	+99	4	12	7	8	12	12
400	22	33	125	+103	3	16	14	14	21	18
800	22	28	106	+84	5	5	10	9	9	13

[1] Mean food consumption over 5 day periods.

Section 7.5.3.1.2-02 **Annex Point IIIA XIII.1.2**	**Short-term toxicity on birds**	
	1. REFERENCE	**Official use only**
1.1. Reference	Xxxxxx, XX and Xxxxxxx, XX. (XXXX). Chlorophacinone: 30-day acute dietary LC_{50} study in mallard ducklings (*Anas platyrhynchos*). XXXXXXXXXXXX., laboratory report number XXXXXXX, 16 May XXXX (unpublished).	
1.2. Data protection	Yes.	
1.2.1. Data owner	LiphaTech S.A.S.	
1.2.2. Companies with letter of access	None.	
1.2.3. Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1. Guideline study	Yes. US EPA FIFRA 71-2 B, comparable to OECD 205.	
2.2. GLP	Yes.	
2.3. Deviations	Report describes taking of samples of diets and despatch to sponsor for analysis. Analytical method and findings not reported.	
	3. METHOD	
3.1. Test material	Chlorophacinone (termed Rozol Technical in the report).	
3.1.1. Lot/Batch number	XXXXXXX	
3.1.2. Purity	XXX% (w/w).	
3.1.3. Method of analysis	UV absorption spectrometry	
3.2. Administration of the test substance	Suspended in corn-oil and incorporated into basal diet at 800 mg/kg, portions further diluted with diet to prepare lower test concentrations.	
3.3. Reference substance	No.	
3.4. Testing procedure		
3.4.1. Test organisms	See Table A7.5.3.1.2-7.	
3.4.2. Test system	See Table A7.5.3.1.2-8.	
3.4.3. Test conditions	See Table A7.5.3.1.2-9.	
3.4.4. Duration of the test	30 days.	
3.4.5. Test parameter	Mortality, body weights, food consumption, sub-lethal effects (observations), gross pathology.	
3.4.6. Examination / Observation	See Table A7.5.3.1.2-8.	
3.4.7. Statistics	Details not reported.	

Section 7.5.3.1.2-02 Annex Point IIIA XIII.1.2	Short-term toxicity on birds	
	4. RESULTS	
4.1. Limit Test / Range finding test	Performed with dietary concentrations of 50, 100, 200, 400, 800 and 1600 mg/kg food.	
4.2. Results test substance		
4.2.1. Effect data (Mortality)	Table A7.5.3.1.2-10.	
4.2.2. Body weight	See Table A7.5.3.1.2-12.	
4.2.3. Food consumption	See Table A7.5.3.1.2-12.	
4.2.4. Other effects	Mortalities occurred in all chlorophacinone treatment groups and were first observed on the third day of exposure among the birds fed with 10 and 400 mg/kg food. Two birds died at 10 mg/kg food and both of these mortalities occurred during the initial treatment period, whilst most deaths at all other treatment levels occurred during the recovery period. The last mortality was on day 23 in the 800 mg/kg food group. Signs of toxicity that included intra-muscular, subcutaneous and internal haemorrhaging, swollen and blood-stained callus-like areas on the feet, anorexia, reduced size, green discolouration of faeces, weakness and lethargy, were noted among birds of all chlorophacinone treatments. Mean body weights of the 800 and 50 mg/kg food treatment groups were reduced by comparison to the controls on days 5 and 30, respectively. Food consumption was severely depressed at 200 mg/kg food for the first five days of the recovery period, but reverted to normal by day 15. Food consumption was reduced during the last five days of the test at 400 mg/kg food and was suppressed throughout the recovery phase at 800 mg/kg food.	
4.3. Results of controls		
4.3.1. Number/ percentage of animals showing adverse effects	No treatment-related effects observed.	
4.4. Test with reference substance	Not performed.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1. Materials and methods	A short-term dietary toxicity test with mallard ducklings (*Anas platyrhynchos*) in accordance with OECD 205. Administration by dietary inclusion at nominal concentrations of 10 to 800 mg/kg food. The test duration was 30 days (five days on test diets, 25 days recovery on basal vitamin K deficient diet).	
5.2. Results and discussion		

Section 7.5.3.1.2-02 **Annex Point IIIA XIII.1.2**	**Short-term toxicity on birds**	
5.2.1. LC_{50}	The 30-day LC_{50} of chlorophacinone to mallard ducklings was 204 mg/kg food (with 95% confidence limits of 67 to 622 mg/kg food).	**X**
5.3. Conclusion	Mortality was less than 10% in the control treatment. Numbers of control groups, period on test diet and total test duration and observation frequency were in accordance with OECD 205.	
5.3.1. Reliability	2	
5.3.2. Deficiencies	No analytical confirmation of dietary concentrations; treatment-related effects occurred at the lowest concentration.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	US EPA FIFRA 71-2 B, comparable to OECD 205. (Avian Dietary Toxicity Test). 5 days fed with the test diet plus 25 days with basal diet. A corn oil suspension of the test material was incorporated into vitamin k deficient diet. Six groups of five-day-old Mallard ducklings (*Anas platyrhynchos*) were fed diets containing 10, 50, 100, 200, 400 and 800 mg a.s. Four vehicle control groups (0 ppm a.i.) each received vitamin k deficient diet which had been mixed with corn oil. Water *ad libitum*. Mean body weight of the 800 mg/kg food treatment group was reduced by comparison to the control on days 5 but recovered by day 30. Food consumption was severely depressed at 200 mg/kg food for the first five days of the recovery periods, but reverted to normal by day 15. Food consumption was reduced during the last five days of the test at 400 mg/kg food and was suppressed throughout the recovery phase at 800 mg/kg food only at day 30. The estimated mean food consumption (g/bird/d) over a 5 and 10-day period differ considerably from the control about a 25 and 40% decrease, but recovered at day 15.	
Results and discussion	The 30-day LC_{50} of chlorophacinone (mortality) to the mallard ducklings was 204 mg/kg food (with 95% confidence limits of 67 to 622 mg/kg food) based on nominal concentrations. NOEC < 10 mg/kg food. **5.2.1.** LC_{16} = 11 mg a.s/kg food and LC_{84} = 3,350 mg a.s/kg food.	
Conclusion		
Reliability	2	
Acceptability	The test is acceptable, although minor deficiencies ocurred; which do not affect significantly the outcome of the test.	
Remarks	24 hour lighting (fluorescent lights). OECD 205 recommends a lighting period of 12-16 h/d.	

Table A7.5.3.1.2-7: Test animals

Criteria	Details
Species/strain	Mallard ducklings (*Anas platyrhynchos*).
Source	Breeding farm (Whistling Wings, IL, USA).
Age (in weeks), sex and initial body weight (bw)	Three-day old birds received from breeding farm and acclimated for one day before test initiation, not sexed, initial group mean body weights 47 – 53 g on day 0 of the test.
Age range within the test	5 to 35 days (from start to end of test).

Table A7.5.3.1.2-8: Test system

Criteria	Details
Test location	Held indoors.
Holding pens	Wire pens (45.7 cm × 61.0 cm × 45.7 cm).
Number of animals	100.
Number of animals per pen [cm²/bird]	10 [279].
Number of animals per dose	10.
Pre-treatment / acclimation	Pre-treatment in wire pens and fed basal diet.
Diet during test	Vitamin K deficient laboratory diet ('Teklad').
Dosage levels (of test substance)	0, 10, 50, 100, 200, 400 and 800 mg/kg food.
Dosing method	Dietary inclusion for first five days of test, then on basal diet only for remainder of test until day30.
Frequency, duration and method of animal monitoring after dosing	Observations: daily; Gross pathology at termination and birds found dead during test; Body weights (days):1, 5 and 30. Food consumption (5 day periods): 5, 10, 15, 20, 25, 30.

Table A7.5.3.1.2-9: Test conditions (housing)

Criteria	Details
Test temperature	Range over entire test duration was 21.1 to 28.3°C.
Relative humidity	43 to 83%.
Photoperiod and lighting	24 hour lighting (fluorescent lights).

