Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Chloroform

ECHA/RAC/DOC No CLH-O-0000001739-64-01/A2

Adopted

10 June 2011

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

[ECHA has compiled the comments received via internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensive as possible. Please note that some of the comments might occur under several headings when splitting the given information is not reasonable.]

Substance name: Chloroform
CAS number: 67-66-3
EC number: 200-663-8

General comments

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
27/05/2010	UK / Desmond Waight / Individual	The classification proposal as indicated on the ECHA website was not the same as found in the document (eg no mention of CARC. The CLP proposals info has spurious brackets. The French dcument fails to how that none of the PHYSICAL classes/categories are applicable (so why then show that Env classes/categories are not applicable?) Industry is to be required under CLI to address all endpoints and so should the CAs. The French document fails to give a proposed CLP Labelling (but does for DSD system) though I accept that P statements would not be appropriate.	The registry of intention is a way to inform third parties on the intention of a member state to work on a substance. The classification proposal is the document in which (and for which) classification is defined after review of available data. Contrarily to biocides and pesticides, classification proposals for chemicals are intended to be targeted. Moreover, covering other endpoints than CMR, Resp sensitiser requires a justification on the need to carry an action at the European level.	No further comments.
11/06/2010	Belgium / Wolfgang Marquardt / ECSA - European Chlorinated Solvent Association /	Important: The specific comments mentioned below are better represented in attached Word-document because our comments include as well tables, references, annexes and corrected typos that were found in the French proposal on C&L for Chloroform. These		

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	Industry or trade association	comments are not shown correctly in the simple text boxes below. We strongly recommend to use the attached 9-page Word-document including two PDF-Annexes as relevant comment from ECSA. *Attachment: 2010-06-10-COMMENTS FROM ECSA ON THE CLASSIFICATION OF CHLOROFORM*		
14/06/2010	Denmark / Peter Hammer / Danish EPA / Denmark	Agreed with the classification agreed by TC C&L. No further comments to that.	Thanks for your support.	Agree.

Carcinogenicity

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
14/06/2010	Denmark / Peter Hammer / Danish EPA / Denmark	Agreed	Thanks for your support.	Agree.

Mutagenicity

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
08/06/2010	Germany / Mark Schwägler / MS	German CA: Some in vivo studies on somatic cells showed positive results (micronucleus, chromosomal aberration). These effects were mainly limited to high doses, weakly expressed and contrasted by negative studies. A mechanism of indirect genotoxicity is considered to be plausible, but a quantitative threshold for genotoxicity is not clear. Overall a weak genotoxic potential in somatic cells should be assumed. In addition chloroform likely reaches the gonads. Therefore chloroform can be suspected to exert genetic effects also in germ cells.	Thanks for your support.	**General comment:** A detailed evaluation (in relation to the test requirements according to the OECD test guidelines for mutagenicity testing) of the studies provided in the dossier was performed by RAC. Based on this evaluation some of the studies were considered, in

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		The proposal to classify with R 68 Muta Cat 3 (67/548/EEC) and Muta. 2 – H341 (CLP) is supported.		contrast with the opinion of the Dossier Submitter, to be of unacceptable quality and were not included in the further evaluation of mutagenicity of the substance. For detailed assessment please see the chapter 5.7.3. of the Background Document. The following studies were chosen as key studies for further assessment: • Fujie et al.1990, study on induction of chromosome aberrations in Long-Evans Rats (seemingly positive according to dossier submitter) • Hoechst et al. 1988, study on induction of chromosome aberrations in Chinese hamster (seemingly positive according to dossier submitter) • Shelby and Witt 1995, study on induction of chromosome aberrations in

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				B6C3F1 mice (negative) • Shelby and Witt 1995, study on induction of micronuclei in B6C3F1 mice (seemingly positive according to dossier submitter) • Whitwell 2009, study on induction of micronuclei in Sprague Dawley Rats (negative) Seemingly positive results are controversial or can be explained by cytotoxicity which is not measured or not reported. The available *in vitro* and *in vivo* data do not provide any clear pattern of strain or species differences in order to justify the role of genetic variations for explanation of negative or positive results Therefore we do not support the classification for mutagenicity. For detailed reasoning, please see in the Table 30 of the Background

