Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at Community level of

leucomalachite green

ECHA/RAC/CLH-O-0000001309-75-03/F

Adopted

23 November 2010

Annankatu 18 | P.O. Box 400 | 00121 Helsinki | Finland
www.echa.eu | Tel.: + 358 9 68.61.80

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

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

Substance Name: ***leucomalachite green***

EC Number: ***204-961-9***

CAS Number: ***129-73-7***

The proposal was submitted by the ***United Kingdom*** and received by RAC on ***21 June 2010***

	CLP Regulation (EC) No 1272/2008	Directive 67/548/EEC (criteria)
Current entry in Annex VI CLP Regulation	No entry	No entry
Current proposal for consideration by RAC	Muta. 2 - H341 Carc. 2 - H351	Muta. Cat. 3; R68 Carc. Cat. 3; R40
Resulting harmonised classification (future entry in Annex VI CLP Regulation)	Muta. 2 - H341 Carc. 2 - H351	Muta. Cat. 3; R68 Carc. Cat. 3; R40

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom 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/consultations/harmonised_cl/harmon_cl_prev_cons_en.asp*** on 21 June 2010. Parties concerned and MSCAs were invited to submit comments and contributions by 05 August 2010.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Norbert Rupprich***

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

The RAC opinion on the proposed harmonised classification and labelling has been reached on ***23 November 2010,*** in accordance with Article 37(4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2.

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

OPINION OF RAC

The RAC adopted the opinion that ***leucomalachite green*** should be classified and labelled as follows[1]:

Classification & Labelling in accordance with the CLP Regulation:

Classification:	**Muta. 2 - H341** **Carc. 2 - H351**
Specific concentration limits:	**None**
M-factor(s):	**None**
Notes:	**None**
Labelling:	**GHS08, Wng, H341, H351**

Classification & labelling in accordance with Directive 67/548/EEC

Classification:	**Muta. Cat. 3; R68** **Carc. Cat. 3; R40**
Specific concentration limits:	**None**
Notes:	**None**
Labelling:	**Xn; R 40-68; S (2-)36/37**

[1] Note that not all hazard classes have been evaluated.

SCIENTIFIC GROUNDS FOR THE OPINION

Introduction

Leucomalachite green is used as a histopathology stain.

The substance is not currently classified in Annex VI of the CLP Regulation.

Substance for which a harmonised C&L has been agreed at TC C&L

For leucomalachite green a harmonised C&L has been agreed at TC C&L. However, this classification proposal does not cover all the hazard classes that have been discussed and decided upon at TC C&L. The dossier submitter decided to put forward a classification proposal specifically for mutagenicity and carcinogenicity.

Mutagenicity

The following information on the mutagenicity of leucomalachite green is a copy of the relevant chapter in the background document:

Original summary of the dossier submitter

"The genotoxicity of leucomalachite green has been investigated in a number of studies, some of which are non-standard tests, including a study in transgenic animals.

Leucomalachite green tested negative in a number of standard *in vitro* (Ames test, COMET assay in CHO cells, and in a mammalian cell gene mutation assay (*Hgprt*) (all +/-S9)) and *in vivo* (two mouse micronucleus tests *in vivo* in bone marrow and blood erythrocytes following oral administration).

One gene mutation test in transgenic animals was positive (based upon liver *lacII* gene mutations), and a second gave equivocal results (based upon liver *lacI* gene mutations). ^{32}P-post-labelling studies in rats and mice exposed for 28 days in the diet demonstrated the formation of DNA adducts in the liver, thus indicating leucomalachite green's ability to covalently bind to DNA.

The findings from standard mutagenic tests do not indicate any mutagenic activity. However, mutations in genes in the liver of transgenic mice and DNA adducts in the liver of rats and mice indicate that leucomalachite green can reach and covalently bind to DNA, and can cause mutations in this organ.

In view of these findings it is considered prudent to presume that leucomalachite green is a potential *in vivo* somatic cell mutagen. Based on the criteria in the CLP Regulation, positive results in at least one *in vivo* assay in mammals, in the absence of germ cell mutagenicity, indicates that a classification as **Muta. 2 - H341** is appropriate. These effects also meet the criteria for classification as **Muta. Cat. 3; R68** under Directive 67/548/EEC (evidence of mutagenic effects *in vivo* in the absence of germ cell mutagenicity or evidence that the substance or its metabolite reaches the germ cells)."

RAC conclusion

The classification proposal of the dossier submitter is in line with the previous corresponding TC C&L recommendation. During public consultation and RAC discussions there were no comments questioning the rationale for the proposed classification for germ cell mutagenicity. Thus, based on the available comparison of mutagenicity data with DSD and CLP classification criteria RAC supports the actual proposal of the dossier submitter (CLP Muta. 2 - H341 respectively DSD Muta. Cat. 3; R68).

Carcinogenicity

The following information on the carcinogenicity of leucomalachite green is a copy of the relevant chapter in the background document:

Original summary of the dossier submitter

The carcinogenicity of leucomalachite green by the oral route has been investigated in good quality studies in mice and rats.

The evidence of possible carcinogenicity was a statistically significant dose-related increase in hepatocellular adenoma or carcinoma (combined) in female mice (the only sex investigated), the incidence of which exceeded historical control ranges. In rats, there were no statistically significant increases in tumour incidence, although the incidence of hepatocellular adenoma and thyroid gland follicular cell adenoma or carcinoma was increased in both sexes and some incidences were above historical controls. Mechanistic studies have shown that leucomalachite green inhibits thyroid peroxidase suggesting that the thyroid tumours were induced by perturbation of thyroid hormone homeostasis. There was also an increase in interstitial (Leydig) cell adenoma of the testes, occurring with a positive trend, in F344 rats (statistically significant in the top dose group), but Leydig cell tumours in this strain of rat are not considered to be relevant for humans.

The evidence for carcinogenicity is not substantial, with limited evidence of tumour induction in the liver in mice (in a strain generally regarded as being particularly sensitive to the induction of such tumours) and only equivocal evidence of induction of liver tumours in female rats. It is recognised that this is only weak evidence for carcinogenicity, and the tumour profile is not typical for a genotoxic agent, but the statistically significant induction of tumours, with genotoxicity possibly involved in their induction, does raise some concern for carcinogenicity. An additional consideration is that the induction of liver tumours in mice was not associated with severe general toxicity.

The limited evidence of carcinogenicity indicates that a classification of **Carc. 2 - H351** according to the CLP Regulation criteria is appropriate. Likewise, the available evidence indicates that a classification with **Carc. Cat. 3; R40** under the Directive 67/548/EEC criteria is justified.

RAC conclusion

The classification proposal of the dossier submitter is in line with the previous corresponding TC C&L recommendation. During public consultation and RAC discussions there were no comments questioning the rationale for the proposed classification for carcinogenicity. Thus, based on the available comparison of carcinogenicity data with DSD and CLP classification criteria RAC supports the actual proposal of the dossier submitter (CLP Carc. 2 - H351 respectively DSD Carc. Cat. 3; R40).

Additional information

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

ANNEXES:

Annex 1 Background Document (BD)[2]

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

[2] The Background Document (BD) supporting the opinion contains scientific justifications for the CLH proposal. The BD is based on the CLH report prepared by a dossier submitter. The original CLH report may need to be changed as a result of the comments and contributions received during the public consultation(s) and the comments by and discussions in the Committees.