**Committee for Risk Assessment**

**RAC**

Annex 1

# Background document

to the Opinion proposing harmonised classification and labelling at Community level of

# Chlorophacinone (ISO); 2-[(4-chlorophenyl)(phenyl)acetyl]-1H-indene-1,3(2H)-dione

**EC number: 223-003-0**
**CAS number: 3691-35-8**

CLH-O-0000003643-75-02/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

**Adopted**

**14 March 2014**

# CLH report

# Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

# Substance Name: CHLOROPHACINONE

**EC Number:** 223-003-0

**CAS Number:** 3691-35-8

**Index Number:** 606-014-00-9

**Contact details for dossier submitter:**

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# Part A.

## 1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

### 1.1 Substance

**Table 1: Substance identity**

| | |
|---|---|
| **Substance name:** | ***CHLOROPHACINONE*** |
| **EC number:** | 223-003-0 |
| **CAS number:** | 3691-35-8 |
| **Annex VI Index number:** | 606-014-00-9 |
| **Degree of purity:** | > 97.8% |
| **Impurities:** | Information on the impurities is considered confidential and it is presented in the confidential attachment to the draft CA-report on Chlorophacinone. |

### 1.2 Harmonised classification and labelling proposal

**Table 2: The current Annex VI entry and the proposed harmonised classification**

| | **CLP Regulation** | | **Directive 67/548/EEC (Dangerous Substances Directive; DSD)** |
|---|---|---|---|
| **Current entry in Annex VI, CLP Regulation** | Acute Tox. 1<br>Acute Tox. 2 (*)<br>Acute Tox. 3 (*)<br>STOT RE 1<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H310<br>H300<br>H331<br>H372(**)<br>H400<br>H410 | T+; R27/28<br>T; R23<br>T; R48/24/25<br>N; R50-53 |
| **Current proposal for consideration by RAC**<br>**(**)** | Acute Tox. 1<br>Acute Tox. 1<br>Acute Tox. 2<br>STOT RE. 1 | H330<br>H310<br>H300<br>H372 | **Hazard symbol:** T+, N<br>**R phrases:**<br>▪ T+; R26/27/28<br>▪ T; R48/23//24/25 |

| | | | |
|---|---|---|---|
| | According to Regulation (EC) No 1272/2008 and Regulation (EU) No 286/2011:<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410<br>Acute M-factor 1<br>Chronic M-factor 1 | ▪ Cn ≥ 25% N; R50-53 |
| **Resulting harmonised classification (future entry in Annex VI, CLP Regulation)** | Aquatic Acute 1 H400<br>Aquatic Chronic 1 H410<br>Acute M-factor 1<br>Chronic M-factor 1 | | Cn ≥ 25% N; R50-53 |

**(**) We sent on 23 March 2009 our Annex XV Intention. The proposal about classification included the risk phrase R61. However, the new OECD 414 study available for warfarin sodium affects the reasons for classification of chlorophacinone. This study addresses a regulatory request to investigate whether all AVK´s should be classified as R61, based on read-across from warfarin sodium data.**

The study carried out with warfarin sodium shows a definitive increase in incidence of subcutaneous and internal foetal haemorrhage (not seen in studies with chlorophacinone), foetal ocular effects and some indications of reduced ossification in skull bone at higher dose levels (see point 6). Similar studies have been carried out with chlorophacinone which was not embryotoxic or teratogenic in rat and rabbit.

The adverse finding detected in a standard OECD 414 study for warfarin sodium, would validate the negative findings in a similar study with chlorophacinone. Therefore, the phrase R61 is not warranted.

**Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria**

1) Based on CLP Regulation:

| CLASSIFICATION | | LABELLING | |
|---|---|---|---|
| Hazard Class and Category Code | Statement codes | Pictogram, Signal Word Code(s) | Hazard statement Code(s) |
| Inhalat: Acute Tox. 1 | H330<br>H310 | GHS06, Danger | H330 |
| Dermal: Acute Tox. 1 | H300<br>H372 | GHS06, Danger<br>GHS06, Danger | H310<br>H300 |
| Oral: Acute Tox. 2<br>STOT RE 1<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410 | Dgr<br>  | H372<br>H410 |

Specific concentration limits:

| | |
|---|---|
| C ≥ 0.1%: | STOT RE 1; H372 |
| 0.01% ≤ C < 0.1%: | STOT RE 2; H373 |

2) Based on DSD criteria:

Classification : $T^+$; R26/27/28, T; R48/23/24/25, N; R50-53

Labelling:

Symbol(s): $T^+$, N
R-phrases: R 26/27/28- R48/23/24/25- R50/53
S-phrases: (1/2)-36/37-45-60-61

Specific concentration limits:

| | |
|---|---|
| C ≥ 0.7%: | $T^+$; R26/27/28- 48/23/24/25 |
| 0.1% ≤ C < 0.7%: | $T^+$; R26/27-25-48/23/24/25 |
| 0.07% ≤ C <0.1%: | $T^+$; R26/27-22-48/20/21/22 |
| 0.01% ≤ C < 0.07%: | T; R23/24-22-48/20/21/22 |
| 0.001% ≤ C < 0.01%: | Xn; R20/21 |

**Table 3: Proposed classification according to the CLP Regulation**

| CLP Annex I ref | Hazard class | Proposed classification | Proposed SCLs and/or M-factors | Current classification [1)] | Reason for no classification [2)] |
|---|---|---|---|---|---|
| **2.1.** | Explosives | - | | None | There are no hazards associated with normal use of the active substance. |
| **2.2.** | Flammable gases | - | | None | Idem |
| **2.3.** | Flammable aerosols | - | | None | Idem |
| **2.4.** | Oxidising gases | - | | None | Idem |
| **2.5.** | Gases under pressure | - | | None | Idem |
| **2.6.** | Flammable liquids | - | | None | Idem |
| **2.7.** | Flammable solids | - | | None | Idem |
| **2.8.** | Self-reactive substances and mixtures | - | | | |
| **2.9.** | Pyrophoric liquids | - | | | |
| **2.10.** | Pyrophoric solids | - | | | |
| **2.11.** | Self-heating substances and mixtures | - | | | |
| **2.12.** | Substances and mixtures which in contact with water emit flammable gases | - | | | |
| **2.13.** | Oxidising liquids | - | | | |
| **2.14.** | Oxidising solids | - | | None | Idem |
| **2.15.** | Organic peroxides | - | | None | Idem |
| **2.16.** | Substance and mixtures corrosive to metals | - | | None | Idem |
| **3.1.** | Acute toxicity - oral | Acute Tox. 2 – H300 | | Acute Tox. 2 – H300 | |
| | Acute toxicity - dermal | Acute Tox. 1– H310 | | Acute Tox. 1– H310 | |
| | Acute toxicity - inhalation | **Acute Tox. 1– H330** | | Acute Tox. 3– H331 | |
| **3.2.** | Skin corrosion / irritation | None | | None | Chlorophacinone does not fulfil the EU criteria for classification as a skin irritant. |
| **3.3.** | Serious eye damage / eye irritation | None | | None | Idem |
| **3.4.** | Respiratory sensitisation | None | | None | No study available. In the acute inhalation study, it was stated there was no evidence of respiratory tract irritation following a 4 h nose only exposure. |
| **3.4.** | Skin sensitisation | None | | None | In a Buehler Test, 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 fulfil the EU criteria for classification as a skin sensitiser. |
| **3.5.** | Germ cell mutagenicity | None | | None | Chlorophacinone, does not fulfil the EU criteria for classification as a mutagenic substance. |
| **3.6.** | Carcinogenicity | None | | None | Based on the avalaible data, no classification for carcinogenicity for Chlorophacinone is proposed. |
| **3.7.** | Reproductive toxicity | None | | None | Based on the available data, no classification for fertility for Chlorophacinone seems to be warranted. |
| **3.8.** | Specific target organ toxicity –single exposure | None | | None | |
| **3.9.** | Specific target organ toxicity – repeated exposure | STOT RE 1 | | STOT RE 1 | |
| **3.10.** | Aspiration hazard | | | | |
| 4.1. | **Hazardous to the aquatic environment** | **According to Regulation (EC) No 1272/2008 and Regulation (EU) No 286/2011** Aquatic Acute 1 H400 **Aquatic Chronic 1 H410** | Acute M-factor 1 Chronic M-factor 1 | Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Acute M-factor 1 **Chronic M-factor 1** | |
| **5.1.** | Hazardous to the ozone layer | | | | |

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

## Labelling:

GHS pictogram: GHS06
GHS09

Signal word: Dgr, Warning
Hazard statements:
Oral:
**H300:** Fatal if swallowed
Dermal:
**H310:** Fatal in contact with skin
Inhalation:
**H330:** Fatal if inhaled

**H372:** Causes damage to organs (blood coagulation system, liver, kidney) through prolonged or repeated exposure

**Aquatic acute 1: H 400** Very toxic to aquatic life
**Aquatic chronic 1: H410** Very toxic to aquatic life with long lasting effects

Precautionary statements:

Prevention:

**P260:** Do not breathe dust/fume/gas/mist/vapours/spray
**P270:** Do not eat, drink or smoke when using this product
**P271:** Use only outdoors or in a well-ventilated area
**P273:** Avoid release to the environment
**P280:** Wear protective gloves/protective clothing/eye protection/face protection

Response:

**P301 + P310:** IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician
**P302 + P350:** IF ON SKIN: Gently wash with plenty of soap and water
**P304 + P340:** IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing
**P391:** Collect spillage

Storage:

**P405:** Store locked up

Disposal:

**P501:** Dispose of contents/container to…

**Proposed notes assigned to an entry:**

–

**Table 4: Proposed classification according to DSD**

| Hazardous property | Proposed classification | Proposed SCLs | Current classification [1)] | Reason for no classification [2)] |
|---|---|---|---|---|
| Explosiveness | Not explosive | | - | |
| Oxidising properties | Not oxidising | | - | |
| Flammability | Not highly flammable | | - | Test material does not have a self ignition temperature below its melting point |
| Other physico-chemical properties | None | | | |
| Thermal stability | Apparently stable up to and beyond its melting point. | | | |
| Acute toxicity | **T+; R26/27/28** | | T+; R27/28<br>T; R23 | |
| Acute toxicity – irreversible damage after single exposure | | | | |
| Repeated dose toxicity | T; R48/23/24/25 | | T; R48/24/25 | |
| Irritation / Corrosion | None | | None | |
| Sensitisation | None | | None | |
| Carcinogenicity | None | | None | |
| Mutagenicity – Genetic toxicity | None | | None | |
| Toxicity to reproduction – fertility | None | | None | |
| Toxicity to reproduction – development | None | | None | |
| Toxicity to reproduction – breastfed babies. Effects on or via lactation | None | | None | |
| Environment | **N; R50/53** | | R50/53 | |

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

## Labelling:

Indication of danger: $T^+$, N, Dangerous for the environment

R-phrases: R26/27/28 - R48/23/24/25 - R50/53

R26/27/28: Very toxic by inhalation 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

R50 Very toxic to aquatic organisms
R53 May cause long-term adverse effects in the aquatic environment

S-phrases: (1/2)-36/37-45-53-60-61

S1/2: Keep locked up and out of reach of children

S36/37: Wear suitable protective clothing and gloves

S45: In case of accident or if you feel unwell, seek medical advice immediately (show the 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

# 2 BACKGROUND TO THE CLH PROPOSAL

## 2.1 History of the previous classification and labelling

On basis of study results from studies presented in the dossier as a active substance in BPD, 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 since 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.

We sent on 23 March 2009 our Annex XV Intention. The proposal about classification included the risk phrase R61. However, the new OECD 414 study available for warfarin sodium affects the reasons for classification of chlorophacinone. This study addresses a regulatory request to investigate whether all AVK´s should be classified as R61, based on read-across from warfarin sodium data.

The study carried out with warfarin sodium shows a definitive increase in incidence of subcutaneous and internal foetal haemorrhage (not seen in studies with chlorophacinone), foetal ocular effects and some indications of reduced ossification in skull bone at higher dose levels (see point 6). Similar studies have been completed with chlorophacinone which was not embryotoxic or teratogenic in rat and rabbit.

The adverse finding detected in a standard OECD 414 study for warfarin sodium, validates the negative findings in a similar study with chlorophacinone. Therefore, the phrase R61 is not warranted.

This MS is not proposing classification of chlorophacinone for reproduction (neither fertility nor development). Therefore, in principle, detail information of studies about reproduction were not required in this Annex XV. However it was considered necessary to include and discuss the available information about teratogenicity for chlorophacinone and compare with existing information on warfarin, due to the previous history of the proposal for R61 for all anti-vitamin K rodenticides by read across from the human embryotoxicity of warfarin. In this way RAC-ECHA, the Commission, Stakeholders and MS will be able to discuss this issue in the context of the proposals for all the antivit-K rodenticides.

## 2.2 Short summary of the scientific justification for the CLH proposal

Carries out an evaluation on an active substance in BP and the evaluation shows that the classification criteria are met.

Regarding the environmental effects, the lowest acute toxicity of chlorophacinone in aquatic organisms was of 0.45 mg a.s/l in fish R50. Chlorophacinone is not biodegradable under environmentally relevant conditions or expected to be biodegradable during sewage treatment processes (R 53).

According to Regulation (EC) No 1272/2008 since the aquatic toxicity is ≤ 1 mg a.s/l and the substance is not rapidly degradable, the substance is classified as Acute Category 1 and Chronic Category 1.

In respect of Regulation (EU) No 286/2011 the classification is Category Acute 1 and Category Chronic 1. Since there is only one NOEC available, a comparison was performed with that NOEC and the surrogate system. The most restrictive classification was chosen. With the algae NOErC = 0.72 mg a.s/l Category Chronic 2 should apply. On the other hand, applying the surrogate systema, Category Chronic 1 should apply. This is the most restrictive one category. Conclusion: chlorophacinone is classified as Category Acute 1 and Category Chronic 1.

## 2.3 Current harmonised classification and labelling

Chlorophacinone is classified in Annex VI to Regulation (EC) No 1272/2008

Class of danger: $T^+$, N

**R Phrases:**

R27/28: Very toxic in contact with skin and if swallowed

R23: Toxic by inhalation

R48/24/25 Toxic: Danger of serious damage to health by prolonged exposure in contact with skin and if swallowed.

R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

**S Phrases:**

S(1/2): Keep locked up and out of reach of children

S36/37: Wear suitable protective clothing and gloves

S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

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

### 2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

| Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|
| Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Acute Tox. 1<br>Acute Tox. 2 *<br>Acute Tox. 3 *<br>STOT RE 1 | H310<br>H300<br>H331<br>H372(**) | GHS06<br>GHS08<br>Dgr | H310<br>H300<br>H331<br>H372 (**) | | | |
| Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410 | GHS09 | H410 | | | |

### 2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

| Classification | Labelling | Conc. Limits | Notes |
|---|---|---|---|
| T+; R27/28<br>T; R23<br>T; R48/24/25 | T+; N<br>R 23<br>R 27/28<br>R 48/24/25<br>S: (1/2-)36/37-45 | | |
| N; R50/53 | N<br><br>R: 50/53<br>S: 60-61 | | |

## 2.4 Current self-classification and labelling

### 2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

GHS pictogram: GHS06; GHS08; GHS09
Signal word: Danger, Warning
Hazard statements:
- Acute Tox. 1: H310 Fatal in contact with skin
- Acute Tox. 2: * H300 Fatal if swallowed
- Acute Tox. 3: * H331 Toxic if inhaled
- STOT RE 1: H372(**) Causes damage top organs through prolonged or repeated exposure
- Aquatic acute 1: H 400 Very toxic to aquatic life

Acute M-factor 1

Aquatic chronic 1: H410 Very toxic to aquatic life with long lasting effects
Chronic M-factor 1

Precautionary statements:

P260**:** Do not breathe dust/fume/gas/mist/vapours/spray
P270**:** Do not eat, drink or smoke when using this product
P271**:** Use only outdoors or in a well-ventilated area
P273 Avoid release to the environment
P280 Wear protective gloves / protective clothing / eye protection / face protection
P301 + P310 If swallowed: immediately call a poison center or a doctor/physician
P302 + P350: IF ON SKIN: Gently wash with plenty of soap and water
P304 + P340: IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing
P391 Collect spillage
P405 Store locked up
P501 Dispose of contents/container to…

### 2.4.2 Current self-classification and labelling based on DSD criteria

| **Classification** | **as detailed in Directive 67/548/EEC** |
|---|---|
| Class of danger | T+<br><br>N |
| R phrases | **R27/28:** Very toxic in contact with skin and if swallowed<br><br>**R23:** Toxic by inhalation<br><br>**R48/24/25** Toxic: Danger of serious damage to health by prolonged exposure in contact with skin and if swallowed.<br><br>**R50/53**: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. |
| S phrases | **S(1/2):** Keep locked up and out of reach of children<br><br>**S36/37:** Wear suitable protective clothing and gloves<br><br>**S45:** In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)<br><br>**S60**: This material and its container must be disposed of as hazardous waste<br><br>**S61**: Avoid release to the environment. Refer to special instructions/safety data sheets. |

## 3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Carries out an evaluation on an active substance in BP and the evaluation shows that the classification criteria are met.

# Part B.

## SCIENTIFIC EVALUATION OF THE DATA

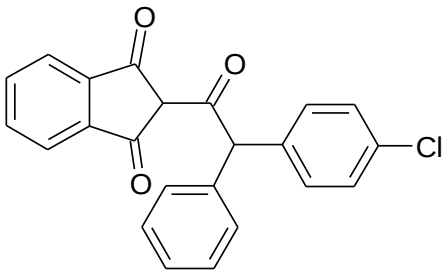
## 1 IDENTITY OF THE SUBSTANCE

### 1.1 Name and other identifiers of the substance

**Table 5: Substance identity**

| | |
|---|---|
| **EC number:** | 223-003-0 |
| **EC name:** | - |
| **CAS number (EC inventory):** | - |
| **CAS number:** | 3691-35-8 |
| **CAS name:** | - |
| **IUPAC name:** | 2-(2-(4-chlorophenyl)-2-phenyl-acetyl)indan-1,3-dione |
| **CLP Annex VI Index number:** | 606-014-00-9 |
| **Molecular formula:** | $C_{23}H_{15}ClO_3$ |
| **Molecular weight range:** | 374.82 |

**Structural formula:**



## 1.2 Composition of the substance

**Table 6: Constituents (non-confidential information)**

| Constituent | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| Chlorophacinone: | > 97.8% %(w/w) | | Chlorophacinone contains one optically active carbon and therefore exists as two enantiomers. The ratio of the enantiomers is provided in the confidential file |

Current Annex VI entry:

**Table 7: Impurities (non-confidential information)**

| Impurity | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| | | | Information on the impurities and additives in the technical grade active substance is confidential |

Current Annex VI entry:

**Table 8: Additives (non-confidential information)**

| Additive | Function | Typical concentration | Concentration range | Remarks |
|---|---|---|---|---|
| - | | | | |

Current Annex VI entry:

### 1.2.1 Composition of test material

Test material used in the **physical-chemical** studies:

| | Method | Test-Material |
|---|---|---|
| Melting point | OECD 102 (≡ EEC A.1) | Batch M121, purity 99.74% |
| Boiling point | OECD 103 (≡ EEC A.2) | BatchM127, purity 99.77% |
| Density | OECD 109/CIPAC MT 3 (≡ EEC A.3) | Batch CLO 217, purity 99.85% |
| Vapour pressure | OECD104 (≡ EEC A.4) | Batch 11328, purity 100% |
| Henry´s Law Constant | Calculation | Calculated from vapour pressure of 4.76 x $10^{-4}$ Pa and water solubility of 13.0 mg/L. |
| Appearance (Physical state, Colour, | Visual, Visual (Munsell colour system), Olfactory - ASTM D1292- | Batch CLO 217, purity 99.85% |

| | | |
|---|---|---|
| Odour) | 80 | |
| Absorption spectra (IR, NMR, MS) | IR: -<br>NMR: Proton and 13C NMR<br>MS: HPLC- APCI MS<br>All spectra are consistent with the structure of the active substance | Batch CLO ANA 479 purity not stated, Batch CLO432 purity not stated, Batch CLO432 purity not stated) |
| Solubility in water | OECD 105 (≡EEC A.6) | Batch M121, purity 99.74% |
| Dissociation constant | OECD 112 | Batch M121, purity 99.74% |
| Solubility in organic solvents, including the effect of temperature on solubility | EPA 40 CFR 158 Subdiv.D, 63.-8<br>(≡EEC A.6 and OECD 105) | Batch CLO2022, purity 101% |
| 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. |
| Partition coefficient n-octanol/water - *including effects of pH (5-9).* | OECD 107 (≡ EEC A.8) | Batch M121, purity 99.74% |
| Thermal stability, identity of relevant breakdown products | OECD 113 | Batch CLOM127, Purity 99.77% |
| Flammability, including auto-flammability and identity of combustion products | EEC A10 (flammability of solids) | Batch CLOM127, Purity 99.77% |
| Surface tension | EEC A5 OECD 115 | Batch CLOM127, purity 99.77% |
| Explosive properties | EEC A14 | Not explosive |
| Oxidising properties | EEC A17 | |

