Committee for Risk Assessment

RAC

Annex 1

Background document

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

3,7-dimethylocta-2,6-dienenitrile

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

CLH-O-0000001412-86-26/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

04 December 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 3,7-dimethylocta-2,6-dienenitrile

EC Number: **225-918-0**

CAS Number: **5146-66-7**

Index Number: **-**

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Part A

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	3,7-dimethylocta-2,6-dienenitrile
EC number:	225-918-0
CAS number:	5146-66-7
Annex VI Index number:	none
Degree of purity:	> 95%
Impurities:	The identity of the impurities is confidential.

No registration dossiers were available for 3,7-dimethylocta-2,6-dienenitrile on 23 October 2013.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	none
Current proposal for consideration by RAC	Muta Cat. 1B H340 May cause genetic defects
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Muta Cat. 1B H340 May cause genetic defects

1.3 Proposed harmonised classification and labelling based on CLP Regulation

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				not relevant for this dossier
2.2.	Flammable gases				not relevant for this dossier
2.3.	Flammable aerosols				not relevant for this dossier
2.4.	Oxidising gases				not relevant for this dossier
2.5.	Gases under pressure				not relevant for this dossier
2.6.	Flammable liquids				not relevant for this dossier
2.7.	Flammable solids				not relevant for this dossier
2.8.	Self-reactive substances and mixtures				not relevant for this dossier
2.9.	Pyrophoric liquids				not relevant for this dossier
2.10.	Pyrophoric solids				not relevant for this dossier
2.11.	Self-heating substances and mixtures				not relevant for this dossier
2.12.	Substances and mixtures which in contact with water emit flammable gases				not relevant for this dossier
2.13.	Oxidising liquids				not relevant for this dossier
2.14.	Oxidising solids				not relevant for this dossier
2.15.	Organic peroxides				not relevant for this dossier
2.16.	Substance and mixtures corrosive to metals				not relevant for this dossier
3.1.	Acute toxicity - oral				not relevant for this dossier
	Acute toxicity - dermal				not relevant for this dossier
	Acute toxicity - inhalation				not relevant for this dossier
3.2.	Skin corrosion / irritation				not relevant for this dossier
3.3.	Serious eye damage / eye				not relevant for

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
	irritation				this dossier
3.4.	Respiratory sensitisation				not relevant for this dossier
3.4.	Skin sensitisation				not relevant for this dossier
3.5.	Germ cell mutagenicity	Muta. 1B			
3.6.	Carcinogenicity				not relevant for this dossier
3.7.	Reproductive toxicity				not relevant for this dossier
3.8.	Specific target organ toxicity –single exposure				not relevant for this dossier
3.9.	Specific target organ toxicity – repeated exposure				not relevant for this dossier
3.10.	Aspiration hazard				not relevant for this dossier
4.1.	Hazardous to the aquatic environment				not relevant for this dossier
5.1.	Hazardous to the ozone layer				not relevant for this dossier

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

Labelling: Signal word: Danger
Hazard statements: H340 May cause genetic defects

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

-

2.2 Short summary of the scientific justification for the CLH proposal

3,7-dimethylocta-2,6-dienenitrile is proposed to be classified as genotoxic Cat. 1B in accordance with the CLP regulation.

Justification:

In mice, 3,7-dimethylocta-2,6-dienenitrile caused chromosomal aberrations in somatic cells in several studies and in spermatogonial cells in one study. Additionally it was shown in a toxicokinetic study that 3,7-dimethylocta-2,6-dienenitrile can reach the reproductive tissues in male and female mice.

2.3 Current harmonised classification and labelling

no entry in Annex VI, CLP Regulation

Part B

SCIENTIFIC EVALUATION OF THE DATA

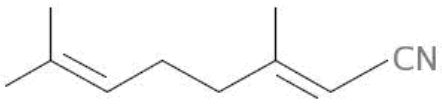
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	225-918-0
EC name:	3,7-dimethylocta-2,6-dienenitrile
CAS number (EC inventory):	5146-66-7
CAS name:	2,6-Octadienenitrile, 3,7-dimethyl-
IUPAC name:	3,7-dimethylocta-2,6-dienenitrile
CLP Annex VI Index number:	none
Molecular formula:	C10H15N
Molecular weight range:	149.2328

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
trans-isomer (CAS No. 5585-39-7)			No information is given about the content of the isomers.
cis-isomer (CAS No. 31983-27-4)			No information is given about the content of the isomers.