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				Document.
11/06/2010	Belgium / Wolfgang Marquardt / ECSA - European Chlorinated Solvent Association / Industry or trade association	MUTAGENICITY CLASSIFICATION Context In 2007, after the submission of the Risk Assessment Report for chloroform, the Rapporteur[1] Member States France has proposed to classify chloroform as mutagen category 3 (R68). Since this proposal was extensively discussed among the Member States and was also challenged by Industry (ECSA), a more deeply critical review was asked to France on the in vitro and in vivo data available for the genotoxicity of chloroform. On the basis of the review provided by the French Authorities, two independent experts commissioned by ECSA showed inconsistencies in the methods and/or results of some studies considered as pivotal for the mutagenicity classification of chloroform (see Annexes 1 and 2). At the Technical Committee IV of December 2007, the executive committee reached to a conclusion (i) giving the opportunity to the Industry to conduct a new study. It was requested that the study design was jointly developed with the French Authorities in order to establish the validity of the positive results. The French Authorities circulated the protocol for the last Technical Committee in April 2008 (i.e. "In vivo micronucleus assay in rat bone marrow after 5 daily administrations of chloroform by oral route") for comments by the other Member States. However, during this meeting and following a long discussion, the commission decided to reverse its decision and concluded that it would not ask Industry to conduct further mutagenicity testing. Nevertheless, ECSA decided to perform the assay using the protocol drafted jointly with the French Authorities. This study has been placed in a well-known laboratory having all necessary techniques in house and working under the Good	As mentioned in your comment, the test was performed against the commission's advice. At the time, most of the countries believed there were sufficient data for classification.	See general comment above

[1] Note that the term "Rapporteur" used in this comment by the ECSA refers to the MSCA and not to the Rapporteur, formally appointed by RAC.

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		Laboratory Practices and OECD guidelines (Covance Laboratories Ltd). The results of this study (Whitwell, 2009) concluded that chloroform did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male and female rats treated up to 480 mg/kg/day (the estimated Maximum Tolerated Dose (MTD)) for five consecutive days, under the experimental conditions employed. However, it appears that the summary written by the submitter France in the CLH report for the "proposal for harmonised classification and labelling of chloroform" leads to a bad interpretation of the study and its results. Thus, Industry would like to comment and correct some findings. Discussion of the Whitwell study results Clinical signs and body weight: the Rapporteur argues that clinical sings are too mild and cites the Annex V of the Directive 67/584/EEC (Part B; Methods for the determination of toxicity and other health; General Introduction). ECSA underlines that the Maximum Tolerated Dose was chosen jointly with the Contract Laboratory Research on the basis of this document and the OECD 474 guideline. Indeed, it is specified "the highest dose is defined as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality. In addition, Covance Laboratories Ltd defines the MTD as the highest tested non-lethal dose level that induces clear evidence of toxicity such that a significantly higher dose (e.g. a fold increase of 1.4) would be expected to cause lethality, morbidity or severe toxicity. A 1.4 fold dose interval is considered sufficiently small to conclude that a completely non-toxic dose could be the MTD if a dose 1.4 fold greater induced lethality, morbidity or severe toxicity. The clinical board observed at the high dose-level of 480 mg/kg/day (i.e. ataxia, bradypnoea, tachypnoea, hunched posture, hypothermia, lethargy, mouth rubbing, decreased activity, ptosis, piloerection and	In the preliminary study, body weights were reduced (D1-6) in high-dose male (-8.8%) and female (-2.2%) rats. This indicates that MTD was not reached within this range-finding study. Moreover, if we use the information provided below in the comment and regarding the oral LD50 of 908 mg/kg, the highest dose used (480 mg/kg/day) is far below LD50/1.4! In the main study, tremors and tachypnea were only observed in the male rat which was moribund. It is unfortunate that no necropsy was performed on this animal to determine if morbidity was treatment-related.	Related to the general comment above.