Test material used in the **(eco-)toxicological** studies:

| | **Method** | **Test-Material / Test Animals** |
|---|---|---|
| **Oral toxicity**, LD50 study in:<br>1. rat<br>2. dog | US EPA Guideline 81-1. In accordance with EC Method B.1 | 1. Batch (E6619), purity 99.36% / Sprague Dawley Rat (IOPS-VAF)<br>2. Batch Lot # 217, purity 100.17% / Dogs (V Purebred Beagle) |
| **Dermal toxicity**, $LD_{50}$ study in:<br>1. rabbits<br>2. range finding study in rabbits<br>3. range finding study in rabbits<br>4. rabbits | 1. US EPA 81-1. Range-finding study in accordance with requirements of EC Method B.3.<br>2. EPA 81-1, Range-finding study in accordance with requirements of EC Method B.3. | Batch Lot #: CLOMO15, purity 100.36% / New Zealand White Rabbits |

| | | |
|---|---|---|
| | 3. EPA 81-1. Range-finding study in accordance with requirements of EC Method B.3.<br><br>4. FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984. EPA 81-1. In accordance with EC Method B.3. | |
| **Inhalation toxicity**, $LD_{50}$ study in:<br>rats | 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 | Batch CLO 2022, purity 101.0% / Albino rat |
| Dermal irritation | FIFRA 40 CFR, Part 158, Subpart F Hazardous Evaluation: Human and Domestic Animals, 1984. EPA 81-4 in accordance with EC Method B.4. | Batch Lot #: CLOM010, purity 99.88% / New Zealand White Rabbits |
| Eye irritation | US EPA Guideline 81-4. In accordance with EC Method B.5. | Batch Lot #: CLOM010 purity 99.88% / New Zealand White Rabbits |
| Skin sensitisation | Method in accordance with EC Method B.6. | Batch Lot #: CLOM010 purity 99.88% / Guinea pigs |
| Absorption, distribution, metabolism and excretion study:<br>1. Single oral dose study in the rat<br><br>2. Single oral dose study in the rat | The study was conducted prior to the availability of guidelines for this study type. However, methodology is similar to US EPA 85-1 guidelines. | 1. Chlorophacinone, Rozol LM-91 , Batch 117<br><br>2. Non-radiolabelled batch – CLOM123 - Radiolabelled batch – SEL/1258-1, purity 99.58% / Rat (Crl:CD(SD)IGSBR) |
| Repeated dose toxicity:<br><br>Dermal: Rabbits (21 days)<br><br>Dermal: Rabbits (21 days)<br><br>Oral: Rats (11 to 16 weeks) | <br><br>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.<br>EPA 82-2. In accordance with EC Method B.9<br>EPA Pesticide Assessment Guidelines, Subdivision F, 82-1. Study design was in accordance with EC Method B.27. | <br><br>Chlorophacinone (Lot # CLO 2022), purity 100%<br><br>Chlorophacinone Lot No: 06691, purity 0.2%<br>Chlorophacinone; 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; |
| In-vitro gene mutation in bacteria (*Bacterial reverse mutation test*) | The study was conducted according to EPA 84-2a and EC Method B13/14 test guidelines. | Chlorophacinone (Analysis No. 1750) O.F.No: 155, purity 99.8% / Salmonella typhimurium strains: TA |

| | | |
|---|---|---|
| | | 98, TA 100, TA 1535, TA 1537, TA 1538 in culture suspension (about $10^9$ bacteria per ml) |
| In-vitro gene mutation in bacteria (*Bacterial reverse mutation test*) | 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.13/14. US EPA 84-2. | Chlorophacinone (Lot # CLO 2022), purity 101%<br>- Salmonella typhimurium strains: TA 98, TA 100, TA 1535, TA 1537, in culture suspension (about $10^9$ bacteria per ml)<br>- Escherichia coli strains: WP2uvrA |
| In-vitro gene mutation in mammalian cells (Induction of gene mutation in CHO cells) | 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). EPA 84-2. In accordance with EC Method B.17. | Chlorophacinone (Batch No: CLOM 027) / mammalian cell lines: Chinese Hamster Ovary (CHO) |
| In-vitro gene mutation in mammalian cells (*In-vitro* mammalian chromosome aberration test) | OECD Guideline 473, US EPA Guideline 84-2. In accordance with EC Method B.10. | Chlorophacinone; Batch Lot No: CLO 2022, purity 96.82% / mammalian cell lines: human lymphocytes cultured from healthy human donor |
| In-vivo mutagenicity (bone marrow) | The test was conducted in accordance with the EPA 84-2 and EC Method B.12. | Chlorophacinone (Lot # CLO2022), Purity 101.00% / Rats VAF CD Sprague- Dawley |
| Carcinogenicity in rats | Justification for non-submission of data | |
| Teratogenicity study:<br>1. In rats<br>2. in rabbits | The study was conducted according to the FIFRA testing guidelines (EPA 83-3, 1988) and EC Method B.31. | 1. Batch Lot # CLO 2022, purity 99.77%<br>2. Lot # CLO 2022 purity 101.00% / Rabbits New Zealand White |

**Table 9: Summary of physico - chemical properties**

| **Property** | **Value** | **Reference** | **Comment (e.g. measured or estimated)** |
|---|---|---|---|
| State of the substance at 20°C and 101,3 kPa | Powder<br>Pale yellow<br>Odourless | | |
| Melting/freezing point | 143 ºC | This information is confidential. See references 12 and 16 of confidential annex. | Capillary tube method |
| Boiling point | Melted at 140°C followed by decomposition without boiling that started at 250ºC. pressure: atmospheric, air. | This information is confidential. See reference 48 of confidential annex. | Differential scanning calorimetry and capillary tube test |
| Relative density | Density = 1.4301 ± 0.01385 g/mL | This information is confidential. See references 16 and 39 confidential annex. | |
| Vapour pressure | 4.76 x $10^{-4}$ Pa<br>temperature: 22.8°C | This information is confidential. See reference 13 of confidential annex. | Gas saturation method |
| Surface tension | 68.9 mN/m at 20.6°C at 90% saturated solution | This information is confidential. See reference 30 of confidential annex. | This value is greater than 60 mN/m and is therefore not considered surface active. |
| Water solubility | Water: 13 µg/mL<br>pH 4: 1 µg/mL<br>pH 7: 344 µg/mL<br>pH 10: 459 µg/mL<br>temperature: 20°C | This information is confidential. See references 14 and 17 of confidential annex. | Column elution<br>Column elution<br>Column elution<br>Column elution<br>Test substance shown to be unstable at pH4.<br>Analysis by HPLC. |
| Partition coefficient n-octanol/water | Log $P_{ow}$ 1.93 (no pH control, 23ºC)<br>pH~4: Log $P_{ow}$ 3.08 temperature: 23°C<br>pH~7: Log $P_{ow}$ 2.42 temperature: 23°C<br>pH~9: Log $P_{ow}$ 2.57 temperature: 23°C | This information is confidential. See references 18 and 33 of confidential annex. | |
| Flash point | | | Not required for a solid active substance. |
| Flammability | Not highly flammable | This information is confidential. See | |

| | | | |
|---|---|---|---|
| | | reference 28 of confidential annex. | |
| Explosive properties | Not explosive | This information is confidential. See reference 31 of confidential annex. | Theoretical assessment in compliance with EEC A14 |
| Self-ignition temperature | Test material does not have a self ignition temperature below its melting point. | This information is confidential. See reference 29 of confidential annex | |
| Oxidising properties | Not oxidising | This information is confidential. See reference 32 of confidential annex. | Theoretical assessment in compliance with EEC A17 |
| Granulometry | | | |
| Stability in organic solvents and identity of relevant degradation 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. |
| Dissociation constant | pKa = 8.0 | This information is confidential. See reference 18 of confidential annex. | 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. |
| Viscosity | | | Not applicable because the active substance is a solid. |

# 2 MANUFACTURE AND USES

## 2.1 Manufacture

The main quantitative method for the determination of chlorophacinone in technical chlorophacinone is a titration. The method of manufacture is confidential.

## 2.2 Identified uses

MG03: Pest control. Product type 14. (Rodenticide)

The content of chlorophacinone in the typical products is 0.005%.

# 3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

**Table 10: Summary table for relevant physico-chemical studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Melting point:**<br>OECD 102 (≡ EEC A.1) | 143.0°C<br>pressure: atmospheric | Capillary tube method | Kramer and Marion, 2002a |
| **Boiling point:**<br>EEC A.2 (OECD 103) | Melted at 140°C followed by decomposition without boiling that started at 250ºC.<br>pressure: atmospheric, air. | Differential scanning calorimetry and capillary tube test | Tognucci, 2002 |
| **Relative density:**<br>OECD 109/CIPAC MT 3 (≡ EEC A.3) | Density = 1.4301 ± 0.01385 g/mL conducted at 20 °C | | Pesselman, 1990a |
| **Vapour pressure:**<br>OECD 104 (≡ EEC A.4) | Vapour pressure = 4.76 x $10^{-4}$ Pa<br>temperature: 22.8°C | Gas saturation method | Hoffman, 1988b |
| **Appearance:**<br>**Physical state**: Visual<br>**Colour**: Visual (Munsell colour system)<br>**Odour:** Olfactory - ASTM D1292-80 | Powder<br>Pale yellow 5Y (9/6)<br>Odourless | | Pesselman, 1990b<br>Pesselman, 1990c<br>Pesselman, 1990d |
| **Spectra active substance:**<br>UV/VIS – No Method<br>IR – No Method<br>NMR – Proton and 13C NMR<br>MS – HPLC- APCI MS | All spectra are consistent with the structure of the active substance | | Queche, 1999 |
| **Solubility in water including effect of pH**<br>OECD 105 (≡EEC A.6) | • Water: 13 µg/mL<br>• pH 4: 1 µg/mL<br>• pH 7: 344 µg/mL<br>• pH 10: 459 µg/mL<br>temperature: 20°C | Column elution<br>Column elution<br>Column elution<br>Column elution<br>Test substance shown to be unstable at pH4.<br>Analysis by HPLC. | Kramer and Marion, 2002b |

| | | | |
|---|---|---|---|
| **Dissociation content**<br>OECD 112 | 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. | Kramer and Marion, 2002c |
| **Solubility in organic solvents**<br>EPA 40 CFR 158 Subdiv.D, 63.-8 (≡EEC A.6 and OECD 105) | Hexane: 854 mg/L<br>Methanol: 786 mg/L<br>temperature: 25°C | Shake flask method<br>Shake flask method | Pesselman, 1991 |
| **Partition coefficient n-octanol / water**<br>OECD 107 (≡ EEC A.8) | pH 4: Log Pow 3.08<br>pH 7: Log Pow 2.4<br>pH 9: Log Pow 2.57<br>temperature: 23°C | Shake flask method | Kramer and Marion, 2002c |
| **Stability in the air, photo-chemical degradation, identity of breakdown product(s)**<br>OECD 113 | 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. | Lindemann, 2004a |
| **Flammability**<br>EEC A10 (flammability of solids)<br>EEC A16 | Not highly flammable<br>Test material does not have a self ignition temperature below its melting point. | | Lindemann, 2004b<br>Lindemann, 2004c |
| **Surface tension**<br>EEC A5 - OECD 115 | 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. | Lindemann, 2003d |
| **Explosive properties**<br>EEC A14 | Not explosive | Theoretical assessment in compliance with EEC A14 | Lindemann, 2003e |
| **Oxidizing properties**<br>EEC A17 | Not oxidising | Theoretical assessment in compliance with EEC A17 | Lindemann, 2003f |
| **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. | | |

### 3.1 *[Insert hazard class when relevant and repeat section if needed]*

None

#### 3.1.1 Summary and discussion of

#### 3.1.2 Comparison with criteria

#### 3.1.3 Conclusions on classification and labelling

Chlorophacinone is thermally stable up to 143°C, its melting point. It is not classified as highly flammable and does not undergo self ignition below its melting point. It is not explosive nor does it have oxidising properties. There is no record that it has reacted with any storage container during many years of industrial production. Therefore, there are no hazards associated with normal use of the active substance. The substance is not classified for physico-chemical properties.

| **RAC evaluation of physical hazards** |
|---|
| **Summary of the Dossier submitter's proposal**<br>Chlorophacinone is not classified as highly flammable and does not undergo self-ignition below its melting point (143 °C, up to which it is thermally stable). It is not explosive and does not possess oxidising properties. According to the Dossier submitter (DS) there are no physical or chemical hazards associated with normal use of the substance, and therefore it should not be classified for physico-chemical properties.<br><br>**Comments received during public consultation**<br>There were no comments on physical hazards.<br><br>**Additional key elements**<br>-<br><br>**Assessment and comparison with the classification criteria**<br>Since Chlorophacinone does not have explosive, oxidising or self-ignition properties, RAC supported the non-classification for physico-chemical properties, as proposed by the dossier submitter.<br><br>**Supplemental information - In depth analyses by RAC**<br>- |

## 4 HUMAN HEALTH HAZARD ASSESSMENT

The active substance belongs to a group of substances, the anti-vitamin K rodenticides (AVKs) where the mode of action is well known. A summary of the mode of action, taken from the WHO IPCS Environmental Health Criteria 175 Anticoagulant Rodenticides (WHO Geneva, 1995 ISBN 92 4 157175 6) is presented here.

Anticoagulant rodenticides 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.

The molecule has significant structural similarity to the forms of vitamin K. This structural similarity is responsible for the ability to interfere with i.e. block the enzymes used to regenerate

vitamin K. The progressive blocking of the regeneration of vitamin K will lead to an increasing probability of a fatal haemorrhage. In general terms, a progressive intake of anticoagulants is causing death.

Blood clots form when the soluble protein fibrinogen, normally present in the blood, is converted by the enzyme thrombin (factor IIa) 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. 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 is dependent upon vitamin K hydroquinone, which 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 blocks the regeneration of vitamin K 2,3-epoxide to vitamin K hydroquinone. The Glu→Gla conversion does not take place.

| **RAC general comment** |
|---|
| Chlorophacinone belongs to a group of compounds known as anticoagulant rodenticides, i.e. those with an anti-vitamin K mode of action (sometimes abbreviated to AVK) which are used mainly as active substances in biocidal products for pest control of rats, mice and other rodents. Some of the substances, like chlorophacinone, had an existing harmonised classification. However, only Warfarin is currently classified for toxicity to reproduction in category 1A.<br><br>The eight substances were previously discussed by the Technical Committee on Classification and Labelling of Dangerous Substances (TC C&L) of the European Chemicals Bureau (ECB) (2006 – 2008). However, the work was referred to be continued at ECHA and to that end Member State Competent Authorities (MSCAs) were requested to prepare CLH proposals.<br><br>CLH proposals for eight AVK rodenticides, Coumatetralyl (Denmark), Difenacoum (Finland),<br>Warfarin (Ireland), Brodifacoum (Italy), Flocoumafen (The Netherlands), Difethialone (Norway) Chlorophacinone (Spain) and Bromodialone (Sweden), were submitted by eight different Dossier Submitters (DS). The dossiers were handled as a group but the Committee for Risk Assessment (RAC) proceeded to evaluate the proposals on a substance by substance basis comparing the human data available for Warfarin (and other AVKs) and relying on a weight-of–evidence approach as required by Regulation 1272/2008 (CLP). |

### 4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The metabolism studies in rats (draft CAR on Chlorophacinone, Doc III-A, 6.2-01, 6.2-02), showed that Chlorophacinone is rapidly absorbed following oral administration. Using radioactive Chlorophacinone it is rapidly absorbed and that 90% radioactivity is excreted in faeces within 48 hours and 100% in 4 days, most as metabolized compounds, with only less than 1% in urine and no excretion via expired air. Compound is absorbed, enters the enterohepatic circulation and then is excreted through the faeces.

Relatively short plasma half-life (10.2 hours), with 100% of the administered material excreted within 4 days after a single low dose. Higher doses (2 mg/kg) showed that a 168 hours excretion is incomplete with 8% of dose was still presented in the carcass.

In a *in vitro* test dermal penetration with human skin, Chlorophacinone showed rapid absorption but with minimal total absorption. Total absorption was estimated to be 1.7% for the human including radioactivity measured in receptor fluid, tape stripping and residual skin values. (Draft CA report on Chlorophacinone, Doc III-A, 6.2-03).

### 4.1.1 Non-human information

Two toxicokinetics studies are available. The first one (A6.2-01) describes the absorption, distribution and excretion using radioactive chlorophacinone and was demonstrated that it is rapidly absorbed and that 90% radioactivity is excreted in faeces within 48 hours most as metabolized compounds, with only less than 1% in urine. In the second study, metabolites are identified and demonstrated that about 19 % of faecal radioactivity is unchanged parent compound. Two main metabolites were identified as hydroxylated metabolite. A metabolite presented as 12% of faecal radioactivity was not identified as well as other minor metabolites. After 168 hours excretion was incomplete and about 8% was detected in carcasses.

**First toxicokinetic study (A6.2-01):** Animals were dosed orally with 1.0 to 1.43mg/animal, either single dose, or three consecutive daily doses.

Chlorophacinone seems to 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. Tissue residue studies on animals sacrificed 48 hours following a single dose show that the liver is by far the organ with the highest concentrations of radioactivity present. The following is the kidneys with the concentration of radioactivity five times lower than in the liver at 4 hours and approximately 2.8 times lower after 48 hours. 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 bilis.

Extracted faeces and bile in TLC indicated that the material is mainly excreted as metabolized 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.

**Second study to identify metabolites** (A6.2-02): In this study, dosing rats with 2 mg/kg b, excretion was incomplete 168 hours after a single oral dose at 2 mg $^{14}$C-chlorophacinone /kg bw to male rats with 8% of dosed radioactivity residues found in carcase at necropsy.

However in a previous study, excretion was reported estimated to 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 faeces.

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

The three identified excreted compounds in faeces (parent compound plus monohydroxylated metabolites) accounted for 66% of the faecal radioactivity, being there maining 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.

In conclusion:

- The in vitro topical application of $^{14}$C-Chlorophacinone as a tracking powder formulation or wheat flour 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.

### 4.1.2 Human information

**An *in vitro* human dermal penetration study** (Section A6.2-03) was performed on a moistened 50 mg/Kg wheat bait formulation and on a 2g/kg tracking powder formulation. The formulations were spiked with radiolabelled Chlorophacinone. Skin samples were exposed for 6 hours and washed with soap. Receptor fluid was collected during the exposure period and for 18 hours post exposure. At 6 h post application, any residual formulation was washed from the surface of the skin. Tape stripping was applied to remove epidermis and residual skin solubilised. The total radioactivity detected in receptor fluid, plus tape strips and residual skin was used as an estimation of total absorption.

The in vitro topical application of $^{14}$C-Chlorophacinone as a tracking powder formulation or wheat flour 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.

Total absorption of $^{14}$C- Chlorophacinone (tracking powder) including receptor fluid (0.1%), residual skin levels (1.0%) and tape stripping values (0.3%) was 1.4%. The study with wheat flour formulation showed 0.44 % in receptor fluid, and 0.236% in tape strips (total 0.676%) and residual skin values was not used due to high adhesion of particles not eliminated by washing; if it is assumed that a similar residual skin value (1.0%) is appropriate then total absorption is circa 1.7%. This last value was adopted as the best estimation of dermal absorption.

In conclusion:

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

### 4.1.3 Summary and discussion on toxicokinetics

| | |
|---|---|
| Oral absorption | Chlorophacinone is **rapidly absorbed** following oral administration.<br><br>Using radioactive Chlorophacinone, it is rapidly absorbed and 90% radioactivity is excreted in faeces within 48 hours and 100% in 4 days, most as metabolized compounds, with less than 1% in urine. The compound is absorbed, enters the enterohepatic circulation and then is excreted through the faeces |
| Plasma half-life | Relatively short plasma half-life (10.2 hours), with 100% of the administered material excreted within 4 days after a single low dose.<br><br>Higher doses (2 mg/kg) showed that at 168 hours **excretion is incomplete** with **8%** of dose still present in the carcass. |
| Elimination | The elimination was mainly **via faeces**, with little or no urinary excretion (< 1%), and no excretion via expired air. Most faecal excretion was as metabolised compounds accompanied with **unchanged parent compound (19.6%** of the faecal radioactivity, equivalent to 15% of dosed radioactivity). Two major **metabolites represented for 45%** of faecal radioactivity (equivalent to 36.2 % of total dosed radioactivity) as hydroxylated metabolites, with some "minor" unidentified metabolites. |
| Metabolites | The three identified excreted compounds in faeces (parent compound plus monohydroxylated metabolites) accounted for 66% of the faecal radioactivity, being the remanding 34% due to other minor unidentified metabolites.<br>Two main metabolites were identified as hydroxylated metabolised, one in the indandione group and the other in the biphenyl portion of the molecule. The two analogues constituted 46% of faecal radioactivity (36.2% of administered dose)<br>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**.<br><br>The industry argues that "none of the metabolites identified for indandione derivatives used as rodenticides have been shown to be toxicologically significant". However no data is presented to justify this statement. So there are not appropriate data to confirm if the metabolites are or not less toxic than the parent compounds. |
| Dermal penetration | **Total absorption in human skin is estimated to be not more than 1.7%, deduced from a test using** topical application of $^{14}$C-Chlorophacinone as a tracking powder formulation or wheat flour bait to human split thickness skin samples maintained ***in vitro***, considering total absorption including radioactivity measured in receptor fluid, tape stripping and residual skin values. |

## 4.2 Acute toxicity

**Table 11: Summary table of relevant acute toxicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| ORAL<br>EPA 81-1<br>Number of animals/Sex/Dose:<br>• Rat Sprague-Dawley IOPS-VAF; 10 male/group, 10 female/group<br>• Single dose at 0, 2, 3.2, 5.2, 8.2, 13.2 and 21 mg/kg bw. Post exposure period, 21 days | • Male: 3.15 mg/kg (1.48 - 6.68)<br>• Female: 10.95 mg/kg (6.46 - 18.57)<br>• Combined: 6.26 mg/kg (3.96 - 9.89)<br>Mortalities mainly on the 4th and 9th day after treatment.<br>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) | • Definitive rat study<br>• High mortality in males at all doses<br>• Male were 3 times more sensitive than females | Mally C., Porret-Blanc G. (1988) |
| DERMAL<br>EPA 81-1<br>Number of animals/Sex/Dose:<br>• Rabbit, New Zealand White; 2 male/group<br>• Applied to 10% body surface at 100, 50, 10, 5 and 1 mg/kg for 24 hours | Male $LD_{50}$ 0.329 mg/kg.<br>Mortalities occurred by days 16 to 19 of post treatment period. | | Lilja H.S., (1990d) |
| INHALATION<br>EPA 81-3<br>Number of animals/Sex/Dose:<br>• Albino Rat, Sprague-Dawley; 7/8 male/group,7/9 female/group<br>• Nose-only exposure at dose levels of 1.33, 10.3, 11.5 and 14.5 µg/L | • Male: 7.0 µg/L (0.83-59.0)<br>• Female: 12.0 µg/L (7.80-18.0)<br>• Combined: 9.3 µg/L (2.30-38.0) | | Holbert M.S., (1991) |

Only the relevant or key studies have been included here. For the rest of studies, please see the CAR.

### 4.2.1 Non-human information

#### 4.2.1.1 Acute toxicity: oral

*Oral acute toxicity in rats*

The critical $LD_{50}$ for oral dosing in rats were 3.15 mg/kg bw for male rats. Male were more sensitive than females with $LD_{50}$ at least 3 times lower in males.

- Male: 3.15 mg/kg (1.48 - 6.68) (< 5 mg/Kg)
- Female: 10.95 mg/kg (6.46 - 18.57)
- Combined: 6.26 mg/kg (3.96 - 9.89) (Not < 5 mg(Kg)

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 goes down to 1.48 mg/kg for $LD_{50}$ in males. Mortalities occurred mainly on the $4^{th}$ and $9^{th}$ day after treatment. Death was a result of internal haemorrhage, which is the known mode of action of anticoagulant active substance.

A pre-test study was performed in dogs with oral dose of 4.0, 25, 50 mg/kg and the main study at 2, 4.6, 10.8 and 25 mg/kg bw. Dogs were fed with a vitamin K deficient diet. (Disturbance in the main effects?). High mortality occurred at all doses in the pre-test study were all animals died and in the main study all males died at all doses and 3/4 females died at 2, 4.6, and 25 mg/kg; and all females at 10.8 mg/kg.

#### 4.2.1.2 Acute toxicity: inhalation

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.

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 as observed in oral or dermal exposure.

