Committee for Risk Assessment

RAC

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

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CLH-O-0000004613-77-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: **Iodomethane**
EC number: **200-819-5**
CAS number: **74-88-4**

The proposal was submitted by the **United Kingdom** and received by the RAC on **4 December 2013**. All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 December 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **20 January 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Agnes Schulte**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **12 September 2014** and the comments received are compiled in Annex 2.

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

OPINION OF THE RAC

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

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)	
Current Entry	602-005-00-9	methyl iodide; iodomethane	200-819-5	74-88-4	Carc. 2 Acute Tox. 4* Acute Tox. 3* Acute Tox. 3* STOT SE 3 Skin Irrit. 2	H351 H312 H331 H301 H335 H315	GHS06 GHS08 Dgr	H351 H312 H331 H301 H335 H315		
Dossier submitters proposal	602-005-00-9	methyl iodide; iodomethane	200-819-5	74-88-4	**Remove** Carc. 2	**Remove** H351	**Remove** GHS08	**Remove** H351		
RAC opinion	602-005-00-9	methyl iodide; iodomethane	200-819-5	74-88-4	Carc. 2	H351	GHS08	H351		
Resulting Annex VI entry if agreed by COM	602-005-00-9	methyl iodide; iodomethane	200-819-5	74-88-4	Carc. 2 Acute Tox. 4* Acute Tox. 3* Acute Tox. 3* STOT SE 3 Skin Irrit. 2	H351 H312 H331 H301 H335 H315	GHS06 GHS08 Dgr	H351 H312 H331 H301 H335 H315		

RAC general comment

RAC has assessed germ cell mutagenicity for classification, although it is not currently included in Annex VI and was not proposed for amendment by the Dossier Submitter (DS). The genotoxicity data provided under that heading were, however, relevant for the assessment of the proposed carcinogenicity declassification and the DS did include an assessment of this hazard class in the CLH report. Furthermore, a number of parties concerned (individuals as well as MSCAs; see below for details) provided comments addressing this hazard class. RAC therefore considered assessment of this hazard class justified.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The DS concluded that a proposal to classify iodomethane for mutagenicity is not justified on the basis of the available genotoxicity data.

Regarding the induction of gene mutations in bacteria as well as in mammalian cell cultures, positive and negative results have been reported. No clearly positive result was available due to the inadequate quality of the test procedures and their limited reporting (in comparison with the regulatory standards in the current technical guidelines). The only negative bacterial gene mutation test (Wagner and Dakoulas, 2001) was characterised as well-conducted by the DS. Although the procedure was not fully in compliance with the corresponding guideline, essential guideline requirements and GLP were considered. Therefore this bacterial gene mutation test adequately addressed gene mutations *in vitro*. The same applies to the test procedure for the chromosomal aberration test *in vitro*. Neither available test was fully in compliance with the respective guideline but essential guideline requirements and GLP were considered. The induction of clastogenic effects with and without S9 in a positive in vitro chromosomal aberration test with CHO cells (Gudi and Brown, 2001) was not confirmed in a guideline-compliant negative *in vivo* micronucleus test in mice (Gudi and Krsmanivic, 2001).

In summary: On the basis of the available mutagenicity tests, the DS came to the conclusion that iodomethane induces no mutagenic effects that are relevant for classification.

Comments received during public consultation

There were three individual comments from the UK that supported the conclusion of the DS.

One individual comment gave a complementary analysis of the available genotoxicity data. Another individual comment considered that the lack of clastogenicity in the *in vivo* mouse bone marrow micronucleus test was a key finding regarding the assessment of the potential of iodomethane to induce genotoxic damage *in vivo*. A further individual comment precluded the induction of genotoxic effects based on the absence of evidence for genotoxic carcinogenicity in the thyroid as well as in the nasal epithelium, the latter being the first site of contact tissue for iodomethane after inhaled exposure.

Different comments were received from two member states regarding the genotoxic potential of idomethane. One agreed with the conclusion of the DS that thyroid tumours are induced by non-genotoxic compounds in rodents. The other considered that the available data provided do not allow a conclusion to be reached on genotoxicity and therefore genotoxic potential cannot be excluded.