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		tremors as well as the loss of weight in a period where the animals should gain weight) cannot be considered as mild even if these effects decreased following several administrations. Any other highest dose-level would have lead to development of severe toxicity or lethality. For information, the oral LD50 is estimated to be 908 mg/kg in rats exposed once to chloroform, as specified in the study of Chu et al. (1980). As evidence that the MTD was achieved, the premature sacrifice of one male of the high-dose level on day 4.	Statement such as "Any other highest dose-level would have lead to development of severe toxicity or lethality" are not acceptable in absence of experimental data to support this statement.	
		Premature sacrifice: the Rapporteur argues that in absence of necropsy of the moribund rat no relationship to the treatment can be established. However, it must be considered that the decision of premature sacrifice was taken on day 4 before treatment accordingly to a deteriorated clinical board (i.e. tremors, tachypnoea and hypothermia) and that during the previous days, the clinical signs observed in this animal (i.e. piloerection, decreased activity, hypothermia, ptosis as well as a body weight loss of 15%) were similar to those noted in the animals of the same group and considered as chloroform treatment-related. At the necropsy, a macroscopic examination of the liver revealed the same findings (paleness and mottling) with increased or similar severity than several other animals treated with chloroform. In this way, there is no doubt that the premature sacrifice of this animal was caused by the treatment with chloroform.	Tremors and tachypnoea were seen only in this animal. We do not understand how you claim this is treatment related? The macroscopic examinations of the livers were not discussed within the study report, neither for the animal killed in extremis nor for the other animals. This finding indicates that this animal was exposed to Chloroform but does not inform on the cause of morbidity.	
		Body temperature: the Rapporteur seems to consider that the hypothermia observed in the chloroform treated groups is too mild. However, we want to underline that the monitoring of body temperature is not required in this kind of study and was presently recorded only to ensure that no important hyper- or hypothermia was observed, which could disturb physiological mechanisms as metabolism or hormonal secretions. In other words, body	We do not consider that the hypothermia observed was too mild. However, using it as an indicator for MTD to be reached is not convincing in our point of view.	Related to the general comment above.

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		temperature was not recorder for assessment of systemic toxicity. Nevertheless, decrease of body temperature was observed in some animals and was attributed to the administration of chloroform. In the range finder, attention was paid that the body temperature did not increase of at least 1°C or decrease of at least 3°C for five or more hours. Otherwise, the dose-levels would have been declared as exceeding the MTD.		
		Percentage of PCE (see Table 1) : the Rapporteur recognizes that in the main study the mean % PCE values decreased in a dose-related manner in the chloroform-treated groups where compared to the controls and that the mean % PCE values of the high dose- level group is markedly lower than the concurrent vehicle control values. However, as these data were within the historical control values (observed range of 21-78%) and the mean % PCE values were not significantly altered during the range finder, the Rapporteur considers that "the bone marrow toxicity of the test article was not convincing".	The fact that mean % PCE values were not significantly altered during the range finder should have been discussed in the study report. The fact that this piece of data was not taken into account as a indication for increasing the dose should at least have been explained. Why doing a dose range finder study, using animals if not considering the results?	Related to the general comment above.
		First of all, it is surprising that the Rapporteur gave more credit to historical values than to concurrent controls. In this case, it has to consider that the decrease in mean % PCE values observed in the positive controls is also not convincing of a bone marrow toxicity and target organ exposure since this decrease is in the same range than that of the high dose-level group, even for the cyclophosphamide (CPA)-treated groups, slightly above.	Comparison with CPA has been discussed within the report. However, we disagree with interpretation provided here: By definition, CPA is used as a positive control because it is known to induce micronucleus in bone marrow: it is not clear whether this occurs in toxic doses or not. Therefore, it is possible that doses of CPA used are genotoxic without being toxic. Regarding the substance tested, guidelines require to go	Related to the general comment above.

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			up to toxic doses.	
		Actually, the historical control values highlight a large variability that can be explained by the different experimental conditions as for example the staining characteristics. For proof of evidence, the difference in the % mean PCE between control males and females (62.40 and 43.82 %, respectively) whereas no sex difference is attended with the vehicle (corn oil) but in contrary some variabilities expected as these assays were not performed at the same time. The Rapporteur used the observed range of 21-78% (all values pooled) instead of the 95% confidence interval for group mean of 5/6 values (i.e. 39-59%) that is a more valid criterion. Compared to this value, the corrected sentence would be " Rats treated with chloroform showed group mean % PCE that decreased in a dose dependent manner with the highest dose (480 mg/kg/day) exhibiting 38% or 27% PCE, which was markedly lower than the concurrent vehicle control values and also lower (for females) or marginally lower (for the males) than the historical values". Otherwise, considering the fact that no significant decrease of the % PCE values was observed during the range finder experiment, it is important to note that there were no concurrent controls and probably not enough animals per sex (i.e. 3) to assess to a firm conclusion. In the main test, the % PCE was clearly dose-related decreased at 480 mg/kg in male and female rats indicating the bone marrow was reached by chloroform. Table 1: Percentage of PCE: summary of group mean data-Micronucleus experiment Treatment group (mg/kg/day) % PCE Males % PCE Females Vehicle Control 62.40 43.82 120 64.45 42.10	The comparison with historical controls was initially performed for the dose range finder study, for which no controls were available. As we question the choice of the doses used, results of the main study were also compared to historical data to place the study within the context of the laboratory. The variability discussed here should have been discussed within the study report. This statement should have been discussed in the study report; The aim of a dose range finding study is to adapt the dose in order to cover minimal toxicity up to MTD. The results obtained in the dose range finding indicated that bone marrow toxicity was not induced: doses should have been increased.	Related to the general comment above. Related to the general comment above.