Mortality was as follows 0/14, 6/14, 13/14 and 5/11 at 1.33, 10.3, 11.5 and 14.5 µg/L group, respectively. 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 $LC_{50}$ with 95 % confidence interval 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) (0.0093 mg/L $<LC_{50}$ 0.05 mg/L for dust)

The critical $LD_{50}$ values in males showed a high uncertainty with a wide confidence interval. The high uncertainty is probably due to the observation that high mortality (6 out of 14) occurred from the second to the fourth dose level and short intervals of dose levels were applied between the different groups from the second to the highest dose level.

#### 4.2.1.3 Acute toxicity: dermal

In a range finding study in rabbits dosed in the range of 1 to 100 mg kg/bw all animals died during days 6 to 11 post-treatment while in other study, in the range of 0.01 to 1 mg/kg bw no animal died. In another range finding study and in the main study, mortalities occurred in the range of 16 to 19 days after application.

In the main study, high but limited mortality was observed at all dose levels tested, including the lowest dose of 0.25 mg/kg bw: 9/10 by day 18 (at 0.25 mg/kg bw); 6/10 by day 16 (0.50 mg/kg bw) and 4/10 by day (0.25 mg/kg bw).

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. No erythema and oedema, following 24 hours of exposure, was observed. 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.

It is concluded that dermal acute exposure of Chlorophacinone elicited limited lethality at all dose levels enabling a determination of the dermal LD50 of 0.329 mg/kg in the acute toxicity study rabbits.

#### 4.2.1.4 Acute toxicity: other routes

-

### 4.2.2 Human information

### 4.2.3 Summary and discussion of acute toxicity

Chlorophacinone was very toxic to rats with the oral combined $LD_{50}$ of **6.26 mg/kg.**

Chlorophacinone was also acutely toxic to rabbit by dermal administration (**$LD_{50}$ of 0.329 mg/kg**) and by inhalation in rats (nose only: **$LC_{50}$: 9.3 µg/l** for males-females)

Therefore, Chlorophacinone is acutely toxic by the oral, dermal and inhalation routes. Death was a result of internal haemorrhage, which is the declared mode of action of the active substance.

### 4.2.4 Comparison with criteria

**A. CLP Regulation:**

Inhalat - Acute Tox. 1, ($LC_{50}$=0.0093 mg/L < 0.05 mg/L)
Dermal - Acute Tox. 1, ($LD_{50}$= 0.329 mg/Kg) (<50 mg/Kg)
Oral - Acute Tox. 2, ($LD_{50}$=6.26) (= range 5-50 mg/Kg)

"Fatal if inhaled, if contact with skin and if swallowed".

**B. DSD:**

Class of danger: T+

R-phrases: **R26/27/28:** Very toxic by inhalation in contact with skin and if swallowed

### 4.2.5 Conclusions on classification and labelling

Classification:

| Hazard Class and Category Code(s) | Hazard Statement Code(s): |
|---|---|
| Acute Tox. 1 | H330 |
| Acute Tox. 1 | H310 |
| Acute Tox. 2 | H300 |

Labelling:

| Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) |
|---|---|---|
| GHS06; Danger | H330: Fatal if inhaled<br>H310: Fatal in contact with skin<br>H300: Fatal if swallowed | |

Precautionary statements:

Prevention:

**P260:** Do not breathe dust/fume/gas/mist/vapours/spray

**P270:** Do not eat, drink or smoke when using this product

**P271:** Use only outdoors or in a well-ventilated area

**P280:** Wear protective gloves/protective clothing/eye protection/face protection

Response:

**P301 + P310:** IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician

**P302 + P350:** IF ON SKIN: Gently wash with plenty of soap and water

**P304 + P340:** IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing

Storage:

**P405:** Store locked up

Disposal:

**P501:** Dispose of contents/container to…

**RAC evaluation of acute toxicity**

**Summary of the Dossier submitter's proposal**

Two acute oral toxicity studies (one in the rat, one in the dog), four acute dermal toxicity studies (in the rabbit; three of them dose-range finding studies), and one acute inhalation toxicity study (in the rat) were available.

According to the DS, the relevant or key acute oral toxicity study was the study in rats performed in accordance with US EPA Guideline 81-1, equivalent to OECD test guideline (TG) 401. (The dog study was performed in animals fed with a vitamin K-deficient diet, which could interfere with the rodenticidal activity of chlorophene.) Chlorophacinone was very toxic to the rats with an acute oral $LD_{50}$–value of 3.15 mg/kg for male rats, 10.95 mg/kg for female rats, and a combined (males and females) oral $LD_{50}$-value of 6.26 mg/kg. Animals died mainly on the $4^{th}$ and $9^{th}$ day after treatment due to internal haemorrhages.

In the main acute dermal toxicity study (performed in accordance with US EPA Guideline 81-2, equivalent to OECD TG 402) only male rabbits were used. The study resulted in an $LD_{50}$ value of 0.329 mg/kg. Mortalities occurred by days 16 to 19 of the post treatment

period, due to internal haemorrhages. There were no signs of skin irritation.

The acute inhalation toxicity study in rats (dust, nose only, performed in accordance with US EPA Guideline 81-3, equivalent to OECD 403) showed an $LC_{50}$ of 7.0 µg/L in male rats, 12.0 µg/L in female rats, and a combined $LC_{50}$ of 9.3 µg/L.

Males of both rats and dogs were more sensitive than females to the acute toxic effects of Chlorophacinone. Male rats had 3.5 times lower acute oral $LD_{50}$ and 1.7 times lower acute inhalation $LC_{50}$ compared to female rats. Female dogs had 25% lower mortality compared to male dogs in the acute oral toxicity study.

The DS concluded that Chlorophacinone was acutely toxic by the oral, dermal and inhalation routes, causing death as a result of internal haemorrhages. , According to the CLP Regulation (EC) No. 1272/2008, it was proposed to classify Chlorophacinone as follows:

- Acute oral toxicity: Category 2; H300(" Fatal if swallowed") (the combined oral $LD_{50}$ was within the interval of 5<ATE≤50 mg/kg bw)
- Acute dermal toxicity: Category 1; H310 ("Fatal in contact with skin") (dermal $LD_{50}$ <50 mg/kg bw)
- Acute inhalation toxicity: Category 1; H330 ("Fatal if inhaled") (male, female and both sexes combined $LC_{50}$ were all <0.05 mg/l, meeting the criteria applicable for dusts and mists).

## Comments received during public consultation

One MSCA supported the classifications for acute toxicity as proposed by the dossier submitter.

## Additional key elements

-

## Assessment and comparison with the classification criteria

Based on a comparison of the available dermal $LD_{50}$ values in rabbits and inhalation $LC_{50}$ values in rats with the classification criteria, RAC supports the conclusion of the dossier submitter that according to the CLP Regulation, Chlorophacinone should be classified in Category 1 for acute dermal and inhalation toxicity (Acute Tox. 1; H310 "Fatal in contact with skin", and Acute Tox. 1; H330 "Fatal if inhaled", respectively).

Regarding the classification for acute oral toxicity, RAC considers that Chlorophacinone should be classified in Category 1 (Acute Tox. 1; H300 "Fatal if swallowed"), based on the acute oral $LD_{50}$ value of 3.15 mg/kg in male rats (which is < 5 mg/kg), instead of Category 2 as proposed by the dossier submitter, who based the proposed classification on the combined $LD_{50}$ value of 6.26 mg/kg. The male rats were 3.5 times more sensitive in the acute oral test than female rats ($LD_{50}$ 3.15 mg/kg *vs.* 10.95 mg/kg, with barely overlapping 95% confidence intervals of 1.48-6.68 and 6.46-18.57, respectively). In addition, males were more sensitive in the acute inhalation study than females, and in the main acute dermal study only male rabbits were tested since in this species males were also more sensitive. Choosing the more sensitive sex for regulatory purposes is supported by the OECD Test Guideline (TG) 425 (Acute Oral Toxicity – Up-and-Down-Procedure; 2006): "*Normally female rats are used. This is because literature surveys of conventional $LD_{50}$ tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive. However, if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive then this sex should be used.*"

**Supplemental information - In depth analyses by RAC**
A clarification of the DS' description of the acute inhalation study in rats, according to Reregistration Eligibility Decision (RED) - Rodenticide Cluster (US EPA Document, July 1998; www.epa.gov/oppsrrd1/REDs/2100red.pdf page 16) is provided: The animals that died within the first 5 hours showed no clinical or morphopathological signs of haemorrhage. These deaths resulted from suffocation, since during 4-hour exposure some animals turned in the restrainers between observations. Substance-related mortalities, which were accompanied by signs of anticoagulant activity, occurred 3-8 days after exposure to chlorophacinone.

## 4.3 Specific target organ toxicity – single exposure (STOT SE)

### 4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

### 4.3.2 Comparison with criteria

### 4.3.3 Conclusions on classification and labelling

**RAC evaluation of specific target organ toxicity – single exposure (STOT SE)**

**Summary of the Dossier submitter's proposal**

There was no proposal on specific target organ toxicity – single exposure because no data was available.

**Comments received during public consultation**

No comments were received for this hazard class.

**Additional key elements**

-

**Assessment and comparison with the classification criteria**

In the opinion of RAC, the blood coagulation system is affected after single exposure since it was the main cause of mortality in acute studies. However, classification for STOT-SE for Chlorophacinone is not warranted since it is considered to be covered by classification as Acute Tox. 1.

## 4.4 Irritation

### 4.4.1 Skin irritation

**Table 12: Summary table of relevant skin irritation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Skin irritation (rabbit)<br>EPA 81-4 | Non irritant<br>Average score 24, 48, 72 h<br>▪ Erythema: 0.00 for non-abraded skin<br>▪ Oedema: 0.00 for non-abraded skin | Reversibility yes/no: No effects therefore not applicable | Lilja H.S., (1989a) |

#### 4.4.1.1 Non-human information

#### 4.4.1.2 Human information

#### 4.4.1.3 Summary and discussion of skin irritation

The test substance's average score or irritant properties for all animals at 24, 48 and 72 h are 0 for erythema and oedema. Chlorophacinone does not fulfil the EU criteria for classification as a skin irritant.

#### 4.4.1.4 Comparison with criteria

#### 4.4.1.5 Conclusions on classification and labelling

Chlorophacinone does not fulfil the EU criteria for classification as a skin irritant.

**RAC evaluation of skin corrosion/irritation**

**Summary of the Dossier submitter's proposal**

The results of a skin irritation study in rabbits (performed in accordance with US EPA Guideline 81-5, equivalent to OECD TG 404) showed an average score of zero for erythema and oedema for all tested animals at 24, 48 and 72 h after exposure. The Dossier submitter concluded that Chlorophacinone did not fulfil the CLP criteria for classification as a skin irritant.

**Comments received during public consultation**

There were no comments on this hazard class.

**Additional key elements**

-

**Assessment and comparison with the classification criteria**

Based on the results of skin irritation study in rabbits and supported by the lack of clinical signs of skin irritation in acute dermal toxicity study in rabbits, RAC supports the conclusion of the dossier submitter that Chlorophacinone should not be classified for skin irritation.

### 4.4.2 Eye irritation

**Table 13: Summary table of relevant eye irritation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Eye irritation (rabbit)<br>EPA 81-4 | Chlorophacinone does not meet EU criteria for classification as an eye irritant.<br>Average Score over 24, 48, 72 h for :<br>▪ Cornea reaction: 0.00<br>▪ Iris reaction: 0.00<br>▪ Conjuntiva redness :<br>▪ Redness 0.00<br>▪ swelling: 0.00 | Reversibility yes/no :<br>No effects therefore not applicable | Lilja H.S., (1989b) |

#### 4.4.2.1 Non-human information

#### 4.4.2.2 Human information

#### 4.4.2.3 Summary and discussion of eye irritation

The test substance's average score for all animals at 24, 48 and 72 h is 0 for the iris and cornea, and for chemosis and redness of the conjunctiva. Chlorophacinone does not fulfill the EU criteria for classification as an eye irritant.

#### 4.4.2.4 Comparison with criteria

#### 4.4.2.5 Conclusions on classification and labelling

Chlorophacinone does not fulfil the EU criteria for classification as an eye irritant.

**RAC evaluation of eye corrosion/irritation**

**Summary of the Dossier submitter's proposal**

The results of an eye irritation study in rabbits (performed in accordance with US EPA Guideline 81-4, equivalent to OECD 405) showed an average score of zero for iris and cornea reaction, and for chemosis and redness of the conjunctiva for all tested animals at 24, 48 and 72 h after exposure. The Dossier submitter concluded that Chlorophacinone did not fulfil the CLP criteria for classification as an eye irritant.

**Comments received during public consultation**

There were no comments on this hazard class.

**Additional key elements**

-

**Assessment and comparison with the classification criteria**

Based on the results of the eye irritation study in rabbits, RAC supports the conclusion of the dossier submitter that Chlorophacinone should not be classified for eye irritation.

**Supplemental information - In depth analyses by RAC**

| |
|---|
| - |

### 4.4.3 Respiratory tract irritation

No study available. In the acute inhalation study, it was stated there was no evidence of respiratory tract irritation following a 4 h nose only exposure.

#### 4.4.3.1 Non-human information

#### 4.4.3.2 Human information

#### 4.4.3.3 Summary and discussion of respiratory tract irritation

#### 4.4.3.4 Comparison with criteria

#### 4.4.3.5 Conclusions on classification and labelling

| **RAC evaluation of respiratory tract irritation** |
|---|
| **Summary of the Dossier submitter's proposal**<br>There was no study in which respiratory tract irritation had been specifically investigated. In the acute inhalation study in rats it was stated that there was no evidence of respiratory tract irritation following a 4-hour nose only exposure. No recommendation for classification with respect to respiratory tract irritation was made by the DS.<br><br>**Comments received during public consultation**<br>There were no comments on respiratory tract irritation.<br><br>**Assessment and comparison with the classification criteria**<br>Based on the absence of respiratory irritation effects in an acute inhalation study in rats, as well as on the absence of skin and eye irritation in rabbits, RAC concludes that classification of Chlorophacinone for respiratory tract irritation is not warranted. |

### 4.5 Corrosivity

Chlorophacinone does not fulfil the EU criteria for classification as a skin and eye irritant.

### 4.5.1 Non-human information

### 4.5.2 Human information

### 4.5.3 Summary and discussion of corrosivity

### 4.5.4 Comparison with criteria

### 4.5.5 Conclusions on classification and labelling

## 4.6 Sensitisation

No signs of irritation were observed. Test substance not classified as a sensitizer.

### 4.6.1 Skin sensitisation

**Table 15: Summary table of relevant skin sensitisation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Skin sensitization<br>EPA 81-6 | - Buehler test design, 10 test and 5 control animals. Six, 6 hour exposure, topical induction applications followed by topical challenge.<br>- No indications of delayed contact hypersensitivity among guinea pigs subject to an induction and challenge regimen with a sublethal dose.<br>- No signs of irritation were observed. Test substance not classified as a sensitizer. | | Shapiro R., (1990) |

#### 4.6.1.1 Non-human information

#### 4.6.1.2 Human information

#### 4.6.1.3 Summary and discussion of skin sensitisation

#### 4.6.1.4 Comparison with criteria

#### 4.6.1.5 Conclusions on classification and labelling

Conclusion for classification: Chlorophacinone is not classified as a skin sensitizer.

| **RAC evaluation of skin sensitisation** |
|---|
| **Summary of the Dossier submitter's proposal**<br>A Buehler test in male guinea pigs was performed (in accordance with US EPA 81-6, |

equivalent to OECD 406, with certain deviations), with six topical induction applications followed by a topical challenge. There were no indications of delayed contact hypersensitivity, and no signs of irritation. The DS concluded that Chlorophacinone should not be classified as a skin sensitiser.

**Comments received during public consultation**

There were no comments on this hazard class

**Assessment and comparison with the classification criteria**

The study design deviated from the Test Guidelines: instead of 20 test and 10 control animals, 10 and 5 were used, respectively. In addition, during the induction phase two animals died in the treatment group, leaving only 8 animals. The number of induction applications was doubled – six topical applications over a 3-week period, instead of three inductions during 2 weeks as recommended in the Guideline.

Since the test results were clearly negative for Chlorophacinone (zero score at 24 and 48h after challenge in all tested animals according to the Magnusson and Kligman grading scale) while all positive control animals showed clearly positive result (moderate to severe erythema at the dose sites 24 and 48 h after challenge), RAC supports the dossier submitter's proposal that the substance should not be classified as a skin sensitiser. However, RAC underlines that the conclusion is based on the study with a markedly lower number of animals than recommended in the test guidelines (2.5 times lower in the treatment group, and 2 times lower in the control group).

### 4.6.2 Respiratory sensitisation

No information

#### 4.6.2.1 Non-human information

#### 4.6.2.2 Human information

#### 4.6.2.3 Summary and discussion of respiratory sensitisation

#### 4.6.2.4 Comparison with criteria

#### 4.6.2.5 Conclusions on classification and labelling

## 4.7 Repeated dose toxicity

### Table 17: Summary table of relevant repeated dose toxicity studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Oral – Rat - EPA 83-3**<br><br>Number of animals/Sex/Dose:<br>• Rat Sprague Dawley OFA IOPS Both sexes, 10 per group.<br>• 5 µg/kg bw daily for 11 weeks;10, 20, 40, 80 or 160 µg/kg bw daily for up to 16 weeks | **Rat** (90 day oral administration)<br>No target organs were identified. The mode of action for anticoagulant rodenticides is well characterised. The critical effect is death arising from persistent or severe haemorrhage. The clinical findings in the study were indicative of internal haemorrhagic events and were consistent with the established pattern of increasing prothrombin times associated with increasing severity of bleeding for orifices or abrasions, pallor, ataxia or weakness/limb paralysis and breathing difficulty. Death rapidly followed development of signs and necropsy confirmed presence of haemothorax and haemoperitoneum among other diffuse, non-specific haemorrhages and haematoma formation. | **Lowest relevant oral NOAEL / LOAEL**<br>LOAEL = 0.010 mg/kg b.w. /day<br>NOAEL = 0.005 mg/kg b.w. /day (11 weeks exposure)<br>(Some uncertainty due to shorter time at the dose of 5 µg/kg b.w. /day and no prothrombin time determination at this dose) | Mally C., Porret-Blanc G., Lorgue G. (1984) |
| **Dermal – Rabbit - EPA 83-3**<br><br>Number of animals/Sex/Dose:<br>• Rabbit New Zealand, 10 (5 M 5 F) per group<br>• Dosage levels were 0.08 mg/kg/day, 0.40 mg/kg/day, and 2.00 mg/kg/day applied as 0.2% w/w tracking powder | **Rabbit** (15 day dermal administration, 5 days/week for 3 weeks)<br>Widespread non-specific haemorrhage was the primary cause of death among rabbits dosed with a 2% formulation of chlorophacinone. Necropsy also revealed centrilobular liver necrosis. In-life signs of haemorrhage were confirmed by necropsy observations of free fluid in many body cavities and pale organs. Increased prothrombin times were measured in-life as an indicator of progressive failure of the clotting cascade arising from non-replenishment of Vitamin K in the liver of intoxicated animals. | **Lowest relevant dermal NOAEL / LOAEL**<br>LOAEL 0.40 mg/kg/day<br>NOAEL 0.08 mg/kg/day (21 day exposure) | Hamada N. (1992b) |
| **Inhalation** | Not established - study not scientifically justified | | |

Only the relevant or key studies have been included here. For the rest of studies, please see the CAR.

### 4.7.1 Non-human information

#### 4.7.1.1 Repeated dose toxicity: oral

An study intended for evaluating the subchronic oral toxicity for a period exceeding 90 days, were performed dosing Chlorophacinone, dissolved in corn oil, administered by gavage (oral intubation) to rats, 7 days/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 study was conducted according to EC Method B.27 guidelines with some deficiencies: limited microscopic examination, clinical signs were not reported for each dose group. The low dose group was terminated after 11 weeks (77 days) (justified by Authors as due to the complete absence of any toxicological effects at this dose). Author and Applicant have considered that the extent of examination is sufficient to characterize the dose-response pattern and that these deficiencies do not impact the NOAEL determination or the study reliability. A main deficiency for a definitive adoption of NOAEL/LOAEL is that coagulation activity was not monitored at the lowest dose of 5 µg/kg bw/day, just the dose proposed for NOAEL. It is justified but in any case some uncertainty is maintained. The study is accepted BUT with the commented uncertainty.

This is a critical study as it is the only subchronic study and no chronic study is available. Therefore, it is analyzed in detail as follows:

*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 and not considered for evaluation.

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 day, and with no clear difference between sexes.

*Macroscopic examination*

Group dosed at 5 µg/kg/day showed no hemorrhagic lesions. Thymusa haemorrhages were observed in some 10% animals dosed at 10 µg/kg/day and in most animals (≥90%) of groups at 20, 40 and 160 µg/kg. and frequent haemorrhages were also noted in haemothorax, haemoperitoneum, and haemorrhages at the stomach, the digestive tract, and the hypophysis, and at 20 µg/kg. At 40 and 80 µg/kg/day also haemorrhages were noted 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 haemorrhages both externally and internally.

*Clinical chemistry*

Biochemical parameters were generally unaffected by chlorophacinone at the lowest levels examined (the level without mortality). However, at 10 and 20 µg/kg/day, 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, which was notably pronounced in groups 20 and 40 µg/kg/day and were minimal in group 10 µg/kg but statistically significant. 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 stopped 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.

*NOAEL/LOAEL deduced from the subchronic study in rats*

The authors of the study and the manufacturer deduced that the NOAEL for this study is 5 µg/kg/day on the basis of no clinical, pathological and histopathological effect observed during the 77 days of the study at this dose (although no haemathological studies on coagulation time were performed at this dose level. The next dose level of 10 µg/kg/day might be considered at the LOAEL at which no death are observed but caused minimal but statistically significant increase in coagulation (quick) time and alterations of some biochemical parameters indicator of hepatic and renal alterations.

As the dose of 10 µg/kg/day only caused minimal increase in coagulation time, in the complete absent of clinical, pathological alterations the dose tested of 5 µg/kg/day, it is reasonable to accept this dose as NOAEL. However the lack of monitoring coagulation parameters and the shorter time of dosing and observation at this dose, involves some uncertainty in the conclusion of the NOAEL. Consequently this uncertainty will have to be considered for risk assessment.

*Summary conclusion of subchronic oral study in rats*

- High mortality is observed at dose 20 µg/kg/d of higher for males and 40 µg/kg/d or higher for females. Males were more sensitive to the lethal effects of Chlorophacinone than females. (At 20 µg/kg/day 4 males died. At 40 µg/kg/day 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/d group and none noted at 5 µg/kg/d. 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/day but significantly different from controls.
- A **LOAEL of 10µg/kg/day** is established 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
- It is concluded that for subchronic oral toxicity **NOAEL value of 5 µg/kg bw/day** can be established based on results from 11 weeks (77 days) administration. An uncertainty is maintained 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.

- The uncertainty in NOAEL/LOAEL will be addressed for risk characterization assuming an additional assessment factor of 3.