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Please see confidential Annex			

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Not relevant				

1.3 Physico-chemical properties

Further physico-chemical properties are not stated in the literature. Furthermore they are not needed for the current classification and labelling.

Table 8: 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	colourless – yellowish liquid	BASF AG, Safety data sheet GERANONITRILE	
Melting/freezing point			
Boiling point			
Relative density			
Vapour pressure			
Surface tension			
Water solubility			
Partition coefficient n-octanol/water			
Flash point	no data available		
Flammability	non flammable	BAM (2013)	Flammability upon ignition (solids, gases): Testing can be waived, substance is a liquid. Flammability in contact with water: The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).
Explosive properties	no explosive properties	BAM (2013)	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive properties.
Self-ignition temperature	no data available		
Oxidising properties	no oxidising properties	BAM (2013)	The classification procedure needs not to be applied because the organic substance does not contain oxygen, fluorine or chlorine.
Granulometry			
Stability in organic solvents			

and identity of relevant degradation products			
Dissociation constant			
Viscosity			

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not classified for physico-chemical properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Type of study:	**Toxicokinetics** Study according to guidelines OECD 417, 87/302/EEC, 40 CFR 870.7485 and GLP
Reference:	RIFM 2005
Test substance:	3,7-dimethylocta-2,6-dienenitrile (^{14}C labeled)
Species and strain:	mouse (CD-1) male and female
Doses, vehicle, duration:	single oral administration (gavage) of 300 or 600 mg/kg bw (bw) in corn oil (4-8 ml/kg bw) The actual mean radioactive doses ranged from 15.8 to 30 μCi/mouse.
Result:	3,7-dimethylocta-2,6-dienenitrile (geranonitrile, GN) undergoes rapid absorption after oral gavage administration. Peak concentrations of 14C residues in plasma occurred within 0.5 to 1 hour. Clearance from the plasma was also rapid as a result of extensive tissue distribution and biotransformation. Kinetic saturation was evident between the 300 and 600 mg/kg dose levels. Three major plasma metabolites were identified as 8-hydroxy-GN, 8-hydroxy-GN-glucuronide, and 8-carboxy-GN. The half-lives of the 14C residues (4.4-5.9 hours) were consistent with those for the metabolites (2.2-6.9 hours). Material balance indicated minimal excretion of 14C residues by exhalation, with the majority of the dose recovered in urine and feces. Total mean absorption ranged from 41 to 88% and appeared to be slightly higher in female than male mice. Minimum residues (<2% of the dose) were retained in tissues by 48 hours after dose administration. The tissue distribution also indicated saturation kinetics with minimal differences between sexes. Lung, liver, kidney, and adrenals of male and female and ovaries of female mice had slightly higher concentrations than plasma (less than 10-fold) by 48 hours after administration whereas testes concentrations were similar to plasma. The overall metabolism of GN was characterized by oxidation on the C8-position to form the alcohol, the aldehyde, the acid, and an epoxide (6,7-GNO). Further phase II metabolism was evident by conjugation with glucuronide, glutathione, and amino acids identified in plasma, urine and feces.

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

3,7-dimethylocta-2,6-dienenitrile is rapidly absorbed and distributed to the tissues. It was detected in lung, liver, kidney, adrenals, ovaries and testes. 3,7-dimethylocta-2,6-dienenitrile is metabolized forming alcohol, the aldehyde, the acid, and an epoxide an and conjugated to glucuronide, glutathione, and amino acids. It is excreted mainly via urine and feces.

4.2 Acute toxicity

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

4.4 Irritation

4.5 Corrosivity

4.6 Sensitisation

4.7 Repeated dose toxicity

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

4.9 Germ cell mutagenicity (Mutagenicity)

Table 9: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Bacterial genotoxicity test	negative	for details see section 4.9.1.1	BASF (1999)
In vitro chromosome aberration assay	positive	for details see section 4.9.1.1	BASF (2002)
In vivo micronucleus assay in bone marrow	positive	for details see section 4.9.1.2	BASF (2003a)
In vivo micronucleus assay in bone marrow	positive	for details see section 4.9.1.2	BASF (2003b)
In vivo micronucleus assay in bone marrow	positive	for details see section 4.9.1.2	BASF (2004a)
In vivo micronucleus assay in bone marrow	positive	Study with (E)-Isomer for details see section 4.9.1.2	BASF (2004b)
In vivo micronucleus assay in bone marrow	positive	Study with (Z)-Isomer for details see section 4.9.1.2	BASF (2004c)
In vivo chromosome aberration assay in spermatogonial cells	positive	for details see section 4.9.1.2	RCC-CCR (2006)