An NGO (European Trade Union Confederation) stated that a genotoxic action of iodomethane cannot be ruled out because in addition to the induction of thyroid tumours, other sites of carcinogenicity have also been identified.

Assessment and comparison with the classification criteria

Comparison with the criteria
Based on an evaluation of the genotoxicity data for iodomethane, the DS and RAC both came to the same conclusion, that considering the reliable mutagenicity data, classification of iodomethane as an *in vivo* mutagen is not warranted.

RAC justification With the exception of the *in vivo* micronucleus test, none of the available genotoxicity studies was fully compliant with the respective current test guidelines and only some mutagenicity tests conducted were comparable with the requirements. Therefore the extent to which conclusions could be drawn from the test results of each study was assessed to be different.

RAC agreed with the statement of the DS that the positive gene mutation tests in bacteria as well as in cells of mammalian cell cultures showed deficiencies in reporting and/or methodology in comparison with the current guidelines. Because the reliability of these studies was considered to be doubtful they were not considered in the evaluation of the mutagenic potential of iodomethane. An *in vitro* chromosomal aberration test was carried out according to GLP and was essentially consistent with guideline requirements. This test, as well as the guideline-compliant *in vivo* micronucleus test, was taken into account by RAC for the assessment of the mutagenic potential of iodomethane.

In vitro
- A bacterial gene mutation test (pre-incubation protocol) was negative with and without S9 up to the highest tested concentration of 5000 µg/plate (Wagner and Dakoulas, 2011).
- A gene mutation test in CHO cells was negative with and without S9 up to the highest tested concentrations of 200 µg/mL and 175 µg/mL, respectively (San and Clarke, 2001).
- A chromosomal aberration test was positive with S9 (at the highest tested concentrations of 150 and 250 µg/mL) and without S9 (at the highest tested concentrations of 100 and 200 mg/mL) (Gudi and Brown, 2001).

In vivo
A micronucleus test with male and female mice was negative up to the highest tested dose of 100 mg/kg bw, which was considered to be the maximum tolerated dose (MTD) after a single intraperitoneal injection of each tested dose (Gudi and Krksmanovic, 2001). No deaths or clinical signs were observed. It can be assumed that the reductions in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) indicated exposure of the bone marrow cells to iodomethane.

Conclusion
Iodomethane did not induce gene mutations in bacteria or in mammalian cell cultures. An induction of clastogenic effects was observed in proliferating CHO cells in a directly exposed cell line. The induction of clastogenicity *in vitro* was not confirmed *in vivo* in a micronucleus assay in which bone marrow was the target organ. According to the current state of knowledge and taking into account its systemic availability, iodomethane is not considered mutagenic *in vivo*.

RAC agreed with the DS proposal that based on the data presented in the dossier, **no classification** for germ cell mutagenicity was warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS proposed to revise the present harmonised classification of iodomethane as carcinogen Cat. 2 (Annex VI of the CLP Regulation). The current classification was agreed to in 1987 when guideline compliant studies were not available, using data from inadequate studies with intradermal or intra-peritoneal injections. The DS briefly described the available information on which the assessment was presumably based. In the study of Duckrey *et al.* (1970), most of the 8-12 rats per group that received 10 or 20 mg/kg bw by weekly subcutaneous injections for an indeterminate period (until necrosis was observed at the injection sites) developed localised carcinomas at the injection site. A further 4/14 rats developed local sarcomas after a single subcutaneous injection. In the second study (Poirier *et al.*, 1975), three groups of 10 female and 10 male A/Heston mice were intraperitoneally injected with iodomethane formulated in tricaprylin 3 times/week for 24 weeks at 0.06, 0.15 and 0.31 mmol/kg bw (and examined for a response on lung adenomas).

New data on toxicokinetics, repeated dose toxicity, mutagenicity and carcinogenicity were documented in the CLH dossier.

From more recent studies (published in 2005 and 2008) there was evidence of statistically significant increases in thyroid follicular adenomas in male rats, of a marginal increase in female rats and an increased incidence in thyroid follicular adenomas and carcinomas (combined) in male mice. A non-genotoxic mode of action was proposed and the DS considered that a perturbation of homeostasis of the hypothalamic-pituitary-thyroid axis was caused by excess circulating iodide derived from the metabolism of iodomethane which induced a reduction in T4 and T3 levels and a compensatory increase in circulating TSH and stimulation of proliferation of the thyroid follicular cells. Hyperplasia of follicular cells can eventually progress to neoplasia.

