Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Iodomethane

EC number: 200-819-5
CAS number: 74-88-4

CLH-O-0000004613-77-03/F

Adopted

12 September 2014

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: methyl iodide; iodomethane
CAS number: 74-88-4
EC number: 200-819-5
Dossier submitter: United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2014	Netherlands		MemberState	1
Comment received				
Only the proposed classification for carcinogenicity was taken into account in this evaluation.				
Dossier Submitter's Response				
This is correct. The existing carcinogenicity classification dates back to 1987, when the database on iodomethane was very much weaker than it is now.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
07.01.2014	United Kingdom		Individual	2
Comment received				
In the past I have been a paid consultant to Arysta LifeScience SAS, France but I am providing these comments based on my independent assessment of the data. Iodomethane is a well studied alkylating agent belonging to a class that does not efficiently induce genetic damage or are not potent genotoxic carcinogens. This may be because the DNA damage it induces is efficiently repaired by a battery of DNA repair systems, that the compound rapidly reacts with proteins before reaching the nucleus and also that the compound is detoxified by reaction with glutathione. The lack of clastogenicity in the in vivo mouse bone marrow micronucleus test is a key finding regarding potential to induce genotoxic damage in vivo and links with the lack of induced tumours in the two modern rodent carcinogenicity studies, apart from thyroid tumours at the highest dose tested. There is overwhelming evidence that the latter are induced through the prolonged exposure of the test animals to excess levels of iodine.				
Dossier Submitter's Response				
We agree that results of the *in vivo* bone marrow micronucleus study and the 2 recent carcinogenicity studies are highly relevant.				
RAC's response				
In the CLH report it is also reported that iodomethane acts as alkylating agent in vitro. But obviously iodomethane does not efficiently induce genetic damage either due to DNA repair systems, detoxification and/or reaction of iodomethane with proteins before reaching the				

cell nucleus. Therefore also RAC agrees with the assessment that the negative in vivo micronucleus test is a key finding regarding potential to induce genotoxic damage in vivo.

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2014	France		MemberState	3

Comment received

FR does not support the proposed declassification of iodomethane for carcinogenicity. Indeed, the data presented do not contradict previous studies (Thyroïd tumours found in the new studies). The new data provided do not show that the MoA is not relevant to human. FR recognises that human might be less sensitive to the modification of thyroid hormone homeostasis induced by Iodide but an effect, even if moderate, cannot be excluded (effects on thyroid are observed at 12 mg/ kg/d in dogs, and from 20 mg/kg/d in mouse in 90d studies, therefore, the potency for Iodide to affect Thyroïd homeostasis is high). Moreover, studies show multiple organ of carcinogenicity. Finally, the substance, that has alkylating properties, has shown contradictory results on clastogenicity/ mutagenicity in vitro and clastogenic effects in vivo and.

FR would appreciate to know from where the statement: "Thyroid" cancer in humans is rare"is coming. Indeed, based on a report published by INVS in 2010, Thyroid cancer was relatively rare 25-30 years ago, but the incidence has been rising in France and in many other countries. The number of diagnoses has increased significantly by 6% per annum between 1980 and 2005 in France. It is about three times more frequent in women than in men, and the most common histological type is papillary cancer.
In 2005 in France, it was at the fifth and 21st rank respectively for incidence and cancer mortality in women and 19th rank for same both indicators in humans.

Dossier Submitter's Response

The previous studies are unreliable, and do not contribute to the assessment of carcinogenicity, as explained in the CLH report. The original classification was not based on thyroid cancer in animal studies.

It was shown in the CLH report that based on the significant quantitative species difference in sensitivity between rodents and humans to thyroid tumour formation by this mode of action, it would not be plausible for humans to achieve sustained elevations of circulating TSH that may be relevant for tumour formation because tolerable levels of iodomethane exposure would have to be exceeded. This is a fundamental point because even in the rat, which is a sensitive species, tumours were only observed at a dose which exceeded an MTD. Therefore, iodomethane should not be classified for carcinogenicity on this basis (see pages 45 and 46).

The data in the CLH report do not support the statement above that "studies show multiple organ carcinogenicity". Convincing evidence is provided that increased thyroid follicular tumours are the only unequivocal treatment related increased tumour incidence associated with iodomethane exposure. There was a slight increase in the incidence of fibroma in the uterus or cervix of mice, although this microscopic lesion was only seen in 1 low dose and 4 high dose animals. However, these were benign and there were no precursor lesions or other signs of toxicity to the uterus or cervix to suggest this was a treatment-related effect. We have made available to RAC a follow-up peer review of these lesions from a Pathology Working Group (Hardisty, 2005).

Genetic toxicity data: when reviewed critically, on the basis of the current state of knowledge about all these assays and the way conducted previously, it is possible to reconcile the various results seen. This is covered in detail in the CLH report. Public domain data from *in vitro* assays using bacteria and cultured mammalian cells do not provide any reliable, reproducible indication of iodomethane being able to induce gene mutations in these assays. Where positive results have been reported, deficiencies in reporting and/or method design are identified when assessed against current standards. The GLP, guideline compliant Ames study more than adequately addresses the gene mutation endpoint (refer to Comment 10). As identified in the CLH report, iodomethane clearly has the potential to induce chromosome aberrations *in vitro*. However, importantly, this *in vitro* clastogenic potential is not realised in the *in vivo* mouse bone marrow micronucleus study.

