Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at Community level of

Chloroform

ECHA/RAC/DOC No CLH-O-0000001739-64-01/F

Adopted

10 June 2011

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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: ***Chloroform***

EC Number: **200-663-8**

CAS Number: **67-66-3**

The proposal was submitted by ***France***
and received by RAC on **30 April 2010**

The proposed harmonised classification originally proposed by the dossier submitter:

	Regulation (EC) No 1272/2008	Directive 67/548/EEC (criteria)
Current entry in Annex VI CLP Regulation	Carc. 2 – H351 Acute Tox 4* – H302 STOT RE 2* – H373** STOT RE 2* – H373** Skin Irrit. 2 – H315	Xn; R22-48/20/22 Xi; R38 Carc. Cat. 3; R40
Specific concentration limits M-factors	*STOT RE 2 – H373: C≥ 5 **%**	Xn; R22: C ≥ 5% Xn; R48/20/22: C ≥ 5%
Proposal for consideration by RAC from dossier submitter	Carc. 2 – H351 Muta. 2 – H341 Repr. 2 – H361d Acute Tox. 3 – H331 Acute Tox. 4 – H 302 STOT RE 1 – H 372 Eye Irrit. 2 – H319 Skin Irrit. 2 – H315	Xn; R20/22 Xn; R48/20 Xi ; R36/38 Muta cat. 3; R68 Carc. Cat. 3; R40 Repr. Cat. 3; R63
Resulting harmonised classification (future entry in Annex VI of CLP Regulation) as proposed by dossier submitter	Carc. 2 – H351 Muta. 2 – H341 Repr. 2 – H361d Acute Tox. 3 – H331 Acute Tox. 4 – H 302 STOT RE 1 – H 372 Eye Irrit. 2 – H319 Skin Irrit. 2 – H315	Xn; R20/22 Xn; R48/20 Xi ; R36/38 Muta cat. 3; R68 Carc. Cat. 3; R40 Repr. Cat. 3; R63
Specific concentration limits, M-factors	None	None

PROCESS FOR ADOPTION OF THE OPINION

France 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 30 April 2010. Parties concerned and MSCAs were invited to submit comments and contributions by 14 June 2010.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Normunds Kadikis***
Co-rapporteur, appointed by RAC: ***Alicja Andersson***

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 ***10 June 2011,*** 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 ***simple majority;*** one RAC member expressed a minority position regarding the RAC assessment for germ cell mutagenicity. The minority position, including its grounds, was made available in a separate document which has been published at the same time as the opinion

OPINION OF RAC

The RAC adopted the opinion that *chloroform* should be classified and labelled as follows:

Classification & Labelling in accordance with the CLP Regulation

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard state-ment Code(s)	Pictogram, Signal Word Code(s)	Hazard state ment Code(s)	Suppl. Hazard statement Code(s)		
602-006-00-4	**chloroform trichloromethane;**	**200-663-8**	**67-66-3**	Carc. 2 Repr. 2 Acute Tox. 3 Acute Tox. 4 STOT RE 1 Eye Irrit. 2 Skin Irrit. 2	H351 H361d H331 H302 H372 H319 H315	GHS06 GHS08 Dgr	H351 H361d H331 H302 H372 H319 H315			

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

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
602-006-00-4	**chloroform trichloromethane;**	**200-663-8**	**67-66-3**	Xn; R20/22 Xn; R48/20 Xi ; R36/38 Carc. Cat. 3; R40 Repr. Cat. 3; R63	Xn R:20/22-36/38-40-48/20-63-S: 2-36/37		

SCIENTIFIC GROUNDS FOR THE OPINION

The opinion relates only to those hazard classes that have been reviewed in the proposal for harmonised classification and labelling, as submitted by *France*. Chloroform was on the 2nd priority list of the Existing Substances Regulation and its classification was reviewed in the context of the Risk Assessment procedure as it was a requirement to harmonise classification for all endpoints. Classification of chloroform in all the hazard classes presented in the CLH dossier except mutagenicity was agreed by TCC&L in September 2007.

During the public consultations the comments received related to the proposed classification for mutagenicity. In addition two comments, one in favour and one not in favour regarded the CLP classification corresponding to Xn; R48/20 as agreed by the TCC&L in 2007.

