**Committee for Risk Assessment**

**RAC**

# Opinion

proposing harmonised classification and labelling
at EU level of

# 3,7-dimethylocta-2,6-dienenitrile

**EC number: 225-918-0**
**CAS number: 5146-66-7**

CLH-O-0000001412-86-26/F

**Adopted**

**04 December 2014**

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4 December 2014

CLH-O-0000001412-86-26/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: 3,7-dimethylocta-2,6-dienenitrile**

**EC number: 225-918-0**

**CAS number: 5146-66-7**

The proposal was submitted by **Germany** and received by the RAC on **7 November 2013.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

## PROCESS FOR ADOPTION OF THE OPINION

**Germany** 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 **26 November 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 January 2014**.

### ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co- rapporteur, appointed by RAC: **Lina Dunauskiene**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation, which are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 December 2014**.

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

## OPINION OF THE RAC

The RAC adopted the opinion that **3,7-dimethylocta-2,6-dienenitrile** should be classified and labelled as follows:

**Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)**

| | Index No | International Chemical Identification | EC No | CAS No | 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) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | TBD | 3,7-dimethylocta-2,6-dienenitrile | 225-918-0 | 5146-66-7 | Muta. 1B | H340 | GHS08 Dgr | H340 | | | |
| RAC opinion | TBD | 3,7-dimethylocta-2,6-dienenitrile | 225-918-0 | 5146-66-7 | Muta. 1B | H340 | GHS08 Dgr | H340 | | | |
| Resulting Annex VI entry if agreed by COM | TBD | 3,7-dimethylocta-2,6-dienenitrile | 225-918-0 | 5146-66-7 | Muta. 1B | H340 | GHS08 Dgr | H340 | | | |

## RAC general comment

The CLH report includes summaries of a series of in vitro and in vivo studies relating to mutagenicity classification and a toxicokinetic study conducted in mice. No endpoints other than mutagenicity are reviewed.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier submitter's proposal

3,7-Dimethylocta-2,6-dienenitrile has currently no harmonised classification in Annex VI of CLP. The Dossier Submitter (DS) has proposed to classify the substance in category 1B for mutagenicity (Muta. 1B) based on its potential to induce chromosomal damages in somatic and germ cells, as seen in studies with mice.

A study conducted in mice has shown that the substance is rapidly absorbed and distributed to the tissues, including lung, liver, kidney, adrenals, ovaries and testes.

A bacterial gene mutation test with 3,7-dimethylocta-2,6-dienenitrile was negative. In an *in vitro* chromosomal aberration test with Chinese hamster V79 cells, a clear positive effect was obtained with S9 mix at all tested doses.

Three independent *in vivo* micronucleus tests were positive for oral doses of 500 mg/kg bw up to the maximum tolerated dose of 1250 mg/kg bw. The increases in micronuclei followed a clear dose response relationship and were reproducible. There was no indication of an aneugenic activity which was however merely based on estimation of the size of micronuclei, i.e. large micronuclei were not observed. 3,7-Dimethylocta-2,6-dienenitrile therefore has the potential to damage chromosomes in bone marrow cells *in vivo*.

Similar positive findings were observed in further mouse bone marrow micronucleus tests with the isolated E- and Z-isomers of 3,7-dimethylocta-2,6-dienenitrile.

The potential of 3,7-dimethylocta-2,6-dienenitrile to induce chromosome aberrations in spermatogonial cells was investigated in a study in mice which was performed according to OECD Test Guideline (TG) 483. At the 24 h sampling time, the administration of the test substance led to evident signs of general toxicity and to a statistically significant and biologically relevant enhancement of the aberration frequencies compared to the vehicle control value. At the 48 h sampling time, there was no statistically significant increase in aberration frequencies. No reduction of the mitotic indices could be observed after treatment with the test item, indicating that the test item was not cytotoxic for spermatogonial cells. In conclusion, 3,7-Dimethylocta-2,6-dienenitrile was considered to be clastogenic to mouse spermatogonial cells under the experimental conditions of the study.

In conclusion, 3,7-dimethylocta-2,6-dienenitrile has been shown to damage chromosomes in bone marrow and spermatogonial cells in vivo. The substance has the potential to reach and interact with the genetic material of the germ cells in vivo.

### Comments received during public consultation

Three MSCAs submitted comments that were all in favour of the classification proposal.

### Assessment and comparison with the classification criteria

RAC excluded a classification in category 1A for mutagenicity based on the absence of human data.

Although this substance gave a negative result when tested for bacterial mutagenicity in *S.typhimurium* and *E.coli*, a clear positive result was obtained with exogenous S9 mix in an *in*

*vitro* chromosome aberration test with Chinese hamster V79 cells. Furthermore, as shown in three mouse bone marrow micronucleus tests, involving single or repeated oral dosing by gavage, this substance clearly has the potential to damage chromosomes in somatic cells. These studies were well conducted and each of them gave a clear, unequivocal positive result. This profile is sufficient to justify classification of 3,7-dimethylocta-2,6-dienenitrile at least in category 2 for mutagenicity.

However, there is further evidence to support a more severe classification. Firstly, following a single gavage dose of 300 or 600 mg/kg of radiolabelled 3,7-dimethyl-2,6-dienenitrile to mice, increased radioactivity was detected in a variety of tissues, including the ovaries and testes. This implies that the substance itself or its metabolite(s) could reach the germ cells and pose a mutagenic hazard there too. Secondly, confirming this, a positive result was obtained in an *in vivo* mouse spermatogonial cell chromosome aberration assay. The doses administered by oral gavage were comparable to those given in the bone marrow assays. The mean aberration frequencies (excluding gaps) at 24 h after dosing were 0.6, 0.2, 0.2, 1.2 and 2.6% with 0, 375, 750, 1250 and 1500 mg/kg 3,7-dimethyl-2,6-dienenitrile, respectively. The increase in aberration frequencies at the top dose was statistically significant ($p<0.02$).

RAC noted that there is no reason to doubt the validity of any of the positive results; no excessive toxicity/cytotoxicity was seen in any study. Given the potential of 3,7-dimethyl-2,6-dienenitrile to damage the chromosomes in germ cells under standard laboratory conditions, RAC concluded along with the proposal from the DS that classification in category 1B for mutagenicity is justified (Muta. 1B, H340).

## 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).