The DS recognised that increased iodide intake may be a risk factor for thyroid cancer in humans, but was of the opinion that humans appear to have a low susceptibility to thyroid cancer, being much less susceptible than rodents to perturbations in thyroid hormone homeostasis. As thyroid tumours in the male rats were only seen at doses above the MTD, the DS considered a sustained elevation of TSH to be extremely unlikely. The DS referred to the EU Specialised Experts paper (1999) which agreed that substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general, do not need to be classified.

According the DS's view, the slightly increased incidences of fibromas in the uterus/cervix of mice were not considered to be a treatment-related effect as they were benign and no precursor lesions or other signs of toxicity to the uterus or cervix were evident.

A slight, non-significant increase in astrocytomas in male rats was found to be close to the historical control incidence. Radioactivity from [^{14}C] iodomethane was detected in the blood, brain and other tissues, but this was not found to provide a convincing explanation for an iodomethane-related tumour response.

In the DS's view tumours are to be expected at the site of first contact, if the alkylating properties were directly acting via a genotoxic MoA.

According to the DS, comparison with the CLP criteria indicates that classification as a carcinogen is not justified. It is to be noted that the original classification was not based on thyroid cancer.

Comments received during public consultation

While some commentors (2 individuals from the UK, DE) supported the declassification of iodomethane for carcinogenicity, two MS and one NGO (ETUC) disagreed with the declassification proposal.

In their arguments, one MS found that quantitative differences in thyroid hormone homeostasis between humans and experimental animals may exist, but stated that it was not shown that the MoA is not relevant to humans. Modifications of the thyroid hormone homeostasis were also seen in dogs at 12 mg/kg bw/d and from 20 mg/kg bw/d in mice and this supported the contention that effects in humans cannot be excluded. The observation of treatment-related increases in incidences of fibromas in the uterus/cervix of mice raised the concern of a 'multi-site carcinogen' and one MS concluded that the benign nature of the tumours is consistent with the criteria for Cat. 2.

In addition to the disturbance in the HPT-axis as a MoA for thyroid tumours, it was mentioned that other MoA could not be excluded. The substance has direct alkylating properties and showed gene mutations *in vitro* that were not ruled out by a negative *in vivo* chromosomal aberration test because this test does not measure gene mutation.

Some information on the two 'old' carcinogenicity studies was given by an individual who reported that after receiving iodomethane by subcutaneous injection, rats developed an increase in local sarcomas (without providing further details on the dosing regime and study design). The local site sarcomas were interpreted as being related to the irritative property of iodomethane. In the second study mentioned to be assessed by IARC in 1986, iodomethane was injected intraperitoneally to strain A mice which were considered to be susceptible to lung tumour development.

One MS questioned whether the dosing in the 78-wk study in CD-1 mice (Harriman, 2005, Kirkpatrick, 2008a) was sufficiently high to reach the MTD as the final BW in high dose males and females was only 7-11% lower than the control values. In the DS's view the MTD was exceeded. At 600 ppm, the BW gain was 24-27% lower than control and the BW was 9-11% lower than control values and thyroid toxicity and local irritation to the upper gastrointestinal tract were observed.

Based on observations in France of a 6% annual increase in diagnoses of thyroid cancer between 1980 and 2005 and (also in France) the incidence in women ranking 5th in 2005, one MS questioned the statement that thyroid cancer is rare in humans, as also did ETUC.

One MS expressed agreement with the EU Specialised Experts (1999) that non-genotoxic substances that cause thyroid tumours after prolonged disturbance in the hypothalamic-pituitary-thyroid axis are not relevant for humans and that the tumour data indicated that the MoA is a disturbance in homeostasis of the HPT axis.

However, with regard to the fibromas in the cervix, this MS found the argumentation insufficient to enable them to concur with the DS's conclusion of their non-relevance for humans.

The DS provided their analysis on the human relevance of the thyroid tumours using the IPCS framework for analysing the relevance of a cancer mode of action to humans (IPCS, 2007).