#### 4.7.1.2 Repeated dose toxicity: inhalation

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#### 4.7.1.3 Repeated dose toxicity: dermal

There are not specific acceptable data available for repeated dose dermal exposure with the active substance.

In the range finding study, Chlorophacinone (100% purity) was topically applied for 6-hour for 5 days/week, for 3 weeks at 1.0, 0.3, 0.1, 0.03, 0.01 and 0.003 mg/kg bw/day in rabbits (2 animal (1 male, 1 female)

Mortalities were observed from 0.1 mg/kg/d (1 females) and both males and females at the two highest doses (1 and 0.3 mg/kg/day) died during the dosing period. All the animals that died during the study either lost weight or had only a slight gain in weight. The necropsy 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.

A full study was carried out with 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.

At 2.00 mg/kg/day dose 4 males died on day 14, 15, 16, 18, and one female on day 21. 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 0.4 mg chlorophacinone/kg/day did not produce any clinical/clinical compound-related signs, mortality, body weight and food consumption changes, or 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 and the low dose (0.08 mg/kg/day) was not causing effect in both males and females.

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 pathological or histopathological changes. The highest dose of 2 mg/kg/day caused high mortality (4/5 males and 1/5 females).

The conclusion has not general value but only for the formulation used as tracking power. The range finding study using the active substance in a limited number of animals (6 doses, 2 animal/group) suggested that the critical dose might be in the range of 0.03 to 0.1 mg/kg. Although that study cannot be used for deducing NOAEL for risk assessment it is useful to confirm that the study with the formulation could be acceptable.

No data of dermal absorption are available in rabbit skin, so the dermal repeated study in rabbit cannot be directly used for estimating the no-effect systemic dose, and consequently no direct use can be done for risk characterization by comparison with systemic dose estimated for human exposure.

#### 4.7.1.4 Repeated dose toxicity: other routes

#### 4.7.1.5 Human information

#### 4.7.1.6 Other relevant information

#### 4.7.1.7 Summary and discussion of repeated dose toxicity

The toxicity response of Chlorophacinone shows very "drastic" dose-effect relationship (high slope in dose-response curve). The toxic doses are lethal showing lethality after some time of repeated dosing. In most studies, the next dose higher than NOAEL is showing high lethality due to haemorrhage, although in some case it is possible to observe a dose with low lethality with altered coagulant parameter (as prothrombin time). The anticoagulant property of Chlorophacinone is responsible of the toxicity and no other effects are significant in comparison with the so relevant anticoagulant property. At higher doses, lethality occurs at shorter time whereas at lower doses lethality only occurs after longer time of repeated dosing but in any case after an accumulative time of repetitive dosing, haemorrhage is causing lethality.

*Repeated dermal toxicity (21 days study):* There are not specific acceptable data available for repeated dose dermal exposure with the active substance. There is a range finding study which is useful to confirm the value of the available study with the formulation tracking power. A full study was performed with the formulation tracking power containing 0.2 % of active substance in New Zealand White rabbits. The study allows obtaining 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. No data of dermal absorption are available in rabbit skin, so the dermal repeated study in rabbit cannot be directly used for estimating the no-effect systemic dose, and consequently no direct use can be done for risk characterization by comparison with systemic dose estimated for human exposure.

*Subchronic oral toxicity in rats:* A study intended for evaluating the subchronic oral toxicity for a period exceeding 90 days, were performed dosing Chlorophacinone, dissolved in corn oil, administered by gavage (oral intubation) to rats, 7 days/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 study was conducted according to EC Method B.27 guidelines with some deficiencies: limited microscopic examination, clinical signs were not reported for each dose group. The low dose group was terminated after 11 weeks (77 days) (justified by Authors as due to the complete absence of any toxicological effects at this dose). An uncertainty for a definitive adoption of NOAEL/LOAEL is that coagulation activity was not monitored at the lowest dose of 5 µg/kg bw/day, just the dose that later is proposed for NOAEL. It is technically and operationally justified but in any case some uncertainty is maintained. The study is accepted but with the commented uncertainty.

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 and not considered for evaluation. Mortality was noted in all dosage groups above 10 µg/kg. High mortality is observed at dose 20 µg/kg/d of higher for males and 40 µg/kg/d or higher for females. The dominant clinical signs were

related to the anticoagulant activity of Chlorophacinone and were responsible for death of animals. Alteration in coagulation parameters (Quick test time) were notably pronounced in groups 20 and 40 µg/kg and were minimal in group 10 µg/kg/day but significantly different from controls. A LOAEL of 10µg/kg/day is established 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. It is concluded that for subchronic oral toxicity NOAEL value of 5 µg/kg bw/day can be established based on results from 11 weeks (77 days) administration. An uncertainty is maintained on this conclusion as no coagulation time was measured at this dose and this group was terminated before the 90 days. The uncertainty in NOAEL/LOAEL is considered for risk characterization.

A *repeat dose inhalation study* is not presented. Applicant argue that considering the acute inhalation toxicity and the anticoagulant properties, an inhalation repeated dose study as it will result in death by induction of a haemorrhagic syndrome including at low dose. Therefore if these arguments for waiving of repeated inhalation study is accepted, it involves accepting that is actually "very toxic by inhalation" and R26 should be applied.

#### 4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

#### 4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

#### 4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Classification with **T; R/48/23/24/25** is warranted based on:

- the repeated **dermal** toxicity and
- subchronic **oral** toxicity
- data plus extrapolation from the acute data for **inhalation** route of exposure

### 4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

#### 4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Category 1 - STOT RE 1

H372: Causes damage to organs (blood coagulation system, liver, kidney) through prolonged or repeated exposure

#### 4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

CLP Regulation:

Category 1 - STOT RE 1

H372: Causes damage to organs (blood coagulation system, liver, kidney) through prolonged or repeated exposure

DSD:

R48/23/24/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed.

### 4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification: Category 1 - STOT RE 1

Labelling:

a. Pictogram: GHS08

b. Signal Word: Danger

c. Hazard Statement:

H372: Causes damage to organs (blood coagulation system, liver, kidney) through prolonged or repeated exposure

No route is specified as it is concluded that all routes (oral, dermal and inhalation) may cause repeated dose toxicity fulfilling the criteria for classification.

**RAC evaluation of specific target organ toxicity (CLP) – repeated exposure (STOT RE)**

**Summary of the Dossier submitter's proposal**

The evaluation of repeated dose toxicity and STOT RE was based on the two most relevant studies: a 90-day oral toxicity study in rats and a 21-day dermal toxicity study in rabbits (Hamada 1992b). In addition, a range finding dermal toxicity study in rabbits (Fitzgerald 1990b) was discussed as supporting evidence.

**90-day oral toxicity study in rats** (Mally et al. 1984)

This oral toxicity study in rats was performed with doses of 10, 20, 40, 80 or 160 µg/kg bw/day for up to 16 weeks, and with 5 µg/kg bw/day for 11 weeks (an earlier termination at this dose was justified by a complete absence of any toxicological effects), in accordance with US EPA 82-1, equivalent to OECD 408, with certain deviations (limited microscopic examination; clinical signs were not reported for each group). In the control group 40 animals (20 males and 20 females) were used, and in Chlorophacinone-exposed groups 20 animals per dose (10 males and 10 females) were used.

Mortality due to haemorrhage was observed at doses ≥20 µg/kg bw/day, with male rats being more sensitive than females.

- At doses of **80** and **160 µg/kg bw/day** all animals died during 7-16 days and 5-8 days after the beginning of the treatment, respectively.
- At **40 µg/kg bw/day** 100% of male rats and 40% of female rats died, with male rats dying earlier, namely during days 29-82 of treatment compared to during days 69-111 in female rats.
- At **20 µg/kg bw/day** 40% of male rats and none of female rats died, with

haemorrhagic lesions of average intensity found in all animals.

- At **10 µg/kg bw/day** there were no substance-related deaths. One male and one female died due to an intubation error. A minimal, but statistically significant, increase in coagulation time (Quick's prothrombin time) was observed; thymus haemorrhage was noted in one of 9 males.

- At **5 µg/kg bw/day** no clinical or pathological alterations were observed. However, at this dose level coagulation parameters were not monitored and animals were dosed for a shorter time period, namely 11 weeks, which introduces uncertainty in determining NOAEL level in this study.

A LOAEL of 0.010 mg/kg bw/day and NOAEL of 0.005 mg/kg bw/day were established by the applicant.

Repeated dose dermal toxicity was evaluated based on two studies in rabbits: one range finding study with Chlorophacinone of 100% purity (Fitzgerald 1990b), and one full study performed with the formulation 'tracking powder' containing 0.2% of active substance (Hamada 1992b), in accordance with US EPA 82-2, equivalent to OECD 410.

**21-day dermal toxicity study in rabbits - range finding study** (Fitzgerald 1990b)
In the range finding study Chlorophacinone was topically applied for 3 weeks (6-hours for 5 days/week) at 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg bw/day to 2 rabbits/per dose (one male, one female).
Mortalities occurred during the dosing period due to internal haemorrhage at doses ≥0.1 mg/kg bw/day. In surviving animals no unusual lesions were found.

- At **0.3** and **1.0 mg/kg bw/day** both males and females died.

- At **0.1 mg/kg bw/day** one female died.

- At **0.003**, **0.01** and **0.03 mg/kg bw/day** no unusual lesions were found.

**21-day dermal toxicity study in rabbits** (Hamada 1992b)
In the full study Chlorophacinone as the formulation tracking powder (clay Chlorophacinone mixture, moistened with distilled water) was topically applied for 3 weeks (6-hours for 5 days/week) at 0.08, 0.40 and 2 mg/kg bw/day to 10 rabbits per group (5 males and 5 females).

- At **2 mg/kg bw/day** 4 males and one female died during the dosing period due to haemorrhage. On necropsy moderate to moderately severe centrilobular liver necrosis was observed in three males and one female.

- At **0.40 mg/kg bw/day** there was an increase in prothrombin values in both males and females, but no mortality. Clinical signs of toxicity or gross pathology or histopathology changes were observed. All animals lost weight during the first week but had recovered by the second week.

At **0.08 mg/kg bw/day** the prothrombin times were not affected and there were no clinical signs of toxicity or any gross pathology or histopathology changes.

The DS considered 0.08 mg Chlorophacinone/kg bw/day as the NOAEL value, based on a LOAEL of 0.4 mg/kg bw/day at which prolongation of prothrombin time (PT) was observed in rabbits dermally exposed to tracking powder formulation. Nevertheless, it was pointed out that this conclusion has no general value but is valid only for the formulation used as tracking powder. The range finding study could not be used for deriving a NOAEL but the results

indicated that the critical dose might be in the range of 0.03 to 0.1 mg/kg bw/day, consistent with the results of the study with the tracking powder formulation.

A repeat dose inhalation study was not presented, with the justification from the applicant that the data from the acute inhalation toxicity study ("fatal if inhaled") and the anticoagulant properties of the substance, would predict that an inhalation repeated dose study would result in death by induction of a haemorrhagic syndrome at a low dose.

It was concluded that despite methodological drawbacks in the studies summarised in the report, Chlorophacinone should be classified as STOT RE, Category 1; H372: Causes damage to organs (blood coagulation system, liver and kidney) through prolonged or repeated exposure, according to the CLP Regulation (EC) No. 1272/2008.

Specific concentration limits for repeated dose toxicity was suggested by the DS according the Dir. 67/548/EEC.

## Comments received during public consultation

One MSCA supported the suggested classification for STOT RE, including the SCLs.

## Assessment and comparison with the classification criteria

In a **90-day oral toxicity study in rats** several deviations from the Test Guideline were noticed: the low dose group (5 µg/kg bw/day) was terminated already after 11 weeks (77 days); a haematological and clinical chemistry evaluation was not performed in the low dose animals; an ophthalmoscopic examination was not performed (according to the CLH Report and CAR IIIA document). Therefore, it could not be excluded that certain adverse substance-related effects were present at the dose level proposed as the NOAEL (5 µg/kg bw/day).

Biochemical parameters were not analysed in animals exposed to 5 µg Chlorophacinone/kg bw/day, but at the dose level of 10 µg/kg bw/day, changes in clinical chemistry observed (decreased bilirubin, phosphorus, magnesium, potassium and ASAT levels in males; decreased bilirubin, triglycerides and ASAT, and increased creatinine, cholesterol and total proteins in females, as reported in the CAR IIIA document) were not statistically significant. As stated in the CAR, the changes "were extremely variable, and for a good number of them, did not correlate with sex or with dose".

The situation was different with the coagulation parameters. According to information in the CAR IIIA document (pages 166-167), an average increase in clotting time (quick) (presumably refers to "Quick's prothrombin time" expressed in seconds) was 3.2 seconds in males (26%) and 0.85 seconds in females (8%), with thymus haemorrhages observed in one out of 9 males (one male died due to intubation error) at a dose level of 10 µg/kg bw/day. No clinical or pathological alterations were observed at a dose level of 5 µg/kg bw/day. Therefore, a LOAEL of 0.010 mg/kg bw/day (based on increased clotting time and thymus haemorrhage in 1/9 males) could be established.

RAC agreed with the dossier submitter's conclusion that the **repeated dose dermal toxicity studies in rabbits** have major drawbacks that seriously limit their usability for evaluation.
The first evaluated study, in which the active substance was tested, is a range finding study (only 2 animals per dose, with no histopathology or clinical chemistry evaluation and therefore could not be used for setting NOAEL and LOAEL values). The second (main) study was performed with a tracking powder formulation (see page 9). When this and a 21-day dermal range-finding study with a formulation (described in CAR IIIA document) are compared to a 21-day dermal range-finding study with the active substance, it seems that toxicity of Chlorophacinone applied as a formulation was lower compared to where it was applied as an active substance (Table 1). Namely, at a similar dose level (0.40 mg/kg/day in the studies with formulation and 0.30 mg/kg/day in study with active substance) different outcomes were

observed: while only prothrombin time was increased without clinical or histopathological signs of haemorrhage at necropsy in the studies with the formulation, mortality occurred in the studies with the active substance. This dose level was, in fact, established as the acute dermal $LD_{50}$ in rabbits (0.329 mg/kg). The NOAEL of 0.08 mg/kg bw/day derived from the main study with the formulation was higher than a dose of 0.03 mg/kg bw/day from range finding study with the active substance at which internal haemorrhage in one animal was observed at necropsy.

Therefore, RAC does not support a NOAEL of 0.08 mg/kg bw/day and a LOAEL of 0.40 mg/kg bw/day derived from the main study in rabbits dermally exposed to a tracking powder formulation. In the range finding study for a 21-day dermal toxicity study with the active substance the lowest dose at which no clinical signs of toxicity and no haemorrhages on necropsy were observed was 0.01 mg/kg bw/day (Table 1). Since in this study only two animals per dose were used and no haematology or clinical chemistry evaluation was performed, RAC considers that the NOAEL and LOAEL could not be set for repeated dose dermal toxicity of Chlorophacinone. In the range finding study of Lilja (1990b) for acute dermal $LD_{50}$ in rabbits, internal haemorrhage at necropsy was found already at 0.01 mg/kg (CAR IIIA document) (Table 1), indicating that a NOAEL for dermal repeated-dose toxicity could be expected to be even below 0.01 mg/kg bw/day.

**CLP classification**
Based on these conclusions, RAC supports the dossier submitter's proposal to classify Chlorophacinone as **Category 1, STOT RE 1 - H372 (Causes damage to the blood through prolonged or repeated exposure)**, according to the criteria in the CLP Regulation ((EC) No. 1272/2008), based on:

- LOAEL of 0.01 mg/kg bw/day in a 90-day oral toxicity study in rats (based on increased clotting time and thymus haemorrhage in 1/9 males) (guidance value (GV): C≤10);

- NOAEL <0.01 mg/kg bw/day in a 21-day repeated dose dermal toxicity study in rabbits (GV: C≤86 with Haber's rule applied);

- extrapolation from the acute toxicity data for the inhalation route of exposure ($LC_{50}$ of 0.009 mg/L in acute inhalation toxicity test is more than 2 times lower than the STOT RE Category 1 guidance value of 0.02 mg/L for a 90-day inhalation study).

The STOT RE 1 classification should apply for all routes of exposure. This is because the oral data can be used for classification for the dermal and inhalatory routes since the acute $LD_{50}$ values for dermal and inhalatory toxicity are below the guidance values for classification as STOT RE 1, and there is a large margin between the oral dose levels indicating severe effects (40% mortality of male rats at 0.02 mg/kg bw/day) and the guidance value for STOT RE 1 (C ≤ 10 mg/kg bw/day).

It is recommended that only blood be stated as a target organ (tissue) since liver and kidney changes observed at the highest doses in oral and dermal repeated dose studies are presumably secondary, due to haemorrhage (namely, hypovolaemic shock due to extensive internal haemorrhage can lead to kidney and liver damage, including acute renal failure, acute tubular necrosis and centrilobular liver necrosis). In the rat oral study "lesions of hepatic degeneration and coagulation necrosis, comparable to necrosis lesions of ischemic origin" were described at 40 µg/kg bw/day, a dose at which also high mortality was found. At a lower dose, 20 µg/kg bw/day, no hepatic or renal histopathological changes were described although mortality was 40% in males and haemorrhagic lesions of average intensity were found in all animals. In the rabbit dermal study, centrilobular liver necrosis was found only at the highest dose, 2 mg/kg bw/day, at which mortality and tissue haemorrhage was observed.

**Specific concentration limit (SCL) derivation**

An SCL is derived from the effective dose of 0.010 µg/kg bw/day (defined as LOAEL in 90-day oral toxicity study in rats at which increased prothrombin time and signs of haemorrhage are observed), and calculated as follows:

SCL, Category 1 = ED/GV1 x 100% = $\frac{0.01 \text{ mg/kg bw/day}}{10 \text{ mg/kg bw/day}}$ x 100 = **0.1%**

SCL, Category 2 = ED/GV2 x 100% = $\frac{0.01 \text{ mg/kg bw/day}}{100 \text{ mg/kg bw/day}}$ x 100 = **0.01%**

These values are in line with the SCLs proposed by the dossier submitter:
C ≥ 0.1%: STOT RE 1; H372
0.01% ≤ C < 0.1%: STOT RE 2; H373

## Supplemental information - In depth analyses by RAC

Table 1. Comparison of repeated-dose dermal toxicity studies in rabbits with Chlorophacinone applied as active substance (100%) and as tracking powder formulation (0.2%)

Range-finding study for 21-day dermal toxicity in rabbits with 100% chlorophacinone (Fitzgerald 1990b)

| Dose (mg/kg/day) | 0.003 | 0.01 | 0.03 | 0.10 | 0.30 | 1.00 |
|---|---|---|---|---|---|---|
| Mortality | 0/2 | 0/2 | 0/2 | 1/2 | 2/2 | 2/2 |
| Clinical signs | - | - | - | -/? | + | + |
| Necropsy | NR | NR | H | H | H | H |

Range-finding study for 21-day dermal toxicity in rabbits with 0.2% chlorophacinone (Hamada 1992a)

| Dose (mg/kg/day) | 0.41 | 0.81 | 1.63 | 3.25 | 6.50 |
|---|---|---|---|---|---|
| Mortality | 0/3 | 0/3 | 0/3 | 1/3 | 2/3 |
| Clinical signs | - | - | + | + | + |
| Prothrombin time | ↑ | ↑ | ↑ | ↑ | ↑ |
| Necropsy | NR | H?, a | H?, b | H | H |

21-day dermal toxicity in rabbits with 0.2% chlorophacinone (Hamada 1992b)

| Dose (mg/kg/day) | 0.08 | 0.40 | 2.00 |
|---|---|---|---|
| Mortality | 0/10 | 0/10 | 5/10 |
| Clinical signs | - | - | + |
| Prothrombin time | N | ↑ | ↑ |
| Necropsy | NR | NR | H |

- = no clinical signs related to haemorrhagic diathesis
+ = clinical signs related to haemorrhagic diathesis
NR = not remarkable
H = haemorrhage
a = mottled atrium, adhesion of abdominal cavity - could indicate haemorrhage
b = mottled atrium
N = normal prothrombin time

## 4.9 Germ cell mutagenicity (Mutagenicity)

In vitro:

| Test system Method Guideline | Organism/ strain(s) | Concentrations tested | Result | | Remarks | Reference |
|---|---|---|---|---|---|---|
| | | | + S9 | - S9 | | |
| | | | +/-/+ | +/-/+ | | |
| Bacterial reverse mutation test EPA 84-2a | Salmonella typhimurium strains: TA 98, TA 100, TA 1535, TA 1537, TA 1538 | 2, 10, 50, 250 µg per plate | - ve | - ve | Chlorophacinone did not induce a mutagenic effect.<br>Cytotoxic at 250 µg without S9 for TA 1535; 250 µg with or without S9 for TA 1537, TA 1538, TA 100; 50 and 250 µg without S9 for TA 98 and 50 µg without S9 for TA 1538 | Betbeder-Matibet A., (1981) |
| Bacterial reverse mutation test EPA 84-2 | Salmonella typhimurium strains: TA 98, TA 100, TA 1535, TA 1537<br>Escherichia coli strain: WP2uvrA | Salmonella typhimurium strains: Without S9 mix: 100, 50, 10, 5, 1, and 0.5 µg per plate. With S9 mix: 500, 100, 50, 10, 5, 1 µg per plate (TA 98 an additional dose level of 0.5 µg per plate)<br>Escherichia coli: Without S9 mix: 5000, 1000, 200, 50, 10, and 5 µg per plate. With S9 mix: 5000, 1000, 500, 100, 50, 10 µg per plate. | - ve | - ve | 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.. Chlorophacinone considered negative for mutagenicity.<br>Cytotoxicity seen in range finding study. Strain TA 100 showed cytotoxicity at concentrations of 100 µg/plate (S9+) and 33.3 µg/plate (S9-). WP2uvrA showed cytotoxicity at 100 µg/plate (S9-). No cytoxicity with WP2uvrA was seen up to the highest level tested (3300 µg/plate).. | Lawlor T.E., (1994) |
| Mammalian chromosome aberration test EPA 84-2 | Human lymphocytes | 6.25, 12.5, 25, 50 µg/ml | - ve | - ve | Test material was considered to be non-genotoxic with or without metabolic activation<br>Severe cytotoxicity was seen at the highest concentration. | Stankowski L.F. (1995) |
| Mammalian cell gene mutation test EPA 84-2 | Chinese Hamster Ovary (CHO) | 5, 10, 50, 100, 200 µg/ml | - ve | - ve | Chlorophacinone did not induce mutagenic effects in CHO cells (HGPRT) locus with or without metabolic activation.<br>Cytogenicity observed at 200 µg/ml in both the presence and the absence of S9. | Weill N., (1990) |

In vivo:

| Type of test Method/ Guideline | Species Strain Sex no/group | Frequency of application | Sampling times | Dose levels | Results | Remarks | Reference |
|---|---|---|---|---|---|---|---|
| Bone marrow mutagenicity EPA 84-2 | Mouse CD1 (ICR) 5 male 5 female Additionally, 5 male and 5 female in mid dose group, 15 male and 15 female in highest dose | 3 days consecutively | 24 hours after the last dose | 3.75, 7.5 and 15 mg/kg bw | Test material induced no significant increase in micronucleated PCEs over the levels observed in vehicle controls for males and females. There were deaths 72 hours after first dosing at 15 mg/kg | Chlorophacinone is considered negative in the mouse bone marrow micronucleus test, even at near-lethal levels. | Murli H., (1994) |

### 4.9.1 Non-human information

#### 4.9.1.1 In vitro data

Results for *in vitro* bacterial gene mutation and *in vitro* mammalian cell gene mutation **tests were negative.** The mouse micronucleus test 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 summarized in point 4.1 indicates that faecal elimination (the only significant route of elimination) shows unchanged Chlorophacinone and two hydroxylated metabolites to account for at least 80% of radioactivity. The hydroxylated metabolites can be assumed to have similar toxicity to the parent material, as they closely resemble the parent material. One other metabolite (unidentified) was present at 8.1% of radioacitivity, and other minor metabolites represented 3.4% of radioactivity. Given that none of these metabolites are likely to be more toxic than the active substance (it is reasonable to assume that in research to find suitable candidate molecules for rodenticides, the industry would have chosen the most toxic substances for the active substance), further genotoxicity testing of metabolites is not absolutely required.

