CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Iodomethane

EC Number: 200-819-5

CAS Number: 74-88-4

Index Number: 602-005-00-9

Contact details for dossier submitter:

UK CLP Competent Authority

Chemicals Regulation Directorate

Health and Safety Executive

United Kingdom

Original dossier prepared by JSC International Limited on behalf of Arysta LifeScience SAS, France, in accordance with Article 37(6) of CLP.

Version number: 1 **Date: November 2013**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Iodomethane
EC number:	200-819-5
CAS number:	74-88-4
Annex VI Index number:	602-005-00-9
Degree of purity:	99.5% w/w
Impurities:	Confidential information, please refer to the technical dossier. The impurities have been taken into consideration and are not thought to be of additional toxicological concern

1.2 Harmonised classification and labelling proposal

Iodomethane was first assigned the harmonised classifications listed in Table 2 in 1987 (8th ATP; Commission Directive 94/69/EC, published 21st August 1987). Records of the data used to support the classification in category 3 for carcinogenicity are not available, but it seems likely that two studies from the 1970s, both involving treatment of rats or mice by non-standard routes of exposure, would have been included. There were no studies at that time for carcinogenicity in rats or mice following oral, dermal or inhalational exposure. Details are provided in Sections 4.10.1.3 and 4.10.3.

Modern GLP and guideline compliant carcinogenicity studies in the rat and mouse by the inhalation and oral routes, respectively, are now available and it is therefore appropriate to update the carcinogenicity assessment for this substance.

A proposal to change the current harmonised classification and labelling of iodomethane was submitted to the UK Competent Authority by JSC International Limited, on behalf of Arysta LifeScience SAS in accordance with Article 37(6) of the CLP Regulation. The proposal was to remove the carcinogenicity classification of iodomethane from Annex VI of the CLP Regulation and it included data from toxicokinetic, repeated dose toxicity, mutagenicity and carcinogenicity studies to justify this.

Therefore, this proposal, submitted by the UK Competent Authority, only seeks to address the classification endpoint: carcinogenicity.

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

	CLP Regulation	Directive 67/548/EEC (Dangerous Substances Directive; DSD)
Current entry in Annex VI, CLP Regulation	Carc. 2; H351 Acute Tox. 4*; H312 Acute Tox. 3*; H331 Acute Tox. 3*; H301 STOT SE. 3; H335	Carc. Cat. 3; R40 Xn; R21 T; R23/25 Xi; R37/38

	Skin Irrit. 2 ; H315	
Current proposal for consideration by RAC	Removal of: Carc. 2; H351 (Suspected of causing cancer)	Removal of: Carc. Cat. 3; R40 (Limited evidence of a carcinogenic effect)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4*; H312 Acute Tox. 3*; H331 Acute Tox. 3*; H301 STOT SE. 3; H335 Skin Irrit. 2 ; H315	Xn; R21 T; R23/25 Xi; R37/38

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

The proposed classification and labelling of iodomethane, based on the removal of the classification for carcinogenicity, is provided in Table 3 and Table 5. Iodomethane is only classified for health hazards, therefore, the tables do not include all other classification endpoints.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
3.1.	Acute toxicity - oral	N ot evaluated	-	**Acute Tox 3*; H301**	-
	Acute toxicity - dermal	Not evaluated	-	**Acute Tox 4*; H312**	-
	Acute toxicity - inhalation	Not evaluated	-	**Acute Tox 3*; H331**	-
3.2.	Skin corrosion / irritation	Not evaluated	-	**Skin Irrit. 2 ; H315**	-
3.3.	Serious eye damage / eye irritation	Not evaluated	-	No classification	Not evaluated
3.4.	Respiratory sensitisation	Not evaluated	-	No classification	Not evaluated
3.4.	Skin sensitisation	Not evaluated	-	No classification	Not evaluated
3.5.	Germ cell mutagenicity	**No classification**	-	No classification	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	**No classification**	**-**	**Carc. 2; H351**	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not evaluated	-	No classification	Not evaluated
3.8.	Specific target organ toxicity –single exposure	Not evaluated	**-**	**STOT SE 3; H335**	**-**
3.9.	Specific target organ toxicity – repeated exposure	Not evaluated	-	No classification	Not evaluated
3.10.	Aspiration hazard	Not evaluated	-	No classification	Not evaluated

Proposed labelling according to the CLP Regulation:

This is based on the current classification, excluding carcinogenicity.

Table 4: Current classification according to CLP

Signal word:	Hazard pictogram:	Hazard statements:
Danger	GHS06: skull and crossbones 	H301: toxic if swallowed H312: harmful in contact with skin H315: causes skin irritation H331: toxic if inhaled H335: may cause respiratory irritation

Proposed notes assigned to an entry:

Not applicable

Table 5: Proposed classification according to DSD

Hazardous property	Proposed classification	Current classification	Reason for no classification
Acute toxicity	Not evaluated	**T; R23/25** **Xn; R21**	-
Acute toxicity – irreversible damage after single exposure	Not evaluated	No classification	Not evaluated
Repeated dose toxicity	Not evaluated	No classification	Not evaluated
Irritation / Corrosion	Not evalutated	**Xi; R37/38**	-
Sensitisation	Not evaluated	No classification	Not evaluated
Carcinogenicity	**No classification**	**Carc. Cat. 3; R40**.	Conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	**No classification**	No classification	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not evaluated	No classification	Not evaluated
Toxicity to reproduction – development	Not evaluated	No classification	Not evaluated
Toxicity to reproduction – breastfed babies. Effects on or *via* lactation	Not evaluated	No classification	Not evaluated

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification; or not evaluated

Proposed labelling according to DSD:

This is based on the current classification, excluding carcinogenicity.

Table 6: Current classification according to DSD

Indication of danger:	R-phrases:	S-phrases:
T: toxic	R21: harmful in contact with skin	(S1/2): keep locked up and out of reach of children
	R23/25: toxic by inhalation and if swallowed	S36/37: wear suitable protective clothing and gloves
	R37/38: irritating to respiratory system and skin	S38: in case of insufficient ventilation, wear suitable respiratory equipment
		S45: in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Iodomethane was first assigned the harmonised classifications listed in Table 2 in 1987 (8th ATP; Commission Directive 94/69/EC, published 21st August, 1987). Records of the data used to support this classification are not available, but it seems likely that two studies from the 1970s, both involving treatment of rats or mice by non-standard routes of exposure, would have been included. There were no studies at that time for carcinogenicity in rats or mice following oral, dermal or inhalational exposure.

2.2 Short summary of the scientific justification for the CLH proposal

The previous studies (Druckrey *et al,* 1970 and Poirier *et al,* 1975) are considered inadequate for assessing the carcinogenicity of iodomethane due to inappropriate study designs and non-relevant routes of exposure (intra-dermal and intra-peritoneal injection, respectively). However, GLP and guideline compliant carcinogenicity studies in the rat and mouse by the inhalation and oral routes, respectively, are now available.