Assessment and comparison with the classification criteria

Category 1A
According to CLP criteria, classification as 1A is appropriate if the substance is known to have carcinogenic potential for humans, and this is largely based on human evidence.

There were no human data that could give information on an association between tumour cases and exposure to iodomethane.

Category 1B
A substance should be classified in Category 1B if a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of a combination of benign and malignant neoplasms in at least two species or in two independent studies in one species.

Although thyroid tumours were observed in two species, the increase in malignant thyroid tumours is weak in male mice and male rats. A clear (benign) tumour response was seen in the male rats. The incidences of cervix fibromas (in female mice) and of astrocytomas (in male rats) were also borderline findings. For both tumours, a treatment-related effect could not be ruled out. However, due to low increased rates compared to controls and the inconsistencies between species, uncertainties remain on the causal relationship to the iodomethane treatment. Consequently, RAC concluded that there is insufficient evidence from experimental animals to justify category 1B.

Category 2
The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence in human studies or from limited evidence of carcinogenicity in animal studies.

The specific considerations for classification define four criteria a)-d) (in Section 3.6.2.2.3 of the CLP Regulation) on limited evidence, of which only criterion b) "there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies" may be applicable to iodomethane.

No classification
By default carcinogenic effects in experimental animals are considered relevant to humans and are considered for classification as carcinogen. Only when there is sufficient evidence showing that a certain type of tumour is not relevant to humans should this tumour type be excluded for classification.

The DS proposed that iodomethane does not need to be classified for carcinogenicity based on the guidance of the Specialised Experts (EC, 1999) and taking into account that the increased thyroid tumour rates in male rats were only seen at a level that exceeded the MTD.

In the view of RAC, the two options – classifying as category 2 and no classification – should be considered using the additional considerations for classification (3.6.2.3.2 CLP guidance). The headings and their numbering below are as they appear in the CLP Regulation.

a. *Tumour type and background incidence*
   High spontaneous tumour incidences were not observed in any of the organs/test species/studies and from this aspect there is no reason to question the relevance of the observed tumours. Where background incidences (laboratory/animal supplier data) were

reported, the tumour incidences of the internal control groups were below the background ranges reported (due to the absence of any tumour in the control groups of mice and rats).

Thyroid tumours are rare tumours in mice. The incidence at the high dose (3/49 = 6.1%) was above the animal supplier's background incidence of maximum of 2% for benign and 2% for malignant follicular cell tumours. However the information on the background incidences is limited as no information on the related number of examined animals and no information on the time-window and study design was available.

Fibromas of the cervix and the uterus are rare tumours. The CLH report indicated an absence of cervix fibromas in the breeder's control data, while incidences up to 2% were reported for fibromas of the uterus (size and time period of the control groups is unknown).

b. *Multi-site responses*
The evidence for iodomethane acting as a multi-site carcinogen was weak, as incidences on tumours at extra-thyroidal sites were small and gave equivocal evidence of a relationship to iodomethane treatment.

c. *Progression of lesions to malignancy*
There were some malignant thyroid tumours in mice and rats, but there was only a marginal increase in their incidence.

d. *Whether responses are in single or both sexes*
Incidences of thyroid tumours were elevated in male mice and male rats. Although some hormonal effects and follicular cell hyperplasia were also seen in female rats and mice (follicular cell hyperplasia only), no tumour response was seen in female animals.

f. *Whether responses are in single species or several species*
Two species were positive, with rats more responsive than mice. Thyroid toxicity (colloid depletion, follicular cell hypertrophy, elevated TSH and decreased thyroid hormones, hyperplasia of *pars distalis* of the pituitary) was also observed in dogs that received capsules at 12 mg/kg bw daily for 52 weeks.

h. *Routes of exposures*
Thyroid tumours were seen after oral and inhalation administration.

j. *The possibility of a confounding effect of excessive toxicity at test doses*
The MTD was not exceeded in the mouse study. The highest dose in the rat study may have exceeded the MTD. However, the lower BW gain, hypoactivity and other clinical signs were not thought to be associated to the development of follicular cell tumours. In general, this tumour type has not been shown to be increased in animals with impaired general health status at the end of the chronic treatment.

k. *Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity*
Indications were not given for any of these modes of actions, including mutagenicity, being involved.
A non-genotoxic mode of action is assumed.