4.9.1 Non-human information

4.9.1.1 In vitro data

Type of study: **Bacterial genotoxicity test**

	Study according to OECD 471 under GLP conditions
Reference:	BASF (1999)
Test substance:	3,7-dimethylocta-2,6-dienenitrile, purity 97.3 %
Species and strain:	Salmonella typhimurium TA1535, TA100, TA1537, TA98, Escherichia coli WP2 uvrA
Doses:	20 – 5,000 µg/plate (SPT) 20 – 2,500 µg/plate (PIT)
Metabolic activation:	With and without S-9 mix
Result:	**negative**
	Standard plate test (SPT) and preincubation test (PIT). No precipitation of the test substance was found. A bacteriotoxic effect was observed under all test conditions (SPT: 500 - 1,000 µg/plate; PIT: 100 – 500 µg/plate)). An increase in the number of revertants was not observed in the SPT or in the PIT with or without S-9 mix.
Type of study:	**Chromosome aberration assay *in vitro*** Study according to OECD 473 under GLP conditions
Reference:	BASF (2002)
Test substance:	3,7-dimethylocta-2,6-dienenitrile, purity 98.9 %
Species and strain:	V79 cells
Doses:	0 – 1,000 µg/ml
Metabolic activation:	With and without S-9 mix
Result:	**positive**
	The test substance caused a clear, statistically significant and dose-dependent increase in the number of structurally aberrant metaphases incl. and excl. gaps after adding a metabolizing system. No increase in the frequency of cells containing numerical aberrations was demonstrated. The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line. Both positive control chemicals, i.e. EMS and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations. 3,7-Dimethylocta-2,6-dienenitrile was considered to be a chromosome-damaging (clastogenic) agent under in vitro conditions in V79 cells.

4.9.1.2 In vivo data

Key studies

Type of study:	**Micronucleus assay (chromosome aberration)** OECD Guideline 474 under GLP
Reference:	BASF (2003a)
Test substance:	3,7-dimethylocta-2,6-dienenitrile, purity 98.8%
Species and strain:	mouse (NMRI), male, 5 animals per group
Doses, vehicle, duration:	single oral administration (gavage) 312.5, 625.0, 1250.0 mg/kg bw in olive oil
Result:	**positive** The administration of the test substance led to evident signs of toxicity (squatting posture, poor general state, eyelid closure, hyperacitivity) and to a statistically significant and dose-dependent increase in the number of polychromatic erythrocytes (PCEs) containing small micronuclei (MN). The positive response was observed in two experiments carried out independently of each other. - 1st experiment (24 h interval): 1.8, 1.2, 4.5** and 6.0** MN in 1,000 PCEs at 0, 312.5, 625 and 1,250 mg/kg (** $p < 0.01$) - 1st experiment (48 h interval): 1.6 and 6.3* MN per 1,000 PCEs at 0 and 1,250 m/kg (* $p < 0.05$). - 2nd experiment (interval 24 h): 1.3 and 6.9** MN per 1,000 PCEs at 0 and 1,250 m/kg (** $p < 0.01$). There was no increase in cells with large micronuclei. An inhibition of erythropoiesis determined from the ratio of PCEs to normochromatic erythrocytes (NCE) was detected at 1,250 mg/kg after a sacrifice interval of 48h. Both of the positive control chemicals (cyclophosphamide and vincristine), led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei.

Type of study:	**Chromosome aberration assay in spermatogonial cells** 9th addendum to the OECD guidelines for testing of chemicals, section 4, Nr. 483, accepted July 21st 1997, "Mammalian Spermatogonial Chromosome Aberration Test" under GLP
Reference:	RCC-CCR (2006)
Test substance:	3,7-dimethylocta-2,6-dienenitrile, purity 99.3 %

Species and strain:	mouse (NMRI) male, 6 animals per group
Doses, vehicle, duration:	single oral administration (gavage) 375, 750, 1,250 and 1,500 mg/kg bw in corn oil
Result:	**positive**

The administration of the test substance led to evident signs of toxicity and to a statistically significant and biologically relevant enhancement of the aberration frequencies as compared to the vehicle control value (0.6% excluding gaps). The mean aberration frequencies (excl. gaps) observed after treatment with 3,7-dimethylocta-2,6-dienenitrile were:

- 24h sampling time:
  0.2, 0.2, 1.2, 2.6* % at 375, 750, 1250 and 1500 mg/kg bw (* $p < 0.02$)

- 48h sampling time:
  1.2 % at 1500 mg/kg bw.