A 2-year inhalation carcinogenicity study in rats (Kirkpatrick, 2005 and Kirkpatrick, 2008b), revealed a statistically significant increase in thyroid follicular adenomas in male rats at the highest dose of 60 ppm. There was a marginal increase in females. There was no effect at 20 ppm. The incidence of thyroid follicular adenomas and carcinomas combined was slightly increased in male mice in an 18-month dietary carcinogenicity study at the highest dose of 600 ppm (Harriman, 2005 and Kirkpatrick, 2008a). There were no other significant treatment related oncogenic findings in either the rat or the mouse. .

A non-genotoxic mode of action (MOA) has been identified with a high degree of confidence for the induction of the thyroid follicular tumours in rats and mice. The proposed MOA involves the perturbation of homeostasis of the hypothalamic-pituitary-thyroid axis caused by excess circulating iodide derived from the metabolism of iodomethane. Excess iodide acts centrally on the thyroid by inhibiting production and release of T_4. Reduced circulating levels of T_4 and T_3 cause a compensatory increase in circulating Thyroid-Stimulating Hormone (TSH) from the pituitary resulting in sustained stimulation of the thyroid to produce thyroid hormone. This leads to enlargement of the thyroid and proliferation of thyroid follicular cells (hyperplasia). Thyroid hyperplasia can eventually progress to neoplasia.

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.

Thyroid cancer in humans is rare. Increased iodide intake may be a risk factor for thyroid cancer, but humans appear to have a low susceptibility to thyroid cancer and the risks arising from disturbances to thyroid hormone homeostasis are low. Humans would be far less sensitive than rodents to the effects of excess iodide on thyroid function and thyroid tumour development owing to species differences in thyroid physiology and biochemistry between rodents and humans. Thyroid tumours in male rats administered iodomethane were only induced at a dose level which resulted in general toxicity and which exceeded an MTD. Consequently, it is extremely unlikely that exposure of humans to iodomethane would lead to sustained elevation of TSH since exposures would have to exceed tolerable levels of iodomethane exposure.

According to the EU Specialised Experts (1999), it was agreed that non-genotoxic carcinogenic 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. There is a convincing weight of evidence to conclude that iodomethane would not present a carcinogenic hazard to humans and should not be classified for carcinogenicity based on the criteria for classification in Regulation EC 1272/2008 and Directive 67/548/EEC. Therefore, removal of the current carcinogenicity classification is proposed.

The information in this dossier is consistent with the information in the registration dossiers available at the date of submission of this proposal. However, additional information (published genotoxicity studies) has also been included.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification:

Acute Tox. 3*:	H301 (toxic if swallowed)
Acute Tox. 4*:	H312 (harmful in contact with skin)
Acute Tox. 3*:	H331 (toxic if inhaled.)
Skin Irrit. 2:	H315 (causes skin irritation.)
Carc. 2:	H351 (suspected of causing cancer)
STOT SE 3:	H335 (may cause respiratory irritation)

Labelling:

Signal word:	Hazard pictogram:	Hazard statements:
Danger	GHS06: skull and crossbones  GHS08: health hazard 	H301: toxic if swallowed H312: harmful in contact with skin H315: causes skin irritation H331: toxic if inhaled H335: may cause respiratory irritation H351: Suspected of causing cancer

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification:

Xn; R21:	harmful in contact with skin
T; R23/25:	toxic by inhalation and if swallowed)
Xi; R37/38:	irritating to respiratory system and skin
Carc. Cat. 3; R40:	limited evidence of a carcinogenic effect

Labelling:

Indication of danger:	R-phrases:	S-phrases:
T: toxic	R21: harmful in contact with skin R23/25: toxic by inhalation and if swallowed R37/38: irritating to respiratory system and skin R40: limited evidence of a carcinogenic effect	(S1/2): keep locked up and out of reach of children S36/37: wear suitable protective clothing and gloves S38: in case of insufficient ventilation, wear suitable respiratory equipment S45: in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification:

As *per* the Annex VI entry, with the addition of:

Eye Dam. 1;	H318 (causes serious eye damage)

Labelling:

As *per* the Annex VI entry with the addition of:

Hazard pictogram:	Hazard statements:
GHS05: corrosion 	H318: causes serious eye damage

2.4.2 Current self-classification and labelling based on DSD criteria

Classification:

As *per* the Annex VI entry with the addition of:

Xi; R41: risk of serious damage to eyes

Labelling:

As *per* the Annex VI entry with the addition of:

R-phrases:	S-phrases:
R41: risk of serious damage to eyes	S26: in case of contact with eyes, rinse immediately with plenty of water and seek medical advice S36/37/39: wear suitable protective clothing, gloves and eye/face protection

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There are data available to show that the existing harmonised classification for iodomethane, Carc. 2 (H351) in accordance with CLP (Carc Cat. 3; R40 in accordance with Dir 67/548/EEC) is incorrect. Therefore, action is required at the Community level and this proposal seeks to amend the existing entry in Annex VI.

This proposal was prepared initially by JSC International Limited on behalf of Arysta LifeScience SAS in accordance with Article 37(6) of CLP and submitted by the UK Competent Authority. Following submission to the European Chemicals Agency (ECHA), it was modified to account for comments received during the Accordance Check made by ECHA's Risk Assessment Committee.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

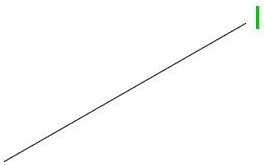
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 7: Substance identity

EC number:	200-819-5
EC name:	iodomethane
CAS number (EC inventory):	74-88-4
CAS number:	74-88-4
CAS name:	iodomethane
IUPAC name:	iodomethane
CLP Annex VI Index number:	602-005-00-9
Molecular formula:	CH_3I
Molecular weight range:	141.939

Structural formula:



1.2 Composition of the substance

Table 8: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
iodomethane EC no.: 200-819-5	99.5 % (w/w)	> 99.0 — < 100.0 % (w/w)	

Current Annex VI entry: Iodomethane Annex VI index number 602-005-00-9.

Details on the current classification are referred to in Part A, Section 2.3. There are no specific concentration limits or M-factors associated with iodomethane and there are no notes associated with its Annex VI entry.

Table 9: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
-	-	-	No impurities of toxicological concern

Current Annex VI entry: Not applicable

The manufacturer has requested that the impurity profile remains confidential, therefore this information is presented in the technical dossier only. The typical purity of iodomethane is >99.8% and there is only 1 process impurity present. This has been taken into consideration in the classification and is not considered to be of additional concern.

Table 10: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None	-	-	-	-

Current Annex VI entry: Not applicable

1.2.1. Composition of test material

Where available, the purity of the tested material is provided in the relevant sections. The reported studies are considered to be representative of the material as specified above.

