Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU 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

Adopted

14 March 2014

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14 March 2014

CLH-O-0000003643-75-02/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: **Chlorophacinone (ISO); 2-[(4-chlorophenyl)(phenyl)acetyl]-1H-indene-1,3(2H)-dione**

EC number: **223-003-0**

CAS number: **3691-35-8**

The proposal was submitted by **Spain** and received by the RAC on **30 November 2012**. All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 March 2013.** Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 April 2013.**

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Veda Varnai**

Co-Rapporteur, appointed by the RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonized classification and labelling was reached on **14 March 2014** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on **Chlorophacinone (ISO)** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	606-014-00-9	chlorophacinone (ISO); 2-(2-(4-chlorophenyl)phenylacetyl)indan-1,3-dione	223-003-0	3691-35-8	Acute Tox. 1 Acute Tox. 2 * Acute Tox. 3 * STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H310 H300 H331 H372** H400 H410	GHS06 GHS08 GHS09 Dgr	H310 H300 H331 H372 ** H410		
Dossier submitters proposal	606-014-00-9	chlorophacinone (ISO); 2-(2-(4-chlorophenyl)phenylacetyl)indan-1,3-dione	223-003-0	3691-35-8	**Modify:** Acute Tox. 1 Acute Tox. 2	**Modify:** H330 H300 (blood coagulation system, liver, kidney) for H372 **Remove:** ** for H372		**Modify:** H330 H300 (blood coagulation system, liver, kidney) for H372 **Remove:** ** for H372		**Add:** STOT RE 1; H372: C ≥ 0,1 % STOT RE 2; H373: 0,01 % ≤ C <0,1 % M (acute) = 1 M (chronic) = 1

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
RAC opinion	606-014-00-9	chlorophacinone (ISO); 2-[(4-chlorophenyl)(phenyl)acetyl]-*1H*-indene-1,3(2H)-dione	223-003-0	3691-35-8	**Add:** Repr. 1B **Modify:** Acute Tox. 1 Acute Tox. 1 STOT RE.1 Aquatic Acute 1 Aquatic Chronic1	**Add:** H360D **Modify:** H330 H300 (blood) for H372 **Remove:** ** for H372		**Add:** H360D **Modify:** H330 H300 (blood) for H372 **Remove:** ** for H372		**Add**: Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,1 % STOT RE 2; H373: 0,01 % ≤ C < 0,1 % M = 1 M = 1
Resulting Annex VI entry if agreed by COM	606-014-00-9	chlorophacinone (ISO); 2-[(4-chlorophenyl)(phenyl)acetyl]-*1H*-indene-1,3(2H)-dione	223-003-0	3691-35-8	Repr. 1B Acute Tox. 1 Acute Tox. 1 Acute Tox. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic1	H360D H300 H310 H330 H372 (blood) H400 H410	GHS06 GHS08 GHS09 Dgr	H360D H300 H310 H330 H372 (blood) H410		Repr. 1B; H360D: C ≥ 0,003 % STOT RE 1; H372: C ≥ 0,1 % STOT RE 2; H373: 0,01 % ≤ C < 0,1 % M = 1 M = 1

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

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.

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.

CLH proposals for eight AVK rodenticides, Coumatetralyl (Denmark), Difenacoum (Finland), 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).

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

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.

Comments received during public consultation

There were no comments on physical hazards.

Assessment and comparison with the classification criteria

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.

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 4th and 9th 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.

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.*"

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.

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.

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.

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.

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.

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.

RAC evaluation of respiratory tract irritation

Summary of the Dossier submitter's proposal

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.

Comments received during public consultation

There were no comments on respiratory tract irritation.

Assessment and comparison with the classification criteria

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.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

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).

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:

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

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

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

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.

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.

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.

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.

ENVIRONMENTAL HAZARD ASSESSMENT

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 (4.76×10^{-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 96hLC_{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 96hLC_{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.**

ADDITIONAL REFERENCES:

Additional references not included in the CLH report

Ashby J, Tennant RW (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the US NTP. Mutat Res 257:229-306

Rady et al (2013). Sub-lethal and teratogenicity action of Bromadiolone and chlorophacinone anticoagulant rodenticides on albino rats. American-Eurasian Journal of Toxicological Sciences 5:7-14

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).