Table A7.5.3.1.2-10: Mortality data after test termination

Test substance nominal dosage level [mg/kg food]	Mortalities after test termination (out of 10 per treatment)		
	Number dead	Percent dead	Time of death (days)
Control (group 1)	0	0	-
Control (group 2)	0	0	-
Control (group 3)	0	0	-
Control (group 4)	0	0	-
10	2	20	3,5
50	4	40	6,7,7,8
100	4	40	4,5,7,7
200	3	30	5,8,10
400	6	60	3,4,6,8,10,12
800	9	90	4,5,7,9,13,13,14,21,23

Table A7.5.3.1.2-11: Validity criteria for short-term toxicity test according to OECD 205

	Fulfilled
Mortality of control animals < 10%	**Yes**
Test substance concentration > 80% of nominal concentration throughout the dosing period	**Not reported**
Lowest treatment level causing no compound-related mortality.	**No**
Lowest treatment level causing no other observable toxic effects	**No**

Table A7.5.3.1.2-12: Summary of body weight and food consumption

Test substance dosage level [mg/kg food]	Mean body weight (g/bird)				Estimated mean food consumption (g/bird/day)[1]					
	Day 1	Day 5	Day 30	Overall gain	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control (I)	49	104	492	443	13	18	27	57	73	103
Control (II)	52	111	544	492	15	29	38	56	73	94
Control (III)	47	120	573	526	14	29	45	63	81	119
Control (IV)	52	114	521	469	23	26	40	65	75	101
Mean control	50	112	533	483	16	26	38	60	76	104
10	51	110	567	516	11	21	41	74	91	90
50	50	102	467	417	17	20	37	52	75	117
100	51	106	582	531	11	18	57	79	84	108
200	53	110	568	515	9	9	49	69	80	90
400	52	101	575	523	11	20	31	61	80	82
800	52	84	486	434	14	6	14	15	27	84

[1] Mean food consumption over 5 day periods.

Section 7.5.3.1.3-01 Annex Point IIIA XIII.1.3	Effects on reproduction of birds	
	1. REFERENCE	**Official use only**
1.1. Reference	Riedel, B., Grün, G. and Clausing, P. (1990). Die subakute und subchronische Toxizität von Chlorophacinon an Japanwachteln (*Coturnix c. japonica*). Institut für Pflanzenschutzforschung Kleinmachnow der Akademie der Landwirtschaftswissenschaften der DDR – Ornithologische Forschungsstelle Seebach. Published: *Arch. exper. Vet.med., Leipzig*. **44** (3): pp 341-346.	
1.2. Data protection	No.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1. Guideline study	None cited. Report refers to a modification based on the Hygienisch-toxikologische Anforderungen für die Zulassung von Pflanzenschutzmitteln und Mitteln zur Steuerung biologischer Prozesse in der DDR und VRP, Kleinmachnow, Pszcyna.	
2.2. GLP	No. GLP did not apply in the former GDR at the time the study was performed.	
2.3. Deviations	Yes. Several deviations with respect to requirements of the current OECD 206. Specification of test substance not given. Diet composition, housing and environmental conditions not reported, bodyweight and food consumption measurements not presented and test results presented selectively, omitting those from the lower chlorophacinone dose groups. No egg fertility/hatch data presented. Nevertheless, this study serves to demonstrate the absence of any long-term reproductive effects on birds, other than haemhorraging typical of anticoagulant poisoning and death.	
	3. METHOD	
3.1. Test material	Chlorophacinone oil concentrate sourced from Lipha, France.	
3.1.1. Lot/Batch number	Not stated.	
3.1.2. Purity	0.25%, nominal.	
3.2. Administration of the test substance	Incorporated in diet.	
3.3. Testing procedure		
3.3.1. Test organisms	See Table A7.5.3.1.3-2	
3.3.2. Test system	See Table A7.5.3.1.3-3	
3.3.3. Diet	Information not reported.	
3.3.4. Test conditions	Conditions not reported.	
3.3.5. Duration of the test	90 days.	
3.3.6. Test parameter	Mortality, sub-lethal physiological and reproductive effects. Reproductive capacity of first generation offspring.	

Section 7.5.3.1.3-01 Annex Point IIIA XIII.1.3		Effects on reproduction of birds	
3.3.7.	Examination / Observation	See Table A7.5.3.1.3-3	
3.3.8.	Statistics	Standard tests of significance for differences between test and control groups, e.g. Mann and Whitney U-test, t-test.	
		4. RESULTS	
4.1. Range finding test		Preliminary sub-acute test performed with 11-day old Japanese quail with 5 day exposure followed by 3-day observation phase gave an LC_{50} of 60 mg chlorophacinone/kg food (with 95% confidence limits of 45 to 75 mg/kg).	
4.1.1.	Concentration	Not reported.	
4.1.2.	Number/ percentage of animals showing adverse effects	Not reported.	
4.1.3.	Nature of adverse effects	Not reported.	
4.2. Results test substance			
4.2.1.	Applied concentrations	0 (control), 0.5, 1, 2, 4 and 8 mg chlorophacinone/kg food.	
4.2.2.	Effect data (Mortality and reproductivity)	Mortalities were recorded in treatment groups fed with dietary chlorophacinone concentrations equal to and higher than 2 mg/kg. Overall group mortality was 17%, 4% and 38% at 2, 4 and 8 mg/kg, respectively, but significantly more females (58%) than males (17%) died at the highest treatment. See Table A7.5.3.1.3-5. Weights of eggs laid by the parental birds were reduced at 8 mg/kg food during the first five weeks of laying. Egg production was reduced in this treatment group, relative to the control, throughout the study. See Table A7.5.3.1.3-6. Eggshell parameters were unaffected at all dietary concentrations tested. The progeny of the 8 mg/kg treatment group initially laid smaller eggs during the first week of their laying period, but subsequent egg weights were not significantly different from those of the control birds. Parental exposure had no discernible impact on the egg production of the offspring. See Table A7.5.3.1.3-7. The NOEC, based on mortality, the most sensitive endpoint, was 1 mg chlorophacinone/kg food.	**X**
4.2.3.	Body weight	Not reported.	
4.2.4.	Food consumption	Not reported.	
4.2.5.	Results of residue analysis	Not performed.	
4.2.6.	Other effects	Dietary inclusion of chlorophacinone had no significant effect on bodyweight gain or food consumption of either sex in all dose groups. A series of birds displayed loss of coordination and lack of movement, symptoms that regularly preceded death. Birds of the high dose group were particularly susceptible to stress. Dissection showed numerous instances and varying degrees of sub-cutaneous and intra-muscular bleeding. Haemorrhaging in the intestinal and breast cavities was regularly seen in dead birds of the 4 and 8 mg/kg food treatments. No changes were detected in the erythrocytes of male	

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	birds, but microcytic anaemia occurred in females at 8 mg/kg food. Leucocyte counts were unaffected. Plasma glucose levels were reduced in females, but this finding was not dose-related. Exposure to chlorophacinone tended to result in liver enlargement, but there was no macroscopic evidence of fat storage. Prothrombin times showed a significant level of blood clotting reduction from 4 mg/kg food upwards.	
4.3. Results of controls		
4.3.1. Number/ percentage of animals showing adverse effects	None.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1. Materials and methods	The long-term effects of chlorophacinone on reproduction of Japanese quail were assessed by a study based on the now obsolete requirements for plant protection product and biologically active substance registration in the former GDR. Administration by dietary inclusion at nominal concentrations of 0.5 to 8 mg/kg food. The test duration was 90 days. The procedure is deficient in several respects by comparison with the equivalent OECD guideline and the report lacks detail. Nevertheless, given the mode of action of chlorophacinone, it is highly unlikely that a different outcome would have been achieved had the study been otherwise conducted.	
5.2. Results and discussion	See Sections 4.2.2 and 4.2.6. The most sensitive endpoint in this study was mortality among the parental birds exposed to chlorophacinone in their diet.	
5.2.1. NOEC	1 mg chlorophacinone/kg food (mortality); 4 mg chlorophacinone/kg food (egg production)	
5.3. Conclusion	Mortality of the parental birds of the control treatment group was less than 10%. The data required to assess other aspects of test validity according to current OECD 206 have not been reported. (See Table A7.3.1.3-10).	
5.3.1. Reliability	3.	
5.3.2. Deficiencies	Yes. Specification of test substance not given. Diet composition, housing and environmental conditions not reported, bodyweight and food consumption measurements not presented and test results presented selectively, omitting those from the lower chlorophacinone dose groups. No egg fertility/hatch data presented. Nevertheless, this study serves to demonstrate that the principal effects of long-term exposure of Japanese quail to chlorophacinone were the pattern of haemhorraging and death typical of anticoagulant poisoning. Given the mode of action of chlorophacinone, it is considered unlikely that these deficiencies influenced the overall outcome of the study.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006.	
Materials and Methods		