We are sorry if the statement "Thyroid cancer in humans is rare" has been misunderstood. It is not intended to be a key statement, open to controversy, and is not central to the proposal. It had been deduced from the publications by Capen (1999) and IARC (2001) which cited very low thyroid cancer rates: IARC noted that "thyroid cancer is relatively rare". It is acknowledged that thyroid cancer rates are rising, however, this needs to viewed in the context of the present mode of action. Papillary carcinoma is the major type of thyroid cancer induced by radiation exposure and there is no evidence that it is causally linked to elevated TSH. The risks of thyroid follicular cancer arising from disturbances to thyroid hormone homeostasis are low.

RAC's response

The evidence on a 'multi-site carcinogen' is considered as weak based on the borderline incidences for tumours in tissues outside the thyroid gland.
The rapporteurs are aware of the more recent development of thyroid cancer in humans. This aspect has been taken into account in the opinion document.

In the CLH report it is also reported that iodomethane acts as alkylating agent in vitro. But obviously iodomethane does not efficiently induce genetic damage either due to DNA repair systems, detoxification and/or reaction of iodomethane with proteins before reaching the cell nucleus. Therefore also RAC agrees with the assessment that the negative in vivo micronucleus test is a key finding regarding potential to induce genotoxic damage in vivo.

Date	Country	Organisation	Type of Organisation	Comment number
17.01.2014	Belgium	European Trade Union Confederation	BehalfOfAnOrganisation	4

Comment received

The European Trade Union Confederation (ETUC) does not support the proposal to remove the carcinogenicity classification of iodomethane from Annex VI of the CLP Regulation.
The mode of action for the thyroid tumors found in rodents is proposed to be a perturbation of homeostasis of the hypothalamic-pituitary-thyroid axis caused by excess circulating iodide derived from the metabolism of iodomethane. However, since multiple sites of carcinogenicity have been identified (i.e., fibromas of the cervix), the existence of other modes of action cannot be rulled out, including genotoxicity.
In our views, the arguments presented are not sufficient to modify the current classification of iodomethane as Carc 2 – H 351.

ETUC also strongly disagrees with the consideration that thyroid cancer in humans is rare (CLH report, p9). Thyroid cancer is one of the few cancers that has increased in incidence rates over recent years.

Also, iodomethane is included in the Trade Union priority List for REACH authorization (http://www.etuc.org/a/6023) and it is also currently listed on California's proposition 65 list of "chemicals known to cause cancer (http://www.oehha.ca.gov/prop65/prop65_list/files/filesp65single110112.pdf)
Dossier Submitter's Response
This is not a "multi-site" carcinogen. See comments above regarding "multiple carcinogenicity" and human thyroid cancer rates. The possibility of other modes of action was discussed in the CLH report (Annex 1, point 1.6). There is no increased incidence of tumours at the site of first contact, which would be expected if iodomethane was genotoxic.
RAC's response
The view of ETUC is noted. With regards to the DS's response, the increase of tumours at the first site of contact is not necessarily expected for all genotoxic carcinogens, e.g. when the metabolite is the carcinogenic agent. For iodomethane no test is available that examines the induction of genotoxic effects in cells at site of first contact (in the case of iodomethane in cells of the nasal epithelium after inhalation). Such a test will usually carried out when the substance to be tested can only act locally in soma cells at site of contact due to their poor systemic availability. This is not the case for iodomethane.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2014	Netherlands		MemberState	5
Comment received				
Current classification: Carc. 2 (H351, suspected of causing cancer) The Netherlands disagrees with the proposed removal of Carc. 2 (H351 suspected of causing cancer) until clarification is provided with regards to the cervical tumors (p. 34-35, CLH Report). The Netherlands agrees with the approach that thyroid tumors induced by non-genotoxic compounds in rodents which demonstrate a prolonged disturbance in the hypothalamic-pituitary-thryroid (HPT)-axis are not considered relevant for human carcinogenic risk (EU 1999, RIVM 2002). Even though conflicting in vitro gene mutation results cannot be fully confirmed with a negative in vivo micronucleus test which measures chromosomal aberrations and not gene mutations, the tumor data are indicative that the mode of action for the thryroid tumors is a disturbance in homeostasis of the HPT-axis caused by excess circulating iodine derived from metabolism of iodomethane. The Netherlands disagrees with some of the arguments provided to conclude that the dose-related trend in the incidence of fibromas in the cervix of female mice is not significant because the observed effects are presumed not to be treatment related (p. 34, CHL Report). The following arguments provided are insufficient to conclude that the presence of fibromas in the cervix is not treatment related: - 'The fibromas were considered not to be associated with treatment due to their low incidence, appearance only at the terminal sacrifice, microscopic in size' (p.34, CHL Report). A low but significant increase in tumors (fibromas in cervix) is indicative of a treatment effect and the fact that the tumors are small and appear only at terminal sacrifice does not				

mean that they are not treatment related. However, these last two arguments could be more important when evaluating the relevance of the tumors.
- The difference between malignant versus benign tumors is normally a factor in deciding between Cat 1B and 2 as an increase in benign tumors fulfils the criteria for limited evidence.
- The difference between tumors arising in one versus two species is normally important for deciding between Cat 1B or 2.
- The absence of fibromas in the cervix of humans would not justify a conclusion that this type of tumors in rats is not relevant to humans.
- 'the detailed sampling and histological examination of the proximal uterine horns, body of the uterus and distal cervix conducted in this study was atypical for routine evaluations of the female reproductive tract' (p.34, CHL Report). Please provide information whether there was a difference in histological examination between treated rats and controls.
- The absence of tumors in the controls may be an indication that this is a rare tumor type in rats but without historical background information, there is no reason to disregard the observed significant dose-related trend. In contrast, the absence of these tumors in historical controls suggests that this may be a rare tumor.