Reduced tumour latency (*d*) and *structural similarity to a substance(s) for which there is good evidence of carcinogenicity* (*g*) were not considered particularly relevant for the assessment of the carcinogenic potential of iodomethane.

In addition, *comparison of absorption, distribution, metabolism and excretion between test animals and humans* (*i*) are considered in the discussion on the mode of action (see below).

Of the mechanisms of tumour formation considered not relevant for humans (CLP guidance, Section 3.6.2.3.2) only one was considered relevant to iodomethane: *Certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (IARC, 1999, EU Specialised Experts 1999).*

Using the IPCS framework for analysing the relevance of a cancer mode of action the DS identified a sequence of key events in the proposed oncogenic MoA of iodomethane (for details see Annex 1 of the CLH report) that includes:

- Excess circulating iodide
- Decreased serum T4 and T3 (hypothyroidism)
- Increased serum TSH
- Thyroid enlargement with thyroid follicular cell hyperplasia

The evidence for each of these key events appeared plausible, although the T4 reduction in rats was only transiently seen at week 26. Overall, RAC concluded that the perturbation of the thyroid hormone homeostasis was likely to be the mode of action for the thyroid tumours.

However, a disturbance in the hypothalamic-pituitary-thyroid axis on its own does not justify the non-classification of a test substance.

While the CLP Guidance refers to UGT induction only as a mechanism that is considered as not relevant for humans and one which could justify non-classification, the guidance given in the Specialised Experts document (EC, 1999) identified a number of non-genotoxic mechanisms (including enhancement of thyroid hormone metabolism) that would justify classification as a Category 2 carcinogen if the carcinogen is of high potency (T25 value < 1 mg/kg bw/d) or non-classification if the carcinogen is of low or medium potency (1 mg/kg bw/d < T25 value < 100 mg/kg bw/d). RAC also noted that the EU Specialised Experts (1999) did not consider excess iodine exposure in their paper.

According to the DS calculation iodomethane does not belong to the high potency carcinogen group and thus does not need to be classified. The Specialised Experts agreed in 1999 that there is convincing scientific evidence that humans are considerably less sensitive than rodents (especially rats) regarding i) perturbation of thyroid hormone homeostasis induced by non-genotoxic xenobiotics and ii) development of epithelial thyroid tumours after long-term exposure to such agents.

<u>MoA considerations in the CLH report</u>

Liver UDP-glucuronyltransferase (UGT) activity and increased degradation of thyroid hormones
Mechanistic studies (2-day inhalation study; Himmelstein, 2004) on the induction of UGT (which would indicate a rat-specific MoA by enhanced metabolism of thyroid hormones and which would justify no classification) were negative. Iodomethane did not induce UGT activity in the rat liver.

Catalytic effects on the ring deiodination of thyroid hormones in extra-thyroidal tissues by 5`-deiodinase activity
The hypothesis of the DS was that prolonged inhibition of type I and type II 5'-deiodinase activities by excess iodide from chronic iodomethane exposure would also lead to reductions in T3 and T4 and compensatory sustained increases in TSH, and may contribute to the primary centrally acting effects of excess iodide.

The activity of D1-, D2-, and D3- 5`-deiodinase was assessed in tissue samples from the mechanistic 2 day inhalation study in rats and in *in vitro* studies on microsomal preparations of liver and kidney from pregnant rats and primary astrocyte cell cultures from neonatal rat brains.

Table 30 of the CLH report (with additional information on the results)

Study	D1 Activity	D2 Activity	D3 Activity
Present study (Farwell, 2004)	Microsomal preparations from liver and kidney Reduction at ≥50 mM	Astrocyte cultures prepared from neonatal brains: Reduction at >100 µM Cell deaths at > 1 mM	-
2-day rat (Himmelstein, 2004)	Homogenised liver and kidney*: 25 ppm: Reduction 15-20% in liver, 10-15% in kidney 100 ppm: Reduction 40% in liver and kidney	Homogenised brain*: 25 ppm: Reduction 35% 100 ppm: Reduction 50-55%	Homogenised brain No effect

* findings significant compared to homogenised tissues from control animals;
- = no data

The results from *in vitro* and *in vivo* studies indicated that D1 and D2 enzyme activities were reduced, which the DS interpreted to be due to a non-specific inactivation (*in vitro*) rather than due to a reversible inhibition of the 5`-deiodinase activity.