1.3 Physico-chemical properties

Table 11: Summary of physico - chemical properties

Property (guideline[1], GLP status)	Value	Reference IUCLID section	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa (US EPA OPPTS 830.6303 & 830.6302; GLP)	Liquid	4.1	Measured
Melting/freezing point (Non-GLP; non guideline)	-66.5°C	4.2	Measured
Boiling point (US EPA OPPTS 830.7220; GLP)	42°C (760 mmHg)	4.3	Measured
Relative density	2.27 (25°C)	4.4	Measured

Property (guideline[1], GLP status)	Value	Reference IUCLID section	Comment (e.g. measured or estimated)
(US EPA 830.7300; GLP)			
Vapour pressure (Non-GLP; non guideline)	405 mmHg (25°C)	4.6	Measured
Surface tension (Brazil guideline: ABNT Agrotoxico-Determinacao da Tensao Superficial NBR 132; GLP)	0.06756 N/M (20°C)	4.10	Measured
Water solubility (OECD 105; GLP – Flask method)	8.66 g/L (20°C)	4.8	Measured
Partition coefficient n-octanol/water (OECD 107; GLP – Shake flask method)	1.57	4.7	Measured
Flash point (EU method A.9; GLP)	No flashpoint up to 32°C	4.11	Measured
Flammability (not applicable)	Not measured	4.13	There are no functional groups that indicate potential reactivity with water or pyrophoric properties. Experience in handling and use indicates that the substance will not spontaneously ignite on contact with air or water.
Explosive properties (not applicable)	Not measured	4.14	There are no functional groups with explosive properties
Self-ignition temperature (EU method A.15; GLP)	350°C (99.418 KPa)	4.12	Measured
Oxidising properties (US EPA OPPTS 830.6314; GLP)	Lack of oxidising potential	4.15	Measured
Dissociation constant (US EPA OPPTS 830.7370; GLP)	No pKa activity	4.21	Measured
Viscosity (US EPA OPPTS 830.7100; GLP)	2.9 mPa (15°C); 2.6 mPa (32°C)	4.22	Measured

1. Where appropriate, methods employed were guideline compliant

2 MANUFACTURE AND USES

2.1 Manufacture

Not manufactured in the EU.

2.2 Identified uses

Iodomethane is believed to be used in the EU as an industrial and pharmaceutical methylating agent and as an intermediate in pharmaceutical and pesticide manufacture. It is also imported into the EU and stored for despatching outside of the EU for the same uses.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Iodomethane is not classified with respect to physico-chemical properties. This is not considered further in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

The focus for this classification proposal is the systematic evaluation of the carcinogenic potential of iodomethane. The following human health hazard assessment is restricted to information relevant to the proposal, including additional information on toxicokinetics, repeat dose toxicity and mutagenicity (genotoxicity)..

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The results of experimental studies on absorption, metabolism, distribution and elimination are summarised in the following table:

Table 12: Overview of experimental studies on absorption, metabolism, distribution and elimination

Method	Results	Remarks	Reference
Rat (Crj: CD(SD)) male (12 animals/group) Oral and inhalation Oral: 1.5, 24 mg/kg (single exposure) Inhalation: 25, 233 ppm (single exposure for 6 hours) EPA OPPTS 870.7485; EPA OPPTS 870.8340; OECD 417, GLP	**Toxicokinetic parameters:** Completely absorbed by oral and inhalation routes. Initial half-life in blood: 5-7hrs **Details on metabolites:** The major metabolite of iodomethane was carbon dioxide, which accounted for approximately 40-60% of the dose irrespective of the route of exposure in the supplementary study. Urine contained two major metabolites, which were identified by LC-MS/MS as N-(methylthioacetyl)glycine and S-methyl glutathione. Small amounts of methylthioacetic acid, methyl mercapturic acid and S-methyl cysteine were detected in some samples. The amounts of metabolites were determined in urine samples that contained ≥ 5% of the dose. Note: Circulating iodide levels were not measured. Neither the route of administration nor dose had any significant effect on the proportions of the metabolites.	purity:99.7%	Sved (2002)
Rat (Crj: CD(SD)) female (12 animals/group) Oral and inhalation Oral: 1.7, 21 mg/kg (single exposure) Inhalation: 24, 250 ppm (single exposure for 6 hours) OECD 417, GLP	**Toxicokinetic parameters**: Completely absorbed by oral and inhalation routes. Initial half-life in blood: 2-6hrs **Details on metabolites:** The major metabolite of iodomethane was carbon dioxide, which accounted for approximately 40-73% of the dose irrespective of the route of exposure. Urine contained two major metabolites which were identified by LC-MS/MS as N-(methylthioacetyl)glycine and S-methyl glutathione. Small amounts of methylthioacetic acid, methylthiopyruvic acid, methylmercapturic acid,	purity:99.7%	Sved (2005)

Method	Results	Remarks	Reference
	methylmercapturic acid sulphoxide, S-methylcysteine and S-methylcysteine sulphoxide were detected in some samples. The amounts of metabolites were determined in urine samples that contained ≥ 5% of the dose but percentages of each of the components were not reported. Neither the route of administration nor dose appeared to have any significant effect on the proportions of the metabolites. The proposed metabolic pathway for iodomethane is either oxidation to carbon dioxide or methylation of glutathione, which undergoes further metabolism to S-methyl cysteine and ultimately methyl mercapturic acid. Although small amounts of additional metabolites were detected in this study the major metabolites in the studies in both male and female rats were N-(methylthioacetyl)glycine and S-methyl glutathione showing that metabolism was the same in both sexes. Note: Circulating iodide levels were not measured.		

4.1.2 Human information

Limited human information on the absorption, metabolism, distribution and elimination of iodomethane is available (HSE, 1996) and is not considered further in this proposal.

4.1.3 Summary and discussion on toxicokinetics

The absorption, distribution, metabolism and excretion of [^{14}C] iodomethane has been studied following oral administration at 1.0, 1.7, 21 and 35 mg/kg (single dose) and inhalation exposure at 21, 25, 209 and 250 ppm (single exposure for 6 hours) in male and female rats. Iodomethane was completely absorbed in both sexes by either route and metabolism and excretion were rapid. The major metabolite was carbon dioxide accounting for approximately 40-73% of the dose, but significant amounts of S-methyl glutathione and N-(methylthioacetyl) glycine were also formed and eliminated in urine. A number of other metabolites, formed by further metabolism of S-methyl glutathione and N-(methylthioacetyl) glycine, were also present in urine, but only as small percentages of the dose. Metabolism and excretion were unaffected by the route of exposure or the dose over the range used. Radioactivity from [^{14}C] iodomethane was detected in tissues and blood at all times after exposure, but as radioactivity entered the one carbon pool it was incorporated into endogenous metabolites and residues would be expected.