Dossier Submitter's Response

Thank you for your support regarding the thyroid tumour findings.

There was a slight increase in the incidence of fibroma in the uterus or cervix (combined) of mice, although this microscopic lesion was only seen in 1 low dose and 4 high dose animals. As discussed, interpretation of these lesions requires expert judgement on the diagnosis of the findings, their relationship to treatment and relevance for hazard assessment. They were benign and no precursor lesions or other signs of toxicity to the uterus or cervix were evident, suggesting this was not a treatment-related effect. There are no other data to suggest these tissues are specific targets for iodomethane toxicity. We have made available to RAC a follow-up peer review of these lesions from an expert Pathology Working Group (Hardisty, 2005). The PWG also commented that, to their knowledge, fibroma of the uterus and cervix had no clinical or biological significance in animals or humans.

Detailed histological examination: additional sections of the uterus and cervix for each animal in the study (i.e. all treated groups and controls) were examined by the PWG.

The DS agrees with the Netherlands with regard to gene mutations. It is deemed that the gene mutation endpoint is adequately addressed through the robust, bacterial gene mutation assay (refer to comment 10).

RAC's response

The RAC tends to support the NL view. In general, benign tumours could not be disregarded from classification. If their incidences are increased as a consequence of the treatment, their occurrence is supportive of classification as Cat. 2. Also the absence of precursors or toxicity is not a precondition for treatment-related tumour growth. At a low level of incidence and in particular if the tumour type has a long latency, a focal hyperplasia of fibrocytes or in situ growth of fibroblasts might not be detectable with standard microscopy.

With regard to the mutagenicity data there are no conflicting gene mutation results in vitro. The available positive data from studies in bacteria and cultured mammalian cells show deficiencies in reporting and/or methodology regarding the current guideline standards. All in all one available robust negative bacterial gene mutation addresses clearly the gene mutation endpoint in vitro.

Date	Country	Organisation	Type of Organisation	Comment number

07.01.2014	United Kingdom		Individual	6
Comment received				

The robust analysis given in the proposal describes convincing evidence that the thyroid tumours seen in the rodent carcinogenicity studies (as described in the proposal) are due to the effects of iodine overload on the thyroid gland following chronic iodomethane exposure. There are many precedents for this non-genotoxic mode of action and the profile of the toxic and histological effects seen do not fit with a genotoxic mode of action as a contributor. If iodomethane acted as a genotoxic carcinogen, the site of contact tissue for inhaled exposures, the nasal epithelium, would be the expected target for carcinogenicity. Although toxic histological damage is observed in the nasal epithelium of animals in the long-term rodent studies of iodomethane, there is no evidence of carcinogenicity in this tissue.

With regards to the key question on the robustness of the lack of a genotoxic mode of action for the induction of thyroid tumours seen in male rats given the highest dose of iodomethane in the inhalational study (and the increasing trend for the same tumours seen in male mice), the following are most pertinent:

i) All the elements for the induction of thyroid tumours due to disturbance of the thyroid-pituitary axis were present in animals dosed with iodomethane i.e. alterations in thyroid and pituitary hormonal levels; follicular cell hyperplasia and cytoplasmic vacuolation; increased weight of the thyroid at the 52 week interim sacrifice; progression of the thyroid follicular changes following prolonged exposure.

ii) Reversibility of effects when test article exposure ceases (after short-term exposure).

iii) Correlation between doses that produce thyroid effects and tumours i.e. statistically significant effects on the pituitary and thyroid were only seen at the dose that induced tumours.

iv) Similar effects have been demonstrated with chronic exposure of rats to iodinated glycerol, which resulted in an increase in thyroid follicular cell adenomas.

v) Thus, as stated by the EPA OPP Cancer Assessment Review Committee when reviewing iodomethane carcinogenicity, 'the key event influencing the thyroid tumour response is the sustained stimulation of cell proliferation by Thyroid Stimulating Hormone (TSH), consistent with the increase in thyroid follicular cells only.' (2)

vi) If iodomethane was acting through a genotoxic mechanism, the most likely site of for tumour formation would be tissues that were at the initial site of contact. One such tissue following inhaled exposure is the nasal epithelium, where it was shown that high levels of methyl iodide deplete glutathione, induces necrosis and metaplasia of nasal epithelial cells, but no tumours. Formaldehyde gas, an irritant and reactive compound like methyl iodide, also targets the nasal epithelium and does induce tumours in this tissue (3).

There are two other older carcinogenicity studies not carried out to modern standards giving positive results for tumour induction by methyl iodide as described by an assessment by IARC (4). In the first of these rats were dosed by subcutaneous injections. Test animals developed an increase in local sarcomas. This was dismissed by Mileson et al (ibid) due to the likely effects of high local concentrations of iodomethane in tissues that overwhelm detoxification pathways. However, the production of local site sarcomas is a well known outcome of exposure to irritating compounds, such that similar effects can be demonstrated in studies of compounds such as hydrochloric acid (5).