Section 7.5.3.1.3-01 Annex Point IIIA XIII.1.3	Effects on reproduction of birds	
Results and discussion	**4.2.2.** Mortalities were recorded in treatment groups fed with dietary chlorophacinone concentrations at least equal to and higher than 2 mg/kg food; below this concentration data have not been reported. Overall group mortality was 17%, 4% (all males) and 38% at 2, 4 and 8 mg a.s/kg food, respectively, but significantly more females (58%) than males (17%) died at the highest treatment. Weights of eggs laid by the parental birds were reduced at 8 mg/kg food during the first five weeks of laying. Egg production was reduced in this treatment group, relative to the control, throughout the study (from week 1 until the end, week 7) at all dosage levels. The progeny of the 8 mg/kg treatment group initially laid smaller eggs during the first week of their laying period, but subsequent egg weights were not significantly different from those of the control birds. Parental exposure had no discernible impact on the egg production of the offspring. Subchronic toxicity test: NOEC (mortality) 1 mg chlorophacinone/kg food; No data reported for 0.5 and 1 mg a.s/kg food NOEC (egg production) =4 mg chlorophacinone/kg food No data reported for 0.5, 1 and 2 mg a.s/kg food Subacute oral toxicity LC_{50} (5+3d) = 60 mg chlorophacinone/kg food (95% confidence limits of 45-75 mg a.s/kg food). 0.25% substance purity. Parental generation five weeks old at start of test. 12 male and 12 female birds pero dose group. Body weight not reported. 0 (control), 0.5, 1, 2 and 8 mg chlorophacinone/kg food nominal concentrations. 0.25% substance purity. Purity of the substance 0.25%, the rest of impurities are unknown. Test animals should be according to OECD206 ±1/2 weeks old not 5. It has not been reported if a acclimation period of 14d was provided to the birds. Not reported: number o animals (male/female), cm2/bird, diet during test. Test temperature, ventilation, relative humidity, photoperiod and lighting. Storing, incubation and hatching conditions for eggs. Reported: general condition, mortality, egg weights and egg production for parental generation (from start of study) and offspring (from start of laying). Blood measurements and leucocyte counts were also made. Mortality data for parental generation. Offspring: egg weights and egg production recorded from the onset of egg-laying. Feed *ad libitum* Table A7.5.3.1.3-3: Test system: Number of animals per dose: 12 male and 12 females per group	
Conclusion		
Reliability	3	
Acceptability	acceptable	
Remarks		

Table A7.5.3.1.3-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	Information not reported.
Organic carrier	Information not reported.
Other vehicle	Information not reported.

Table A7.5.3.1.3-2: Test animals

Criteria	Details
Species/strain	Japanese quail (*Coturnix coturnix japonica*).
Source	Test facility stock.
Age (in weeks), sex and initial body weight (bw)	Parental generation five weeks old at start of test. 12 male and 12 female birds per dose group. Body weight data not reported.
Age range within the test	Five weeks (35 days) old at start, +90 days at end.
Breeding population	Flock maintained at test facility.
Amount of food	Available *ad libitum*.
Age at time of first dosing	Five weeks.
Health condition / medication	Not reported.
Pre-treatment	Not reported.

Table A7.5.3.1.3-3: Test system

Criteria	Details
Test location	Not reported.
Holding pens	Not reported.
Number of animals (male/female)	Not reported.
Number of animals per pen [cm²/bird]	Not reported.
Number of animals per dose	Not reported.
Pre-treatment / acclimation	Not reported.
Diet during test	Not reported.
Dosage levels (of test substance)	0 (control), 0.5, 1, 2, 4 and 8 mg chlorophacinone/kg food.
Replicate/dosage level	Not reported.
Dosing method	Dietary inclusion.
Frequency, duration and method of animal monitoring after dosing	Parental generation: General condition, mortality, egg weights and egg production were recorded daily. Bodyweights and food consumption were measured weekly. Eggs collected on weeks 11 and 12 were incubated and hatched. Eggs collected on week 13 were used to measure eggshell index and surface area and to obtain eggshell thickness data. At test-end, all survivors were terminated and dead birds necropsied to determine overall condition of internal organs and

	absolute and relative weights of heart, liver, spleen and testes. Blood measurements and leucocyte counts were also made Offspring: Egg weights and egg production recorded from the onset of egg-laying.
Time and intervals of body weight determination	Weekly.
Incubation, storing and hatching	Not reported.
Test period after egg-laying	Eggs laid throughout, from week 1 of test to termination.
Turning of eggs	Not reported.
Collection period for eggs	Weeks 1 to 13.

Table A7.5.3.1.3-4: Test conditions (housing)

Criteria	Details
Test temperature	Not reported.
Shielding of the animals	Not reported.
Ventilation	Not reported.
Relative humidity	Not reported.
Photoperiod and lighting	Not reported.
Storing, incubation and hatching conditions for eggs	Not reported.
Environmental conditions for young birds	Not reported.

Table A7.5.3.1.3-5: Mortality data for parental generation

Test substance nominal dosage level [mg a.s./kg food]	Mortality, % (out of 12 birds of each sex per treatment)	
	Male	Female
Control	0	0
0.5	nr	nr
1	nr	nr
2	17	17
4	0	8
8	17	58

nr: not reported.

Table A7.5.3.1.3-6: Egg production data for parental generation

Test substance nominal dosage level [mg/kg food]	Week number (from start of study)						
	1	2	3	4	5	6	7
Egg weights (g)							
Control	8.0 (8)	8.5 (17)	8.9 (30)	9.4 (42)	9.6 (55)	9.6 (57)	9.8 (62)
0.5	nr	nr	nr	nr	nr	nr	nr
1	nr	nr	nr	nr	nr	nr	nr
2	nr	nr	nr	nr	nr	nr	nr
4	7.7 (5)	8.5 (6)	9.1 (13)	5.6 (21)	9.3 (42)	9.7 (57)	10.0 (60)
8	6.8 (2)	7.9 (4)	8.0 (7)*	7.8 (10)*	9.1 (7)*	9.1 (11)	9.3 (14)
Eggs/hen/day							
Control	0.095	0.202	0.357	0.545	0.655	0.600	0.738
0.5	nr	nr	nr	nr	nr	nr	nr
1	nr	nr	nr	nr	nr	nr	nr
2	nr	nr	nr	nr	nr	nr	nr
4	0.062	0.078	0.169	0.273	0.558	0.753	0.779
8	0.024	0.048	0.083	0.119	0.104	0.161	0.250

nr: not reported
Values in brackets indicate numbers of eggs;
* Statistically significant difference from control ($p < 0.05$)

Table A7.5.3.1.3-7: Egg production data for offspring

Test substance nominal dosage level [mg/kg food]	Week number (from start of laying)	
	1	2
Egg weights (g)		
Control	8.2 (20)	8.9 (76)
0.5	nr	nr
1	nr	nr
2	nr	nr
4	7.8 (8)	8.9 (67)
8	7.4 (7)*	9.0 (31)
Eggs/hen/day		
Control	0.124	0.472
0.5	nr	nr
1	nr	nr
2	nr	nr
4	0.054	0.320
8	0.100	0.443

nr: not reported
* Statistically significant difference from control ($p < 0.05$)