The importance of glutathione in the metabolism of iodomethane was confirmed in an additional study in which male rats were exposed to 0, 25 and 100 ppm iodomethane by inhalation for 6 hours/day for 2 days (see Section 4.12.1.3). Dose and time dependent reductions in glutathione concentrations were found in olfactory and respiratory tissue and to a lesser extent in blood, liver and kidney. S-methyl cysteine adducts were also detected in haemoglobin showing that iodomethane had also methylated thiol groups in globin. Serum iodide concentrations were also elevated and were at a maximum at the end of both exposure periods, which is consistent with a rapid rate of metabolism of iodomethane.

4.2 Acute toxicity

Not evaluated for this proposal.

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

Not evaluated for this proposal.

4.4 Irritation

Not evaluated for this proposal.

4.5 Corrosivity

Not evaluated for this proposal.

4.6 Sensitisation

Not evaluated for this proposal.

4.7 Repeated dose toxicity

The results of the repeat dose toxicity studies considered relevant to this proposal are summarised in Sections 4.7.1.1 and 4.7.1.2. For convenience, the toxicity results in the carcinogenicity studies are summarised in Section 4.10 Carcinogenicity.

4.7.1 Non-human

4.7.1.1 Repeated dose toxicity: oral

Table 13: Summary of experimental studies on repeated dose toxicity after oral administration

Method	Results (significant effects on tissues)	Remarks	Reference
90d, rat (Sprague-Dawley) (10 animals/sex/group) oral: gavage 0, 5, 10, 25, 50 mg/kg/day (all doses within 15% of nominal) EPA OPPTS 870.3100; JMAFF, 12 NouSan No. 8147, GLP	At 50 mg/kg: Four females died between days 81-86. Males, but not females, showed reduced body weight gain. At 10 and 50 mg/kg: Hyperkeratosis and hyperplasia of the squamous epithelium of the forestomach; squamous meaplasia of the ductal epithelium of the submandibular salivary glands. At 5 mg/kg: no observed adverse effects.	Purity: 99.9%	Nishimura (2003)
90d mouse (CD-1) (10 animals/sex/group) oral: feed 0, 133, 400, 1200 ppm [equiv. to 0, 23.6, 65.3, 212 (M) and 0, 26.8, 79.2, 221.6 mg/kg/day (F)]	At 1200 ppm (*ca.* 210-220 mg/kg): Decreased body weight of males and females. At 400 (65-80 mg/kg) and above; oesophageal hyperkeratosis in the majority of animals At 133 ppm (*ca.* 25 mg/kg) and above: mean absolute and relative thyroid/parathyroid weight; accumulation of follicular colloid with corresponding attenuation of follicular lining cells in the thyroid.	Purity: 99.7%	Harriman (2003)

Method	Results (significant effects on tissues)	Remarks	Reference
OECD 408, GLP			
90d dog (Beagle) (4 animals/sex/group) oral: capsule 0, 1.5, 6, 15 mg/kg/day (analytical conc.) OECD 409, GLP	At 15 mg/kg: 1 male was in a moribund condition and killed during the study. Microscopic changes were seen in the stomach, oesophagus and/or rectum (ulceration, chronic active inflammation and/or haemorrhage); degeneration of olfactory epithelium and cysts of the respiratory epithelium at nasal level two (females only). At 6 mg/kg: gastric unlceration and olfactory degeneration (1 female). Decreases in mean albumin and total protein levels at 15 mg/kg/day and increased incidence of emesis in both the 6 and 15 mg/kg/day At 1.5 mg/kg: no observed adverse effects.	Purity 99.7%	Harriman (2002)
52wk dog (Beagle) (4 animals/sex/group) oral: capsule 0, 1.5, 6, 12 mg/kg/day (analytical conc.) OECD 452, GLP	At 12 mg/kg and 6 mg/kg: at both dose levels, 1 dog euthanised *in extremis,* showing lesions in the oesophagus, salivary gland and stomach. At 12 mg/kg: 1 male and 1 female showed mild to severe colloid depletion and moderate follicular hypertrophy of the thyroid gland; associated with elevated TSH. At 12 mg/kg: increased severity of vacuolar change in the liver; bilateral tubule degeneration in the testes. At 1.5 mg/kg: no observed adverse effects.	Purity: 99.7%	Harriman (2004) Harriman and Armstrong (2005)

90-day oral study in rats followed by 28-day recovery (Nishimura, 2003)

In a 90-day study, groups of Sprague-Dawley rats (10/sex/dose) were administered 0, 5, 10, 25 and 50 mg/kg/day iodomethane by gavage. To establish the reversibility of the observed effects, a further 10 animals/sex were allocated to the control, 25 and 50 mg/kg/day dose groups and observed for a further 28-day recovery period.

Clinical signs of toxicity included salivation in the majority of animals treated at doses of 10 mg/kg/day and greater. Four females in the 50 mg/kg/day dose group died between days 81 to 86 of the study, these deaths were considered test material-related. Test material related changes in body weight consisted of decreased body weight in males dosed at 50 mg/kg/day during the study. Body weight gain relative to controls increased transiently during the second week of the recovery period. No differences were observed in females at the same dose.

No test-material related effects were observed on haematology, serum chemistry, urinalysis parameters or sensorimotor reaction to irritation.

Histopathological changes were sporadic in nature, however, a dose related increase in hyperkeratosis and hyperplasia of the squamous epithelium in the forestomach were observed in animals treated with ≥10 mg/kg/day group; with all animals in the 50 mg/kg/day dose group exhibiting such signs. An increased incidence of squamous metaplasia of the ductal epithelium of the submandibular salivary glands was noted at 10 mg/kg/day and above.

Based on the results of this study, the NOAEL for males and females was 5 mg/kg/day.

90-day oral study in mice (Harriman, 2003)

Microencapsulated iodomethane was administered on a continuous basis in the diet for a minimum of 90 days to male and female CD-1 mice. Dosage levels were 133, 400 and 1200 ppm (equivalent to a maximum dose of 212 or 221.6 mg/kg/day for male and females respectively). A concurrent control group received placebo microcapsules on a comparable regimen. All groups consisted of 10 animals/sex/group.

Haematology, serum chemistry and urinalysis parameters were unaffected by test material administration and no test material-related macroscopic changes were observed at the scheduled necropsy.

Test material-related effects in the 400 and 1200 ppm groups consisted of decreased defecation (during the first 5 weeks of the study), decreased body weights (1200 ppm M: 17%; F: 11% lower than the controls) and food consumption (9% lower than the controls). In all three treatment groups, findings in the thyroid included increased mean absolute and relative thyroid/parathyroid weights along with accumulations of follicular colloid with corresponding attenuation of follicular lining cells in the thyroid gland. Hyperkeratosis in the oesophagus was observed in the majority of animals at 400 and 1200 ppm, which was attributed to local irritation from iodomethane.