Carcinogenicity

Studies in animals reveal that chloroform can cause an increased incidence of kidney tumors in male rats or mice and an increased incidence of liver tumors in mice of either sex. These induced tumors responses are postulated to be secondary to sustained or repeated cytotoxicity and secondary regenerative hyperplasia, according to the dose levels tested. The weight of evidence in genotoxicity studies is consistent with the hypothesis that the liver and kidney tumors induced depend on persistent cytotoxic and regenerative cell proliferation responses. The persistent cell proliferation presumably would lead to higher probabilities of spontaneous cell mutation and subsequent cancer (US EPA, 2001).

RAC supports the proposal from the dossier submitter to classify chloroform as Carc Cat 2 – H351 (Carc Cat 3; R40). This classification was agreed at TC C&L in September 2007.

Germ Cell Mutagenicity

Assessment of the original information presented by the dossier submitter

Results from studies *in vitro* are generally negative, although studies that produced positive results occur sporadically for the different endpoints tested in assays for gene mutation in bacteria, gene mutation in fungi, gene mutation in mammalian cells, chromosome aberration in plants, aneuploidy in fungi, aneuploidy in mammalian cells, DNA repair in bacteria, DNA repair in mammalian cells, primary DNA damage in plants and primary DNA damage in mammalian cells.

The 17 *in vivo* key studies presented in the dossier were chosen by the dossier submitter on the basis that they could be ascribed a reliability code of 1 or 2 according to the Klimisch scoring system. Ten of these studies measured permanent transmissible changes, i.e. mutations (micronuclei, 6 studies; chromosome aberrations, 3 studies; gene mutations, 1 study), and such studies are generally considered to be of higher significance in weight-of-evidence analyses of mutagenicity than the results of indicator tests measuring induced damage to DNA (but not direct evidence of mutation) via effects such as DNA repair or DNA strand breaks, and studies measuring DNA-binding ability. Six of the *in vivo* studies in the

dossier use indicator tests (DNA strand breaks, 1 study; sister-chromatid exchange, 1 study; DNA repair, 1 study; DNA-binding ability, 3 studies). Finally, the dossier includes an *in vivo* study of regenerative cell proliferation in liver and kidney, but these data are not relevant for the evaluation of the mutagenicity of chloroform

According to the dossier submitter two of the six *in vivo* micronucleus studies were positive. The study by Shelby and Witt (1995) involved two experiments in which mice were exposed to chloroform by intraperitoneal injection. In both experiments a statistically significant dose-related increase in micronucleated polychromatic erythrocytes (PCEs) was observed in the bone marrow. The study by Robbiano et al. (1998) established a statistically significant 3.3-fold increase in micronucleated cells in the kidney of rats after a single oral administration of chloroform (472 mg/kg bw). Four *in vivo* micronucleus studies were negative. The studies by Gocke et al. (1981), Tsuchimoto and Matter (1981) and Salamone et al (1981) showed no increase in micronucleated PCEs in the bone marrow of mice following intraperitoneal injection of chloroform, and the study by Whitwell (2009) showed no increase in micronucleated PCEs in the bone marrow of rats following oral administration of chloroform.

According to the dossier submitter two of the three *in vivo* chromosome aberration studies produced positive results. The study by Fujie et al. (1990) involved experiments in which chloroform was administered to rats either intraperitoneally or orally. For both routes of administration a statistically significant dose-related increase in the frequency of cells with chromosome aberrations was established. The study by Hoechst et al. (1988) showed a statistically significant dose-related increase in the frequency of cells with chromosome aberrations in the bone marrow of Chinese hamsters in one of the two experiments performed, and an accumulation of heavily damaged cells (exchanges, multiple aberrations, chromosome disintegration) at higher doses in both experiments. One *in vivo* chromosome aberration study was considered to be negative (Shelby and Witt, 1995), although a statistically significant dose-related increase was observed in one of the two experiments in which mice were exposed to chloroform by intraperitoneal injection.

The *in vivo* gene mutation study by Butterworth et al. (1998) in hepatocytes of mice exposed to chloroform by inhalation showed no increase in *LacI* mutant frequency.