However, there are no evidences in the data summarized in the report of chlorophacinone demonstrating if metabolites are or not less toxic than the parent compounds. Therefore, some uncertainty remains with the evaluation of the metabolites.

#### 4.9.1.2 In vivo data

The study *in vivo* bone marrow and *in vitro* tests are negative.

### 4.9.2 Human information

### 4.9.3 Other relevant information

Given these study results and the no evidence of any metabolites of concern in mammals, further genotoxicity testing does not seem to be required.

### 4.9.4 Summary and discussion of mutagenicity

Chlorophacinone, does not fulfill the EU criteria for classification as a mutagenic substance.

### 4.9.5 Comparison with criteria

### 4.9.6 Conclusions on classification and labelling

Chlorophacinone, does not fulfill the EU criteria for classification as a mutagenic substance.

**RAC evaluation of germ cell mutagenicity**

**Summary of the Dossier submitter's proposal**

In *in vitro* genotoxicity studies (Ames test, mammalian chromosome aberration test, and mammalian cell gene mutation test (CHO-HGPRT)), Chlorophacinone did not induce any mutagenic effects, with or without metabolic activation.

In an *in vivo* bone marrow chromosome aberration test in CD1 mice, Chlorophacinone did not induce a significant increase in micronucleated bone marrow polychromatic erythrocytes. It was concluded that no classification for mutagenicity was required according to the CLP Regulation.

However, it was pointed out that the *in vitro* mutagenicity studies were performed on the parent compound, i.e. Chlorophacinone, and not on its metabolites. The applicant suggested that the metabolites can be assumed to have similar toxicity to the parent compound and that the metabolites were not expected to be more toxic than the parent compound (since it could be reasonably assumed that industry chose the most toxic candidate molecules identified during research for a candidate rodenticidal molecule). However, there were no data in the report demonstrating that Chlorophacinone's metabolites are more or less toxic than the parent compound. Therefore, some uncertainty regarding the mutagenic potential of Chlorophacinone's metabolites remains.

**Comments received during public consultation**

No comments were received for this hazard class.

**Assessment and comparison with the classification criteria**

According to the rapporteur member state (RMS) under the Biocidal Products Regulation, the justification stated above for non-testing of metabolites was not valid. Chlorophacinone is extensively metabolised, with only 20% excreted as the parent compound. While only the parent compound and two monohydroxylated metabolites were identified in the excreta in ADME studies (66% of faecal radioactivity), 34% of the remaining faecal radioactivity was considered to relate to other, non-identified metabolites. (Further genotoxicity testing may be considered if tumours are found in rodents, *in vitro* metabolic activation system is not considered optimal, extrahepatic metabolism is expected or if there are human-specific metabolites (Guidance on a strategy for genotoxicity testing of chemical substances, COM 2011).

The highest tissue concentration of Chlorophacinone is found in the liver. After absorption Chlorophacinone enters the enterohepatic circulation and is eliminated mainly through faeces via biliary excretion. Within 48h after oral administration approximately 90% of applied dose was eliminated via faeces, only ≤1% was excreted in urine, and there was no excretion via expired air (ADME studies). Therefore, there are no strong indications of extensive extrahepatic metabolism. On the other hand, a carcinogenicity study has not

been performed for Chlorophacinone, so its tumorigenic potential has not been evaluated. The metabolic S9 system used in *in vitro* studies was prepared from rat liver as recommended by the Test Guideline, so whether it adequately reflects human hepatic metabolism of the substance may be questioned.

Ashby-Tennant structural alerts (Ashby et al., 1991) are not present in the parent compound or in its two main metabolites. Regarding the 34% portion of metabolites with a non-identified structure, it could be speculated that they would be present at very low concentrations at Chlorophacinone doses which do not induce significant toxicity on blood coagulation system.

RAC concludes, therefore, that based on clearly negative results of *in vitro* and *in vivo* genotoxicity studies, no classification for mutagenicity is warranted for Chlorophacinone.

## 4.10 Carcinogenicity

**Table 19: Summary table of relevant carcinogenicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | Species/type of tumors: The closely related molecule warfarin is not carcinogenic to humans. Study on chlorophacinone is not available. Applicant argument for non submission of data was accepted.<br>Lowest dose with tumors: Not appropriate | | |

### 4.10.1 Non-human information

Carcinogenicity and long-term toxicity studies have not been performed.

Chlorophacinone is structurally and functionally similar to the molecule warfarin. The long history of therapeutic use of warfarin gives evidences that warfarin is not carcinogen to human. The repeated doses studies showed no indications of either hyperplasia or hypertrophy at near lethal levels of administration. Chlorophacinone have been proved that is not mutagenic.

#### 4.10.1.1 Carcinogenicity: oral

#### 4.10.1.2 Carcinogenicity: inhalation

#### 4.10.1.3 Carcinogenicity: dermal

### 4.10.2 Human information

### 4.10.3 Other relevant information

### 4.10.4 Summary and discussion of carcinogenicity

Study not required. Long term use of the structurally similar active substance warfarin in humans has shown. Based on the available data, no classification for carcinogenicity for Chlorophacinone is proposed.

### 4.10.5 Comparison with criteria

### 4.10.6 Conclusions on classification and labelling

Based on the available data, no classification for carcinogenicity for Chlorophacinone is proposed.

## RAC evaluation of carcinogenicity

### Summary of the Dossier submitter's proposal

The dossier submitter accepted the applicant's arguments that Chlorophacinone is structurally and functionally similar to warfarin, and that a long history of therapeutic use of warfarin has not provided any evidence of human carcinogenicity. The repeated dose studies showed no indications of hyperplasia or hypertrophy, even at near lethal levels of administration. Chlorophacinone has been shown to not be mutagenic.
Therefore, no classification for carcinogenicity was proposed.

### Comments received during public consultation

No comments were received for this hazard class.

### Assessment and comparison with the classification criteria

In the CAR IIIA document, the applicant presented several arguments for non-submission of a long term toxicity and carcinogenicity study, such as practical difficulties of performing long-term studies (difficulty in obtaining MTD for AVKs; route of AVK administration is problematic - oral gavage is not appropriate for a 2-year study and it is not feasible to accurately prepare homogenous rodent test diets at the very low concentrations needed for the MTD), non-mutagenicity in *in vitro* and *in vivo* tests, no evidence for carcinogenicity in patients treated with warfarin, which is a molecule structurally and functionally similar to Chlorophacinone.

RAC supports the dossier submitter's proposal of no classification for carcinogenicity due to negative results of *in vitro* and *in vivo* mutagenic studies, absence of Ashby-Tennant structural alerts in the parent compound and its two main metabolites, and no indications of hyperplasia or hypertrophy in the repeated-dose studies, even at near lethal doses of Chlorophacinone.

## 4.11 Toxicity for reproduction

### Table 20: Summary table of relevant reproductive toxicity studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Species/ Reproduction target / critical effect: | The closely related molecule warfarin shows no adverse effects on human fertility. Study on chlorophacinone is not available. Applicant argument for non submission of data was accepted. | Lowest relevant reproductive NOAEL / LOAEL: Not appropriate | |
| Species/Developmental target / critical effect (EPA 83-3) | Rat<br>High maternal toxicity (18 of 25) at the highest dose: (72% at 100 µg/kg bw) No mortalities at other doses. Pregnancy rate was high and equivalent across groups. 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 signs of maternal toxicity seems to be related with the mode of action as antiVit-K anticoagulant.<br><br>No indication of developmental toxicity (embryotoxicity /teratogenicity) including in foetus of dams at the higher dose.Rabbit<br>High maternal lethality 81% (13/16) and 100% (16/16) at 25 and 75 µg/kg bw. Pregnancy rate was high and equivalent across groups and there were no treatment-related effects on any gestational parameters<br>All other females at lower doses survived and were pregnant. All pregnant does had one or more live foetuses 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. All sign of maternal toxicity seems to be related with the mode of action as antiVit-K anticoagulant.<br><br>No indication of developmental toxicity (embryotoxicity /teratogenicity) including in foetus of dams at the higher dose. | NOAEL for maternal toxicity of 50 µg/kg bw/day was adopted on the basis of mortality at higher dose (LOAEL 100 µg/kg bw/day)<br>NOAEL for developmental toxicity of 100 µg/kg bw/day<br><br>Number of animals/Sex/Dose:<br>• Rat VAF CD Sprague-Dawley Female 25 per group<br>• 0, 12.5, 25, 50, 100 µg/kg bw per day<br><br>NOAEL for maternal toxicity of 50 µg/kg bw/day was adopted on the basis of mortality<br><br>NOAEL for developmental toxicity of 25 µg/kg bw/day<br><br>Number of animals/Sex/Dose:<br>• Rabbit New Zealand White Female 16 per group<br>• 0, 5, 10, 25, 75 µg/kg bw per day | Tyl R.W., Marr M.C., Myers C.B., (1994a)<br><br>Tyl R.W., Marr M.C., Myers C.B., (1994b) |

This MS is not proposing classification of chlorophacinone for reproduction (neither fertility nor development). Therefore, in principle, detail information of studies about reproduction were not required in this Annex XV. However it was considered necessary to include and discuss the available information about teratogenicity for chlorophacinone and compare with existing information on warfarin, due to the previous history of the proposal for R61 for all anti-vitamin K rodenticides by read across from the human embryotoxicity of warfarin.

### 4.11.1 Effects on fertility

#### 4.11.1.1 Non-human information

#### 4.11.1.2 Human information

### 4.11.2 Developmental toxicity

Teratogenicity studies show no teratogenic effect. Embryogenesis period not tested. Chlorophacinone administered orally during major organogenesis in rats and in rabbits gave no indication of developmental toxicity at all evaluated tested dosed, including the highest doses which had enough surviving pregnant animals for evaluation of embryotoxicity and teratogenicity (100 µg/kg/day in rats and 25 µg/kg/day).

*Study in Rats*

- *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 100µg/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.

- *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.

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.

*Study in Rabbits*

Chlorophacinone was tested to produce maternal and developmental toxicity (including teratogenicity) in rabbits by gavage once daily, on gestational days 7 through 19 at doses 0, 5, 10, 25, 75µg/kg/day.

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

- *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 25µg/kg/day, 13 out of 16 does died (81%). All other females at lower doses survived and were pregnant. All pregnant does had one or more live foetuses 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 5µg/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 evaluated doses (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 foetuses per litter, sex ratio or foetal body weight per litter, when calculated as all foetuses, 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 foetal external variations observed. Foetal visceral and skeletal variations were equally distributed across groups

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 for developmental toxicity of 25 µg/kg bw/day is adopted.

#### 4.11.2.1 Non-human information

#### 4.11.2.2 Human information

### 4.11.3 Other relevant information

### 4.11.4 Summary and discussion of reproductive toxicity

For maternal toxicity a NOAEL of 10 µg/kg bw/day on the basis of mortality in rabbit was adopted. Clinical of toxicity and necropsy pathology demonstrated that mortality in rats and rabbits was due to internal haemorrhage caused by the anticoagulant properties of the substance. Treatment-related clinical observations were limited to does causing mortality prior to death. There were no treatment-related clinical signs of toxicity at lower doses. At scheduled necropsy, there were no treatment-related findings in surviving pregnant animals.

No developmental effects were noted at any tested evaluated dose in two studies in rats and rabbits. 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 rabbit , 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 observed but no significant effect were detected in the foetus of the surviving does.

So it is concluded that no developmental effect was observed at the highest possible dose with surviving pregnant animals in experimental guideline tests. Strictly NOAEL for developmental toxicity cannot be established. For a practical point of view for further risk characterization, a NOAEL for developmental toxicity of 25 µg/kg bw/day is adopted on the basis of no embryotoxic and teratogenic effect at the highest evaluated dose in the teratogenicity study in rabbit.

It is a matter of discussion if the standard teratogenicity test is appropriate for anticoagulant rodenticides, in particular when data is intended to be used to deduce no classification for reproduction-development as the embryogenesis period is not tested and no study of two generation reproduction may be tested. Classification of all anticoagulant rodenticides from read across from warfarin has been suggested on the basic of the teratogenic/embryotoxicity properties of warfarin in human pregnancy, when administered as a pharmaceutical. Non-guideline non-GLP studies with warfarin in animals have shown equivocal effects, this circumstance, together with the absence of adverse findings in the rat and rabbit teratogenicity studies with other AVKs lead the TC C&L to conclude that a study according to the OECD 414 guideline was not capable of detecting effects with AVKs, concluding that it was appropriate to read across from the known human effects of

warfarin to other AVKs, and classify all of them as Cat 1 or Cat 2 R61. However, the new OECD 414 study available for warfarin sodium affects the reasons for classification of chlorophacinone.

The study carried out with warfarin sodium shows a definitive increase in incidence of subcutaneous and internal foetal haemorrhage (not seen in studies with chlorophacinone), foetal ocular effects and some indications of reduced ossification in skull bone at higher dose levels (see point 6). This study is relevant but not essential for the classification of warfarin, because human data supporting classification are available. However, it is relevant for chlorophacinone as it demonstrates that the test is able to show the possible teratogenicity of AVK. On the other hand, the different results in the test between warfarin and chlorophacinone with regard to foetal haemorrhages could indicate a difference in foetal availability. Therefore, some differences in relation to the developmental effects may be expected between warfarin and chlorophacinone.

The adverse finding detected in a standard OECD 414 study for warfarin sodium, would validate the negative findings in a similar study with chlorophacinone. Therefore, the phrase R61 is not warranted. Nevertheless, taking into account the comparable chemical structure and toxicological activity of warfarin and chlorophacinone, it may be possible to think that chlorophacinone could induce the effects observed with warfarin if chlorophacinone is present at or above a certain level in the foetus. However, this would be unlikely because the highest concentration recorded in the test was 100 µg/kg bw per day and this caused 72% mortality without any significant observed effects in foetus on the surviving dams. Similar situation is confirmed in rabbit study with 81 and 100 % mortality at 25 and 75 µg/kg bw per day and clinical signs of external and internal bleeding related with it mode of action as antiVitK anticoagulant effects.

Chlorophacinone causes in adults similar toxic effects with haemorrhages by the same mode of action (antivit K) as other related rodenticides including warfarin and similar maternal toxicity in the teratogenicity test. However at doses causing high maternal toxicity and lethality no haemorrhages and no cataracts are observed in foetus in contrast with the effects by warfarin

The different results in the test between warfarin and chlorophacinone with regard to foetal haemorrhages suggest a clear difference in foetal availability. Therefore, differences in relation to the developmental effects may be expected.

The main argument for classifying all AVK rodenticides as warfarin is based on the common mode of action.

We agree that chlorophacinone has the same mode of action but just this justify that it cannot be classified because with this mode of action it cannot cause effect at non-lethal dose. No anticoagulant level of the compound seems to be available in the foetus at doses that are causing very severe and lethal effects in the mother thorough the same mode of action. Therefore the prediction is that embryotoxic developmental effects are not possible to be caused by chlorophacinone at any non-lethal maternal dose.

Therefore classification as for warfarin is not possible.

The only uncertainty is due to the lack of studies comparing levels of the substance in the foetus with the mother blood. No different clotting mechanism is expected in the foetus. Therefore, it seems obvious that availability is much lower in the foetus as no blooding is observed at doses causing severe maternal haemorrhages. Moreover there are no reasons to believe that the availability of chlorophacinone might be higher to human foetus than in rats or rabbits. Therefore, that uncertainty is doubtful to justify classification including for the lowest level of Cat 2, and clearly not supporting Cat 1B or 1A

### 4.11.5 Comparison with criteria

### 4.11.6 Conclusions on classification and labelling

Based on the available data, **no classification** for fertility neither for developmental toxicity for Chlorophacinone seems to be warranted.

**RAC evaluation of reproductive toxicity**

**Summary of the Dossier submitter's proposal**

Effects on sexual function and fertility were not addressed by the dossier submitter in the CLH dossier. Therefore, RAC did not assess this endpoint.

**Developmental toxicity**

In the dossier submitter's Annex XV Intention sent in March 2009, classification for developmental toxicity in category 2 according to Dir. 67/548/EEC (with the risk phrase for developmental toxicity: R61) was included, based on a read-across approach for anticoagulant rodenticides.

In the meantime, a new OECD TG 414 –compliant study with warfarin became available (Kubaszky 2009), in which developmental effects were observed in rats, including an increased incidence of subcutaneous and internal foetal haemorrhage, foetal ocular effects and some indications of reduced ossification of skull bones at higher dose levels. Such effects were not observed after exposure to Chlorophacinone in teratogenicity studies of similar design. The dossier submitter considered that the adverse findings detected in the standard OECD 414 study for warfarin validated the negative findings in a similar study with Chlorophacinone and, therefore, proposes no classification for reproductive toxicity of Chlorophacinone.

Developmental toxicity of chlorophacinone was evaluated in two teratogenicity studies (consistent with EPA 83-3), one in the rat and the second one in the rabbit.

**Rat developmental toxicity study** (Tyl et al. 1994a)
Chlorophacinone was administered orally (by gavage) during the period of major organogenesis (gestational days 6 through 15) to 25 Sprague-Dawley female rats per dose, at doses of 0.0, 12.5, 25.0, 50.0 and 100.0 µg/kg/day.

Maternal effects
Mortality (72%) and clinical and pathological signs of haemorrhage were limited to animals dosed at 100 µg/kg/day. All other females were pregnant and survived, and showed no signs of treatment-related toxicity. Maternal body weight, body weight gain, food consumption, gravid uterine weight and absolute and relative liver weights showed no treatment-related changes.

Chlorophacinone treatment did not influence pregnancy rate, the number of corpora lutea, implantations, resorptions, live and dead foetuses per litter, foetal weight or foetal sex ratio. There were no abortions or early deliveries. 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.

Developmental effects
No indication of developmental toxicity, including teratogenicity, up to the highest dose (100 µg/kg/day) was noted.

**Rabbit developmental toxicity study** (Tyl et al. 1994b)
Chlorophacinone was administered orally (by gavage) during the period of major organogenesis (gestational days 7 through 19) to New Zealand white rabbits at doses of 0, 5, 10, 25 or 75 µg/kg bw/day (16 animals per dose).

Maternal effects
Mortality was 100% at 75 µg/kg/day and 81% at 25 µg/kg/day (established as the LOAEL), all attributable to haemorrhage. All other females at lower doses were pregnant, survived, and had no treatment-related clinical signs or pathological signs at necropsy.
Although maternal body weights and body weight gains were similar across all groups, a significant dose-related downward trend was observed (with no significant differences noted in pairwise comparisons to the control group). Maternal food consumption was significantly reduced at 75 µg/kg/day.
Maternal gravid uterine weights and liver weights were statistically and biologically equivalent across all groups.
Pregnancy rate was high and similar in all groups (93.3-100.0%), and there were no significant effects of treatment on gestational parameters.

Developmental effects
There were no indications of developmental toxicity, including teratogenicity, up to the highest dose that could be evaluated (25 µg/kg bw/day). There were no treatment-related changes in the incidence of individual or pooled external, visceral, skeletal or total malformations or variations.

No classification of Chlorophacinone for reproduction (developmental toxicity) was proposed in the CLH dossier.

## Comments received during public consultation

Three industry organisations supported no classification for reproductive toxicity with the justification that a new OECD TG 414 –compliant study (Kubaszky 2009) with warfarin showed foetotoxicity and adequate evidence of teratogenicity according to them, while animal studies with Chlorophacinone were negative regarding developmental effects.
Five Member States opposed the proposal for no classification, and instead proposed Category 1A (H360D) due to the same MoA operating as with warfarin and the other AVKs, the inability of new and old OECD 414-compliant studies with warfarin to detect bone malformations, and insufficient differences in placental transfer and toxic potency in mammals among AVKs in order to exclude the possibility of developmental effects, as seen with warfarin in humans. Furthermore, the absence of reported bleeding in the foetuses treated with Chlorophacinone (and the other six AVK inhibitors; in contrast to the foetal bleedings seen in the Kubaszky study with warfarin) could be due to a very narrow margin between the foetal effect dose and maternal lethal dose.

## Additional key elements

In the open literature, a non-guideline study on developmental effects of sublethal doses of Bromadiolone and Chlorophacinone was found (Rady et al., 2013). In the study, Chlorophacinone was orally applied to 10 pregnant dams per group on the 3$^{rd}$ gestational day at a dose level of 1/4 (1.578 mg/kg bw) and 1/10 (0.631 mg/kg bw) of the acute oral $LD_{50}$ (purity of the test substance not stated). An unspecified number of dams (probably 3-4 per time point) were sacrificed on the 9$^{th}$ and 18$^{th}$ day of gestation, and the others were allowed to deliver spontaneously. In the dams, a dose-dependent increase in liver enzymes, bilirubin, creatinine, urea and cholesterol, and histopathological changes in liver (degenerative changes at lower dose and degenerative changes and inflammation at higher dose) and kidneys (oedema, inflammation, swelling of the glomerular lining endothelia, proliferation) were observed, indicating maternal toxicity, although presumably not to the level of mortality. Developmental effects included generalised oedema and haemorrhage in

foetuses and a dose-dependent prolongation of the gestational period, a decreased number of foetuses and newborn rats, decreased foetal body weight, prolonged time to eye opening and fur coating, and shortened survival time after birth. The majority of these effects were statistically significant (Fisher's Least Significant Difference (LSD) test).
Since the study did not follow OECD or other international guidelines, the active substance used was of unknown purity, and since the study is poorly reported (e.g. number of dams at each time point is not stated, number of pups is unknown), it can be used only as supportive evidence indicating that the offspring can be more severely affected than the dams after a single administration of Chlorophacinone during gestation at maternally sublethal doses.