D1 and D2 enzymes are activating enzymes that catalyse the outer-ring or 5`deiodination of T4 to produce T3, the biologically active form. The formation of T3 in tissues is primarily dependent on these enzymes.

In vivo, it is to be expected that inhibitory effects on D1 and D2 enzymes should induce subnormal T3 serum values as the production of T3 will be inhibited (this was seen in the mechanistic 2 day study at 100 ppm, but not in the cancer studies on rats up to 60 ppm) and could increase the conversion of T4 to reverse T3 (the biologically inactive form, this was seen in the rat cancer study, but not in mechanistic study).

RAC found that the reduced T4 values in rodent studies could not be attributed to the reductions in D1 and D2 enzyme activities (because then T4 would be increased or normal). Iodomethane may have affected the D1 and D2 5`-deiodinase activities, but the data did not allow a clear-cut conclusion on its contribution.

Inhibition of synthesis and release of thyroid hormones by excess serum concentration of iodide
The DS assessed other non-deiodination pathways as the critical initial effect of iodomethane administration. According to the CLH report, the excess of iodide was assumed to block the thyroid peroxidase and inhibit the oxidation of iodide and binding of iodine to thyroglobulin and ultimately block the synthesis of thyroid hormone. Elevated iodide was also noted to inhibit thyroid hormone release (T3 and T4), possibly through the proteolysis of thyroglobulin. Excess iodide may also reduce the effects of TSH stimulation by reducing the cAMP response to TSH receptor binding.

No mechanistic studies were available on iodomethane that demonstrated that this chain of effects had occurred in the rodent studies. Instead human data on effects associated with excess iodide were taken into account by RAC.

From human patients with hyperthyroidism it is known that an excess of iodide inhibits the secretion of thyroid hormones from the thyroid gland. Through an excess of iodide, the circulating levels of T4, T3 and rT3 would be decreased. Results from the rat carcinogenicity study showed that T4 was reduced, but iodomethane had no effect on T3, and rT3 was elevated. Thus, the blocking of the secretion of <u>all</u> thyroid hormones by iodomethane was not observed. This part of the postulated MoA appears not to be plausible

> (For information: T4 is produced only in the thyroid gland, T3 is primarily produced by extrathyroidal deiodination in the liver, kidney, brain, pituitary and brown fat and some

(20% in man) by deiodination in the thyroid. In the rat deiodination of T4 takes place mainly in the thyroid[1]).

The Scientific Committee on food (SCF) summarised in their opinion from 2002[2] general observations on the response to excess iodine (underlining by RAC):

> '*Disturbed thyroid gland activity as a result of excessive iodine intake may manifest itself either as a goitre, as hypothyroidism with/without goitre, or as hyperthyroidism (0.01-0.6% in populations on iodine prophylaxis, 0.25% in West Germany [JECFA, 1989]), the outcome depending on the initial and current iodine status and current thyroid dysfunction. Other effects may be sensitivity reactions (0.4-5%) (JECFA, 1989) and poisoning through ingestion of large quantities of iodine. Modest excessive iodine intake causes a temporary increase in iodide uptake by the thyroid with formation of more organic iodine and large hormone stores. Somewhat larger excessive intake inhibits the iodide release from thyrotoxic thyroids or from TSH stimulated glands and in 0.01-0.06% of exposed people leads to hypothyroidism. Greater excessive intake inhibits the formation of iodinated tyrosine, lowers the T4 and T3 plasma levels and raises the plasma TSH (Wolff-Charkoff iodine effect). These effects may be transient and in many individuals the thyroid can escape this Wolff-Charkoff effect. Individuals not escaping the Wolff-Charkoff effect develop goitre and become hypothyroid. The inhibiting effects of excess iodine occurs via unknown organic compounds, probably iodolipids (Cavalieri, 1997). TSH effects are blunted while the Wolff-Charkoff effect occurs. Other effects include the down-regulation of iodide transport, a raised ratio of iodotyrosines to iodothyronines in thyroglobulin, inhibition of pinocytosis and proteolyis with reduced hormone secretion (EGVM, 2000). The Wolff-Charkoff effect is the basis for the treatment of thyrotoxicosis with iodide. Very high intakes of iodide saturate the active transport system thereby preventing the uptake of radioactive iodine isotopes. If excess intake occurs during pregnancy, the foetal thyroid is unable to escape the Wolff-Charkoff effect. The newborn therefore develops a goitre, is hypothyroid and may suffer possible tracheal compression. Alternatively, the condition may regress spontaneously postnatally after several months.*
>
> *Some subpopulations such as those suffering from autoimmune thyroid disease, from iodine deficiency disorders (IDD) or nodular goitre with autonomous functioning nodules are sensitive to external iodine supply. They tend to respond adversely to levels of iodide which are without adverse effects in the general population. These persons may develop thyroiditis, goitre, hypothyroidism, hyperthyroidism, sensitivity reactions, papillary thyroid cancer and acute effects following exposure to iodide. Iodine-induced hypothyroidism occurs particularly in underlying thyroid disease especially in women (Braverman, 1990).*'