The report concluded that the systemic NOAEL was 400 ppm (~ 65 mg/kg/day) based on less than 10% reduction in body weight and adaptive changes in the thyroid at this dose level. The findings in the oesophagus were considered to have been a sign of the local irritant nature of the test substance and the method of dosing

90-day oral study in dogs (Harriman, 2002)

In a 90-day study, groups of beagle dogs (4/sex/dose) were administered iodomethane *via* oral capsules once daily, 7 days/week for a minimum of 90 days at doses of 1.5, 6 and 15 mg/kg/day. A concurrent control group received capsules containing corn oil.

Test material related effects noted in the 15 mg/kg/day group included: euthanasia (1 male) due to moribund condition; increased clinical signs of toxicity (emesis, salivation, head shaking, soft or mucoid faeces); lower mean albumin and total protein (weeks 6 and 12) and microscopic changes in the stomach, oesophagus and/or caecum and rectum (ulceration, chronic active inflammation and/or haemorrhage) and olfactory epithelium degeneration and cysts of the respiratory epithelium at nasal level 2 (females only).

Test material related effects noted in the 6 mg/kg/day group included: increased emesis, salivation prior and post dosing. Microscopic changes included gastric ulceration and olfactory degeneration (1 female).

For animals dosed at 1.5 mg/kg/day, clinical signs of toxicity were limited to increases in injected sclera (also apparent at the other two doses). This finding represents dilation of the blood vessels in the eye and was most likely pharmacological but not considered toxicologically relevant. A subsequent evaluation of injected sclera in the 12-month toxicity study revealed that the incidence of injected sclera (at the same dose levels employed in the present study) was unlikely to be treatment related (see below). The incidence of injected sclera in the present study was very variable and did not show a consistent dose response.

Based on the result of this study the NOAEL was considered 1.5 mg/kg/day for both genders.

1-year oral study in dogs (Harriman, 2004; Harriman and Armstrong, 2005)

In a 12 month study, groups of beagle dogs (4/sex/dose) were administered 1.5, 6 and 12 mg/kg/day iodomethane *via* oral capsules once daily, 7 days/week for a minimum of 52 weeks. A concurrent control group received capsules containing corn oil.

Test material related effects included 1 male (6 mg/kg/day) and 1 female (12 mg/kg/day) euthanized in extremis during the study. All surviving animals in the 6 and 12 mg/kg/day groups throughout the study exhibited test material related clinical signs including decreased defecation, diarrhoea, emesis, excessive salivation (which extended into the 1.5 mg/kg/day groups), head shaking, hypoactivity and soft faeces.

Haematological and clinical chemistry changes (higher mean platelet counts and cholesterol, lower mean serum albumin, total protein and serum calcium level) were observed in the 6 and 12 mg/kg/day groups.

Macroscopic, test material related findings including thickening and discolouration of the oesophagus, firmness and enlargement of the mandibular salivary gland and thickening of the stomach were observed in the two animals euthanised *in extremis* and in one 12 mg/kg/day group male. These changes were accompanied by microscopic changes including extensive ulceration of the oesophagus, mucous cell hypertrophy and/or decreased secretion of the mandibular salivary gland and hyperplasia and/or decreased secretion of the stomach. These effects were considered secondary to the irritant nature of iodomethane.

Mild to severe colloid depletion and moderate follicular cell hypertrophy of the thyroid gland (with minimal to mild hyperplasia of basophilic cells within the *pars distalis* of the pituitary gland) were noted in one male and one female in the 12 mg/kg/day group. These changes corresponded to elevated thyroid stimulating hormone (TSH) levels observed in both animals together with decreased T_3 and T_4 levels and decreased T_4 and reverse T_3 levels in the male and female, respectively. Bilateral seminiferous tubule degeneration in the testes and increased incidence and severity of vacuolar change in the liver were also noted in the 12 mg/kg/day group. Unilateral seminiferous tubule degeneration noted in one male in each of the 1.5 and 6 mg/kg/day groups is considered unlikely to be treatment related based on historical control data.

There was a variable incidence of injected sclera amongst dogs in the control and treated groups, however, following a detailed assessment they were considered to reflect natural variability in susceptibility among dogs and the incidences in treated groups were not related to iodomethane.

Based on the results of this study, the NOAEL was considered to be 1.5 mg/kg/day in both genders based on mortality, clinical signs and clinical pathology changes at 6 mg/kg/day.

4.7.1.2 Repeated dose toxicity: inhalation

The results of experimental studies are summarised in the following table:

Table 14: Summary of experimental studies on repeated dose toxicity after inhalation exposure

Method	Results	Remarks	Reference
4 or 13wk (dosed 5d/wk) rat (Sprague-Dawley) (10 animals/sex/group) inhalation: vapour (whole body) 0, 5, 21, 70 ppm/6 hr/day (analytical conc.) Vehicle: clean air EPA OPPTS 870.3465; GLP	At 70 ppm (*approx: 0.4 mg/L): reduced body weight gain, increased degeneration and regeneration of the olfactory and respiratory epithelial metaplasia in the nasal cavity. No pituitary or thyroid measurements were performed in this study. NOAEC: ca. 21 ppm (male/female) (based on reduced body weight and olfactory changes at 70 ppm.)	Purity: 99.7%	Kirkpatrick (2002)
4-w (dosed 5 days a week) rat (Sprague-Dawley) (10 animals/sex/group) inhalation: vapour (whole body) 0,25,75,100 ppm (nominal conc.) 0,25,72,99 ppm (analytical conc.) Vehicle: clean air Not guideline, GLP	100 ppm: increased thyroid weight in males and females, correlating with follicular hyperplasia and degeneration of the thyroid follicle and hypertrophy of the pars distalis in the pituitary gland. Degeneration of the olfactory epithelium in males and females. 75 and 100 ppm: Reduced body weight gain (NB: this study was designed as a dose range-finding study; histopathological investigations were not conducted at the low and intermediate dose levels to enable a NOAEL to be defined.)	Purity: 99.7%	Nemec (2004a)

Note: 1 ppm = 5.81 mg/m^3 iodomethane

90-day inhalation study in rats (Kirkpatrick, 2002)

Iodomethane was administered by inhalation (whole body exposure) to Sprague Dawley rats (10 animals/sex/treatment duration) for either 4 (minimum of 20 exposures) or 13 weeks (minimum of 65 exposures) at target concentrations of 5, 20 and 70 ppm. Animals were exposed to the test material daily for 5 days/week.

Test material related effects were limited to animals treated at 70 ppm and included (but not limited to) lower mean body weight gains during the exposure period; increased mean serum cholesterol, higher liver weight relative to final body weights (however as no microscopic changes were observed in the liver these changes were considered adaptive); increased degeneration/regeneration of the olfactory epithelium and respiratory epithelial metaplasia in the nasal cavity. These effects were observed in both sexes. Pituitary and thyroid hormone measurements were not performed in this study.