## Assessment and comparison with the classification criteria

**Foetal haemorrhage**
In teratogenicity studies with Chlorophacinone in the rat (Tyl et al. 1994a) and rabbit (Tyl et al. 1994b), performed in accordance with the OECD 414 Guideline, blood in vagina and amniotic sacs were described at the highest doses tested (100 µg/kg bw/day in the rat study, 75 and 25 µg/kg bw/day in the rabbit study). Placental and foetal haemorrhages were not noted, either in controls or exposed groups. In the Kubaszky (2009) study with warfarin, which was specifically focused on revealing potential anticoagulant effects on foetuses and in which a dose-related increase in foetal haemorrhage was found, a 2% incidence of foetal haemorrhage was observed in the control group as well. In teratogenicity studies with other AVKs, no foetal haemorrhage in control animals was reported.

In addition, in a preliminary rat teratogenicity study with Chlorophacinone on the same rat strain and in the same laboratory, it was shown that the prothrombin time (PT) was prolonged by only 0.4 s (3.3%) and 0.8 s (6.6%) at doses of 25 and 50 µg/kg bw/day, respectively (please see Table 20 under "Supplemental information - In depth analyses by RAC" in the Background Document). In a preliminary study in pregnant rabbits, no effect on PT was found at doses below 10 µg/kg/day, at which the PT was increased 1.4 –fold relative to the control value (Table 22 in "Supplemental information - In depth analyses by RAC" in the Background Document).

In an intraperitoneal study (Kronick et al. 1974) with warfarin in mice exposed during various stages of pregnancy, a very high incidence of haemorrhaged placentae and foetal death was observed in animals treated with 2 and 4 mg/kg bw/day, with PT prolonged by 3.5-5 –fold relative to the controls. In animals treated with 1 mg/kg bw/day, a dose at which no significant increase in the PT was observed, there was no evidence of haemorrhaged placentae or foetal deaths.

These results are consistent with the conclusion that the dose causing foetal toxicity in rodents is close to the dose inducing significant maternal toxicity. Therefore the steep dose-response curve, especially in the case of rodenticides with higher anticoagulant potency compared to warfarin, makes foetal toxicity difficult to evaluate in a standard OECD 414 study. Dose spacing could be an important factor in targeting a narrow range within which AVKs could exert their potential developmental toxicity without inducing significant maternal morbidity and mortality. In the Kubaszky study (2009) with warfarin, the dose increment was 1.2 to 1.3 fold (0.125, 0.150, 0.200 and 0.250 mg/kg bw/day). In teratogenicity studies with Chlorophacinone, a wider dose spacing was applied, with dose increment of 2 fold in the rat study (12.5, 25, 50 and 100 µg/kg bw/day), and 2 to 3 fold in the rabbit study (5, 10, 25 and 75 µg/kg bw/day).

**Skeletal changes**
Skeletal malformations had very low incidences in the rat (0.5 to 1%) and the rabbit study

(one affected foetus in the control group) with Chlorophacinone. Skeletal variations did not show a dose response relationship, but one type of skeletal variations (e.g. incidence of reduced and incomplete ossification) was not reported at different dose levels.

Skeletal malformations (nasal hypoplasia, stippled epiphyses, growth retardation) are the most often reported malformations in neonates of mothers on coumarin therapy during gestation. However, in animal studies with warfarin that generally followed the OECD 414 design, clear effects on foetal bone and cartilage were not routinely observed:

- in the study of Mirkova and Antov in rats (1983) increased incidences of structural malformations of the rear limbs (*pes varus*) and delayed ossification of the parietal skull bones was observed;

- in the Feteih et al. (1990) studies in rats, no external structural malformations were reported and there was no significant increase in the number of ossification centres at a dose that induced 43% maternal mortality. Histological analysis of tibial growth showed widened hypertrophic zones, increased calcification of these zones and disorganisation of the hypertrophic chondrocytes, suggesting growth plate abnormalities in warfarin treated foetuses. These morphologic defects were associated with biochemical effects in bones (decreased y-carboxyglutamic acid and osteocalcin levels);

- in the first part of the Kronick et al. (1974) study in mice, which generally followed the OECD 414 guideline, no increase in the frequency of malformations was noted, possibly due to high foetal death rates;

- In the second, OECD TG 414 non-compliant part of the Kronick et al. (1974) study in mice (single daily i.p. injections of warfarin at 4 mg/kg were applied on various gestational days), co-administration of 8 mg/kg vitamin K together with 4 mg/kg warfarin on gestational day 10 (a protocol designed to prevent warfarin-induced foetal deaths) induced slight increase in incidence of gross foetal malformations (cleft lip and/or cleft palate);

- in the OECD 414 study with warfarin in rats (Kubazsky, 2009), skeletal malformations were also not a prominent teratogenic feature. Only one litter at mid-dose (0.150 mg/kg bw/day) had foetuses showing facial skeletal malformations (malformed skulls with wide nasal and/or frontal bone/cartilage), unossified nasal bone, malformed vertebra and malformed sternum.

**Foetal ocular effects**
In warfarin exposed foetuses in the Kubaszky study (2009), yellowish discolouration of the lens was observed, which was shown to be a central cataract which is an extremely rare malformation in rats. Ocular effects of this type were not observed in teratogenicity studies with Chlorophacinone, but neither were they seen in other studies with warfarin.

**Visceral malformations**
A dose-related increase in the foetal incidence of hydroureter was observed in the Chlorophacinone study in rats (Table 2 and Table 21), but was not considered by RAC to be a toxicologically relevant effect (please see the justification in "Supplemental information - In depth analyses by RAC", in the Background Document).

**Foetal toxicity**
Foetal toxicity was found in the Kubaszky (2009) warfarin study in one subgroup, at a maternally toxic dose (8% mortality). In the teratogenicity studies with Chlorophacinone presented in the CLH dossier, foetal toxicity was not observed. However, although poorly reported, the study of Rady et al. (2013) indicated that *in utero* Chlorophacinone exposure

could lead to foetotoxic effects in rats even at a dose levels that do not induce mortality in mothers.

**Overall conclusion on classification for developmental toxicity**

Based on the known developmental toxicity of the AVK rodenticide Warfarin in humans (Repr. Cat 1A), the reproductive toxicity of Chlorophacinone has been analysed in detail. It is acknowledged that the animal developmental toxicity studies with Warfarin are weakly positive and that the animal developmental toxicity studies with Chlorophacinone are negative. However, in comparison with Warfarin, Chlorophacinone and other 2nd generation AVKs have higher acute and repeated dose toxicity, steeper dose-response curves, and much longer half-lives in the exposed organisms, making the evaluation of developmental effects of all 2nd generation AVK rodenticides difficult. Thus, repeated exposure to relatively low doses during gestation lead to maternal toxicity and lethality which hinders the detection of developmental toxicity at higher doses.

As there were no data available on the outcome of maternal exposure to Chlorophacinone in humans, classification as Repr.1A was not considered to be applicable for Chlorophacinone.

Based on the assumption that all AVK rodenticides, including Warfarin and other anticoagulant coumarin-based pharmaceuticals (see below) share the same MoA, namely inhibition of vitamin K epoxide reductase (VKOR), the assessment of Chlorophacinone includes consideration of the total database for the AVKs. A weight of evidence assessment resulted in the conclusion that Chlorophacinone has the capacity to adversely affect human development *in utero*. Therefore, classification as Repr. 1B is proposed with the reasoning given below.

The reasons for this conclusion are:

- Chlorophacinone shares the same MoA as expressed by other anticoagulant AVK rodenticides and coumarin-based pharmaceuticals (inhibition of vitamin K epoxide reductase, an enzyme involved with blood coagulation and foetal tissues development, including bone formation, CNS development and angiogenesis)
- Warfarin and 2 other coumarin pharmaceuticals (acenocoumarol, phenprocoumon) have been shown to cause developmental toxicity in humans.
- One of the 2nd generation AVK rodenticides (Brodifacoum) has been shown to cause foetal effects in humans, possibly after one or a few exposures.
- For AVK rodenticides with a long half-life in the body, even single exposures might suffice to trigger developmental effects. However, such studies are normally not conducted and effects of single dose exposure cannot be detected in a standard OECD 414 test where instead the repeated exposure may lead to maternal mortality with steep dose-response.
- The standard animal studies do not pick up all developmental toxicity effects of the AVK rodenticides, most notably the face and CNS malformations that are characteristic for Warfarin and other AVK coumarin pharmaceuticals.
- The most sensitive window for face malformations in humans is the first trimester. Thus, even if some AVK rodenticides may have a lower degree of placental transfer than Warfarin, this will not affect the face malformation hazard.

Not all steps of the MoA in the target tissues liver and bone have been proven, thus introducing some uncertainty in the assessment. However, RAC is of the opinion that the uncertainty is not sufficient to warrant a Repr. 2 classification.

Reliable evidence of an adverse effect on reproduction in humans, which is required for Repro 1A, was not available for Chlorophacinone, but a potential for human developmental toxicity is presumed based on the weight of evidence assessment above, and RAC thus

proposes classification as **Repr. 1B**, i.e. "presumed human reproductive toxicant".

**Specific concentration limits (SCLs):**
Regarding a SCL for Chlorophacinone, it is acknowledged that the specific data on developmental toxicity of Chlorophacinone are too scarce to guide the setting of the SCL.

Classification as Repr. 1B for developmental toxicity for Chlorophacinone is supported by the RAC. However, only for Warfarin is there sufficient data to set an SCL for developmental toxicity. Thus, based on human data, doses of 2.5-5 mg/person/day (equivalent to 0.04-0.08 mg/kg/day) may cause developmental toxicity and could be regarded as an ED10 level. This human ED10 value would, if using the guidance for setting SCLs based on animal data, belong to the high potency group (<4 mg/kg/day). The CLP guidance states that for an ED10 <4 mg/kg/day, the SCL is 0.03%, and for an ED10 below 0.4 mg/kg/day the SCL becomes 0.003%. Also if starting from an ED10 value obtained from animal studies (0.125 mg/kg/day; Kubaszky et al 2009), it would qualify Warfarin for the high potency group and result in a SCL of 0.003%. Thus, the RAC concluded on a SCL on 0.003% for the developmental toxicity of Warfarin.

As the other AVK rodenticides are equally or more toxic than Warfarin, it is not considered appropriate to apply the generic concentration limit for these substances (0.3%), but rather to base the SCLs on the SCL proposed for Warfarin. Thus, the RAC is of the opinion that the SCL for Warfarin can be used as a surrogate SCL for the other AVK rodenticides, resulting in an **SCL of 0.003%** for all 7 AVK rodenticides evaluated at this time, including Chlorophacinone.

## Supplemental information - In depth analyses by RAC

**Rat developmental toxicity study** (Tyl *et al.* 1994a)

The study was performed in accordance with the US EPA Guideline 83-3, equivalent to OECD 414. The experimental work was done from September 1993 to March 1994.

Table 2. Maternal and developmental effects in Sprague-Dawley rats

| | **Control data** | | **Chlorophacinone (µg/kg bw/day)** | | | |
|---|---|---|---|---|---|---|
| | Historical | Study | **12.5** | **25.0** | **50.0** | **100.0** |
| **Maternal effects** | | | | | | |
| N of dams | 29 | 25 | 25 | 25 | 25 | 25 |
| Clinical signs | no data | - | - | reddish colour of urine | alopecia at limbs | related to bleeding |
| Mortality | 0 | 0 | 0 | 0 | 0 | 72% |
| Body weight gain (g) | | | | | | |
| before treatment: | | | | | | |
| 0-6 day | no data | 45.5 | 46.0 | 42.3 | 41.2 | 38.8 |
| during treatment: | | | | | | |
| 6-15 day | no data | 52.7 | 52.4 | 56.0 | 56.2 | 54.2 |
| 15-20 day | no data | 77.2 | 73.1 | 81.8 | 78.3 | 78.8 |
| total weight gain | 164.1 | 175.4 | 171.5 | 180.1 | 175.7 | 171.8 |
| Food consumption (g/day) | no data | 75.8 | 76.2 | 78.4 | 77.7 | 77.0 |
| Necropsy | no data | NR | NR | NR | NR | H |
| Pregnancies | no data | 25/25 | 24/25 | 25/25 | 25/25 | 25/25 |
| Abortions | 0 | 0 | 0 | 0 | 0 | 0 |

| **Developmental effects** | | | | | | |
|---|---|---|---|---|---|---|
| N of litters | 29 | 25 | 24 | 25 | 24[a] | 7[b] |
| N of foetuses/litter | | | | | | |
| live | 15.3 | 16.2 | 15.5 | 16.4 | 16.5 | 15.7 |
| dead | 3.6 | 0 | 0 | 0 | 0 | 0 |
| Mean foetal wt (g) | 3.642 | 3.579 | 3.541 | 3.631 | 3.604 | 3.658 |
| N of foetuses with variations or malformations/examined (%): | | | | | | |
| external malformations | 0 | 0/406 | 0/373 | 1/410 (0.2) | 0/395 | 0/110 |
| external variations | no data | 0/406 | 0/373 | 1/410 (0.2) | 0/395 | 0/110 |
| skeletal malformations | 0 | 1/201 (0.5) | 1/187 (0.5) | 2/204 (1) | 0/199 | 0/55 |
| skeletal variations | no data | 19/201 (9.5) | 21/197 (11.2) | 18/204 (8.8) | 25/199 (12.7) | 1/55 (1.8) |
| visceral malformations (hydroureter) | 21.6% foetuses | 6/205 (2.9) | 12/186 (6.5) | 26/206 (12.6)* | 27/196 (13.8)* | 13/55 (23.6)* |
| visceral variations | no data | 149/205 (72.8) | 144/186 (77.4) | 137/206 (66.5) | 138/196 (70.4) | 55/55 (100) |

NR = not remarkable
H = haemorrhage
[a] one litter was fully resorbed
[b] 18 pregnant rats died at this dose
*Significantly different from controls (Pearson's $\chi^2$ of Fisher's test, $P<0.05$); analysed by RAC

The total weight gain in study animals is calculated by summing the average weight gains during three periods (0-6, 6-15, 15-20 days) stated in the CAR IIIA document.
The number of foetuses with malformations or variations is calculated from percentages and number of examined animals stated in the CAR IIIA document.

***Maternal effects***
If reddish colour of the urine in one animal at a dose of 25 µg/kg bw/day is not considered substance-related (since no other relevant clinical signs were observed at this dose and at the higher dose of 50 µg/kg bw/day), maternal effects only occurred at the highest dose (100 µg/kg bw/day). At this dose, 72% mortality was observed, due to haemorrhage. At the lower doses no substance-related effects were noted, but necropsy was performed only on dams that died before the end of the study (highest dose) (Table 2).

Based on available results, RAC proposes an LOAEL of 0.100 mg/kg bw/day and NOAEL of 0.050 mg/kg bw/day for maternal toxicity in the rat study.

**Table 20 - Prothrombin (PT) and Activated Partial Thromboplastin Times (APTT) in a Preliminary Rat Developmental Toxicity Study**

| | Dose Level (µg/kg/day) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 5 | 25 | 50 |
| Prothrombin Time (sec)[a] | 12.2 ± 0.6 N=4[b] | 12.9 ± 1.3 N=2[b] | 12.8 ± 0.3 N=4[b] | 12.6 ± 0.4 N=3[b] | 13.0 ± 0.2 N=5 |
| Activated Partial Thromboplastin Time (sec)[a] | 15.5±2.1 | 23.9±12.4 | 16.1±1.4 | 16.2±1.3 | 17.0±0.9 |

[a]Reported as the mean ± S.E.M.
[b]decrease in N is due to the clotting of some of the samples on which the analysis could not be done.

In a preliminary rat developmental toxicity study on the same rat strain and in the same laboratory as the main study, it was shown that the prothrombin time was prolonged with only 0.4 s (3.3%) and 0.8 s (6.6%) at doses of 25 and 50 µg/kg bw/day, respectively. Activated partial thromboplastin time (APTT) did not show a clear dose response.

These data were not presented in the CLH dosser or in the CAR IIIA document, but were found during open literature search in Reregistration Eligibility Decision (RED) - Rodenticide Cluster (US EPA Document, July 1998; www.epa.gov/oppsrrd1/REDs/2100red.pdf page 32).

***Developmental effects***

Malformations and variations
Regarding external and skeletal malformations and variations, no *significant* effect of Chlorophacinone treatment was observed.
The type of external malformations, external variations and skeletal malformations was not stated in available documents.
Skeletal variations included 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.
Visceral malformations included mainly hydroureter.
Visceral variations included distended ureter and enlarged lateral cerebral ventricles.

Regarding **visceral malformations**, the dossier submitter reports a statistically significant increasing dose-related trend in all foetuses and in males and females separately, although no pairwise comparison to the control group was significant. In the CAR IIIA document it is stated that "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." 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 resulted in profound maternal mortality" (CAR IIIA document). The applicant concluded that there is no indication of developmental toxicity including teratogenicity and proposed the NOAEL for developmental toxicity greater than 100.0 µg/kg/day.

**Rabbit developmental toxicity study** (Tyl *et al*, 1994b)

The study was performed in accordance with the US EPA Guideline 83-3, equivalent to OECD 414. The experimental work was done from September 1993 till April 1994.

Table 3. Maternal and developmental effects in New Zealand white rabbits

| | Control data | | Chlorophacinone (µg/kg bw/day) | | | |
|---|---|---|---|---|---|---|
| | Historical | Study | **5** | **10** | **25** | **75** |
| **Maternal effects** | | | | | | |
| N of does | 50 | 16 | 16 | 16 | 16 | 16 |
| Clinical signs related to haemorrhage | no data | - | - | - | + | + |
| Mortality | 0 | 0 | 0 | 0 | 81% | 100% |
| Body weight gain (g) | | | | | | |
| 7-19 day | | 81 | 165 | 153 | 110 | 191 |
| 19-30 day | | 193 | 168 | 199 | 189 | |
| 3-30 day (0-30 for historical control) | 658 | 526 | 599 | 589 | 472 | |
| Food consumption (g/day) | no data | 158.9 | 169.2 | 165.6 | 151.2 | - |
| Necropsy | no data | NR | NR | NR | H | H |
| Pregnancies on GD 30 | no data | 16/16 | 14/16 | 16/16 | 2/16 | - |
| Abortions | 0 | 0 | 1/16[a] | 0 | 1/16[b] | 0 |
| **Developmental effects** | | | | | | |
| N of litters | 50 | 16 | 14 | 16 | 2 | - |
| N of foetuses/litter | | | | | | |
| live | 7.67 | 8.44 | 8.21 | 7.81 | 8.00 | |
| dead | 0 | 0.06 | 0.14 | 0.13 | 0 | |
| Mean foetal wt (g) | 53.3 | 48.0 | 49.7 | 50.0 | 50.6 | |
| N of examined foetuses | | 135 | 115 | 125 | 16 | - |
| % of foetuses with variations or malformations: | | | | | | |
| external malformations | 0.27 | 0.74 | 0 | 0.80 | 0 | |
| external variations | 0.27 | 0 | 0 | 0 | 0 | |
| skeletal malformations | 1.86 | 0.74 | 0 | 0 | 0 | |
| skeletal variations | 36.44 | 43.0 | 57.4 | 45.6 | 100 | |
| visceral malformations | 0.53 | 0.74 | 1.74 | 0 | 0 | |
| visceral variations | 9.04 | 14.1 | 14.8 | 13.6 | 18.8 | |

NR = not remarkable
H = haemorrhage
[a] = early delivery on GD 29
[b] = aborted on GD 23

***Maternal effects***

A high mortality (≥81%) of does occurred at the doses of 25 and 75 µg/kg bw/day. All mortalities occurred before GD 24 at 75 µg/kg bw/day, and before GD 22 at 25 µg/kg/day. At the dose of 75 µg/kg bw/day 100% mortality prevented further developmental evaluation, and at 25 µg/kg bw/day only 2 litters survived, which reduced the adequacy of the evaluation of findings at this dose level.

There was no effect of Chlorophacinone on body weight gain, food consumption, clinical observations or necropsy findings below 25 µg/kg bw/day.
In the CAR IIIA document it is stated that in a range-finding study in pregnant rabbits no effects on PT and activated partial thromboplastin time (APTT) were found at doses of 1, 2 or 5 µg/kg/day. At 10 µg/kg/day both parameters were significantly increased (PT 1.4 times and APTT 2 times the control value).

The values are not numerically presented in the CLH dossier or in the CAR IIIA document, but are stated in RED - Rodenticide Cluster (US EPA Document, July 1998; www.epa.gov/oppsrrd1/REDs/2100red.pdf) on page 33:

**Table 22 - Prothrombin (PT) and Activated Partial Thromboplastin Times (APTT) in a Preliminary Rabbit Developmental Toxicity Study** Chlorophacinone (µg/kg/day)

| | 0 | 1 | 2 | 5 | 10 |
|---|---|---|---|---|---|
| No. of female rabbits bled | 3 | 3 | 3 | 3 | 3 |
| Prothrombin Time (sec)[a] | 8.1 ± 0.5 | 7.8 ± 0.2 | 7.9 ± 0.1 | 8.7 ± 0.6 | 11.6 ± 2.1 |
| Activated Partial thromboplastin Time (sec)[a] | 26.5 ± 5.7* | 26.6 ± 3.7 | 23.2 ± 1.5 | 26.4± 4.9 | 53.0 ±14.3 |

[a]Reported as the mean ± S.E.M.
*$p < 0.05$; Jonckheere's Test (significant by trend test)
Table from page 167 of MRID 43570801.

Based on these results, RAC proposes a LOAEL of 0.010 mg/kg bw/day (based on increased PT and APTT in pregnant rabbits in preliminary study) and a NOAEL of 0.005 mg/kg bw/day for maternal toxicity in the rabbit study.

***Developmental effects***

Placental and foetal haemorrhage
Placental and foetal haemorrhages were not stated. At 75 and 25 µg/kg bw/day blood in vagina, uterus and amniotic sacs was found.

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 foetuses: one at 0 µg/kg/day - agnathia and aglossia, and one at 10 µg/kg/day - exophthalmia.

**External variations** were not observed.

A **skeletal malformation** was found in one foetus at 0 µg/kg/day - fused sternebrae.

**Skeletal variations** were equally distributed across groups and included predominantly an extra (13th) rib, and/or an extra sternebral ossification site, floating extra rib and/or bipartite center in thoracic centrum.

**Visceral malformations** were observed in three foetuses, one at 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.

**Visceral 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 the gall bladder.

The RAC supports the dossier submitter's conclusion that no real developmental LOAEL could be established for the rabbit teratogenicity study with Chlorophacinone.

### 4.12 Other effects

#### 4.12.1 Non-human information

##### 4.12.1.1 Neurotoxicity

The closely-related active substance Difethialone was investigated, in various screening tests (Refer to the study summaries (Doc. IIIA 6.9-01) in the draft CA- report on Chlorophacinone), for potential pharmacological activity other than its known anticoagulant properties. Specifically, the following endpoints were investigated: antianginal activity *in vivo* or *in vitro*; antihypertensive activity; sedative activity; anticonvulsant activity; antidepressant activity; antispasmodic activity in a variety of *in vitro* tests; analgesic, anti-inflammatory or gastric antiacid activity. The absence of sedative activity, anticonvulsant activity, antidepressant activity and the absence of any clinical signs in rodent and dog toxicity tests with Chlorophacinone also support the conclusion that there are not evidences that Chlorophacinone could show specific neurotoxic effects.