In the second study, iodomethane was injected intraperitoneally in strain A mice, this is known to be susceptible to the development of lung tumours at an early age. This study was carried out to an inadequate protocol with small numbers of animals and although the IARC assessment of this study stated that 'a marginally increased incidence of lung tumours was observed' this was not sufficient to define carcinogenicity (6).

As discussed in the proposal there is considerable data to show that iodomethane is metabolised by conjugation with glutathione (primarily in the liver) in a detoxification reaction to form S-methylglutathione. Cleavage of the glutamic acid and glycine moieties of glutathione yields S-methylcysteine and further metabolism yields mercapturic acid and methylthioacetic acid. Free iodine is also released during metabolism. Conjugation of iodomethane with glutathione is predominantly non-enzymatic and thus is not dependent to the same extent on levels of glutathione S-transferases including the theta isozymes (7). This is important as there are human subpopulations that lack this enzyme and could be at potential risk, if conjugation was primarily enzymatic (8).
As discussed above, iodomethane induced toxicity to nasal epithelium is known to be associated with glutathione depletion from this tissue (9), yet even when glutathione levels are largely depleted following chronic exposure to iodomethane, nasal tumours are not induced in this tissue.
1. Gansewendt, B, Xu, D, Foest, U, Hallier, E, Bolt, HM, Peter, H. (1991). DNA binding of methyl iodide in male and female F344 rats. Carcinogenesis, 12, 463-467.
2. EPA, Office of Prevention, Pesticides and Toxic Substances (2005) Iodomethane: Report of the Cancer Assessment Review Committee, PC Code: 000011, November 10th 2005, TXR Number: 0053852.
3. Kerns, W.D., K.L. Pavkov, D.J. Donofrio, E.J. Gralla and J.A. Swenberg. 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res. 43: 4382-4392
4. Agency for Research on Cancer (1986) Methyl iodide. In IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol 41, pp 213-227.
5. International Programme on Chemical Safety (IPCS) (1982). Chlorine and Hydrogen Chloride. Environmental Health Criteria 21. WHO. Geneva.
6. Hallier, E, Deutschmann S, Reichel, C, Bolt, HM, Peter, H. (1990). A comparative investigation of methyl bromide and methyl iodide in human erythrocytes. Int. Arch. Occup. Environ. Health, 62, 221-225.
7. Strange, RC, Fryer, AA. (1999) The glutathione S-transferases: influence of polymorphism on cancer susceptibility. IARC Sci. Publ. 148, 231-249.
8. Chamberlain, MP, Lock, EA, Reed, CJ (1998) Investigations of the pathways of toxicity of methyl iodide in the rat nasal cavity. Toxicology, 129, 169-181.

Dossier Submitter's Response

Thank you for this supportive summary of the data, including the older carcinogenicity studies.

RAC's response

As Iodomethane is classified as irritant to the skin and the respiratory tract, irritation properties may have occurred at the injection sites in the old rat studies.

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2014	France		MemberState	7

Comment received

p. 33: In the 78wk study in mouse (CD-1)by Harriman (2005) and Kirkpatrick (2008a), FR wonders if the MTD was reached. Indeed, there were no treatment related effects on survival and all groups had 79% or higher survival. At the end of the study body weights for males at 60 and 200 ppm and males and females at 600 ppm were approximately 7-11% lower than the control group. Details would be appreciated.
Only cumulative body weight gains was affected, which is not a criteria for defining the MTD.
This study confirms the effect of the substance on adenoma and carcinoma follicular cell

tumor.
Interestingly, this study displayed findings on another tissue: fibroma of the cervix and uterus. No further data has been provided to discard this finding.

p.36: The inhalation study conducted by Kirkpatrick (2005) Kirkpatrick (2008b) should be regarded with caution: indeed the hormonal results from the week 52 are incoherent. It might be welcome to have an explanation. Concerning table 23, the number of animals would also be appreciated.

p. 39: It is mentioned other tumours that have not statistically significant incidence: the list would be appreciated.

In conclusion, FR believes that the uncertainties around the mode of action of Iodomethane (in particular its genotoxicity that might lead to more than one site of carcinogenicity), together with the uncertainty on which extent its direct effect on thyroid hormone homeostasis leading to carcinogenic effects is relevant to human justify the Carc2 classification to be maintained.

Dossier Submitter's Response

78-week mouse study (pages 33-36)

The effects in the highest dose group (600 ppm) in this carcinogenicity study provided evidence of toxicity in the form of reduced body weight gain (24-27% lower than control) and body weights (9-11% lower than control), toxicity to the thyroid, and local irritation to the upper gastrointestinal tract. Mean body weights were consistently statistically significantly lower than in the control group throughout the study. Therefore, 600 ppm is considered a MTD and may have exceeded it based on the degree of reduction in body weight gain.

Additional support for this interpretation comes from the 90-day dose range finding study. This showed one male and one female death at the highest dose (1200 ppm), both of which were considered treatment related, and mean bodyweights were reduced by 11-17%. This dose level would not have been sustainable for 78-weeks. It is likely that 600 ppm was chosen as the highest dose level for the carcinogenicity study as it was half the dose that resulted in mortality and was well above the NOAEL of 400 ppm, where a 6-8% decrease in final bodyweights had been observed relative to controls. Note that there were no dose levels between 400 and 1200 ppm in the 90-day study.