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		240 49.10 40.58 480 38.22 26.58 CPA, 20 47.83 27.53 CBZ, 1500 36.43 22.82 CBZ, 2000 39.08 26.55 Positive control slides: the Rapporteur points out the fact that slides from CPA-treated animals were initially checked to ensure the system was operating satisfactorily implying that they were not blindly read. However, since a second positive control was included in the study and that all the other groups than CPA were coded and blinded read, this deviation has no impact on the results of the study.	Regarding positive control slides and their reading, the study report is not clear. The second positive control, namely, the CBZ was used to evaluate the aneugenic (whole chromosome loss) potential of chloroform should a positive induction of micronuclei have been observed. However, even if significantly elevated aneugenic (CBZ) positive control responses were noted, these were of a lower magnitude than the clastogenic response with a degree of heterogeneity (both genders). Having another positive control does not seem a sufficient argument to separate the reading of the CPA slides. Here again, it should have been reported clearly and discussed in the study report.	
		(Typing) Errors in the text: please correct also in the text the following points: As shown in table 18 17, high-dose male rats (according to our own calculations: - 9.1% based on the mean of five animals since male 470 died at Day 5) "please delete this sentence as the % change in	These remarks have been taken into account and the report	Related to the general comment above.

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		body weight in the high-dose male group is well 10.5% without taking account the animal sacrificed on day 4. The value would be 9.1% if the body weight of animal 470 would have been taking into account on day 1)" and in mid- and ??" please finish this sentence" An increase in severity of observations was noted in high-dose female animals on Days 3 and 4 compared to males. One male animal of the high dose group was killed in extremis on Day 4 but was not necropsied "please specify that necropsy of the liver was performed ". Clinical signs in both genders were noted to be less severe by Day 5. High- and mid-dose female rats (- 1.5% and - 8.3%, respectively) lost weight. Most of the males having lost weight gained weight from day 5 (not shown in the table).	modified accordingly. (see pg 30)	
		Negative (vehicle) control male rats exhibited a group mean frequency of polychromatic erythrocytes (PCE) to normochromatic ... the bone marrow toxicity of the test article was not convincing. " for a better appreciation, it would be useful to indicate and discuss the % PCE of the positive controls".	This information has been added. However, it appeared nowhere in the study report, only in the table of appendix 8 with no mention to it or explanation!	Noted.
		The groups mean frequencies of MN PCE observed in test article treated groups (male and female data) were not significantly ($p \leq 0.05$) different to the vehicle controls (see table 1918).	Comparison added and discussed.	
		Discussion of the positive studies We emphasize that the strictness with which the Whitwell study was performed was not present in the non GLP studies considered as pivotal by the Rapporteur for the mutagen classification of chloroform (i.e. Fujie, 1990 and Robbiano, 1998). As a reminder, the independent experts pointed out the several bias (see Annexes 1 and 2):	Although GLP, we are still convinced that the study was not reported correctly (see above) and that a few divergences to the standard protocol lighten the pivotal status of this study.	