Based on the results of this study, the NOAEL was considered to be 21 ppm (mean analysed concentration) for both sexes based on reduced body weight and olfactory changes at 70 ppm.

28-day inhalation study in rats (Nemec, 2004a)

This range finding study was designed to determine atmospheric concentrations of iodomethane for a developmental toxicity study in rats, a two-generation reproductive toxicity study in rats and for a 13-week sub-chronic toxicity study in rats. The study design consisted of

three separate phases, a pre-mating exposure phase, a reproductive toxicity phase and a sub-chronic toxicity phase. The latter phase only will be discussed in this section.

Sprague-Dawley rats (10/sex/group) were exposed whole body to iodomethane for 6 hours daily for 4 weeks (5 days/week) for a total exposure period of 20 or 21 days. Intended exposure concentrations were 25, 75 and 100 ppm.

All animals survived to the scheduled necropsy; no test material related internal findings were observed at necropsy. Findings included increased arousal level for 1 male (100 ppm) and 1/10 female (75 ppm) and circling in 1/10 male (75 ppm). Reductions in body weight gains were observed for animals in the 75 and 100 ppm groups. No effect was observed for animals in the 25 ppm group. No haematology effects were observed in any treatment animal. Changes in serum chemistry (increases in albumin, total protein and cholesterol) were limited to animals treated in the 75 and 100 ppm groups.

Test material related increases in thyroid weights were observed in both males and females in the 100 ppm group. These increases were correlated to follicular hyperplasia observed microscopically, with degeneration of the thyroid follicle and hypertrophy of the *pars distalis* in the pituitary gland. The change in the pituitary may be secondary to the thyroid alterations, since thyroid stimulating hormone is produced by the basophilic cells of the *pars distalis*. Pituitary and thyroid hormone measurements were not performed in this study. Test material related degeneration of the olfactory epithelium was observed in the nasal tissues in the 100 ppm males and females.

This study was designed as a dose range-finding study for a subsequent 90-day study and histopathological investigations were not conducted at the low and intermediate dose levels to enable a NOAEL to be defined.

4.7.1.3 Repeated dose toxicity: dermal

Not evaluated in this proposal.

4.7.1.4 Repeated dose toxicity: other routes

No relevant information.

4.7.1.5 Human information

No relevant information.

4.7.1.6 Other relevant information

None.

4.7.1.7 Summary and discussion of repeated dose toxicity

The summaries reported above are not discussed further here because they are discussed in the context of the carcinogenicity classification in Section 4.10.4.

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

Not relevant for this proposal.

4.9 Mutagenicity (genotoxicity)

The information provided in this section of the CLH report is provided to help clarify the possible mode of action behind the treatment-related increased tumour frequencies seen when rats or mice were treated repeatedly with iodomethane (see Section 4.10).

4.9.1 Non-human information

4.9.1.1 *In vitro* data

The results of the available studies are summarised in Table 15 and Table 16 with a detailed discussion of the studies provided under the relevant tables.

Table 15: Overview of (experimental) *in vitro* genotoxicity studies

Method	Results	Remarks	Reference
BACTERIAL (AMES) DATA			
S. typhimurium TA1535, TA1537, TA98 and TA100 ; *E. coli* WP2*uvr*A 0 - 5000 µg/plate +/-S9 all strains (pre-incubation assay) EPA OPP 84-2, GLP	Using the pre-incubation protocol, negative for TA1535, TA1537, TA98, TA100 and WP2 *uvr*A +/-S9; cytotoxicity: in most strains up to the maximum recommended dose (5000 µg/plate)	Well conducted, GLP-compliant study. Purity: 99.7%	Wagner and Dakoulas (2001)
S. typhimurium TA1538; TA1535 *E. coli* (DNA polymerase deficient (polA$^-$; polA$^+$)) Doses: TA1535; TA1538 +/-S9: 0, 10 uL/plate; polA$^-$ and polA$^+$: -S9: 10 µL/plate Non-GLP, non-guideline	Given the volatility of methyl iodide, a disc diffusion procedure (and not plate incorporation) was used to maximise exposure. In this spot test, a dose-related increase in the number of revertants was seen with TA1535 (2-10 ug/plate) with and without S9. No increase was seen with TA1538 (up to 10 ug/plate). Given the limited data presented, it was not possible to determine whether the result with *E. coli* was positive or negative.	A large scale, multi-substance trial, partly aimed at method development. Although a clear mutagenic response was seen with TA1535, the results are of uncertain relevance when assessed against current criteria due to the non-standard method used. It is unclear from the available information whether iodomethane applied in a concentrated form to a small part of the agar plate was cytotoxic. The assays were in duplicate only; no independent, confirmatory assays were conducted. Purity: not stated	Rosenkranz and Poirier (1979)
S. typhimurium: TA1535; TA1536 ; TA1537; TA1538; TA98; TA 100 (+/-S9) Doses: Std, non-desiccator test:	Using the plate incorporation method, negative for TA1535, TA1536, TA1537, TA1538, TA98 and TA100. up to 500 µg/plate. In contrast, with TA100 in the absence of S9, a clear increase in the number of revertants was seen when the test was	A large scale, multi-substance trial; only limited data were presented for each substance. The results are of uncertain relevance when assessed against current criteria due to the lack of information on the potential cytotoxicity of the air concentrations	Simmon (1979a)

Method	Results	Remarks	Reference
all strains: up to 500 µg/plate (equivalent to 3.52 µmol/plate) Desiccator tests: all strains 0 – 50 µL Non-GLP, non-guideline	modified so that exposure (7 hours) took place in a desiccator. The result with S9 was less clear.	of iodomethane used in the assay; lack of information on the variability in mutation frequency among treated cultures. Purity: not stated**.**	
S. typhimurium: TA100 (-S9) Doses from 1977 study: Desiccator test with TA100: 0 – 12.5 mg/L Non-GLP, non-guideline	A dose-dependent increase in the number of revertants was reported in an assay in which exposure (8h) occurred in a desiccator.	The results are of uncertain relevance when assessed against current criteria due to poor reporting (lack of cytotoxicity data; lack of information on the variability in mutation frequency (data also missing); validity of vehicle controls not defined; no positive control data). Purity: not stated	Simmon *et al*. (1977)
MAMMALIAN CELL GENE MUTATION (MCGM) TESTS			
Chinese hamster Ovary (CHO) cells -S9 (5hr): 0 - 175 µg/mL +S9 (5hr): 0 - 200 µg/mL EPA OPP 84-2	Negative -S9; with RCE reduced by 25%. Negative +S9; with RCE reduced by 59%.	This appears to be the best available MCGM test and it was conducted according to GLP. The levels of toxicity seen were above those recommended in guidance published subsequently, which recommends a reduction of RCE 10-20% (Moore *et al.,* 2002. Purity: 99.7%	San and Clarke (2001)
Chinese hamster ovary (CHO) cells *hprt* assay (-S9 only): 0.1-3 µg/mL –S9 Non GLP, non-guideline	A clear dose-related increase in mutant fraction (5- 30 mutants/10^6 clonable cells) was seen up to 1.5 µg/mL, Toxicity (relative cell survival) at these doses was not marked (<50%).	Only graphical data are presented in the paper. Whilst it is evident that a dose related increase in mutant frequency (MF) was observed in the absence of overt toxicity, marked heterogeneity at each dose were observed (as evidenced by the error bars). Furthermore, the reported background MF of CHO *hprt* cells is between 2-50 mutants/10^6 clonable cells (as reported by Johnson, 2012). The maximum increase in MF reported in this paper with CH_3I was ~30 mutants x 10^6 clonable cells. This level of MF is within the spontaneous background range reported for this cell line. The results should therefore be viewed with caution. Purity: 99%	Amachar and Zelijadt (1984)
Mouse lymphoma L5178Y *tk* and *hprt* assays: 0 - 46 µg/mL -S9	A very slight, dose-related increase in *tk* mutant fraction was observed with S9, and a "borderline" increase seen without S9. In both cases, severe cytotoxicity was seen marked (i.e. viability down to <10There was no	Multi-substance trial. The positive data should be viewed against the extreme cytotoxicity observed and inappropriate expression times (48, 72 and 144h used in this assay).	Clive *et al*. (1979)