Table A7.5.3.1.3-8: Blood analysis data for parental generation

Test substance nominal dosage level [mg/kg food]	Blood parameter						
	Haematocrit (%)	Haemoglobin (g/l)	Mean corpuscle volume (μm^3)	Glucose concentration (g/l)		Prothrombin time (min)	
	f	f	f	m	f	m	f
Control	42.2	134	13.9	2.87	2.71	2.1 – 6.0	2.3 – 7.2
0.5	nr	nr	nr	nr	nr	nr	nr
1	nr	nr	nr	nr	nr	nr	nr
2	42.8	125	13.9	2.85	2.61	1.5 - 10	2.2 – 5.4
4	42.8	130	13.2	2.64	2.43	2.2 - 10	5.3 – 10*
8	35.6*	113*	11.2*	2.68	2.69	2.3 – 10*	10*

m: male, f: female;
nr: not reported
* Statistically significant difference from control ($p < 0.05$)

Table A7.5.3.1.3-9: Liver weights for parental generation

Test substance nominal dosage level [mg/kg food]	Liver weights			
	Mean absolute (mg)		Mean relative (‰)	
	Male	Female	Male	Female
Control	1888	4085	17.5	27.7
0.5	nr	nr	nr	nr
1	nr	nr	nr	nr
2	1715	4131	16.3	28.0
4	1912	4567	17.8	30.6
8	2354*	4289	20.2*	30.0

nr: not reported
* Statistically significant difference from control ($p < 0.05$)

Table A7.5.3.1.3-10: Validity criteria for bird reproduction test according to OECD 206

	Fulfilled	Not fulfilled
Mortality of control animals <10%	**Yes**	**-**
Average number of 14-day-old survivors per hen in controls ≥ 14, 12 and 24 for mallard duck, bobwhite quail and Japanese quail	**Not reported**	
Average eggshell thickness for the control group ≥ 0.34, 0.19 and 0.19 mm for mallard duck, bobwhite quail and Japanese quail	**Not reported**	
Concentration of the test substance in the diet ≥ 80 % of the nominal concentration throughout the test period	**Not analysed**	

Section A7.5.3.1.3-02 Annex Point IIIA XIII.1.3	Effects on reproduction of birds	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [×] **Limited exposure [×]**	**Technically not feasible [×]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Although an avian reproduction study with chlorophacinone has been submitted and summarised under section A7.5.3.1.3-01, the study was deficient in several aspects. The following waiver is therefore presented to address this data requirement fully. The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted*…". The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". It should be noted that this waiver applies to anticoagulant rodenticides, including chlorophacinone, but not necessarily to rodenticides with other modes of action. All anticoagulant molecules are structurally similar. They act to form a stable complex with vitamin K. Vitamin K cannot be synthesised by the body. It is involved in the coagulation cascade that leads to blood clotting in response to haemorrhage (WHO IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides WHO Geneva 1995). Vitamin K is continually recycled in a loop system in the liver, in the activation of clotting factors that are then released into the bloodstream. The loop system employs enzymes, known as vitamin K reductases, to regenerate (recycle) the vitamin K. The anticoagulant rodenticides, also known as antivitamin K or AvK compounds, block the reductase enzymes. The resulting vitamin K/avK/reductase complex is bound to hepatocyte organelle membranes. The finite supply of vitamin K is used up, the production of the activated clotting factor ceases, and the coagulation cascade is interrupted. The organism dies by lethal haemorrhage. It is difficult to demonstrate that this is the sole mode of action, as administration is acutely lethal, but it is supported by the available short-term toxicology data. Short-term studies in mammals where vitamin K has been administered at the same time as several daily doses of anticoagulant,	

Section A7.5.3.1.3-02 Annex Point IIIA XIII.1.3	Effects on reproduction of birds	
	sufficient to be lethal in normal circumstances (referred to as "antidotal" administration, although this is not strictly accurate use of the word), have not shown any other toxic effects at doses that would have otherwise been lethal. However, such antidotal administration of vitamin K is not practical for the longer duration of the reproduction study in birds, as the vitamin K/avK complex will affect the liver cell organelle membranes to which they are bound. Short-term studies (up to 90-days duration) in rats and dogs have shown no adverse effects on the reproductive organs (macroscopic condition, organ weight analysis and histology). The absence of effects on the reproductive organs of mammals indicates that a direct effect on reproduction and fertility is unlikely. **Exposure** A high proportion of rodenticides are used indoors in urban situations, in factories, restaurants, offices, etc., in specially designed bait stations by professional pest control operators. Open field use is not permitted under the BPD, but use is permitted in and around farm buildings. Wild birds present in and around farm buildings may possibly feed on grain baits. Predators and scavenging birds are not at risk of primary poisoning, as they do not generally feed on foods used in rodenticide baits, but they are potentially at risk from consuming rodents that have themselves taken baits, either as dead animals (scavengers) or as prey (secondary exposure). In practice, as recommended in various guidance documents issued by manufacturers for good working practices, the presence of a specific anticoagulant-based product should not be available for prolonged periods. Thus, products are limited to use during baiting campaigns in response to infestations. The avoidance of prolonged use thus avoids the development of resistence in target rodents but also prevents long-term exposure to non-target birds. Monitoring data shows that some predator populations contain a proportion of individuals with very low levels of second generation AvKs. However, there are no indications that there is an effect at the population level, as populations of raptors and other predators and scavengers are static, or in some cases increasing, and not declining. (English Nature, pers. comm.). **Technical feasibility** The more lipophilic second-generation molecules have long half-lives in the liver, the site of action. Progressive daily doses accumulate in the liver until the	

Section A7.5.3.1.3-02 Annex Point IIIA XIII.1.3	Effects on reproduction of birds	
	coagulation cascade is compromised and death occurs. While use of the materials as rodenticides in baits is lethal after one or two exposures, it is theoretically possible to administer low, non-lethal doses in the experimental situation. However, no matter how low the dose, the avK still accumulates with time, until lethal levels are reached. It is possible to conduct short-term avian studies provided the accumulated dose never reaches lethal levels, but as the LD_{50} of these molecules is very low, the level for low lethality (e.g. LD_{10}) can be anticipated to be even lower, such that the amount administered daily over the three months of an avian reproduction study would be very low indeed. This argument has been made for long-term mammalian studies, and applies equally to avian studies. As with mammals, dietary admixture is the only practical repeat-dose route. The stress of handling birds for any other route would increase the chances of haemorrhage, leading to reduced survival. The avian reproduction study assesses the consequences of medium to long-term exposure by administering typically three dose levels to groups of sexually mature birds (generally Japanese or Bobwhite quail or Mallard duck), in comparison to a similar group of untreated birds (the control group) daily via the diet. The birds are maintained on a long-day photoperiod, to stimulate sexual activity, and allowed to mate. Egg production is measured, fertility of the eggs is estimated (by candling) and the eggs incubated to hatching. Eggshell thickness is measured for a subsample of eggs. Hatching success and offspring viability are assessed. As stated above, the progressive accumulation of the anticoagulant rodenticides leads to an increased probability of death by haemorrhage. There are several events in the reproductive cycle that either has incidental or inevitable haemorrhage in birds. Mallards (one of the test species) have a relatively violent courtship, with the male frequently grabbing the nape feathers of the female during copulation. Under normal circumstances, this appears to be trivial (or at least a generally accepted behaviour), not resulting in any injury, but the loss of feathers and associated bruising in an untreated bird may result in haemorrhage in a bird exposed to a test diet containing rodenticides. Ovulation causes minor haemorrhage within the ovary, and the act of egg-laying is frequently accompanied by haemorrhage in the oviduct/cloaca. It is generally recognised that short-term studies with anticoagulant rodenticides display a lack of dose responsiveness, due undoubtedly to haemorrhaging of test birds. As the dosing period is extended, as in a reproduction study, the likelihood of non dose-related death from	