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				Noted.
			Moreover, this study reproduces existing studies, not enabling to deepen the understanding of the mode of action of Chloroform.	
			The negative results obtained within this study do not overrule the other (positive) results, even when considering the bias of the positive studies.	Noted.
		Robbiano, 1998 - First expert: "A micronucleus test carried out on the kidney of Sprague-Dawley rat was found to be positive for a single dose of 4 mmol/kg (equivalent to approx. 480 mg/kg) p.o. (Robbiano et al. 1998). Any information about the toxicity were not given, but it can be seen from the literature for the F344 rat that tubulus damages had occurred even with a single dose of 34 mg/kg. With 477 mg/kg, extended necroses were found in the kidney (Larson et al. 1993). Only slight vacuolization of the epithelial cells in the proximal tubulus were found in another study in which, however, 477 mg/kg were administered to the F344 rat and 180 mg/kg to the Osborne-Mendel rat (Templin et al. 1996). Under these conditions, it is difficult to exclude an interference of an excessive level of cytotoxicity. This test was performed after a partial nephrectomy could be a confounding factor. Such methodology is not validated enough to be taken into account for human risk assessment." - Second expert: "The protocol used was similar to the OECD guideline 474, but with a single dose level and with the kidney as the target. Because it is necessary for the cells to have gone through a division, one kidney was removed and then, one day later the proportion of dividing cells in the remaining kidney was increased by i.v. administration of folic acid at 250 mg/kg bw. Chloroform was administered 2 days later. The frequency of micronucleated		Related to the general comment above. Related to the general comment above.

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		cells in the chloroform treated rat kidneys was 4.42/1000 (n = 7), compared with 1.33/1000 (n = 16) in the vehicle controls group. According to the statistical analysis used, this difference was significant. Weaknesses in the study are: (1) a single experiment was performed, there being no attempt to confirm the observation, (2) it was not possible to observe a dose-response, because there was a single dose administered, and (3) there was no adjustment of the statistical analysis to take account of the single control group being used for the 7 different chemicals (including the positive control) being tested. Fujie, 1990 - First expert: concerning the results obtained by Fujie et al., differences of results were observed between the 2 studies particularly at the low dose of 10-2 mM/kg and the kinetic of chromosomal aberrations at 10 mM/kg is not traditional: positive 12 and 18 hours after five daily administration and negative 6 and 24 hours after the same treatment. Such a kinetic is not compatible with the kinetic of specific chromosomal aberrations but more with a cytotoxic activity, unfortunately, no information about the cytotoxic activity in the bone marrow is available in the 2 papers.		Related to the general comment on page 3.

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		Such positive results should then be considered as equivocal. - Second expert: There are two points of concern regarding this study that, in other respects, provides evidence for a clastogenic effect of chloroform on rat bone marrow cells. The first is that, in both the i.p. and p.o. segments of the study, the data that are presented after a single harvest time (12h i.p. and 18h p.o.) are precisely the same as the data presented in the multiple harvest time tables at the same dose level. Therefore, there is a false impression given regarding the reproducibility of the effects. Nevertheless, there is qualitative reproducibility of the effects to be seen when the i.p. and the p.o. dosing results are compared. The difference in sensitivities observed (only 1 mmole/kg bw being effective after p.o. dosing) is not too surprising because i.p. dosing does produce higher concentrations in blood and other tissues (Wang et al., 1995; Gemma et al., 1996). What is surprising, however, is the sensitivity of the rats in this study in comparison with other published studies. Thus, a statistically significant effect was observed with only 1.2 mg/kg bw i.p. at 12h, whereas no effect was observed in a (micronucleus) study with mice at an almost 1000-fold higher dose level (Gocke et al., 1981). Conclusion In light of this additional information, ECSA would like: - the summary of the Whitwell study (2009) be reviewed and corrected especially concerning the evidence of the bone marrow toxicity and target organ exposure after the administration of chloroform. - the abandonment of the proposal of classification of chloroform as	Protocol used different, for eg. not the same specie The summary of the Whitwell study has been modified. The results of this study do not overrule the other positive	Related to the general comment on page 3.