Only the observed increase at 24h / 1500 mg/kg bw was statistically significant.

The positive control (Adriblastin, 5 mg/kg bw) showed the expected statistically significant response (aberration rate excl. gaps 5.2%).

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.

Supporting studies

Type of study:	**Micronucleus assay (chromosome aberration)** OECD Guideline 474 under GLP conditions, however report does not have GLP status (screening study with a different batch of 3,7-dimethylocta-2,6-dienenitrile).
Reference:	BASF (2003b)
Test substance:	3,7-dimethylocta-2,6-dienenitrile, purity 98.6 %
Species and strain:	mouse (NMRI) male, 5 animals per group
Doses, vehicle, duration:	single oral administration (gavage) 625 and 1,250 mg/kg bw in olive oil
Result:	**positive**

The administration of the test substance led to evident signs of toxicity and to a statistically significant and dose-dependent increase in the number of polychromatic erythrocytes (PCEs) containing small micronuclei (only 24 h interval investigated):
2.5, 3.5 and 8.1** MN in 1,000 PCEs at 0, 625 and 1,250 mg/kg
(** $p < 0.01$)
There was no increase in cells with large micronuclei.
No inhibition of erythropoiesis determined from the ratio of PCEs to normochromatic erythrocytes (NCE) was detected. The positive control (cyclophosphamide) led to the expected increase in the rate of polychromatic

erythrocytes containing small micronuclei.

Type of study:	**Micronucleus assay (chromosome aberration)** OECD Guideline 474 under GLP, only two doses tested
Reference:	BASF (2004a)
Test substance:	3,7-dimethylocta-2,6-dienenitrile, purity 99.3 %
Species and strain:	mouse (NMRI) male, 5 animals per group
Doses, vehicle, duration:	1) 600, 1200 mg/kg bw; 3 administrations within 24 hours at intervals of 12 hours (3x200 mg/kg or 3x400 mg/kg) in olive oil 2) single oral administration (gavage) of 0, 1,200 mg/kg bw in olive oil
Result:	**positive** The administration of the test substance led to evident signs of toxicity. A slight, but statistically significant increase in the number of polychromatic erythrocytes (PCEs) containing small micronuclei was observed: 1) 3 Applications (6 h interval): 1.4, 4.4** and 4.3 MN in 1,000 PCEs at 0, 200 and 400 mg/kg (** $p < 0.01$) - 3 Applications (24 h interval): 1.5, 4.5** and 4.6** MN in 1,000 PCEs at 0, 200 and 400 mg/kg (** $p < 0.01$) 2) Single application (24 h interval): 1.5 and 6.6** MN in 1,000 PCEs at 0 and 1,200 (** $p < 0.01$) There was no increase in cells with large micronuclei. The positive controls (cyclophosphamide, vincristine) led to the expected increase in the rate of PCEs containing small and large micronuclei. Inhibition of erythropoiesis determined from the ratio of PCEs to normochromatic erythrocytes (NCE) was detected at 200 and 400 mg/kg bw.

Type of study:	**Micronucleus assay (chromosome aberration)** OECD Guideline 474 under GLP
Reference:	BASF (2004b)
Test substance:	E-Geranonitrile, (E)-3,7-Dimethyl-2,6-octadienenitrile (CAS No.: 5585-39-7), purity 99.2 %
Species and strain:	mouse (NMRI) male, 5 animals per group
Doses, vehicle, duration:	single oral administration (gavage) 250, 500, 1000 mg/kg bw in olive oil
Result:	**positive** The administration of the test substance led to evident signs of toxicity and to a statistically significant and dose-dependent increase in the number of polychromatic erythrocytes (PCEs) containing small micronuclei: - 24 h interval: 1.9, 2.0, 5.4* and 6.2** MN in 1,000 PCEs at 0, 250, 500 and 1,000 mg/kg (* $p < 0.05$, ** $p < 0.01$) - 48 h interval: 1.2 and 5.8** MN in 1,000 PCEs at 0 and 1,000 mg/kg