##### 4.12.1.2 Immunotoxicity

##### 4.12.1.3 Specific investigations: other studies

##### 4.12.1.4 Human information

There are no published data on specific cases of Chlorophacinone intoxication, and no case reports from the manufacturer concerning adverse effects in users applying the products. The closely-related active substance warfarin has been in use for over forty years as an anticoagulant drug in human medicine. Its use is described in more detail in 3, but in summary it has been used in millions of patients with clotting disorders, heart disease, atrial valve replacement, and more recently, deep vein thrombosis. Use is life-long for most patients with heart disease, clotting disorders or valve replacement. There have been no reports of any increase in tumors incidence or of any adverse effects on human fertility. There have been no reports of neurotoxic or neurodegenerative disease, or neuromuscular disease associated with the use of warfarin. Use during pregnancy is contraindicated.

#### 4.12.2 Summary and discussion

#### 4.12.3 Comparison with criteria

#### 4.12.4 Conclusions on classification and labelling

## 5 ENVIRONMENTAL HAZARD ASSESSMENT

A detailed summary of the available studies has been reviewed under the Biocidal Products Directive (98/8/EC), see Document IIA attached to the technical dossier. The key information pertinent to determining a classification position is presented below.

## 5.1 Degradation

**Table 21: Summary of relevant information on degradation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Hydrolysis<br>OECD 111<br>EPA OPPTS 835.2100. | $DT_{50}$ value equivalent to > 1 year (0.6 mg a.s/l; pH~4, 7 and 9) at temperatures up to 70°C | | Adam, D., 2003, |
| Photolysis in water<br><br>Commission directive 95/26/EC; Annex II: 2.9.2 and 7.2.1.2 Photochemical degradation<br><br>OECD draft August 2000 and EPA OPPTS 835.2210 | $DT_{50}$ = 0.78 d (0.82 ug a.s/l; buffer pH 7; Photolysis rate constant ($k^c_p$) = 0.88712; 25ºC) | In aqueous solution (at 25ºC), chlorophacinone is photolysed with a mean $DT_{50}$ value of 0.62 days under artificial sunlight. | Diehl, M., (2004) |
| Photolysis in water<br>OECD and EPA OPPTS 835.2210 | $DT_{50}$ = 0.45 d (0.82 ug a.s/l; pond water pH~8.4; Photolysis rate constant ($k^c_p$) = 1.52564. 25 ºC) | | Diehl, M., (2004) |
| Photo-degradation on a soil surface<br>US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3 | 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. | | Spare, W., 1992 |
| Transformation and fate in air<br>Atmospheric Oxidation Program v1.90 (AOPWIN) using the Atkins method | In conclusion, significant amounts of chlorophacinone are not likely to volatilise or persist in air. | | Curl, M.G., 2004 |
| Biodegradation under aerobic conditions. Biodegradability (ready), manometric respirometry test (OECD 301 F) | chlorophacinone is not biodegradable under environmentally relevant conditions or expected to be biodegradable during sewage treatment processes | | Peither, A., 2003 |
| Biotic degradation in soil. Aerobic degradation in soil (initial study).<br>US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1 | 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.<br>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 phenyl acetic acid) do not exceed 10% AR at any sampling interval. Soil non-extractable residue (NER) comprises 9.0% AR after *ca* 100 days. | 2 soil types. Aerobic conditions | Spare, W., 1994 |

| | | | |
|---|---|---|---|
| Adsorption/desorption screening test<br>US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 163-1 | Chlorophacinone is strongly adsorbed to soil. The amount of chlorophacinone adsorbed to soil was > 36.6 to > 85.2% during the adsorption phase. The Freundlich soil sorption coefficient normalised for organic carbon content ($K_{oc}$) was 15,600 to 136,000 ml/g. This result indicates chlorophacinone as 'non mobile' according to the SSLRC classification index. The Freundlich exponent (1/n) ranged from 1.145 to 1.231. In the case of chlorophacinone, adsorption to soil does not depend only on the organic carbon content. | | Spare, W., 1993 |

### 5.1.1 Stability

A **HYDROLYSIS** study is available according to OECD 111 EPA OPPTS 835.2100.The abiotic degradation of chlorophacinone in the dark (i.e. hydrolysis) was investigated in aqueous solutions at three different pH values. Initially, a Pre-Test was performed at a concentration about 0.6 mg a.s/l for 5 days at 50ºC in the dark using buffer solutions at pH~4, 7 and 9. Additionally, a Main Test was performed for pH~4 at 60 and 70ºC. 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 as insufficient degradation was observed at lower temperatures. The recovery of the applied radioactivity ranged from 93.9 to 104.6% throughout the investigation. The pH of the buffer solutions (citrate, phosphate and borate buffer 0.01M) 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.

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. Although in the hydrolysis pre-test, 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. Hydrolysis of chlorophacinone is not expected to be a significant process in the environment.

Chlorophacinone exhibited little hydrolytic degradation under sterile aqueous conditions (pH~4, 7 and 9) at temperatures up to 70°C. Chlorophacinone is considered stable to hydrolysis with a $DT_{50}$ value equivalent to > 1 year at environmentally relevant temperatures therefore hydrolytic degradation is not expected to be a significant process in the environment. Reliability 1.

An **AQUEOUS PHOTOLYSIS** study under laboratory conditions was performed, according to OECD and EPA guidelines (Commission directive 95/26/EC; Annex II: 2.9.2 and 7.2.1.2 Photochemical degradation OECD draft August 2000 and EPA OPPTS 835.2210) (phtototransformation in water study with $^{14}$C-chlorophacinone). 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 rate of photolysis of chlorophacinone in aqueous solution was investigated under artificial sunlight, Hanau Suntest, in sterile pH~7 buffer and in sterile pond water, the latter with a pH of 8.4 post sterilisation, at 25.0±0.1ºC. Test solutions were prepared and their duplicates, controls and dark control samples. The sterile solutions were continuously irradiated through their borosilicate lids, which absorb radiation < 290 nm, similar to the natural cut-off by ozone. Sterile, filtered, humidified air was drawn through the incubation vessels over the solutions at approximately 10 ml/minute. Any radioactive carbon dioxide or organic volatiles in the purged air was captured in traps of ethylenglycol followed by 2N NaOH, respectively.

The definitive phase of the study was conducted over 13 days. Sampling irradiated 0, 4 hours, 1, 3, 4, 7 and 13 d. 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. A preliminary test was performed as a range finding test. A chemical actinometer was not used for this study.

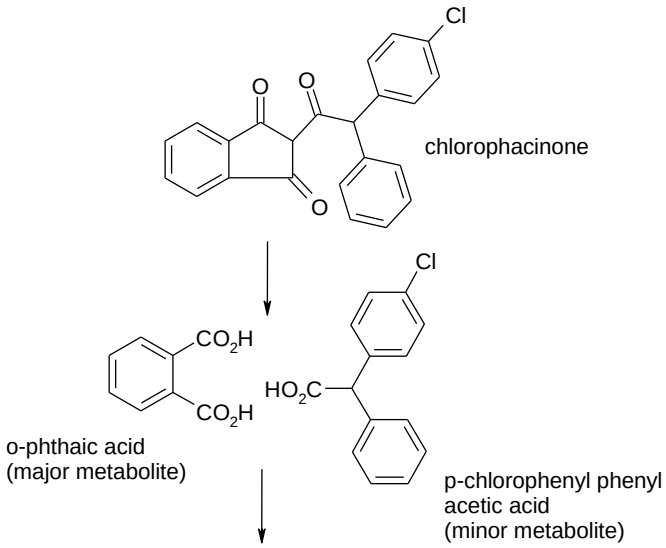
The best fit $DT_{50}$ value for the photolysis of chlorophacinone in sterile buffer solution (pH~7) was determined to be 0.78 days ($DT_{90}$=2.60 days; $R^2$=0.99881) and in sterile pond water (pH~8.4) 0.45 days ($DT_{90}$=1.51 days; $R^2$=0.99018). The buffer solution $DT_{50}$ (0.78 days) following 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. Concerning 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. The photolysis of chlorophacinone, under artificial sunlight, gave a good correlation to pseudo first order kinetics ($R^2$ values were $\geq$ 0.99) rate constants ($k^c_p$) were 0.88712 and 1.52564 $days^{-1}$ for the buffer and pond water samples, respectively. The sunlight reaction quantum yield ($\phi^c_E$) of the test substance was not determined. 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), separated by HPLC, were observed in the buffer solution 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). Levels of M2 and M3 were not significant (i.e. < 10% AR). In pond water, 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. Photolysis only happens between 10% and 50% (worst case) of the water body, the upper layer. The risk arising from M1 is considered covered by the parent. The maximum ocurrance is 23.4% in the fourth day. With this assumption if the chlorophacinone metabolised to M1, it would be necessary that the toxicity of the metabolite was between 8 (50% of the water body suffering photolysis) and 40 times (10% of the water body were photolysis was a relevant abiotic degradation process) more toxic than the active substance for the risk not to be covered. In buffer solution, M1 was a minor component observed at a maximum of 7.5% AR at day 4. 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. The pH and sterility of the test solutions were maintained throughout the incubation period.

In aqueous solution (at 25ºC), chlorophacinone is photolysed with a mean $DT_{50}$ value of 0.62 days under artificial sunlight. Based on the artificial-sunlight $DT_{50}$ values for buffer solution $DT_{50}$ (0.78 days) following continuous "Suntest" irradiation corresponded to **2.2 days natural** summer sunlight at latitude 50°N and in relation to the pond water $DT_{50}$ (0.45 days), following continuous "Suntest" irradiation, corresponded to **1.3 days** natural summer sunlight at latitude 50°N, both based on standard calculations. Reliability 1.

## PHOTO-DEGRADATION ON A SOIL SURFACE

Soil photolysis according to US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-3. 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. 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$^2$. Exposure cycle consisted of a 12 hour light/dark exposure periods. 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, 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 Doc. IIIA 7.2.2.4-01). 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%). Chlorophacinone quickly photo-degraded on a soil surface when exposed to an artificial light source, with 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. 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.

**Figure 1:** Postulated photo-degradation pathway for chlorophacinone on a soil surface.




Soil NER (minor) and carbon dioxide (major)

Photolysis of chlorophacinone on a soil surface proceeds rapidly with a $DT_{50}$ of 11.1 days at an equivalent temperature of 12°C (based on $DT_{50}$ at 25ºC). 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). Chlorophacinone is steadily degraded in soil under aerobic conditions in the dark, with degradation leading primarily to the formation of carbon dioxide. In the presence of light, soil degradation is more rapid however the route of degradation is unaffected. Reliability 2.

**TRANSFORMATION AND FATE IN AIR**

The estimation of photochemical oxidative degradation of chlorophacinone (unpublished). QSAR estimation performed using the Atmospheric Oxidation Program v1.90 (AOPWIN) using the Atkins method based on structural relationships. 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 $9.00 \times 10^{-12}$ $cm^3$ $molecule^{-1}$ $s^{-1}$, respectively (based on $1.5 \times 10^6$ 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.

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 $4.76 \times 10^{-4}$ 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 a.s/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.

### 5.1.2 Biodegradation

#### 5.1.2.1 Biodegradation estimation

Not relevant for this dossier.

#### 5.1.2.2 Screening tests

The biodegradation of chlorophacinone has been investigated in laboratory studies under aerobic conditions. A summary of the studies conducted is provided in the table below:

The ready biodegradability of chlorophacinone (purity 99.77%) was investigated under aerobic conditions at a mean temperature of 22°C in the dark over a period of 28 days. The inoculum used was aerobic activated sewage sludge from a treatment plant (Füllinsdorf, Switzerland) treating predominantly domestic wastewater. The ThODs of the reference and test materials were calculated to be 1.67 and 2.13 mg $O_2$/mg, respectively. The percentage of biodegradation of the test material was calculated based on the ThOD of 2.13 mg $O_2$/mg. In the procedural control, the reference material (sodium benzoate) 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 (pH~7.4-7.9). No significant biodegradation of chlorophacinone was observed after an incubation period of 28 days, consequently, chlorophacinone can not be considered readily biodegradable under the conditions of the test.

In the environment, chlorophacinone is not readily biodegradable according to the conditions of test OECD 301F (manometric respirometry test). An investigation into the inherent biodegradability was not carried out since the notifier assumed that chlorophacinone is not inherently biodegradable. It has also been assumed by the notifier that chlorophacinone is not likely to be biodegradable in biological sewage treatments either under aerobic or under anaerobic conditions.

As a conclusion, chlorophacinone is not biodegradable under environmentally relevant conditions or expected to be biodegradable during sewage treatment processes. Reliability 1.

#### 5.1.2.3 Simulation tests

### 5.1.3 Summary and discussion of degradation

## 5.2 Environmental distribution

**Biotic degradation in soil**

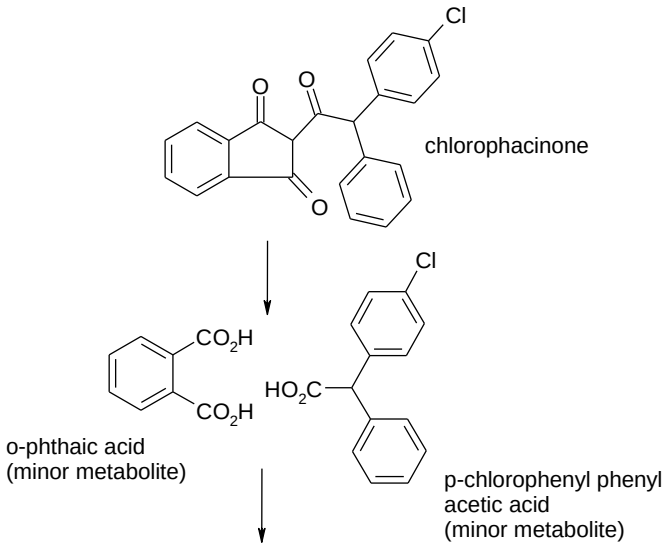
**Aerobic degradation in soil**

The aerobic degradation in soil (initial study) was performed following US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1. As reference materials phthalic acid and chlorophenyl-phenyl acetic acid were utilized. The route and rate of aerobic degradation of Indan-$^{14}$C-chlorophacinone (purity above 99%) was investigated in one soil (sandy clay loam: sand 56%, silt 21% and clay 23%; o.c. 1.2%) of US origin in the dark under laboratory conditions at a temperature of 24 to 26ºC and moisture content of 75% field capacity (1/3 bar moisture). The microbial viability of the soils was determined at the start and end of the study. In order 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 sand 71%, silt 22.3% and clay 7%; 1.0%).

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 (Non Extractable Residues) 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.

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 at 25ºC and 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, in this fashion, the $DT_{50}$ value for Buckeystown sandy clay loam soil was 128 days.

**Figure 2: Postulated degradation pathway for cholorophacinone in aerobic soil**



The biotic degradation of chlorophacinone in moist soil has been investigated in two laboratory studies. A summary of the studies conducted is provided in the table below:

**Laboratory studies investigating the degradation of chlorophacinone in soil (aerobic conditions)**

| Guideline / Test method | Soil type | Data range (days) | Moisture content | Temperature | Degradation parameters (days) | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | $DT_{50}$ | $DT_{90}$ | Regression parameters | |
| US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1 | Buckeystown (US) Sandy clay loam (pH~7.0, oc 1.18%) | 0-182 | 75% 1/3 bar | 25°C | 47.3 | > 200[1] | 1ST order ($Y = C_O$ x exp(-kt)) $C_O$ = 93.508, k = 0.01466, $R^2$ = 0.967 | Spare, W., 1994 |
| | | | -- | **(12°C calc)** | **128** | -- | -- | -- |
| | Buckeystown (US) Sandy loam (pH~7.2, oc 0.99%) | 0-70 | 75% 1/3 bar | 25ºC | 17.1 | -- | 1ST order ($Y = C_O$ x exp(-kt)) $C_O$ = 88.633, k = 0.04063, $R^2$ = 0.955 | Spare, W., 1994 |
| | | 30-70 | | | -- | 125[1] | 1ST order ($Y = C_O$ x exp(-kt)) $C_O$ = 45.745, k = 0.01846, $R^2$ = 0.999 | |

[1]$DT_{50}$ (or $DT_{90}$) value was not demonstrated experimentally, result obtained by extrapolation.

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 phenyl acetic acid) do not exceed 10% AR at any sampling interval. Soil non-extractable residue (NER) comprises 9.0% AR after *ca* 100 days. Reliability 2.

### 5.2.1 Adsorption/Desorption

**Adsorption/desorption screening test**

Four soil types were used (clay, sand, sandy clay loam and loam). 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.

Freundlich adsorption isotherms were determined for all soils over an actual concentration range of 0.17 to 2.56 µg/ml. The adsorption of chlorophacinone to soil gave a good correlation to the Freundlich equation (correlation 0.99 to 1.00).

The sorption properties of chlorophacinone have been investigated in a laboratory adsorption/desorption study and a summary of the results is provided in the table below:

Chlorophacinone is strongly adsorbed to soil. The amount of chlorophacinone adsorbed to soil was > 36.6 to > 85.2% during the adsorption phase. The Freundlich soil sorption coefficient normalised for organic carbon content ($K_{oc}$) was 15,600 to 136,000 ml/g. This result indicates chlorophacinone

as 'non mobile' according to the SSLRC classification index. The Freundlich exponent (1/n) ranged from 1.145 to 1.231.

The log *n*-octanol-water partition coefficient (log $K_{ow}$) is a measure of the hydrophobicity of a chemical. As such, log $K_{ow}$ is a key parameter in the assessment of environmental fate. Estimations of the $K_{oc}$ based on the $K_{ow}$ applying (Q)SARs 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. Reliability 2.

### 5.2.2 Volatilisation

Its vapour pressure is low, $4.76 \cdot 10^{-4}$ Pa at 23ºC and its Henry's Law Constant as well (0.013725 $Pa \cdot m^3 \cdot mol^{-1}$), hence the atmospheric compartment is not usually contaminated.

### 5.2.3 Distribution modelling

Not relevant for this dossier.

## 5.3 Aquatic Bioaccumulation

**Table 22: Summary of relevant information on aquatic bioaccumulation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Absorption, distribution, metabolism and excretion study<br>Single oral dose study in the rat<br>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 | 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 compound is absorbed, enters the enterohepatic circulation and then is excreted through the faeces. The urinary elimination and $CO_2$ elimination is less than 1% after 4 days. | No metabolite identification was undertaken in this study | Belleville M.J., (1977) |
| Absorption, distribution, metabolism and excretion study<br>Single oral dose study in the rat | 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 (19.6% faecal radioactivity). Two major metabolites, accounting for 46% faecal radioactivity were identified as mono-hydroxylated analogues of chlorophacinone | | Needham, D and Russell, N., (2004) |

### 5.3.1 Aquatic bioaccumulation

#### 5.3.1.1 Bioaccumulation estimation

Two studies on absorption, distribution, metabolism and excretion were performed with chlorophacinone in rats (see section 3.1.). A summary is given below.

Absorption, distribution, metabolism and excretion studies in the rat using $^{14}$C-labeled chlorophacinone. Single oral dose study in the rat. Methodology similar to US EPA 85-1. Purity of the test material not stated in the report. 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 blood half-life for elimination was 10.2 h. Excretion predominantly faecal. The study of the degradation of the compound from extracted faeces indicates that the material is excreted principally as metabolized rodenticide. Chromatographic evidence indicates that unchanged parent accounted for only a small component of the faecally eliminated radioactivity. No metabolite identification was undertaken in this study. The compound is absorbed, enters the enterohepatic circulation and then is excreted through the faeces. The urinary elimination and $CO_2$ elimination is less than 1% after 4 days. Reliability 2.

Another study was conducted with $^{14}$C-chlorophacinone: Metabolism in the rat following oral dosing. Absorption, distribution, metabolism and excretion study. Purity of the test material (99.58%). Single oral dose study in rat. A single group of eight male rats were fed by oral gavage a nominal dose of 2 mg/kg food with a post-exposure period of 168 hours. Three rats were killed on health grounds 72 hours after dosing. None of the other five showed adverse reactions to dose administration. 78% of the radioactivity appeared in faeces and less 1% in urine after 7 days. 8% of dose was found in the carcass at necropsy 7 days after dosing indicating that excretion was incomplete. Overall recovery was 91%; the discrepancy with the 100% excretion at 96 h from the previous study (6.2-01) was not explained.

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. Solvent concentrates were analysed by HPLC, which showed up five metabolites, the major one co-eluting with the chlorophacinone standard. Some of the minor metabolites co-eluted with impurities present in the radiolabelled chlorophacinone. An aliquot of faeces was extracted three times in methanol whose extract contained 85.6% of total radioactive residues. After a further clean-up it became 90% of total radioactive residues and a further non-extractable 12.5% remaining in the faecal pellet. Aliquots of the methanol extract were evaporated to near dryness to remove organic solvents, the results showed no significant difference in metabolite profile suggesting that there were no glucuronide or sulphate conjugates present. A second MTBE extract was prepared. The process produced three ions for the three major radiolabelled compounds found in faecal extracts. 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 and the other two metabolites hydroxylated analogues of chlorophacinone (the first one of the analogues with hydroxylation occurring on the indandione portion of the molecule and the second one on the biphenyl portion of the molecule).

Metabolite quantification. Faecal samples from the five surviving animals to termination were pooled and extracted in methanol. The extract contained 81.8% of the faecal radioactivity with 18.2% remaining in the residue. The three major metabolites identified were chlorophacinone and hydroxylated products accounting for 80.2% of the radioactivity in the faeces (unchanged chlorophacinone accounted for 19.6% of faecal radioactivity and indicated that 15.5% of

administered radioactivity was preent in faeces as unchanged parent molecule; the hydroxylated analogues accounted for 46% of radioactivity eliminated in faeces which corresponds to a 36.2% of the administered dose). Further attempts to identify the minor metabolites were unsuccessful since no meaningful spectra could be obtained.

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 (19.6% faecal radioactivity). Two major metabolites, accounting for 46% faecal radioactivity were identified as mono-hydroxylated analogues of chlorophacinone. Reliability 2.

#### 5.3.1.2 Measured bioaccumulation data

Measurements of aquatic bioconcentration of chlorophacinone have not been performed. According to the TGD; ECB 2003, the BCF for fish can be predicted from the relationship between $K_{ow}$ and BCF in cases where measured BCF values are not available. For substances with a log $K_{ow}$ lower than 6 is generally agreed that a linear relationship exists for chemicals which are not biotransformed. The linear model developed by Veith *et al*. (1979) can be used:

$$\log BCF = 0.85 \cdot \log Kow - 0.70$$

$\log BCF_{fish} = 0.85 \cdot 2.42 - 0.70$; $BCF_{fish} = 22.75$ l/kg

Having checked these studies and acknowledging that chlorophacinone has a log $P_{ow}$ = 2.42 (pH~7 at 23ºC); value below 3, it is accepted that chlorophacinone has a relatively low potential to bioaccumulate. No studies on the bioconcentration potential of chlorophacinone in aquatic and terrestrial environment have been supply by the applicant to confirm its low bioaccumulation potential.