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		mutagen category 3. Finally, we wish to express our astonishment in the fact that within the same institution (AFSSET) representing the French Authorities, conflicting opinions can be issued and published. Indeed, AFSSET published in 2009 a document for the development of the Toxicological Reference Values (TRVs) for chloroform in which AFSSET ruled that "the available genotoxicity data indicate that neither chloroform nor its metabolites (including phosgene) seem able to interact directly with DNA". This assumption was crucial for the development of the TRV for chloroform. References: Chu I., Secours V., Marino I., Villeneuve DC. (1980) The acute toxicity of four trihalomethanes in male and female rats, Toxicol. Appl. Pharmacol. 52, 351-353. Fujie K, Aoki T and Wada M (1990) Acute and subacute cytogenetic effects of the trihalomethanes on rat bone marrow cells in vivo. Mutat. Res. 242, 111-119. Gemma, S., Faccioli, S., Chieco, P., Sbraccia, M., Testai, E. & Vittozzi, L. (1996) In vivo CHCl3 bioactivation, toxicokinetics, toxicity, and induced compensatory cell proliferation in B6C3F1 male mice. Toxicol. Appl. Pharmacol. 141, 394-402. Gocke E, King MT, Eckhardt K, Wild D (1981) Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat. Res., 90, 91-109. Larson JL, Wolf DC and Butterworth BE (1993) Acute hepatotoxic and nephrotoxic effects of chloroform in male F-344 rats and female B6C3F1 mice. Fund. Applied Toxicol, 20, 302-315. Robbiano L, Mereto E, Migliazzi-Morando A, Pastore P, Brambilla	results. As mentioned, AFSSET ruled that "the available genotoxicity data indicate that neither chloroform nor its metabolites (including phosgene) seem able to interact **directly** with DNA" in the document for the development of TRV whereas in our CLH report, we state, in the conclusion for mutagenicity: "Mentioned results and chloroform metabolism via oxidative or reductive pathways suggest that chloroform is a slightly genotoxic compound *in vivo*, based on **indirect** genotoxic mechanism." These two statements are therefore not conflicting.	Noted. See also the general comment on page 3.

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		G (1998) Increased frequency of micronucleated kidney cells in rats exposed to halogenated anaesthetics. Mutation Research, 413, 1 6. Templin MV, Jamison KC, Wolf DC, Morgan KY, Butterworth BE (1996b) Comparison of chloroform-induced toxicity in the kidneys, liver, and nasal passages of male Osborne-Mendel and F-344 rats. Cancer Letters, 104:71–78. Valeurs Toxicologiques de Référence (VTRs) : Élaboration de VTR fondées sur les effets cancérogènes pour le chloroforme, le tétrachlorure de carbone et le 1,2-dichloroéthane. Publication AFSSET juin 2009. Wang Pei-Yu, Kaneko T, Sato A, Charboneau M and Plaa L (1995) Dose- and Route-Dependant Alteration of Metabolism and Toxicity of Chloroform in Fed and Fasting Rats. Toxicol. Appl. Pharmacol. 135, 119-126. <u>Annexes</u> Annexe 1: Dr. Marzin expert opinion on chloroform genotoxicity Annexe 2 : Dr. Mc Gregor expert opinion on chloroform genotoxicity *Attachment: 2010-06-10-COMMENTS FROM ECSA ON THE CLASSIFICATION OF CHLOROFORM*		
11/06/2010	Ireland / Health & Safety Authority / National Authority	The Irish CA is not in agreement with the proposed classification of Mut. Cat 3 R68 (Dir 67/548/EEC) or Muta 2 H341 (CLP Regulation). In accordance with para 3.5.2.3.9 of Annex I of CLP: "The classification of individual substances shall be based on the total weight of evidence available, using expert judgement…" The Irish CA is of the opinion that the total weight of evidence does not support classification for mutagenicity.	Weight of evidence has been evaluated by expert judgment and we consider that a classification for mutagenicity is warranted on this basis.	Related to the general comment on page 3.

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		In our opinion, there are conflicting data from the in vivo mutagenicity assays presented. With respect to in vivo micronucleus studies, data from studies by Robbiano et al. (1998) and Shelby & Whitt (1995) demonstrate increased frequency of micronucleated cells in Sprague-Dawley rat kidney following oral administration of chloroform and in mouse bone marrow following i.p. administration of chloroform respectively. As indicated in the Annex VI report however, Robbiano et al. (1998) employed kidney cells as opposed to erythrocytes and only one test concentration. In addition, the positive results obtained by Shelby & Whitt (1995) were from one of two trials conducted, the first of which was negative. The Irish CA is of the opinion that the evidence presented from studies conducted to OECD guidelines by Whitwell et al. (2009) and Gocke et al. (1981), reporting that oral and i.p. administration of chloroform to rats, respectively, did not increase the frequency of micronucleated PCE cells deserve further consideration.	We agree that there is conflicting data from the *in vivo* mutagenicity assays presented. So far, the difference of sensitivity for micronucleus test of the bone marrow/ target organ (kidney) is not clear. However, despite the fact that the protocol used in kidney is not standardised, the study was considered as valid. All the data available have been evaluated, their reliability taken into account and all the results integrated. Negative results cannot overrule the positive ones, only if they could explain mechanistically these differences, showing that positive results are specific to the tested conditions.	Related to the general comment on page 3.
		The Whitwell study (2009) is conducted to current guidelines and GLP, which includes detailed information regarding the protocol and results, such as justification of the MTD and formulation analyses. In contrast, it is noted that the results from other in vivo micronucleus studies reported, both positive and negative, are derived from published scientific papers, and while these are valid studies, they do not include information on the GLP status and or detailed reporting of results. The Annex VI report comments that the highest dose used in the Whitwell study is less than the MTD, however the highest dose chosen is in line with oral LD50 values	The choice of doses tested in the dose-range finding study was consistent with LD50 and previous literature. However, the results of this dose range finding should have leaded the study director to propose to increase the highest dose.	Related to the general comment on page 3.