Method	Results	Remarks	Reference
0 - 70 µg/mL +S9 Non-GLP, non-guideline	increase in mutant fraction in the *hprt* assay (+S9 only).	Purity: not stated	
Mouse lymphoma L5178Y cells *hprt* assay (-S9 only): 10 µg/mL *tk* assay (-S9 only) : 0, 7.5, 10 & 12.5 ug/mL Non GLP, non-guideline	The authors concluded that the results were positive at both the *tk* and *hprt* loci. However levels of cytotoxicity were high (60,20 and 6% survival at 7.5, 10 and 12.5 ug/mL, respectively. The *tk* assay showed an increase in small colonies (as well as total colonies) which was considered by the authors as a possible indicator of a clastogenic response.	This was essentially a method development study (same *tk* data reported in different papers), exploring variables such as culture conditions, expression times and mutant colony size for the *tk* assay. The results of the mutagenicity assays are difficult to interpret when assessed against current criteria (e.g. Moore et al (2000, 2002, 2006), given the high cytotoxicity and (for the *hprt* assay) the low mutant fraction reported. Purity: not stated.	Moore and Clive (1982) Moore *et al* (1985a,b)
Mouse lymphoma L5178Y cells Ouabain-resistance assay (-S9 only): 1.9 - 10 µg/mL Non GLP, non-guideline	The authors reported a dose-related increase in the frequency of mutant colonies at the lower doses tested (< 4 µg/mL), although the variation seen between duplicate independent trials was high. At higher exposure levels the total relative growth (a measure of cytotoxicity) fell below 50% and the observed mutation rate also decreased (to the control level).	Limited details of the results were presented making an independent assessment of this non-standard assay difficult. The dose-response, shown graphically, was not clearly positive given the high variability in response from different cultures. The results were presented in an unconventional way (mean +/- half the range from duplicate cultures, rather than SEM or SD). Purity: not stated.	Amacher and Dunn (1985)
MAMMALIAN CHROMOSOMAL ABERRATION DATA			
Chinese hamster Ovary (CHO) cells 4 and 20 hr -S9: 0 – 250 µg/mL; 4 hr +S9: 0 – 200 µg/mL EPA OPP 84-2, GLP	Positive for structural chromosome aberrations with and without S9. A reduced mitotic index was seen in each treatment group: (52% 4h exposure without S9; 77% 4 exposure with S9; (53% 20h without S9).	Well conducted, GLP-compliant study. Purity: 99.7%	Gudi and Brown (2001)

Bacterial mutagenicity assays

Iodomethane was first tested for bacterial mutagenicity in the 1970s, when laboratories in the US especially, were developing methods that would be suitable for the routine testing of substances with various different physico-chemical characteristics and requirements for metabolic activation (Simmon *et al*., 1977; Simmon, 1979a; Rosenkranz and Poirier, 1979). When the standard plate incorporation assay was modified (e.g. spot test; exposure to vapour in a desiccator), these authors demonstrated that iodomethane could produce a mutagenic response in *S typhimurium*. However, as explained in Table 15, the interpretation of these positive results according to current regulatory standards is not straightforward, and doubts are

raised by the absence of evidence that cytotoxicity was adequately controlled and the limited reporting of the studies overall.

In a more recent study (Wagner and Dakoulas, 2001), a conventional pre-incubation protocol was used to assess the mutagenicity of iodomethane. *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E coli* WP2*uvr*A were employed in this study. Following a preliminary toxicity-mutation assay, doses of 15, 50, 150, 500, 1500 and 5000 µg/plate iodomethane (in aq. solution) were assayed in the presence and absence of S9 in the confirmatory mutagenicity assay.

There was a reduction in the bacterial background lawn at the highest dose tested, this toxicity serving to confirm the bioavailability of the test substance to the bacteria (see detailed results, below). No precipitate was observed. There was a clear negative result for all the tested strains, with and without S9 when tested up to a maximum recommended dose in accordance with current regulatory requirements for this assay type.

Mammalian cell gene mutation assays

CHO cells

San and Clarke (2001) provide the best available study of the mutagenicity of iodomethane in mammalian cell cultures, in which mutation at the hypoxanthine guanine phosphoribosyl transferase (*hprt*) gene locus was measured in Chinese hamster ovary (CHO) cells. A preliminary toxicity test was undertaken using iodomethane (aq.) up to 1430 µg/mL (10 mM) in the absence and presence of S9 with a 5 hour exposure period. No visible precipitate in the treatment medium was observed at any concentration. Toxicity (relative cloning efficiency RCE <50%) was observed at concentrations of ≥505 µg/mL with and without S9.

In the main test, there was no dose-related increase in mutant frequency either in the presence or absence of S9.

In the absence of S9, relative cloning efficiency (RCE) was reduced to 19% at the top dose of 150 µg/mL iodomethane. However, mutant frequency at this concentration was not determined due to contamination in the mutation plates. At the next lowest concentration, 125 µg/mL, at which the RCE was reduced to 25%, no increased mutagenicity was seen. Although the toxicity at this level was just outside the preferred range (RCE 10-20%), the negative result is considered valid (in accordance with guidance provided by Moore *et al.,* 2002).

In the presence of S9, RCE was only reduced to 41% at the highest tested concentration of 200 µg/mL iodomethane. The authors concluded that this level of toxicity was acceptable, but it is not consistent with current recommendations and guidance, or indeed with guidance published at the time the study was conducted. Strictly, the desired level of toxicity was an RCE of 10-20%. Consequently, it is not possible to conclude that the result with S9 was definitively negative; it is possible that significantly higher doses could have been tested.