Section A7.5.3.1.3-02 Annex Point IIIA XIII.1.3	Effects on reproduction of birds	
	haemorrhaging is highly likely to increase as a result of the factors presented above. Therefore, the interpretation and utility of the data from such studies is likely to be very limited and contribute little to the assessment of risk. There are, therefore, practical difficulties in performing an avian reproduction study with anticoagulant rodenticides. This conclusion is supported by a reproduction study with chlorophacinone summarised in Doc. III-A Section 7.5.3.1.3, Annex Point IIIA XIII.1.3. Despite shortcomings, the study demonstrated the absence of any long-term reproductive effects on birds, other than haemorrhaging typical of anticoagulant poisoning and death. **Conclusions** In conclusion, a waiver for avian reproduction studies on anticoagulant rodenticides is scientifically justified, based on lack of adverse effects on reproductive tissues in short-term mammalian studies and on the absence of population effects of UK raptors where low levels of rodenticides are routinely detected. A waiver is further supported by the practical difficulties of performing a study to determine reproduction endpoints. The practical difficulties of long-term administration of anticoagulants, already demonstrated with a study with chlorophacinone in which mortality was the most sensitive endpoint, are such that a further avian reproduction study would very likely fail to provide specific information on reproduction. Commissioning further avian reproduction studies in spite of the fact that sufficient is known to be able to predict an unsatisfactory outcome in advance is both unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Evaluation of applicant's justification	Acceptable	
Conclusion		
Remarks		

Section A7.5.4.1-01 Annex Point IIIA XIII.1.3	Acute toxicity to honeybees and other beneficial arthropods, for example predators	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [×]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** Rodenticidal baits are not applied outdoors by spraying, neither are they deployed in a manner likely to result in the widespread occurrence of active residues in soil that may subsequently be taken up by plant roots and translocated to the nectaries of flowers. Contact and oral exposure of honeybees to chlorophacinone will therefore not occur. The same applies to all other beneficial arthropods, including predatory species. Studies of the acute toxicity of chlorophacinone to honeybees and other beneficial arthropods are consequently unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Give date of action	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	
Conclusion	Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	July 2007	

Section A7.5.4.1-01 Annex Point IIIA XIII.1.3	Acute toxicity to honeybees and other beneficial arthropods, for example predators
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	This test is not necessary for the risk assessment of chlorophacinone in its use as biocide.
Remarks	

Section A7.5.5.1-01 Annex Point IIIA XIII.1.3	Bioconcentration (terrestrial), further studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [x]**	
Detailed justification:	The Directive 98/8/EC states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". **Exposure** According to the 'Emission scenario document for biocides used as rodenticides' (EUBEES 2), exposure of the soil compartment to active substances contained in rodenticidal baits is feasible, with concentrations dependent on deployment practices and product type. Exposure of the terrestrial compartment will be extremely localised and confined to small concentration 'hotspots' within a few cm of bait points located outdoors. The scope for widespread and significant exposure of the terrestrial compartment is therefore negligible. In addition, the potential for bioconcentration in terrestrial organisms is considered to be negligible for compounds with a log k_{ow} value of less than 3.0. The log k_{ow} value for chlorophacinone is 2.42, indicating that chlorophacinone is unlikely to accumulate in the tissues of terrestrial organisms. Based on the negligible exposure and the low bioaccumulation potential, further studies of the bioconcentration of chlorophacinone in the terrestrial compartment are unnecessary.	
Undertaking of intended data submission [ ]	Not applicable.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2007	
Evaluation of applicant's justification	Discuss applicant's justification and, if applicable, deviating view	

Section A7.5.5.1-01 **Annex Point IIIA XIII.1.3**	**Bioconcentration (terrestrial), further studies**
Conclusion	No bioconcentration is expected.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE (specify)
Date	
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	This test is not considered necessary.
Remarks	

Section 7.5.6-01 **Annex Point IIIA**	**Effects on other non-target terrestrial organisms** **Simulated field testing of secondary poisoning of birds**	
	1. REFERENCE	**Official use only**
1.1 Reference	Xxxxxx, X. (XXXX). Secondary hazard study using chlorophacinone-killed laboratory rats fed to black-billed magpies (*Pica pica*). XXXXXXXXXXXXXX., laboratory report number XXXXX, 4 June XXXX (unpublished).	
1.2Data protection	Yes.	
1.2.1Data owner	LiphaTech S.A.S.	
1.2.2Companies with letter of access	None.	
1.2.3Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1. Guideline study	Yes. US EPA FIFRA 71-5 (no OECD or EU equivalent).	
2.2. GLP	Yes.	
2.3. Deviations	None.	
	3 METHOD	
3.1. Test material	Rozol paraffinized pellets ground-squirrel bait, nominally 50 mg chlorophacinone/kg product.	
3.1.1. Lot/Batch number	XXXX	
3.1.2. Purity	Analysed a.s. content: 58.68 mg chlorophacinone/kg.	
3.1.3. Method of analysis	Not reported.	
3.2. Administration of the test substance	During the primary phase, bait pellets containing 0.005% (w/w) chlorophacinone were fed for up to 5 days to 46 individually caged, ex-laboratory breeder rats (Sprague Dawley). Rats (15) that died during the presentation period were collected, bagged and frozen. 24 control rats from the same source received a proprietary diet uncontaminated by chlorophacinone. All survivors of both groups were euthanised immediately after their last meal at the end of the presentation period, then individually bagged and frozen. Rat carcasses with and without chlorophacinone residues were provided as the sole source of food to test and control birds, respectively, in the secondary phase of the study. See Table A7.5.6-2.	
3.3. Reference substance	No.	
3.4. Testing procedure		
3.4.1. Test organisms	Table A7.5.6-1.	
3.4.2. Test system	Table A7.5.6-2.	
3.4.3 Test conditions	Table A7.5.6-3.	
3.4.4Duration of the test	26 days.	

Section 7.5.6-01 **Annex Point IIIA**	**Effects on other non-target terrestrial organisms** **Simulated field testing of secondary poisoning of birds**	
3.4.5Test parameter	Mortality, body weights, food consumption, sub-lethal effects (observations), gross pathology.	
3.4.6Examination / Observation	See Table A7.5.6-2.	
3.4.7Statistics	Not applied.	
	4 RESULTS	
4.1Limit Test / Range finding test	Not performed.	
4.2Results test substance		
4.2.1Effect data (Mortality)	Table A7.5.6-6.	
4.2.2Body weight	See Table A7.5.6-5.	
4.2.3Food consumption	See Table A7.5.6-5. Pattern of carcass consumption most commonly seen was that the major organs of the abdomen and thorax were eaten first, followed by the large limb muscles. Heads, skins and bones were generally not eaten.	
4.2.4Other effects	No treatment-related behavioural effects were observed and no mortalities occurred during the test. No haemorrhagic effects typical of anticoagulant rodenticide poisoning were found during *post mortem* examination. The liver of four treated birds showed slight discolouration or yellowing and the spleen of one of these birds was also non-uniformly coloured. One bird that consumed bait-fed rat carcasses passed green, dye-stained faeces on day 3 of the exposure phase. At the end of the study eight control magpies showed small gains in body weight, ranging from 3.4 to 9.0%, whereas losses of comparable magnitude were recorded for the other two birds (overall mean: 4.3%). Mean body weight gains in both chlorophacinone replicate groups were similar (3.0 and 4.7%), and 1-2 individuals showed weight losses.	
4.3Results of controls		
4.3.1Number/ percentage of animals showing adverse effects	None.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1Materials and methods	A test of secondary poisoning of magpies (*Pica pica*) in accordance with US EPA FIFRA 71-5. Administration by dietary inclusion in the carcasses of rats that had fed *ad libitum* on bait pellets containing 0.005% chlorophacinone up to the point at which they died of the effects of the rodenticide or until survivors were euthanised on day 5. The test duration was 26 days (five days on test diets, 21 days recovery on proprietary pet food).	