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		referenced earlier in the Annex VI report. The lack of bone marrow toxicity in this study is also discussed, but it is noted that the available toxicological data indicates that bone marrow is not the target organ which results in death (liver/kidney) and that bone marrow toxicity is not reported for any of the other positive studies. Further in vivo mutagenicity assays reported include data from studies by Fujie et al. (1990) demonstrating increased chromosomal aberrations in Long Evans rats following i.p. and oral administration of chloroform, and Hoechst (1998), demonstrating increased chromosomal aberrations in Chinese hamsters following oral administration of chloroform. The choice of strain or species by both authors is questionable, given that the Long-Evans rat is susceptible to clastogenic effects from chemicals in bone marrow (Yoshiaki et al. 1994, see attached journal reference) and that the hamster is a non-conventional choice for this assay. Moreover, the study by Hoechst (1998) was not conducted to test guidelines, and positive results, within the range of the historical negative controls were observed only at the high dose when male and female results were combined. With respect to in vivo genotoxicity, two of the ten tests conducted (as referenced in Table 29: Summary of the key studies) displayed positive or weakly positive results for DNA damage [Morimoto and Koizumi (1983), Pereira et al. (1982)]. However, an overwhelming body of evidence from the remaining DNA repair, DNA damage and germ cell assays demonstrates that chloroform is non-genotoxic. This data includes UDS studies by Mirsalis et al. (1982) conducted in accordance with OECD test guidelines at doses up to 400mg/kg and further DNA damage assays including a study by	We agree that studies performed on target organs, eg ; kidney (Robbiano) appear more relevant. These arguments have been taken into account. Although the genotoxicty assays discussed by the Irish CA are not conducted according to GLP and standard protocols, they are considered of sufficient reliability to provide scientifically valid indications of Chloroform mutagenicity. The discrepancies described by Irish CA show that Chloroform genotoxicity arises in specific conditions (conditions reached in the positive studies). This is not incompatible with the fact that other conditions, species and/or organs would not be sensitive to Chloroform	Related to the general comment on page 3. Related to the general comment on page 3. Related to the general comment on page 3.

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		Reitz et al. (1982) demonstrating that chloroform is non-genotoxic in mice and rats at 240 mg/kg bw. Based on a weight of evidence and in accordance with the classification criteria, the Irish CA is of the opinion that there are insufficient data to classify chloroform as mutagenic.	genotoxicity. The overall database did not allow understanding and defining precisely these conditions: therefore, it cannot be overruled that genotoxicity would occur in humans.	
14/06/2010	Sweden / Helena Kramer / MS	As regards mutagenicity, SE agrees with the rapporteur in concluding that chloroform is a slightly genotoxic compound in vivo and that classification of chloroform for mutagenicity in Category 3 with the risk phrases R68 possible risk of irreversible effects (CLP Muta 2 – H341) should be proposed. We think that particularly the well conducted studies of high reliability by Fujie et al. (1990) on induction of chromosome aberrations in the bone marrow of rats, Robbiano et al. (1988) on induction of micronuclei in the kidney of rats and Shelby and Witt (1995) on induction on micronuclei in the bone marrow of rats add considerable weight-of-evidence for a mutagenic potential of chloroform. In the recently conducted study in rats employing the bone marrow micronucleus assay, no induction of micronuclei was observed (Whitwell, 2009). However, due to limitations noted, particularly as regards the level of the highest dose for which there are indications that it was below the MTD and did not induce bone-marrow toxicity (i.e. the target cells were not sufficiently exposed), the reliability of the negative result of this study is, in our view, not sufficient to overrule the positive results of the studies referred to above.	Thanks for your support.	Related to general comment on page 3.
14/06/2010	Denmark / Peter Hammer / Danish EPA / Denmark	Regarding the proposal, Muta. Cat. 3; R68, Denmark agrees with the conclusion that chloroform should be regarded as genotoxic in vivo and classified. It has been shown by well-conducted in vivo studies that chloroform causes CAs in the bone mar-row of rats even at the low dose of 1.2 mg/kg (Fujie et al., 1990) and micronuclei formation in bone marrow in mice (Shelby and Witt 1995) and kidney of rats (Robbiano et al.1988). Mechanistic in-formation indicates that an indirect mechanism might be involved which implies that the	Thanks for your support.	Related to general comment on page 3.