### 5.3.2 Summary and discussion of aquatic bioaccumulation

## 5.4 Aquatic toxicity

**Table 23: Summary of relevant information on aquatic toxicity**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Short-term toxicity to fish<br>US EPA FIFRA 72-1 comparable to OECD 203 | $LC_0$ (96h) = 0.22 mg a.s/l<br>$LC_{50}$ (96h) = 0.45 mg a.s/l<br>$LC_{100}$ (96h) = 1.0 mg a.s/l | Flow-through<br>*Onchorhycnchus mykiss* | Machado, M.W. (1992a) |
| Short-term toxicity to fish<br>OECD 203 | $LC_0$ (96h) = 0.36 mg a.s/l<br>$LC_{50}$ (96h) = 0.71 mg a.s/l | Flow-through<br>*Lepomis macrochirus* | Machado, M.W. (1992b). |
| Acute toxicity to invertebrates | $EC_0$ (48 h) = 0.31 mg a.s/l<br>$EC_{50}$ (48 h) = 0.64 mg a.s/l | *D. magna*<br>Measured | Putt, A.E. (1992). |

| | | | |
|---|---|---|---|
| OECD 202 | | concentrations | |
| Acute and long-term toxicity to algae<br>OECD 201 | $NOE_rC = NOE_bC = 0.72$ mg a.s/l<br>$E_bC_{50} = 1.7$ mg a.s/l (calculated from the area under the growth curve)<br>$E_bC_{50} = 2.2$ mg a.s/l (calculated from growth rate) | Static. Endpoint: growth inhibition<br>*Desmodesmus subspicatus* (former *S. subspicatus* | Peither, A. (2003). |
| Activated sludge<br>OECD 209 | $EC_{50}$ (3 h) > 1000 mg a.s/l<br>$EC_{80}$ (3 h) > 1000 mg a.s/l<br>NOEC ($EC_{15}$) (3 h) = 775 mg a.s/l | Static. Respiration inhibition.<br>All EC and NOEC values above the water solubility limit | *Peither, A. (2003).* |

### 5.4.1 Fish

#### 5.4.1.1 Short-term toxicity to fish

In two tests performed under flow-through conditions, the 96-hour $LC_{50}$ were 0.45 and 0.71 mg a.s/l for *Oncorhynchus mykiss* and *Lepomis macrochirus*, respectively (mean measured concentrations). These two studies conform to current guideline requirements and exposure concentrations were verified analytically.

In the first test the author determined an $LC_{50}$ for rainbow trout (*Onchorhynchus mykiss)* of 0.45 mg a.s/l (95% confidence limits of 0.42 to 0.49 mg a.s/l) based on mean measured concentrations and using probit analysis. The acute toxicity test was performed according to US EPA FIFRA 72-1, comparable to OECD guideline nº 203.

Twenty fish (Rainbow trout) (ten per replicate) were tested for 96 h under flow-through conditions. A preliminary test was conducted in order to determine the toxically relevant range. All fish were fed a dry commercial pelleted food daily ad libitum except during the 48 h prior to and during the definitive test. No mortality occurred in the fish test population during the two days prior to testing. The test was performed at five nominal concentrations of the test material (1.0, 0.60, 0.36, 0.22 and 0.13 mg a.s/l), a solvent control and a dilution water control. All the mean measured concentrations were above 80% of the nominal concentrations. The 96-hour LC50 value was 0.45 mg a.s/l based on mean measured concentrations; LC0 (96 h) = 0.22 mg a.s/l and LC100 (96 h) = 1.0 mg a.s/l. Temperature between 11 and 13ºC, pH~6.7-7.2, dissolved oxygen above 60% and a 16-h photoperiod. Test fish were 36-54 mm and a mean wet weight of 1.1 g. The age of the organism was not reported. All relevant parameters were within the ranges dictated by the guideline. Reliability 1.

In the second test, the author determined an $LC_{50}$ for bluegill sunfish (*Lepomis macrochirus)* of 0.71 mg a.s/l (95% confidence limits of 0.63 to 0.83 mg a.s/l) based on mean measured concentrations and using probit analysis. The acute toxicity test was performed according to US EPA FIFRA 72-1, comparable to OECD guideline nº 203.

US EPA FIFRA 72-1, comparable to OECD 203 (Fish, acute toxicity test). Twenty fish (ten per replicate) Bluegill sunfish were tested for 96 h under flow-through conditions. A preliminary test was conducted 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 in the fish test population during the two days prior to testing. The test was performed at five nominal concentrations of the test material (1.2, 0.72, 0.43, 0.26 and 0.16 mg a.s/l), a solvent control and a dilution water control. Mean measured concentrations (0.82, 0.52, 0.36, 0.24 and 0.11 mg a.s/l) were ranged from 68-92 % of the nominal concentrations. The 96-hour $LC_{50}$ value was 0.71 mg a.s/l based on mean measured concentrations. $LC_0$ = 0.36 mg a.s/l. 100% mortality was not reached. Temperature between 21 and 22ºC, pH~7.0-7.3, dissolved oxygen above 60% and a 16-h photoperiod. Test fish were 36-54 mm and a mean wet weight of 1.1 g. The age of the organism was not reported. All relevant parameters were within the ranges dictated by the guideline. Reliability 1.

#### 5.4.1.2 Long-term toxicity to fish

Information on long-term toxicity on aquatic vertebrates has not been provided but, the available information is considered enough for carrying out a proper risk assessment.

### 5.4.2 Aquatic invertebrates

#### 5.4.2.1 Short-term toxicity to aquatic invertebrates

The author determined an $EC_{50}$ (24 h) > 820 µg/l and an $EC_{50}$ (48 h) = 640 µg/l (95% confidence limits of 540 to 820 µg/l) based on mean measured concentrations and using probit analysis. The acute toxicity test was performed according to US EPA FIFRA 72-2, comparable to OECD guideline nº 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 in order to determine the toxically relevant range. Daphnids were not fed during the 48-hour definitive exposure. 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 concentrations. The 48-hour $EC_{50}$ value was 0.64 mg a.s/l. Temperature of the test ranged between 19 and 22ºC, pH~8.0-8.2, dissolved oxygen above 60% and a 16-h photoperiod. Test aquatic invertebrates were ≤ 24 hours old at the beginning of the test. All relevant parameters were within the ranges dictated by the guideline.

*D. magna* was similar in sensitivity to fish, with a 48-hour $EC_{50}$ of 0.64 mg a.s/l recorded under flow-through conditions. The endpoint was based on immobilisation and on mean measured concentrations of chlorophacinone in the test media. Reliability 1.

#### 5.4.2.2 Long-term toxicity to aquatic invertebrates

No data have been provided on long-term toxicity to aquatic invertebrates but, the available information is considered enough for carrying out a proper risk assessment.

### 5.4.3 Algae and aquatic plants

The author determined in a 72-hour algal growth inhibition test with *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) an $E_bC_{50}$ (72 h) = 1.7 mg chlorophacinone/l and an $E_rC_{50}$ (72 h) = 2.2 mg a.s/l (95% confidence limits of 0.7 – 9.1 mg/l). The NOEC was 0.72 mg a.s/l

with respect to both biomass yield, $NOE_bC$, and specific growth rates, $NOE_rC$ based on mean measured concentrations and using probit analysis for the $EC_{50}$s and the Dunnett's t-test to determine significant differences from solvent control to locate the NOECs. The growth of the green algal species *Desmodesmus subspicatus* was investigated in a 72-hour static test according to OECD 201 (Alga, Growth Inhibition Test) and EU method C.3.

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 a.s/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 a.s/l and the value of $E_rC_{50}$ was 2.2 mg a.s/l based on the nominal concentrations of the active substance. Temperature of the test was between 22-23ºC, pH~7.9 to 8.0 at start to 8.1 to 8.6 at end. All relevant parameters were within the ranges dictated by the guideline.

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. The second one is "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%. And the third one "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*". The coefficient of variation for the control replicates over the entire study duration was 0.81%. The algal study performed with chlorophacinone may therefore be considered valid according to all three criteria. Reliability 1.

From all the aquatic toxicity endpoints for chlorophacinone before mentioned, fish represent the most sensitive of the three aquatic trophic levels tested and the $LC_{50}$ (96h) = 0.45 mg a.s/l for *O. mykiss* serves as the key endpoint for the aquatic risk assessment.

### 5.4.4 Other aquatic organisms (including sediment)

The effect of chlorophacinone on aerobic biological sewage treatment processes was assessed by determining inhibition of respiration of the micro-organisms present in activated sludge following three-hour contact. The following nominal concentrations of the active substance were tested: 10, 32, 100, 320 and 1,000 mg a.s/l. In addition, two controls and three different concentrations of the reference substance 3,5-dichlorophenol (5, 16 and 50 mg a.s/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. Therefore, the water solubility limit has been used for the $PNEC_{microorganisms}$ derivation instead of the nominal concentration.

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.

## 5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Chlorophacinone is classified for the environment in Annex 1 of Council Directive 67/548/EEC.

**Current classification of Chlorophacinone for the environment under the DSD**

| **Classification** | **as detailed in Directive 67/548/EEC** |
|---|---|
| Class of danger | N |
| R phrases | **R50/53**: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. |
| S phrases | **S60**: This material and its container must be disposed of as hazardous waste<br><br>**S61**: Avoid release to the environment. Refer to special instructions/safety data sheets. |

**According to Regulation (EC) No 1272/2008 and Regulation (EU) No 286/2011**

GHS pictogram: GHS09
Signal word: Warning
Hazard statements:

Aquatic acute 1: H 400 Very toxic to aquatic life
Acute M-factor 1

Aquatic chronic 1: H410 Very toxic to aquatic life with long lasting effects
Chronic M-factor 1

Precautionary statements:

P273 Avoid release to the environment
P391 Collect spillage
P501 Dispose of contents/container to…

### 5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

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).

Regarding the environmental effects, the acute toxicity of chlorophacinone in fish, daphnia and algae was investigated, so that sufficient data are available to allow classification and labelling of the active ingredient according to the requirements of Annex VI of directive 67/548/EEC. The proposed classification and labelling (R50) is based upon the lowest acute toxicity testing in aquatic organisms (fish $LC_{50}$ (96 h) = 0.45 mg a.s/l). Chlorophacinone was also tested for ready biodegradability under aerobic conditions at a mean temperature of 22°C in the dark over a period of 28 days (inoculum used: aerobic activated sewage sludge from a treatment plant). It was concluded not being readily biodegradable. An investigation into the inherent biodegradability was not carried out since the notifier assumed that chlorophacinone is not inherently biodegradable. It has also been assumed by the notifier that chlorophacinone is not likely to be biodegradable in biological sewage treatments either under aerobic or under anaerobic conditions. The conclusion

was that chlorophacinone is not biodegradable under environmentally relevant conditions or expected to be biodegradable during sewage treatment processes (R 53).

According to Regulation (EC) No 1272/2008 since the aquatic toxicity is ≤ 1 mg a.s/l and the substance is not rapidly degradable, the substance is classified as Acute Category 1 and Chronic Category 1.

In respect of Regulation (EU) No 286/2011 the classification is Category Acute 1 and Category Chronic 1. Since there is only one NOEC available, a comparison was performed with that NOEC and the surrogate system. The most restrictive classification was chosen. With the algae NOErC = 0.72 mg a.s/l Category Chronic 2 should apply. On the other hand, applying the surrogate system, Category Chronic 1 should apply. This is the most restrictive one category. Conclusion: chlorophacinone is classified as Category Acute 1 and Category Chronic 1.

**RAC evaluation of environmental hazards**

**Summary of Dossier submitter's proposal**

There is a current entry in Annex VI of CLP Regulation with an environmental classification as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 under CLP and N; R50-53 under DSD. The dossier submitter (DS) proposed to add M-factors for Aquatic Acute 1, H400 (M=1) and Aquatic Chronic 1, H410 (M=1) according to CLP.

*Degradation*

Degradation was studied in a hydrolysis test, two photolysis tests in water, one photodegradation on soil test, one ready biodegradability test, and one degradation test in soil.

The DS considered chlorophacinone as hydrolytically stable ($DT_{50}$ > 1 year, 70ºC) and rapidly photodegradable in water with an experimental half-life between 0.45 – 0.78 days (25ºC). Chlorophacinone quickly photo-degraded on a soil surface when exposed to an artificial light source, with a $DT_{50}$ value of 4.1 days at 25 ºC. It is degraded rapidly in the atmosphere by reaction with OH radicals ($DT_{50}$ = 14.3 hours), although the presence of this compound in air is not expected due to its low vapour pressure ($4.76 \times 10^{-4}$ Pa (22.8ºC)).

Chlorophacinone is not readily biodegradable under test conditions (OECD TG 301F), with no significant biodegradation of chlorophacinone observed after an incubation period of 28 days. An investigation into the inherent biodegradability was not carried out since the notifier assumed that chlorophacinone is not inherently biodegradable.

Chlorophacinone showed moderate degradation under aerobic conditions in the soil. The best fit first order $DT_{50}$ value of chlorophacinone was determined to be 47.3 days for a sandy clay loam soil at 25 ºC. Degradation of chlorophacinone did not lead to the formation of any significant metabolites (i.e. > 10%).

Based on the available data a non-rapid/ready degradation was proposed for chlorophacinone.

*Bioaccumulation*

The experimental log $K_{ow}$ of chlorophacinone was 2.42 at pH = 7 and 23ºC, which is lower than the cut-off values of log $K_{ow}$≥4 (CLP). Measurements of aquatic bioconcentration of chlorophacinone have not been performed.

In conclusion, based on the low log $K_{ow}$, the DS concluded that chlorophacinone does not

have potential for bioaccumulation.

*Aquatic toxicity*

Two acute toxicity studies in fish (*Oncorhynchus mykiss,* $LC_{50}$ = 0.45 mg/L and *Lepomis macrochirus,* $LC_{50}$ = 0.71 mg/L), one in invertebrates (*Daphnia magna,* $EC_{50}$ 0.64 mg/L) and one in algae (*Pseudokirchneriella subcapitata,* $E_rC_{50}$ = 2.2 mg/L) were reported by the DS. Long-term tests in fish and invertebrates were not available, but for algae the test submitted in the CLH report can be considered as a chronic test ($NOE_rC$ = 0.72 mg/L). All the toxicity values for these tests were based on mean measured concentrations.

Fish (*Oncorhynchus mykiss*) was the most sensitive taxonomic group in acute tests, with an $LC_{50}$ value of 0.45 mg/L, while in the chronic test the $NOE_rC$ value of 0.72 mg/L was determined for *Pseudokirchneriella subcapitata*. However, no adequate chronic data is available for all trophic levels, and in this case the surrogate approach from fish was chosen as the most stringent outcome to propose the aquatic chronic classification, taking into account that the substance is no rapidly/readily biodegradable and the $E(L)C_{50}$ (for fish) was ≤1 mg/L ($LC_{50}$= 0.45 mg/L).

## Comments received during public consultation

Three member states supported the environmental classification proposed by the dossier submitter.

In their post public consultation response to the comments received, the DS explained how the M-factors had been derived, as follows:

"*The proposed M-factor for acute toxicity of 1 is based on the most sensitive species, Onchorhynchus mykiss, with a 96h$LC_{50}$ = 0.45 mg/l; toxicity band between 0.1 mg/l and 1 mg/l).*

*Based on the most stringent outcome for Aquatic Chronic toxicity (on the basis of the Algae NOEC and the $LC_{50}$ for the other trophic levels) an M-factor for chronic toxicity of 1 could be assigned, based on the fish 96h$LC_{50}$=0.45 mg/l and the fact that the substance is not rapidly degradable*."

## RAC assessment and comparison with criteria

Degradation

RAC agreed that chlorophacinone can be considered hydrolytically stable and rapidly photodegradable in water and soil based on the information provided in the CLH report but
was not readily biodegradable under the test conditions; no significant biodegradation of chlorophacinone was observed after an incubation period of 28 days. Furthermore, in an aerobic soil study, chlorophacinone showed moderate degradation ($DT_{50}$ = 47.3 days, 25ºC). Therefore, based on these data, RAC agreed with the DS that chlorophacinone should be considered **not rapidly degradable** according to CLP.

Bioaccumulation

The experimental log $K_{ow}$ for chlorophacinone is 2.47 at pH 7 which is lower than the cut-off values of log $K_{ow}$≥4 (CLP), therefore RAC agrees with the DS, chlorophacinone has **not potential for bioaccumulation.**

Aquatic toxicity
Classification for acute toxicity should be based on the lowest $EC_{50}$ of 0.45 mg/L from a test with *Oncorhynchus mykiss* (OECD 203). This value is ≤ 1 mg/L, therefore

chlorophacinone classifies as Aquatic Acute 1 (H400), with an M-factor of 1, because the $LC_{50}$ is between 0.1 and 1 mg/L.

No adequate chronic data was available for all three trophic levels and only chronic data from algae were submitted in the CLH report. According to this, classification as Aquatic Chronic 2 (H411) is applicable for chlorophacinone based on a $NOE_rC$ of 0.72 mg/L. However, the surrogate approach should be taken into account due to the lack of chronic data for fish and invertebrates. Therefore, as the substance is not rapidly degradable and the $E(L)C_{50}$ (fish) was 0.45 mg/L (i.e. ≤ 1mg/L), which was the highest acute toxicity value among invertebrates and fish, classification as Aquatic Chronic 1 (H410) with an M-factor of 1 is justified. This classification results from selecting the most stringent outcome and therefore it must be applied to chlorophacinone.

In conclusion, RAC agrees with the DS's proposal to classify chlorophacinone according to CLP criteria as **Aquatic Acute 1 (H400) with M-factor 1** and **Aquatic Chronic 1 (H410) with M-Factor 1.**

# 6 OTHER INFORMATION

Teratology study of the test item Warfarin sodium with rats (Kubaszky, R., 2009)

The objective of the study was to assess the effects of Warfarin Sodium on pregnant females and on the developing conceptuses during pregnancy in Crl:(Wi) BR-Wistar Rats after oral administration at three dose levels (0.125, 0.150, 0.200, mg/kg/day) from day 6 up to and including day 15 post coitum (T.P. 1) and from day 6 up to and including day 19 post coitum (T.P. 2). Two additional extra high dose groups of 0.250 mg/kg/day of 12 dams were added for both T.P. 1 and T.P. 2, to demonstrate clear maternal toxicity. The dose volume was 5 ml/kg.

The clinical symptoms, mortality, necropsy findings; body weight, body weight gain, and reproduction data of the dams were recorded and evaluated. The body weight of the foetuses was measured; the placentas were examined externally; the external, visceral and skeletal abnormalities of foetuses were recorded and evaluated. This nonclinical exploratory laboratory study was performed in compliance with OECD Guideline for Testing of Chemicals (OECD 414/22 January 2001).

Nine dams died in the T.P.1 treatment period group; two at 0.150 mg/kg, two at 0.200 mg/kg and five at 0.250 mg/kg. There were five dead and four moribund dams in the T.P. 2 group. One moribund dam was in the 0.150 mg/kg dose group. Five dead and three moribund dams were in the 0.250 mg/kg dose group. The death or euthanasia of the dams occurred between gestation days 14 and 17 and in one case on gestation day 19. The mortality was ascribed to treatment.

Clinical signs such as piloerection, paleness, reduced activity and vaginal bleeding and open vaginal orifice were recorded for the animals subsequently found dead or sacrificed. Vaginal bleeding, open vaginal orifice, paleness, haemorrhage or piloerection were recorded for some surviving females in the 0.150 mg/kg, 0.200 mg/kg and 0.250 mg/kg T.P. 1 groups and 0.200 mg/kg T.P.2. These changes were attributed to treatment. The body weight, body weight gain, gravid uterine weight, corrected body weight, corrected body weight gain of dams were not affected by treatment.

At necropsy, treatment related gross pathology alterations included uterus filled with blood, bloody staining around the vaginal orifice, intestinal bleeding, pale organs.

There was no difference between the experimental groups in the number of corpora lutea, pre- or post implantation loss, number of implantation or viable foetuses. Sex ratio was not adversely affected by maternal treatment. Mean foetal weight was similar in all groups.

External examination of foetuses showed one litter with 4 foetuses with short nose and wide frontal bone in the 0.150 mg/kg T.P.1 group (confirmed at skeletal examination). This litter was not included in the statistical evaluation. It is concluded that these malformations were attributable to treatment with Warfarin Sodium.

The incidence of greenish discolouration of placentas was significantly higher in the 0.150 mg/kg, 0.200 mg/kg and 0.250mg/kg T.P.1 groups and was significantly higher in the T.P. 2 in the 0.200 mg/kg dose group. The incidence of pale placentas was significantly higher in the T.P. 1 0.250 mg/kg dose group. These alterations were considered to be due to treatment.

The incidence of external and visceral foetal haemorrhages was higher in all treated groups than the respective control groups; this was attributed to the known anticoagulant effect of the test item.

At visceral examination, an increased incidence of central cataract (confirmed by histopathology) was observed (one foetus in the T.P. 1 0.200 mg/kg dose group, 2 at 0.150 mg/kg and 4 at 0.200 mg/kg dose levels in the T.P. 2 group). This rare malformation was considered to be an effect of treatment with Warfarin Sodium. No statistical differences in skeletal observations were attributed to treatment. However, one litter in the 0.150 mg/kg T.P.1 group showed skull malformations which were attributed to a teratological effect of the test item. At 250 mg/kg in the T.P.2 group, there was an indication of reduced skull ossification.

In conclusion, under the conditions of this study it is considered that warfarin sodium administered orally to the pregnant Crl:(Wi) BR-Wistar Rats caused adverse effects in the foetus. These included subcutaneous haemorrhage at all dose levels and visceral haemorrhage at 0.200 mg/kg in T.P.2 group. Foetal abnormalities ascribed to treatment included central ocular cataract in the 0.150 mg/kg T.P.2 and 0.200 mg/kg T.P.1 and T.P.2 groups, skull malformations of short nose and wide frontal bones in the 0.150 mg/kg dose T.P.1 group and an indication of reduced ossification of skull bones in the T.P.2 group at 250 mg/kg. Treatment was associated with increased incidence of maternal death and bleeding from the vagina in later pregnancy, with necropsy findings of blood in the uterus, at 0.150 mg/kg and above.

NO(A)EL maternal toxicity: 0.125 mg/kg bw/day
NOEL developmental toxicity: <0.125 mg/kg bw/day
NOEL teratogenicity: 0.125 mg/kg bw/day
STUDY CODE: 07/396-105P Page 11 of 363

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