Please refer also to the responses to comments 3 and 5 about the findings of fibroma in the uterus or cervix. These were discussed in the CLH report and a PWG concluded that the lesions were not treatment related after extensive histopathological examination of additional sections of the tissues. Therefore, it is not correct to say that "No further data has been provided to discard this finding".

2-year rat study (pages 36-40)

It is considered that the results from this study can be reliably interpreted for the evaluation of the carcinogenic potential of iodomethane and effects on the thyroid gland. The results of the thyroid hormone measurements are shown below in Table 23 from the CLH report and include the number of animals examined at each time point in square parentheses.

The pattern of changes in serum thyroid hormone levels and TSH were discussed in Annex 1 of the CLH report. Serum levels of T_3 and T_4 in rats at 60 ppm were slightly lower than

those in controls at week 26, however, the levels were generally similar to those in controls at weeks 52 and 104. This, together with the reduced magnitude of the increase in TSH at weeks 52 and 104 compared with week 26, suggests that compensatory mechanisms were operating after prolonged exposure to iodomethane. Thus the results at week 52 are considered not to be incoherent. For T_3 and rT_3 at study weeks 26 and 52, the low number of samples analysed for some groups probably affected differences from control group means and findings of statistical significance. The results of the 2-day inhalation study (Himmelstein, 2004) revealed significantly lower serum T_3 and T_4 levels and significantly increased serum TSH after 6-hour exposures each day at 100 ppm.

Overall, the changes in serum hormone levels are consistent with the mode of action for thyroid tumour formation in the rat and the 2-year inhalation study does not need to be regarded with caution.

The CLH report clearly states that apart from the thyroid there were no other statistically significant differences in tumour incidences. The only finding of note which was not statistically significant was the astrocytoma incidence, this was discussed in the CLH report. An additional table is provided below listing the only other tumour incidences that were above control incidences. The single incidences of each tumour type are considered to be unrelated to treatment

Table 23: Summary of thyroid hormone data

Parameter	Males				Females			
Dose level (ppm)	0	5	20	60	0	5	20	60
TSH (ng/dL)								
Week 26	2.46±1.2 [10]	3.78±1.9 [10]	4.92±3.9 [9]	30.53±13.7** [9]	1.76±0.6 [10]	1.76±0.5 [10]	2.09±0.7 [9]	12.92±13.4** [10]
Week 52	2.25±0.9 [10]	2.26±0.6 [10]	3.60±2.8 [10]	9.11±11.4 [20]	2.61±0.7 [9]	3.33±1.9 [10]	2.87±1.3 [10]	5.49±6.4 [18]
Week 104	2.38±1.1 [17]	3.29±1.6 [23]	3.48±1.8 [24]	11.29±14.9** [17]	2.52±1.0 [19]	2.93±1.8 [23]	3.78±2.9 [23]	3.98±6.3 [18]
T_3 (ng/dL)								
Week 26	57.50±5.8 [5]	51.40±18.6 [8]	57.12±21.1 [4]	38.08±16.3 [4]	67.54±28.3 [8]	55.38±17.1 [10]	80.12±21.9 [8]	49.44±19.7 [7]
Week 52	43.23±11.4 [2]	38.95±15.6 [8]	51.34±40.4 [8]	38.29±11.4 [16]	81.78±33.1 [7]	78.70±20.5 [7]	60.10±9.8 [5]	72.55±15.7 [12]
Week 104	49.79±21.0 [17]	52.77±21.0 [23]	50.01±20.8 [24]	44.28±15.9 [17]	72.72±32.4 [19]	70.90±19.3 [23]	65.93±24.0 [23]	64.82±22.2 [18]
T_4 (ng/dL)								
Week 26	3.87±1.0 [10]	3.38±0.4 [10]	3.24±0.5 [7]	1.71±3.4** [9]	2.03±0.6 [9]	1.68±0.6 [10]	1.93±0.5 [8]	1.78±0.7 [9]
Week 52	2.56±0.8 [9]	2.45±0.9 [10]	3.44±0.7 [10]	3.42±0.8* [20]	2.02±0.3 [10]	2.16±0.4 [9]	1.74±0.3 [9]	2.23±0.6 [16]
Week 104	2.25±0.7 [17]	2.27±0.7 [23]	2.24±1.0 [24]	2.50±0.6 [17]	1.55±1.0 [19]	1.56±0.7 [23]	1.96±0.8 [23]	2.47±1.0** [18]
rT_3 (ng/dL)								
Week 26	0.13±0.05 [5]	0.12±0.05 [7]	0.11±0.05 [4]	0.15±0.03 [2]	0.10±0.05 [7]	0.11±0.03 [6]	0.15±0.05 [8]	0.19±0.09 [8]
Week 52	0.09±0.03 [5]	0.09±0.05 [5]	0.09±0.04 [8]	0.19±0.05** [6]	0.12±0.04 [6]	0.14±0.06 [3]	0.09±0.02 [4]	0.33±0.16** [9]
Week 104	0.03±0.03 [17]	0.04±0.03 [23]	0.04±0.03 [24]	0.07±0.05** [17]	0.05±0.03 [19]	0.09±0.04 [23]	0.20±0.12** [23]	0.24±0.12** [18]

Male data: Note: Weeks 26 and 52 total T_3 and reverse T_3 compared using the Kruskal-Wallis test. All total T4 and TSH and week 104 total T_3 and reverse T_3 compared using Dunnett's test.
* Significantly different from the control group at 0.05.
** Significantly different from the control group at 0.01.
Female data: ** Significantly different from the control group at 0.01 using Dunnett's test.
Values in square parenthesis refer to number of animals analysed. Wk 26 10 animals/sex/gp; wk 26 and 52 10/animals/sex/gp for groups 1-3; and 20 animals/sex/gp for group 4. Serum collected from all remaining animals at wk 104.