	(** $p < 0.01$) There was no increase in cells with large micronuclei. Inhibition of erythropoiesis determined from the ratio of PCEs to normochromatic erythrocytes (NCE) was detected at 250 mg/kg (24 h interval) and 500 mg/kg (48 h interval). The positive controls (cyclophosphamide, vincristine) led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei.
Type of study:	**Micronucleus assay (chromosome aberration)** OECD Guideline 474 under GLP
Reference:	BASF (2004c)
Test substance:	Z-Geranonitrile, (Z)-3,7-dimethylocta-2,6-dienenitrile (CAS No.: 31983-27-4), purity 99.5 %
Species and strain:	mouse (NMRI) male, 5 animals per group
Doses, vehicle, duration:	single oral administration (gavage) 500, 1,000 and 2,000 mg/kg bw in olive oil
Result:	**positive** The administration of the substance led to evident signs of toxicity and to a statistically significant and dose-dependent increase in the number of polychromatic erythrocytes (PCEs) containing small micronuclei: - 24 h interval: 1.9, 2.8, 5.0* and 8.2** MN in 1,000 PCEs at 0, 500, 1,000 and 2,000 mg/kg bw (* $p < 0.05$, ** $p < 0.01$) - 48 h interval: 1.2 and 7.8** MN in 1,000 PCEs at 0 and 2,000 mg/kg bw (** $p < 0.01$) There was no increase in cells with large micronuclei. Inhibition of erythropoiesis determined from the ratio of PCEs to normochromatic erythrocytes (NCE) was detected at 2,000 mg/kg bw (48 h interval). The positive controls (cyclophosphamide and vincristine) led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei.

4.9.2 Human information

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

A bacterial gene mutation test with 3,7-dimethylocta-2,6-dienenitrile was negative. In an in vitro chromosomal aberration test a clear positive effect was obtained with S-9 mix at all tested doses. Cytotoxicity was not observed.

Three valid 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 clastogenic effects followed a clear dose response and were reproducible. There were no indications of an aneugenic activity which was however merely based on estimation of the size of micronuclei, i.e. large micronuclei were not observed.

It is concluded that 3,7-Dimethylocta-2,6-dienenitrile is clastogenic (chromosome-damaging) in bone marrow cells in vivo.

Similar effects were observed in further valid tests with the isolated E- and Z-isomers which were as well proved to be clastogenic in bone marrow cells in vivo.

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 TG 483. At 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 as compared to the vehicle control value. At 48 h sampling time, there was no statistically significant increase. No cytotoxicity occurred. In conclusion, 3,7-Dimethylocta-2,6-dienenitrile was considered to be clastogenic to mouse spermatogonial cells under the experimental conditions of the study.

3,7-dimethylocta-2,6-dienenitrile is clastogenic (chromosome-damaging) agent in bone marrow and spermatogonial cells in vivo.

4.9.5 Comparison with criteria

A toxicokinetic investigation indicated that 3,7-dimethylocta-2,6-dienenitrile is metabolized leading to formation of the corresponding alcohol, aldehyde and an epoxide. It was also shown that 3,7-dimethylocta-2,6-dienenitrile can reach the reproductive tissues.

The available information on genotoxicity indicates that 3,7-dimethylocta-2,6-dienenitrile can cause chromosomal aberrations in somatic cells in vivo. In addition to the in vivo somatic cell genotoxicity it was clearly shown that 3,7-Dimethylocta-2,6-dienenitrile can cause chromosomal aberrations in spermatogonial cells in vivo and is therefore considered to be genotoxic to germ cells.

Classification as genotoxic Cat. 2 would be insufficient as there is evidence that 3,7-dimethylocta-2,6-dienenitrile can cause not only mutations in somatic cells but also in germ cells *in vivo*. 3,7-Dimethylocta-2,6-dienenitrile has the potential to reach and interact with the genetic material of germ cells *in vivo* and should be regarded as if it causes heritable mutations in germ cells in humans. Therefore classification as genotoxic Cat. 1B is warranted.

Evidence from human epidemiological studies is not available. Therefore classification is as Cat. 1A is not warranted.

4.9.6 Conclusions on classification and labelling

3,7-dimethylocta-2,6-dienenitrile is proposed to be classified as genotoxic Cat. 1B according to the regulation EC No.1272/2008 (H340 May cause genetic defects).

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).

4.10 Carcinogenicity

4.11 Toxicity for reproduction

4.12 Other effects

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

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8 ANNEXES

APPENDIX I: confidential data