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		mutagenic-ity of chloroform is likely to have a dose threshold; the available data however do not allow its iden-tification. *Attachment: Danish comments for Chloroform - cas.no. 67-66-3*		

Toxicity to reproduction

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment

Respiratory sensitisation

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment

Other hazard classes

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
11/06/2010	Belgium / Wolfgang Marquardt / ECSA - European Chlorinated Solvent Association / Industry or trade association	SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE A classification STOT RE1- H372 (cause damage organs) has been proposed by the Rapporteur Member States France. It is specified that this classification was agreed in September 2007 during the TC C&L meeting. However the classification agreed during this meeting was SOT RE2-H373 : may cause damage organs. Indeed the classification STOT RE1- H372 seems not justified as the category 1 is proposed for substances that have produced significant toxicity in humans, or on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans following	A classification STOT RE 2 is mentioned in the follow-up document of the TC C&L. However, its origin is not clear as the French proposal for this classification was already STOT RE1- H372 at that time and there is no track of discussions on the CLP classification during the	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

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		repeated exposure. If the guidance values are considered to assist in the category classification, the NOAELs and LOAELs concerning the liver or kidney findings in the 90-day oral or inhalation studies would lead to a classification category 2. The only value would lead to a classification category 1 is the LOAEL observed in Templin et al, 1996a and attributed to generalized atrophy of the ethmoid turbinates. However since no information (human incidents, epidemiology...) suggests relevance in humans, a classification category 2 seems more pertinent. *Attachment: 2010-06-10-COMMENTS FROM ECSA ON THE CLASSIFICATION OF CHLOROFORM*	meeting. This may have been inserted by ECB using the conversion table. Classification in category 1 is applicable when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur at or below the guidance values as indicated in table 3.9.2 of CLP. Most of the LOAEL are below this guidance value, 0.2 mg/L for vapour, justifying a cat.1.	CLP (see table 3.9.2) the criteria for STOT RE 1 – H372 1 are met.
11/06/2010	Ireland / Health & Safety Authority / National Authority	The Irish CA agrees with the proposed additional classifications for human health: Xn; 22 (Dir 67/548/EEC) or Acute Tox. 3 (CLP Regulation), and the removal of the stated concentration limit values; Xi; R36 (Dir 67/548/EEC) or Eye Irrit. 2 (CLP Regulation); Xn; R48/20 (Dir 67/548/EEC).The Irish CA notes an amendment in corresponding CLP classification for this endpoint in Annex VI, from STOT RE 2 H373 to STOT RE 1 H372. Based on renal and severe nasal effects observed in rats and mice at concentrations below 0.2 mg/litre/6h/day, we agree that the criteria for category 1 are met. We propose that the justification for STOT RE 1 could be strengthened by making reference to the cut-off values in para 3.9.2.9.6 of Annex I to CLP, since these values are slightly different to the cut off values under Dir 67/548/EEC for this endpoint. We note the amendment of the existing entry to remove the specific concentration limit of 5% for this endpoint. We suggest that justification for this amendment be provided in the CLH report.	Thanks for your support. The report has been modified as proposed.	Agree with response

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CHLOROFORM

Date	Country/ Person/Organisation/ MSCA	Comment	MSCA Response to comment	Rapporteurs' response to comment
		Attachment: Yoshiaki Ito et al 1994		

Attachments provided from :

MSCA Ireland:	*Yoshiaki Ito et al 1994*
MSCA Denmark:	*Danish comments for Chloroform - cas.no. 67-66-3*
ECSA - European Chlorinated Solvent Association*:*	*2010-06-10-COMMENTS FROM ECSA ON THE CLASSIFICATION OF CHLOROFORM* *Annexe 1: Dr. Marzin expert opinion on chloroform genotoxicity* *Annexe 2 : Dr. Mc Gregor expert opinion on chloroform genotoxicity*