Additional table: 2-year rat: summary of tumour incidences where incidence exceeds concurrent control (incidence / no. of animals examined)

Organ	Finding	0 ppm	5 ppm	20 ppm	60 ppm
Males					
Duodenum	Malignant carcinoma	0/17	0/0	1/1*	0/17
	scheduled				
Females					
Lungs	Metast. carcinoma, follicular cell	0/31	0/27	0/26	1/32
	scheduled				

* Gross macroscopic abnormality detected therefore tissue examined

For these tumour incidences no historical control data were available.

RAC's response
FR remarks and the additional information were considered.

Date	Country	Organisation	Type of Organisation	Comment number
15.01.2014	Germany		MemberState	8
Comment received				
The German CA supports the proposal to remove Carc 2 – H351 (Suspected of causing cancer) of the current classification. Based on the data reported iodomethane does not present a carcinogenic hazard to human and consequently it should not be classified for carcinogenicity. The proposal to remove the carcinogenicity classification of iodomethane from Annex VI of the CLP Regulation included data from toxicokinetic, repeated dose toxicity, mutagenicity, and experimental studies on carcinogenicity after oral administration in mice and after inhalation exposure in rats. Iodomethane induced benign follicular adenomas of the thyroid gland in male rats and male mice. There is evidence to conclude that tumour induction in the thyroid glands is based on inhibition of T4 release which is listed as one of the clearly established mechanisms for perturbation of the pituitary-thyroid hormone axis				
Dossier Submitter's Response				
Thank you for supporting the proposal.				
RAC's response				
The fact that the inhibition of T4 release indicated a perturbation of the pituitary-thyroid hormone axis is not a sufficient argument on its own to support declassification.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
07.01.2014	United Kingdom		Individual	9
Comment received				
Iodomethane, an established, direct alkylating agent, is positive in some in vitro genotoxicity assays, as discussed in the proposal. In some, but not all bacterial mutation assays, this compound is weakly positive in strains that detect base-change mutagenicity. The finding that mutagenic activity in bacteria is weak may be related to its potency as an inducer of several efficient DNA repair pathways that remove the methyl groups from alkylated DNA bases and the direct removal of the alkylated bases themselves. Iodomethane is also positive in assays to measure chromosome damage in Chinese hamster ovary (CHO) cells in culture and in some mouse lymphoma tk assays, also as a result of chromosome damage. These positive findings generally occur at cytotoxic doses. DNA damage induced by iodomethane can be repaired by a number of enzymes including O6 methylguanine-DNA-methytransferase (MGMT) (1), an enzyme that transfers methyl groups from DNA lesions onto a cysteine residue within its own molecule. This enzyme is highly efficient, is conserved in nature and is present in human cells. The adaptive response in bacteria is a cellular process to induce DNA repair enzymes in response to challenge by alkylating agents. The Ada protein plays a major role in this response. It accepts methyl groups from methylated DNA at cysteine residues as per the MGMT protein. The methylated Ada protein not only induces its own gene transcription but				

also that of the alkB, alkA and aidB genes, all involved in the removal and/or repair of alkylated DNA, by a variety of mechanisms (2). It also appears that particular methylating agents, like iodomethane, can directly methylate the Ada protein causing induction of the repair process and promoting resistance to alkylation damage (3). These DNA repair activities are conserved from bacteria to man and thus there are homologues of the some of these bacterial genes identified in the human genome (4). In most of the references cited above, iodomethane has been used as a model compound to study this process. There are additional DNA repair systems that can repair alkylated DNA, including base excision repair, mismatch repair and nucleoside excision repair (as discussed in reference 4).
Iodomethane is known to be an SN2 class alkylating agent (1), which like all members of this class, preferentially induces non-mutagenic N7 methylguanine adducts and very much lower levels of the highly mutagenic O6- methylguanine. This contrasts with SN1 class alkylating agents which induce much higher levels of O6 –methylguanine (5). It is SN1 alkylating agents that tend to be potent genotoxic carcinogens and some are used in chemotherapy for anti-cancer treatments (6).

Studies of iodomethane in chromosome damage studies in vitro and in mouse lymphoma assays, have produced a consistent picture of structural chromosome damage as reviewed in Mileson et al (7) and in the proposal . As stated, most of the positive responses seen were at cytotoxic doses. This potential is not realised in a GLP in vivo mouse bone marrow micronucleus test carried out to modern protocols, at high doses (MTD) given by the intraperitoneal route. A study in rats given 14C radiolabelled iodomethane purported to show the appearance of methylated DNA bases in a variety of tissues (mostly not the target tissues for toxicity) (8). However, as stated in the proposal and the Mileson paper (ibid), it is apparent that the radio labelled carbon appears in the one carbon pool and is thus incorporated into purine bases through de novo synthesis.