The dossier submitter also presented a possible explanation for the mutagenic effect of chloroform suggesting an indirect genotoxic mechanism that requires metabolism for its toxicity. Chloroform can undergo both oxidative and reductive metabolism in the human liver depending on oxygen and substrate concentration. The required step for $CHCl_3$-induced toxicity is the cytochrome P450 (P450)-mediated bioactivation to reactive metabolites. Extensive *in vitro* and *in vivo* studies on rodents have demonstrated that chloroform may be metabolised oxidatively to trichloromethanol, which spontaneously decomposes to the electrophilic **phosgene** ($COCl_2$). $COCl_2$ is highly reactive and binds covalently to cell components containing nucleophilic groups, including proteins, phospholipid's polar heads, and reduces glutathione (Gemma *et al*., 2003). The decrease of GSH levels by chloroform and/or phosgene will decrease protective levels of GSH. This could increase oxidative stress and

probably reactive oxygen species production. These free radicals could bind to DNA and contribute to genotoxicity.

Assessment of the information received during the public consultation

No new information was submitted. Only comments reflecting different interpretation of the already existing information were provided.

Four Member States have submitted comments during the public consultation on the proposal to classify chloroform with R68 Muta Cat 3 (67/548/EEC) and Muta. 2 – H341 (CLP). Denmark, Germany and Sweden support the proposal, while Ireland is not in agreement with the proposed classification. Industry (ECSA) has also submitted comments and does not support the proposed classification.

Comparison of available information with the criteria for Germ Cell Mutagenicity

RAC conclusion

RAC has performed a detailed evaluation of the *in vivo* studies in the dossier which reduced the number of studies to be considered as relevant and reliable for the evaluation of the *in vivo* mutagenicity of chloroform (see the Background Document for detailed evaluation) to the ones:

- Fujie *et al.*1990, study on induction of chromosome aberrations in Long-Evans Rats
- Hoechst *et al.* 1988, study on induction of chromosome aberrations in Chinese hamster
- Shelby and Witt 1995, study on induction of chromosome aberrations in B6C3F1 mice
- Shelby and Witt 1995, study on induction of micronuclei in B6C3F1 mice
- Whitwell 2009, study on induction of micronuclei in Sprague Dawley Rats.

The following studies were subject to the weight of evidence assessment summarized in the table below.

Reference	Study	Route of exposure	Doses	Animal species and strain	Cyto-toxicity	Results	Comments
Fujie *et al.*1990	Induction of chromosome aberrations	i.p.	0, 1.2, 11.9 and 119.4 mg/kg bw	Long-Evans Rats	Not measured	Dose-related effect within the range 0-11.9 mg/kg bw (Experiment I) and within the range 0-119.4 mg/kg bw	Effects in other studies were induced by concentration of a few magnitudes higher. High doses in a

						(Experiment II)	number of negative studies gave no effect. The conditions of the experiment do not allow determination of clear time and dose related relationships. Could be some effect of cytotoxicity.
Hoechst *et al.* 1988	Induction of chromosome aberrations	Oral	0, 40, 120 and 400 mg/kg bw	Chinese hamster	Not reported	Occurrence of heavily damaged cells without determination of dose-related relationships; weak effect within the range 0-400 mg/kg bw (Experiment II)	The presence of heavily damaged cells was not replicated in other tests. The conditions of the experiment do not allow determination of clear time and dose related relationships.
Shelby and Witt 1995	Induction of chromosome aberrations	i.p.	0, 200, 400, 800, 1000 mg/kg bw	B6C3F1 mice	Not measured	**No effect** in two experiments of three	In one positive experiment within the range 0-400 mg/kg bw untypically low value of the untreated control group
Shelby and Witt 1995	Induction of micronuclei	i.p.	0, 200, 400 and 800 mg/kg bw	B6C3F1 mice	Not reported	**Effect** in all concentration ranges tested with dose-related relationships however very weak response	Confirmed in two experiments but the effect very weak and could be the response to cytotoxicity.
Whitwell 2009	Induction of micronuclei	Oral	0, 120, 240 and 480 mg/kg bw	Sprague Dawley Rats	Measured and demonstrated at >480 mg/kg bw level	**No effect**	One experiment performed

RAC acknowledges that results from studies *in vitro* are generally negative and that data on *vivo* studies are not coherent, as shown in the table above.