Section 7.5.6-01 Annex Point IIIA	Effects on other non-target terrestrial organisms Simulated field testing of secondary poisoning of birds	
5.2 Results and discussion		
5.3 Conclusion	Black-billed magpies (*P. pica*) that fed exclusively on chlorophacinone-poisoned rat carcasses for five days were monitored for a further 21 days during which uncontaminated food was provided. No mortalities occurred and no evidence of toxicosis was seen. Discolouration of the liver and/or spleen was noted at post mortem examination in four out of 20 treated birds, but haemorrhagic effects were absent.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Low recovery of chlorophacinone from storage stability samples (chlorophacinone spiked into control rat homogenate and stored frozen).	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	US EPA FIFRA 71-5 (no OECD or EU equivalent). 5 days exposure plus 21 days recovery on proprietary pet food.	
Results and discussion		
Conclusion	Black-billed magpies (*P. pica*) that fed exclusively on chlorophacinone-poisoned rat carcasses for five days were monitored for a further 21 days during which uncontaminated food was provided. No mortalities occurred and no evidence of toxicosis was seen. Discolouration of the liver and/or spleen was noted at post mortem examination in four out of 20 treated birds, but haemorrhagic effects were absent.	
Reliability	2	
Acceptability	Acceptable	
Remarks		

Table A7.5.6-1: Test animals

Criteria	Details
Species/strain	Black-billed magpies (*Pica pica*).
Source	XXXXXXXXXXXXXXXXXX, USA.
Age, sex and initial body weight (bw)	Ages not determined. 20 female + 16 male birds caught, 15 f + 15 m used in test. Bodyweight ranges of test birds (after pre-conditioning) were 138.4 to 161.8 g (f) and 162.1 to 210.2 g (m).

Table A7.5.6-2: Test system

Criteria	Details
Test location	Held indoors.

Holding pens	Constructed of plastic-coated wire, 61 × 76 × 46 cm, floor area (4,636 cm^2) covered with wood shavings.
Number of animals	30.
Number of animals per pen [cm^2/bird]	1 (4,636 cm^2/bird)
Pre-treatment / acclimation	Birds were individually caged indoors under test conditions and provided with water and food (moistened dog food pellets) *ad libitum* for 17 days. Food was withdrawn prior to pre-conditioning.
Pre-conditioning and selection of test birds	After fasting for 17.5 hours, all birds (initially 36) each received a single pre-weighed carcass of a rat that had been fed on a diet of uncontaminated feed. A slit was cut in the abdominal skin of the carcasses to help the birds feed on them. After 3 days the carcass remains were re-weighed and weight losses used to assess individual birds' acceptance of the carrion diet. Five birds that ate markedly less carrion than the others, and a sixth bird with an injured foot, were withdrawn. The remaining birds (15 f + 15 m) were used in the secondary poisoning study.
Diet during test	At the start of the secondary phase of the study, a single, thawed, weighed carcass of a rat that had fed on chlorophacinone bait pellets was presented to each magpie of two replicate test groups. Each control bird (single group) received a carcass of a rat that had eaten the control diet. On day 3, when substantial quantities of the first carcass had been consumed, each bird received a second carcass of the appropriate group alongside the remains of the first. At the end of the 5-day secondary exposure period, remains of rat carcasses were retrieved and re-weighed to determine quantities consumed. Five bait-fed rat carcasses and two (+ one blank) control carcasses were randomly selected to analyse homogenates of whole-body tissues for residues of chlorophacinone.
Dosage levels (of test substance)	0 and 0.467 mg chlorophacinone/kg diet (rat carcasses), based on mean analysed whole-body content (see Table A7.5.6-4). On average, total body residues represented 2.65% of the chlorophacinone ingested by individual rats during the primary feeding phase (Section 3.2).

	Mean intakes by magpies in treatment replicates 1 and 2 were 700.0 and 763.4 µg chlorophacinone/kg body weight, respectively. See Table A7.5.6-5.
Frequency, duration and method of animal monitoring after dosing	Monitored at least once daily for intoxication and mortality during 5 day exposure period and 21 day post-exposure observation period. Body weights measured at start and end of exposure phase and at end of the observation period.

Table A7.5.6-3: Test conditions (housing)

Criteria	Details
Test temperature	Generally 13 to 22°C, minimum of 13°C occurred during post-test observation period.
Relative humidity	20 to 52%.
Photoperiod and lighting	10 hour photoperiod, fluorescent lighting.

Table A7.5.6-4: Chlorophacinone concentrations measured in homogenised whole-body tissues of rat carcasses representative of those used to feed *P. pica*

Treatment group	Analysed concentration (µg chlorophacinone/g)
Rats (× 3) fed with untreated control diet	<LOD[a], 0.0647[b], <LOD[c]
Rats (× 5) fed with pellets (0.005% chlorophacinone)[d]	0.4248, 0.2107, 0.9272, 0.3030, 0.4709 (mean: 0.467)

[a] Limit of detection: 0.0387 µg/g;
[b] Sample probably contaminated during collection;
[c] A third carcass of the control group was used in a blank determination;
[d] Mean intake by rats during 5 day exposure period was 16.74 µg chlorophacinone/g bodyweight.

Table A7.5.6-5: Summary of body weights, carcass consumption and estimated chlorophacinone intake of *P. pica*.

Control magpies		Magpies fed with carcasses containing chlorophacinone					
		Replicate 1			Replicate 2		
body weight (g)[1]	total carcass intake (g)[2]	body weight (g)	total carcass intake (g)	total a.s. intake (µg/kg bw)[3]	body weight (g)	total carcass intake (g)	total a.s. intake (µg/kg bw)
186.2 (m)	340.8	185.7 (m)	240.2	604.1	138.4 (f)	302.1	1,019.4
153.4 (f)	340.1	187.5 (m)	329.3	820.2	150.4 (f)	159.4	494.9
162.1 (m)	290.8	161.6 (f)	235.0	679.1	172.4 (m)	317.1	859.0
173.0 (m)	318.0	161.7 (f)	205.8	594.4	153.7 (f)	317.3	964.1
152.9 (f)	327.3	142.0 (f)	259.3	852.8	142.4 (f)	244.0	800.2
170.8 (m)	303.7	184.9 (m)	247.8	625.9	178.4 (m)	281.8	737.7
139.5 (f)	272.7	180.8 (m)	282.8	730.5	210.2 (m)	261.1	580.1
169.6 (m)	259.4	185.6 (m)	215.3	541.7	149.7 (f)	284.4	887.2
187.7 (m)	399.4	182.3 (m)	306.7	785.7	151.5 (f)	220.5	679.7
145.7 (f)	250.3	161.8 (f)	265.4	766.0	151.7 (f)	198.8	612.0

[1] At the end of pre-conditioning;
[2] Cumulative 5-day total;
[3] Based on a mean concentration of 0.467 µg chlorophacinone/g carcass;
(m): male, (f): female.

Table A7.5.6-6: Mortality of *P. pica* after test termination

Treatment	Mortalities after test termination (out of 10 birds per treatment replicate)		
	Number dead	Percent dead	Time of death (days)
Control	0	0	-
Chlorophacinone (replicate 1)	0	0	-
Chlorophacinone (replicate 2)	0	0	-

Section 7.5.6-02 **Annex Point IIIA**	**Effects on other non-target terrestrial organisms** **Simulated field testing of secondary poisoning of non-target mammals**	
	1. REFERENCE	**Official use only**
1.1. Reference	Xxxxxx, XX., Xxxxxxx, X., Xxxxx, X. and Xxxxxx, X. (XXX). Secondary hazard study using chlorophacinone-killed laboratory rats fed to domestic ferrets (*Mustela putorius furo*). XXXXXXXXXXXXX., laboratory report number XXXX, 22 October XXXX (unpublished).	
1.2. Data protection	Yes.	
1.2.1. Data owner	LiphaTech S.A.S.	
1.2.2. Companies with letter of access	None.	
1.2.3. Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1. Guideline study	Yes. US EPA FIFRA 71-5 (no OECD or EU equivalent).	
2.2. GLP	Yes.	
2.3. Deviations	None.	
	3. METHOD	
3.1. Test material	Rozol paraffinized pellets, nominally 50 mg chlorophacinone/kg.	
3.1.1. Lot/Batch number	XXXXX.	
3.1.2. Purity	Analysed a.s. content: 55.96 mg chlorophacinone/kg product.	
3.1.3. Method of analysis	Not reported.	
3.2. Administration of the test substance	During the primary phase, bait pellets containing 0.005% (w/w) chlorophacinone were fed for up to 5 days to 44 individually caged, ex-laboratory breeder rats (Sprague Dawley). Rats (7) that died during the presentation period were collected, bagged and frozen. 22 control rats from the same source received a proprietary diet uncontaminated by chlorophacinone. All survivors of both groups were euthanised immediately after their last meal at the end of the presentation period, then individually bagged and frozen. Rat carcasses with and without chlorophacinone residues were provided as the sole source of food to test and control ferrets, respectively, in the secondary phase of the study. See Table A7.5.6-8.	
3.3. Reference substance	No.	
3.4. Testing procedure		
3.4.1. Test organisms	Table A7.5.6-7.	
3.4.2. Test system	Table A7.5.6-8.	
3.4.3. Test conditions	Table A7.5.6-9.	