1. Oh, HK, Teo, AK, Ai, RB, Lim, A, Ayi, TC, Yarosh, DB, Li, BF (1996) Conformational change in human DNA repair enzyme O6-methylguanine-DNA methyltransferase upon alkylation of its active site by SN1 (indirect-acting) and SN2 (direct-acting) alkylating agents: breaking a 'salt-link'. Biochemistry, 35, 12259-12266.
2. Sedgwick, B, Lindahl, T(2002) Recent progress on the Ada response for inducible repair of DNA alkylation damage. Oncogene, 21, 8886-8894
3. He, C, Wei, H, Verdine, GL (2003) Converting the sacrificial DNA repair protein N-Ada into a catalytic methyl phosphotriester repair enzyme. J. Am. Chem. Soc., 125, 1450-1451.
4. Nieminuszczy, J, Grzesiuk, E (2007) Bacterial DNA repair genes and their eukaryotic homologues: 3. AlkB dioxygenase and Ada methyltransferase in the direct repair of alkylated DNA. Acta Biochimica Polonica, 54, 459-468.
5. Beranek, DT (1990) Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. Mutat Res, 231, 11-30.
6. Fu, D, Calvo, JA, Samson, LD (2012) Balancing repair and tolerance of DNA damage caused by alkylating agents. Nature Reviews Cancer, 12, 104-120.
7. Mileson, BE, Sweeney, LM, Gargas, ML, Kinzell, J. (2009) Iodomethane human health risk characterisation. Inhalation Toxicology, 21, 583-605.
8. Gansewendt, B, Xu, D, Foest, U, Hallier, E, Bolt, HM, Peter, H. (1991). DNA binding of methyl iodide in male and female F344 rats. Carcinogenesis, 12, 463-467.

Dossier Submitter's Response
Thank you for this additional analysis of the data.
RAC's response
Thank you for this additional detailed information.

Date	Country	Organisation	Type of Organisation	Comment

				number
20.01.2014	France		MemberState	10
Comment received				
p.9: DS states that " Iodomethane is assessed not to be genotoxic in vivo and, given the target organ specificity of the tumour response in rats and mice, a genotoxic MoA is considered implausible. " when pg 27, it is stated: " both positive and negative findings have been reported in the assays for gene mutation. A definitive conclusion about the mutagenicity of iodomethane in mammalian cells is not possible from these studies". FR believes that the second statement is much more coherent with the bunch of data presented, although FR also agrees that "this genotoxic activity was not confirmed in a well-conducted in vivo mouse bone marrow micronucleus assay (with target organ exposure)". p.29: FR would appreciate having the data of the "small, dose-related decrease in group mean P/N ratio" from the Gudi and Krsmanovic study (2001) Therefore, FR believes that the data provided do not allow to state on the genotoxicity. Indeed, this mechanism cannot be excluded and should be taken into account while evaluating carcinogenicity of the substance.				
Dossier Submitter's Response				
The conclusions stated by France are considered in isolation. The conclusions drawn on page 27 discuss the findings from the *in vitro* gene mutation studies only. The GLP compliant OECD 476 mouse lymphoma (MLA) study that was undertaken was conducted prior to the complete published recommendations of Moore *et al*. Consequently, the gene mutation data generated should be viewed with caution. On page 29 an overall summary of the gene mutation data (bacterial and mammalian) is provided. Whilst it is clearly stated that "*a definitive conclusion about the mutagenicity of iodomethane in mammalian cells is not possible*", the robust negative Ames study more than adequately addresses the gene mutation endpoint. This conclusion is supported by an evaluation by Kirkland *et al* (2005, 2011) on combinations of two or three assays, which has shown that the inclusion of *in vitro* mammalian gene mutation cell into the basic *in vitro* battery does not enhance either the specificity[1] or sensitivity[2] of the test battery. The *in vitro* chromosomal aberration data confirmed evidence of increased frequency of chromosomal aberrations, which was not realised in the robust *in vivo* mouse bone marrow micronucleus study. Regarding the request to provide data on the mean P/N ratio from the Gudi and Krsmanovic study, this is included in the CLH report (Table 18, page 31). At doses of 0, 25, 50 and 100 mg iodomethane/kg bw/d, PCE ratios of 0.451, 0.518, 0.443, 0.384 and 0.470, 0.484, 0.406, 0.374 were reported for males and females, respectively following the 24 h harvest. As stated in the CLH report, with reasonable certainty in the absence of analytical confirmation, systemic exposure of bone marrow to iodomethane following *ip* injection was deemed to have resulted. "*Following oral administration iodomethane is completely absorbed. Due to the rich blood supply in the intraperitoneal cavity, absorption is expected to be rapid and complete following the administration of an aqueous solution of iodomethane into an aqueous environment. The bone marrow is a well perfused tissue and it can be deduced therefore that iodomethane levels here will have been comparable to those in blood or plasma*." The genotoxicity data has been considered when addressing the carcinogenicity data. If the direct alkylating agent iodomethane were to be acting *via* a genotoxic mode of action the most likely site of tumour formation would the site of first contact. In two rodent				

carcinogenicity studies (performed by the oral and inhalatory routes), evidence of nasal epithelial necrosis and metaplasia resulting from depleted glutathione was observed following exposure *via* the inhalation route, which did not lead to neoplastic lesions. Following oral exposure hyperkeratosis, limited to the squamous regions of the upper gastrointestinal tract were observed, without development of any neoplastic lesions. This was considered most likely to be an irritant effect of iodomethane.