Based on generally negative results in *in vitro* studies, negative DNA binding experiments as well as non coherent results from *in vivo* studies regarding chromosome aberration and micronuclei, RAC concludes that body of evidence does not support the classification of chloroform as a mutagen according to CLP and DSD criteria.

Reproductive Toxicity

Concerning developmental toxicity, epidemiological studies of chloroform in drinking water suggest an association between exposure to chloroform and reduced foetal weight, stillbirth, chromosomal abnormalities and cleft defects. Otherwise, we need to keep in mind that many epidemiological studies present limitations like the use of water concentration as the measure of exposure, co-exposure with other THM or Disinfection By-Product, which can lead to exposure misclassification.

By inhalation, the effects of chloroform on the various animals tested include effects on pregnancy rate, resorption rate, litter size and live foetuses, foetal weight and CRL, as well as skeletal and gross abnormalities or variations. However, maternal toxicity has been evidenced with the developmental effects reported in these studies.

Considering the effects evidenced in human and animal studies, RAC supports the proposal from the dossier submitter to classify chloroform as Repr Cat2 – H361d (Repr Cat 3; R63). This classification was agreed at TC C&L in September 2007.

Acute Toxicity: oral

Kidney damage induced in male mice is related to very sensitive strain (C3H/Tif), thus it is not considered relevant for acute toxicity classification. Due to oral $200 < LD_{50} \leq 2000$mg/kg for rats, female mice (C3H/Tif) or mice of other strains, classification of chloroform as Acute Tox 4 is justified.

There is no need to maintain the specific concentration limits of the 19th ATP.

RAC supports the proposal from the dossier submitter to classify chloroform as Acute Tox 4 – H302 (R22) and deletion of the specific concentration limits (SCLs). Both the classification and the deletion of SCLs were agreed at TC C&L in September 2007.

Acute Toxicity: Inhalation

Based on inhalation $2 < LC_{50} \leq 20$ mg/l for mice and rats classification of chloroform as Acute Tox 3 (R20) is justified.

RAC supports the proposal from the dossier submitter to classify chloroform as Acute Tox 3 – H331. This classification was agreed at TC C&L in September 2007.

The dossier submitter proposed to classify chloroform also for STOT SE 3 H336 to cover the narcotic effects of the substance. Although these effects are well recognised specific data related to this effect were not presented in the CLH dossier.

Skin irritation

Based on the rabbit study and on the previous classification, classification of chloroform as Skin Irrit.2 (R38) is justified.

RAC supports the proposal from the dossier submitter to classify chloroform as Skin Irrit 2 – H315. This classification was agreed at TC C&L in September 2007.

Eye Irritation

Based on the rabbit studies reporting corneal injury and human data showing reversible corneal effects, classification of chloroform as Eye Irrit 2 (R36) is justified.

RAC supports the proposal from the dossier submitter to classify chloroform as Eye Irrit 2 – H319. This classification was agreed at TC C&L in September 2007.

Repeated Dose Toxicity: Inhalation

Based on renal and severe nasal effects observed in rats and mice at concentrations below 0.2 mg/litre/6h/day, which is the cut-off values given in paragraph 3.9.2.9.6 of Annex I of CLP (see table 3.9.2) the criteria for STOT RE 1 –H372 1 are met.

According to Directive 67/548/EEC renal and severe nasal effects on mice and rats at concentrations $\leq$ 250 mg/m^3, justify application of R48/20: danger of serious damage to health by prolonged inhalation exposure.

There is no need to maintain the specific concentration limits of the 19th ATP.

RAC supports the proposal from the dossier submitter to classify chloroform as STOT RE 1 –H372 (R48/20) and delete SCLs. Both the classification and deletion of SCLs were agreed at TC C&L in September 2007.

Additional information

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

ANNEXES:

Annex 1	Background Document (BD)[1]
Annex 2	Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information)

[1] 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.