Section 7.5.6-02 Annex Point IIIA	Effects on other non-target terrestrial organisms Simulated field testing of secondary poisoning of non-target mammals	
3.4.4. Duration of the test	26 days.	
3.4.5. Test parameter	Mortality, body weights, food consumption, sub-lethal effects (observations), gross pathology, chlorophacinone residues in livers.	
3.4.6. Examination / Observation	See Table A7.5.6-8.	
3.4.7. Statistics	Not applied.	
	4. RESULTS	
4.1. Limit Test / Range finding test	Not performed.	
4.2. Results test substance		
4.2.1. Effect data (Mortality)	Table A7.5.6-12. There were no deaths among the control animals. Mortality among the two groups of ferrets fed with carcasses of chlorophacinone-poisoned rats were 50% and 60% (mean: 55%). Apart from one death on the last day of the exposure period, mortalities occurred during the observation phase, after the contaminated rat carcasses were withdrawn. The last mortality was on day 14.	
4.2.2. Body weight	See Table A7.5.6-11.	
4.2.3. Food consumption	See Table A7.5.6-11.	
4.2.4. Other effects	No treatment-related behavioural effects were recorded. Haemorrhagic effects typical of anticoagulant rodenticide poisoning were observed during the study and found at *post mortem* examination of the throat, thorax and abdomen of ferrets that died during the study. Analysis of the liver of one female and one male ferret (representative of those that died during the study) showed accumulations of chlorophacinone, with concentrations of 0.600 and 0.483 µg/g respectively (mean: 0.542 µg/g). At the end of the study all control ferrets showed gains in body weight, ranging from 15.4 to 56.5% (mean: 32.5%). Overall mean body weight gains in both chlorophacinone replicate groups were reduced (12.4 and 10.9%), and some individuals showed weight losses during the study.	
4.3. Results of controls		
4.3.1. Number/ percentage of animals showing adverse effects	None.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1. Materials and methods	A test of secondary poisoning of ferrets (*Mustela putorius furo*) in accordance with US EPA FIFRA 71-5. Administration by dietary inclusion in the carcasses of rats	

Section 7.5.6-02 **Annex Point IIIA**	**Effects on other non-target terrestrial organisms** **Simulated field testing of secondary poisoning of non-target mammals**	
	that had fed *ad libitum* on bait pellets containing 0.005% chlorophacinone up to the point at which they died of the effects of the rodenticide or until survivors were euthanised on day 5. The test duration was 26 days (five days on test diets, 21 days recovery on proprietary ferret food).	
5.2. Results and discussion	*Summarize relevant results; discuss relevant test material-specific properties (e.g. solubility, stability, adsorption behaviour, volatility).*	
5.3. Conclusion	Domestic ferrets (*M. putorius furo*) that fed exclusively on chlorophacinone-poisoned rat carcasses for five days were monitored for a further 21 days during which uncontaminated food was provided. No behavioural symptoms of toxicosis were recorded, but 55% mean mortality occurred. Haemorrhaging typical of anticoagulant rodenticide poisoning was seen in some ferrets during the study and was noted at *post mortem* in all the ferrets that died during the test.	
5.3.1. Reliability	2.	
5.3.2. Deficiencies	No verification of storage stability of chlorophacinone in frozen rat carcasses or ferret livers.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2006	
Materials and Methods	US EPA FIFRA 71-5 (no OECD or EU equivalent). 5 days exposure plus 21 days recovery on proprietary pet food.	
Results and discussion		
Conclusion	Domestic ferrets (*M. putorius furo*) that fed exclusively on chlorophacinone-poisoned rat carcasses for five days were monitored for a further 21 days during which uncontaminated food was provided. No behavioural symptoms of toxicosis were recorded, but 55% mean mortality occurred. Haemorrhaging typical of anticoagulant rodenticide poisoning was seen in some ferrets during the study and was noted at *post mortem* in all the ferrets that died during the test.	
Reliability	2	
Acceptability	Acceptable	
Remarks		

Table A7.5.6-7: Test animals

Criteria	Details
Species/strain	Domestic ferrets (*Mustela putorius furo*).
Source	XXXXXXXXXXX, USA.
Age, sex and initial body weight (bw)	Eight to 11 weeks old on receipt. 16 female + 16 males received, 15 f + 15 m used in test. Bodyweight ranges of test ferrets (after pre-conditioning) were 549 to 712 g (f) and 867 to 1,082 g (m).

Table A7.5.6-8: Test system

Criteria	Details
Test location	Held indoors.
Holding pens	Constructed of plastic-coated wire, 61 × 76 × 46 cm, floor area (4,636 cm^2) covered with wood shavings.
Number of animals	30.
Number of animals per pen [cm^2/ferret]	1 (4,636 cm^2/ferret)
Pre-treatment / acclimation	Ferrets were individually caged indoors under test conditions and provided with water and food (proprietary ferret diet) *ad libitum* for a minimum of 7 days. Food was withdrawn prior to pre-conditioning.
Pre-conditioning and selection of test animals	After fasting for 3.5 hours, all ferrets (initially 32) each received a single pre-weighed carcass of a rat that had been fed on a diet of uncontaminated feed. Additional carcasses were provided, as necessary. After 3 days the carcass remains were re-weighed and weight losses used to assess individual ferrets' acceptance of the carrion diet. Two ferrets that ate less carrion than the others were withdrawn and another three that ate reduced quantities were pre-selected for the control group. The remaining ferrets were randomly allocated to the control and chlorophacinone treatments. 30 animals were used in the secondary poisoning study.
Diet during test	At the start of the secondary phase of the study, a single, thawed, weighed carcass of a rat that had fed on bait pellets was presented to each ferret of two replicate test groups. Each control animal (single group) received a carcass of a rat that had eaten the control diet. An additional carcass was provided when substantial quantities of the previous one had been consumed. Remains of rat carcasses were retrieved and re-weighed to

	determine quantities consumed. Four bait-fed rat carcasses and one control carcass were randomly selected to analyse homogenates of whole-body tissues by HPLC/UV detection for residues of chlorophacinone.
Dosage levels (of test substance)	0 and 0.453 mg chlorophacinone/kg diet (rat carcasses), based on mean analysed whole-body content. See Table A7.5.6-10. On average, total body residues represented 4.12% of the chlorophacinone ingested by individual rats during the primary feeding phase (Section 47.2). Mean intakes by ferrets in treatment replicates 1 and 2 were 308.7 and 327.0 µg chlorophacinone/kg body weight, respectively. See Table A7.5.6-11.
Frequency, duration and method of animal monitoring after dosing	Monitored at least once daily for intoxication and mortality during 5 day exposure period and 21 day post-exposure observation period. Body weights measured at start and end of exposure phase and at end of the observation period. Necropsy of all ferrets that died during test. Livers were removed and stored frozen for analysis of chlorophacinone residues by HPLC/UV detection.

Table A7.5.6-9: Test conditions (housing)

Criteria	Details
Test temperature	18 to 25°C.
Relative humidity	31 to 96%.
Photoperiod and lighting	12 hour photoperiod, fluorescent lighting.