References:
Kirkland, D., Aardema, M., Henderson, L. & Muller, L. (2005). Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. 1. Sensitivity, specificity and relative predictivity. *Mutation Research,* **584**, pp 1-256.
Kirkland, D., Reeve, L., Gatehouse, D. & Vanparys, P. (2011). A core *in vitro* genotoxicity battery comprising the Ames test plus the *in vitro* micronucleus test is sufficient to detect rodent carcinogens and *in vivo* genotoxins. *Mutation Research*, **721**, pp 27-73.

Footnotes:
1. In the context of genotoxicity testing specificity refers to the correct prediction of non-carcinogens
2. In the context of genotoxicity testing sensitivity refers to the correct prediction of rodent carcinogens which are *in vivo* genotoxins

RAC's response

RAC supports the statement on the mutagenic aspects given by the DS.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2014	France		MemberState	11

Comment received

FR would appreciate the dossier submitter to give more information related to the 90-day oral study in dogs (Harriman, 2002). Indeed, the description of the findings at 6 mg/kg/d is pretty scarce. We would appreciate to have a circumstantial description of the findings, especially on Thyroïd.
Similarly, FR would appreciate the dossier submitter to give more information related to the 1-year oral study in dogs (Harriman, 2004; Harriman and Armstrong, 2005). Indeed, there is no indication if any findings related to the Thyroïd were observed (or not) at 6 and 1.5 mg/kg/d.

FR would appreciate the dossier submitter to give more information related to the 90-day inhalation study in rats (Kirkpatrick, 2002). Indeed, there is no indication if there were no or any findings on Thyroïd.
From the, 28-day inhalation study in rats (Nemec, 2004a), can DS specify if the findings observed on Thyroid at 100ppm were or were not observed at 25 and 75 ppm?

Dossier Submitter's Response

<u>90-day dog study</u>:
<u>Effects observed at 6 mg/kg bw/day (in addition in those reported in CLH report)</u>:

- *Clinical signs*: increased frequency of emesis occurred throughout the study. The severity gradually improved beginning ~2 wks after initiation of dosing indicating some acclimation to an apparent GI irritation caused by the test article. Three individual animals were also affected by periods of emesis, accompanied by weight loss and decreased food consumption.
- *Body weight*: no effects on mean body weight or body weight changes were observed

in any of the treated groups, compared to the control. Body weight losses were noted for individual animals during periods of frequent emesis, decreased food consumption and hypoactivity.

- *Food consumption*: There were no test article-related effects on mean food consumption values. Decreased food consumption was noted for individual animals during periods of frequent emesis, body weight loss and/or hypoactivity.
- *Haematology*: There were no test article-related effects on haematology parameters.
- *Chemistry:* significant differences were observed, but were considered not to be treatment-related due to lack of a dose response or temporal-related trends or similar effects in the opposite sex.
- *Serum hormones*: No test material related effects on serum hormones (TSH, T_3, T_4) were observed.
- Urinalysis: There were no test article-related effects on urinalysis parameters.
- *Macroscopic examinations*: at 6.0 mg/kg bw/day no test article-related macroscopic findings were observed at the scheduled necropsy.
- *Organ weights*: There were no test article-related effects on organ weights.
- *Microscopic examination*: test article related findings were observed in the stomach and in one male at the scheduled necropsy. Possible test article related changes in the olfactory epithelium (degeneration observed at nasal level 4) were noted in the 6.0 mg/kg bw/day group females.
-

Thyroid observations: due to the nature of the test article the thyroid glands were examined carefully. Although some variety in the size and number of thyroid follicles was observed, there did not appear to be any evidence of thyroid follicular hyperplasia/degeneration. Colloid within thyroid follicles of the iodomethane-treated dogs also appeared adequate. No treatment related changes were noted in thyroid hormone levels or the thyroid gland at any dose level in this study. The highest dose level (15 mg/kg bw/day) resulted in significant toxicity, including one male sacrificed in moribund condition.

1 year dog study:
Thyroid observations: whilst higher mean thyroid stimulating hormone (TSH) levels were noted in the 12 mg/kg bw/day group males and females, this was attributed to one animal of each sex. Both these animals had accompanying microscopic changes in the thyroid as described in the CLH report. No alterations in TSH, T_3 or T_4 levels or microscopic changes in the thyroid gland were observed in the mid or low dose groups (6 and 1.5 mg/kg bw/day). Note that thyroid effects were only observed at the highest dose level (12 mg/kg bw/day) which resulted in mortality; it was therefore considered to exceed a MTD.

90-day inhalation study in rats
There were considered to be no treatment related histopathological findings in the thyroid. One out of 10 males at the highest dose (70 ppm) had mild thyroid follicular cell hypertrophy. Thyroid glands of animals in the low and intermediate dose groups were not examined in the absence of treatment related findings. In light of the findings in the subsequent 2-year inhalation study the observation of one animal with follicular cell hypertrophy may be related to iodomethane exposure.

28-day inhalation study in rats
This was a dose range-finding study for further studies, including the 90-day study, and as noted in the CLH report no histopathological examinations were performed on low and intermediate dose animals.

RAC's response

Additional information noted.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON METHYL IODIDE; IODOMETHANE

Confidential Appendix 1 – Pathology Working Group Peer Review of Proliferative Lesions Reported in the Uterus and Cervix. Supplemental to 18month Carcinogenicity Study of Microencapsulated Iodomethane in the Female CD-1 Mice – Hardisty 2005