(and, *with regard to goitre and thyroid cancer)*

> '*Some 70% of the epithelial tumours of the thyroid are papillary carcinomas, 15% are follicular carcinomas, >5% are anaplastic carcinomas, while some 5-10% arise from medullary calcitonin-producing C-cells. The papillary carcinomas are less aggressive while the follicular carcinomas have a worse prognosis. Carcinomas are more frequent in females than males, occur especially in the aged and the mortality ranges from 0.2-0.7/100 000 females. Thyroid cancer incidence is increasing in many countries, particularly Norway and Denmark, but mortality rates are decreasing (NNT, 2002). The incidence shows great geographical variation between and within countries indicating an influence of exogenous factors. In man the only well established cause of thyroid cancer is external radiation to the thyroid (NNT, 2002). Goitre predisposes to thyroid papillary*

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1 http://ec.europa.eu/food/fs/sc/scf/out146_en.pdf

2 http://ec.europa.eu/food/fs/sc/scf/out146_en.pdf

cancer as diffuse hyperplasia may be followed by nodular hyperplasia, benign tumour formation and eventual follicular papillary cancer, the risk being related to the presence of goitre and not the functional state of the thyroid. There is no animal evidence for this cancerogenic effect of goitre. The effect of iodine prophylaxis on the incidence of thyroid cancer in an IDD area of Argentine was examined by comparing the incidence in the 15 years before introduction of iodised salt with the incidence in the next 16 years. The incidence of papillary carcinoma increased but there was no effect on the incidence of follicular or medullary cancer. The papillary carcinomas were associated with a higher occurrence of lymphocytic thyroiditis (Harach and Williams, 1995).'

EFSA (2014[1]) did not assess the cancer risks of excess iodine, but concluded with regards to iodine excess as follows:

'*Chronic excessive iodine supply can also lead to goitre, as has, for example, been observed following chronic excessive iodine intakes through water in China (Zhao et al., 2000). Long-term follow-up suggests that chronic excessive iodine intakes may accelerate the development of sub-clinical thyroid disorders to overt hypothyroidism or hyperthyroidism, increase the incidence of autoimmune thyroiditis and increase the risk of thyroid cancer (Laurberg et al., 1998; Teng et al., 2006).'*

EFSA referred to the opinion of WCRF/AICR (2007), that iodine deficiency (via higher serum TSH concentrations) is a "probable" cause and iodine excess is a "possible" cause of thyroid cancer. Iodine deficiency or excess has been linked to various clinical outcomes such as goitre, thyroid cancer or sub-clinical and overt thyroid disorders. At a population level, the association of thyroid disorders with iodine intakes appears to be U-shaped and the range for the lowest prevalence of thyroid disorders could be relatively narrow (Laurberg *et al.*, 2010).

Bürgi (2010) indicated that the consequences of iodine excess may differ in the population: 'Persons with a normal thyroid gland may respond with a persistent drop of T4 and T3 and a rise of TSH without clinical symptoms, in persons who respond with clinical hypothyroidism and in persons who respond with hyperthyroidism.'