Table A7.5.6-10: Chlorophacinone concentrations measured in homogenised whole-body tissues of rat carcasses representative of those used to feed *M. putorius furo*

Treatment group	Analysed concentration (µg chlorophacinone/g)
Rats (× 2) fed with untreated control diet	<LOD[a], <LOD
Rats (× 4) fed with pellets (0.005% chlorophacinone)[b]	0.175, 0.805, 0.218, 0.614 (mean: 0.453)

[a] Limit of detection: 0.0387 µg/g;
[b] Mean intake by rats during 5 day exposure period was 10.62 µg chlorophacinone/g bodyweight.

Table A7.5.6-11: Summary of body weights, carcass consumption and estimated chlorophacinone intake of *M. putorius furo*.

Control ferrets		Ferrets fed with carcasses containing chlorophacinone					
		Replicate 1			Replicate 2		
body weight (g)[1]	total carcass intake (g)[2]	body weight (g)	total carcass intake (g)	total a.s. intake (µg/kg bw)[3]	body weight (g)	total carcass intake (g)	total a.s. intake (µg/kg bw)
males							
1,026	962.5	1,044	355.0	154.0	1,020	742.9	329.9[d]
950	984.5	1,026	701.8	309.9[d]	1,064	995.5	423.8[d]
905	1,033.2	990	869.2	397.7[d]	911	683.2	339.7[d]
1,052	950.5	1,082	859.0	359.6	1,023	869.4	385.0[d]
928	946.0	1,023	872.6	386.4[d]	867	696.6	364.0
females							
712	604.4	655	422.5	292.2	632	416.0	298.2
613	601.3	614	257.0	189.6	583	420.7	326.9[d]
620	417.3	575	469.6	370.0	643	534.4	376.5
645	97.2	606	385.7	288.3[d]	663	571.8	390.7[d]
549	449.7	593	443.9	339.1[d]	654	315.5	218.5

[1] At the end of pre-conditioning;
[2] Cumulative 5-day total;
[3] Based on a mean concentration of 0.453 µg chlorophacinone/g carcass;
[d] Died during study.

Table A7.5.6-12: Mortality of *M. putorius furo* at test termination

Treatment group	Mortalities after test termination (out of 10 ferrets per treatment replicate)		
	Number dead	Percent dead	Time of death (days)
Control	0	0	-
Chlorophacinone (replicate 1)	5	50	7,8,9,11,14 (3 males; 2 females)
Chlorophacinone (replicate 2)	6	60	5,7,8,10,10,11 (4 males; 2 females)

Section A 8 Measures necessary to protect man, animals and the environment

Annex Point IIA VIII.8.1 to VIII.8.6 and IIIA VIII.1

Subsection (Annex Point)			Official use only
5.4	**Recommended methods and precautions concerning handling, use, storage, transport or fire**		
Handling and storage:		Exposure controls/Personal protection: Wear coveralls or long-sleeved protective clothing, gloves, apron and shoes. Wear goggles or face shield and anti dust mask. If possible handle the material under aspiration. General handling precautions: When using do not eat, drink or smoke. Keep only in the original container. Wash hands before eating, drinking, chewing gum, smoking or using the toilet. Wash contaminated clothing before re-use. There are no known materials which are incompatible with the product nor evidence of reactions with containers. Generation of dusts must be avoided. Storage: Store in tightly closed containers in a cool and dark place. Keep locked up and out of reach of children. Keep away from food, drink and animal feedingstuffs.	
Transport:		Refer to material safety data sheet in Document I.2.	
Fire:		Chlorophacinone is not classified as highly flammable, oxidising or explosive. There are therefore no special fire or explosion hazards. Suitable extinguishing media: water or foam.	
5.5	**In case of fire, nature of reaction products, combustion gases, etc.**	Chlorophacinone is not highly flammable but decomposes on heating and may therefore combust under incinerating conditions. The molecule contains mostly carbon, hydrogen and oxygen and so the major combustion products are likely to be water and oxides of carbon. The molecule also incorporates one atom of chlorine and so minor combustion or pyrolysis products may include hydrogen chloride/hydrochloric acid.	
5.6	**Emergency measures in case of an accident**	Advice on emergency treatment in cases of anti-coagulant rodenticide poisoning is presented as an Annex to this document. The active ingredient is manufactured and shipped in unit quantities no greater than 10 kg and securely packaged in sealed hard plastic containers, compliant with ADR regulations, to prevent accidental release.	
5.7	**Possibility of destruction or**	There are no special procedures for destruction or	

	decontamination following release in or on the following: (a) air (b) water, including drinking water (c) soil	decontamination following release into the environment. It is recommended that spilled material is swept up ensuring the operator is wearing appropriate personal protective equipment. Contamination of air or water is unlikely because the active substance is not volatile and is poorly soluble in water.
5.8	**Procedures for waste management of the active substance for industry or professional users**	The active substance is only handled and used within the manufacturing facilities and is not supplied to users in an un-formulated state.
5.8.1	Possibility of re-use or recycling	The active substance is not manufactured in bulk quantities and opportunities for re-use or recycling are minimal.
5.8.2	Possibility of neutralisation of effects	No specific neutralisation procedures are known. Unused active substance should be disposed of by incineration.
5.8.3	Conditions for controlled discharge including leachate qualities on disposal	The active substance is not disposed of using controlled discharges or landfill. Unused active substance should be disposed of by incineration.
5.8.4	Conditions for controlled incineration	The active substance may be destroyed by controlled incineration in accordance with local or national legislation. The oxygen content is 13% and the halogen content is 9.5%, both being at levels not expected to form furans or polychlorinated dioxins.
5.9	**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms**	Discussion about the possibility and prevention of affects on non-target organisms is presented in the Documents II-C. Apart from the target effect, mortality, no other adverse effects on organisms or non-living items are expected. The active substance is not volatile and will not therefore migrate into the upper atmosphere and contribute to ozone depletion. Its photochemical oxidative degradation half-life is less than one 24 hour day.
5.10	**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances**	The active substance is an organic compound which contains a covalently bonded halogen atom, consequently the active substance is considered for List 1 of the Annex to Directive 80/68/EEC.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	December 2005
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	The applicant's version is acceptable
Remarks	

ANNEX

Advice to Physicians – the treatment of anticoagulant rodenicide poisoning

Section A 9 Annex IX	Classification and labelling	

Classification	as detailed in Directive 67/548/EEC
Class of danger	T+, N
R phrases	**R26/27/28**: Very toxic in contact with skin and if swallowed.**R48/23/24/25** Toxic: Danger of serious damage to health by prolonged exposure through inhalation; in contact with skin and if swallowed. R61 May cause harm to the unborn child **R50/53**: Very toxic to aquatic organisms may cause long-term adverse effects in aquatic environments.
S phrases	**S1/2:** Keep locked up and out of reach of children. **S36/37** Wear suitable protective clothing and gloves **S45**: If you feel unwell, seek medical advice immediately (show label where possible). **S53:** Avoid exposure – obtain special instructions before use. **S60**: This material and its container must be disposed of as hazardous waste **S61**: Avoid release to the environment. Refer to special instructions/safety data sheets.

The safety phrases proposed are based on the classification and risk phrases. On basis of study results from studies presented in the dossier classification of chlorophacinone was proposed according to principles detailed in Annex VI of Council Directive 67/548/EEC (with amendments and adaptations). The classification for human health effects of chlorophacinone is in May 2007 still under discussion. For anticoagulant rodenticides, regarding human health effects, a provisional classification with R61 was decided in November 2006 by the C & L, but without a final decision on the category to be used (Repr. Cat.1 or Repr. Cat. 2). The proposed classification for chlorophacinone for acute and repeated dose toxicity was agreed in May 2007. At that moment, the provisionally classification for reprotoxicity was not confirmed as the TC C& L decided to await further results from studies on anticoagulant rodenticides before finalising the discussion on reprotoxicity. Specific concentration limits for chlorophacinone are proposed, but there are still under consideration.