Conclusion on the relevance for humans

RAC considered that the statement 'thyroid cancer in humans is rare' is no longer valid. The DS clarified that it was connected to the disturbances in thyroid hormone homeostasis.
One MSCA informed that thyroid cancer incidence has been rising in France and in many other countries.

In addition, data from IARC's release on the latest global cancer trends[1] (October, 2013) were consistent with an increase in thyroid cancer rates in humans in various parts of the world.

The DS acknowledged that the same fundamental mechanisms were acting in rodents and humans, but also addressed major quantitative species differences in thyroid physiology and biochemistry between rodents and humans:

'*Both humans and rodents have nonspecific protein carriers of thyroid hormones, however, rodents lack thyroxine-binding globulin (TBG) which has a high affinity for binding T4 and to a lesser extent T3 in humans. As a result T4 bound to lower affinity proteins in rodents (albumins) is more susceptible to removal from the blood, metabolism and excretion from the body. This correlates with the much shorter half-lives of both T4 and T3 in rodents compared with humans. Consequently, thyroid hormone synthetic activity in rodents is much higher than in humans with a correspondingly higher level of circulating TSH (by*

1 http://www.efsa.europa.eu/de/efsajournal/pub/3660.htm

approximately 25-fold in the rat). The morphology of the rodent thyroid gland is similar to that of the stimulated human gland, indicating that the rodent thyroid is much more active in the normal state. Thus, it follows that increases in TSH levels above basal levels in rodents may render the thyroid more susceptible to increased growth and potential neoplasia than in humans. Modest increases in TSH will promote tumour formation in rats. This is supported by evidence that adult male rats have higher serum TSH levels than females and they are often more sensitive to thyroid growth and neoplasia, as is the case for iodomethane.'

RAC noted that the contributions of extrathyroidal parameters (e.g. lack of thyroxine-binding globuline for T4 binding in rats) that are thought to show differences between rodents and humans have not been examined (except UGT induction). Also their impact on the tumour formation is unknown (the major effect is proposed to be a disruption of thyroid hormone synthesis and/or secretion in the follicular cell).

The excess of iodide produced by iodomethane and its consequences are not considered to be a rodent-specific MoA. The metabolism of inhaled methyl iodide to inorganic iodide has been confirmed for humans (Bolt and Gansewendt, 1993). All routes of exposure including the dermal route, (MAK, 2007) are relevant. The thyroid was also a target organ in dogs. Some details of the MoA were examined, but overall it is concluded by RAC that the MoA – one or more – were not clarified. By comparing the effects in iodomethane-exposed animals with data from humans who had an excess iodine uptake, similarities in the observed effects were identified and similarities in the underlying mechanism were assumed.

Excess of iodine uptake is a relevant condition in humans that can cause several diseases. Increased TSH levels and chronic stimulation of thyroid hormone production is assumed to be associated with a higher risk for thyroid cancer in humans (Boelaert *et al.*, 2006).

For humans there is an ongoing debate and currently available data do not allow a firm conclusion to be formed on the dose-response and association of excessive iodide with thyroid tumours in general. A relevant observation is that during the last decades the incidences of thyroid tumours have increased.

Due to a number of similarities observed in rodent studies and the human responses known from conditions with iodide excess it is concluded that uncertainties with regards to the MoA remain and the relevance of the observed thyroid effects and possibly of the tumour responses could not be excluded.

RAC concluded that iodomethane is not acting via a genotoxic mode of action. RAC noted that the CLP criteria require that by default carcinogenic effects in experimental animals are considered relevant to humans and are considered for classification as carcinogens. **As there is not sufficient evidence showing that the observed thyroid tumours are not relevant to humans, iodomethane should be classified as category 2 carcinogen.**

The Specialised Experts' recommendation was not applicable since a species-specific mechanism was not identified. RAC found the MoA (chronic hypothyroidism causes follicular cell hyperplasia and tumours via increased TSH level) to be plausible. However, their initial events leading to lower thyroid hormone levels and their underlying mechanisms were not established. Also, taking the similarities of the thyroid hormone regulation and in particular the observation that excess iodine can cause increased TSH levels in humans into account, the relevance for humans could not be ruled out on the basis of a general assumption of a lower sensitivity of humans.

Additional references

Additional references not included in the CLH report.

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