In contrast to this study, an earlier study had shown a dose-related increase in CHO cells mutant at the *hprt* locus following exposure to iodomethane in the absence of S9 (Amacher and Zelijadt, 1984). In this study, concentrations of between 0.1 and 1.5µg/mL produced a dose-related increase in mutants (mean values from triplicate cultures ranging from approx. 0.5 to 30 mutants per million clonable cells). In this assay, the level of cytotoxicity reported was relatively modest: mean relative cell survival > 50%.

Mouse lymphoma L5178Y cells

Iodomethane was tested as part of an extensive research project aimed at better understanding the nature of the mutagenic responses seen in the mouse lymphoma L5178Y cell *tk* assay (Moore and Clive, 1982, Moore *et al*, 1985a, Moore *et al*, 1985b). As summarised in Table 15, the authors reported "positive" results at both the *hprt* and *tk* loci. However, the methods employed and criteria used to evaluate these studies are now somewhat dated and, accordingly, the results are not regarded as being sufficiently robust for regulatory purposes.

The recent publications by Moore *et al* (2000, 2002 and 2006) have described the evolution of the mouse lymphoma test methodology and the findings presented by Moore *et al* (2010) contribute in part to a major review of mouse lymphoma data generated in the 1980s. The conclusion from this paper confirmed that only about 17% of the experiments conducted for the US NTP met all the current acceptance criteria, with 60% of the NTP 'positive' results not meeting the current global evaluation factor criteria for a positive response. Overall, more than 60% of the chemicals could not be classified as positive, negative or equivocal. It is therefore considered prudent to view the older mouse lymphoma data (*tk* locus), typified by the studies with iodomethane with caution.

In summary, 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 due to the variable results obtained, the limited reporting, and the non-standardised nature of the methods employed.

Mammalian cell chromosome aberration assay

In a mammalian chromosomal aberration assay, CHO cells were exposed to iodomethane using water as the solvent in either the presence (+S9, 4 hours) or absence (-S9, 4 and 20 hours) of metabolic activation.

Following a range finding trial, the doses chosen for the main test ranged from 50 to 350 µg/mL (-S9) and 25 to 350 µg/mL (+S9) for the 4 hour treatments. For the continuous (20 hour) treatment, concentrations ranged from 25 to 250 µg/mL. The maximum dose was selected with the intention of reducing cell growth (mitotic inhibition, MI) by at least 50%.

For the 4 hour treatment –S9, doses selected for chromosome aberration assessment were 50, 150 and 250 µg/mL. At 250 µg/mL, MI was reduced to 52%. Statistically significant increases ($p \leq 0.01$) in structural aberrations were observed at 150 and 250 µg/mL.

For the 4 hour treatment +S9, doses selected for chromosome aberration assessment were 25, 100 and 200 µg/mL. At 200 µg/mL, MI was reduced to 77%. Statistically significant increases in structural aberrations were observed at 100 and 200 µg/mL ($p \leq 0.05$ and $p \leq 0.01$ respectively).

For the 20 hour treatment -S9, doses selected for chromosome aberration assessment were 50, 150 and 250 µg/mL. At 250 µg/mL, MI was reduced to 53%. Statistically significant ($p \leq 0.01$) increases in structural aberrations were observed at 150 and 250 µg/mL.

No biologically relevant increases in polyploidy were observed in any of the treatment conditions tested. Positive controls induced the appropriate response.

Based on the results of this assay, iodomethane induced chromosome aberrations in the *in vitro* chromosome aberration study using CHO cells following sampling at 4 (+ and –S9) and 20 (-S9) hours, when tested up to cytotoxic concentrations.

Other available studies

Presented in Table 16 are additional, published *in vitro* studies. They are included to provide a full picture of the available data but, as remarked in the table, are not considered to further inform on the *in vitro* mutagenicity of iodomethane.

Table 16: Overview of other published (experimental) *in vitro* genotoxicity studies

Method	Results	Remarks	Reference
Mutation assay *E. coli* WP2*uvr*A Doses: Not stated clearly; possibly 20 – 10000 uM (–S9) Non-GLP, non-guideline	The authors concluded that this study showed a positive result with respect to DNA alkylation and mutagenicity.	Uninterpretable – limited weight can be given to this study due to inaccurate determination of viability data; unconventional mutant frequency ratio reported; poor reporting (lack of cytotoxicity data; validity of vehicle controls not defined; no positive control data); no dosing information reported. Purity: not stated	Hemminki *et al*. (1980)
Mutation (SOS[#]) assay *E. coli* WP2 Doses: 5 – 20 mM –S9 Non-GLP, non-guideline	In a pre-incubation assay, a dose-related increase in Try+ revertants/plate was reported at high concentrations that also induced cell death.	Unclear whether the individual experiments were repeated; very high concentrations were used in this assay (10-40 mM): compared to the study of Wagner and Dakoulas (2001) where cytotoxicity was seen at 0.697 mM; poor reporting of data (only graphical representations were made and no indication of reproducibility); lack of cytotoxicity data; validity of vehicle controls not defined; no positive control data); doses that were clearly cytotoxic were interpreted as mutagenic. Purity: not stated	Takahashi and Kawazoe (1987a) Takahashi and Kawazoe (1987b)
Mitotic recombination assay *Saccharomyces cerevisiae D3* Doses: 0.1% (v/v) -S9 Non-GLP, non-guideline	The author concluded that the result with respect to mitotic recombination was positive.	Uninterpretable when assessed against current guidance (EPA OPPTS 870.5575 recommends a different strain of *S.cerevisiae*); authors encountered a number of problems with the assay (2-3 fold variation in cell concentrations treated, variation (up to 10-fold) in spontaneous mitotic recombinants; mitotic recombinant activity occurred in a narrow concentration that coincided with toxicity; the fewer cells that were plated (i.e. due to increased toxicity); the number of spontaneous recombinants did not decrease proportionally); criteria for vehicle controls not stated, therefore validity not unknown. Purity: not stated.	Simmon (1979b)
Gene mutation assay *Aspergillus nidulans* Doses: 0.01 - 0.1M Non-GLP, non-guideline.	The authors concluded that the result of this study was negative.	Translated from a limited report written in Spanish. The volatility of compound may not have been adequately controlled as survival was 100%. Purity: not stated.	Moura, Duarte (1972) IARC (1988)

SOS response is a global response to DNA damage in which the cell cycle is arrested and DNA repair or mutagenesis is induced. The RecA protein is up regulated and inactivates the LexA repressor gene thereby inducing the response

Overall summary of bacterial and *in vitro* mammalian genotoxicity data

From the one Ames study conducted under strict guideline requirements and to GLP, when iodomethane was tested up to 5 mg/plate (maximum recommended dose) a negative result was returned in both the absence and presence of metabolic activation. Evidence of toxicity (reduction in the bacterial background lawn) was observed in most test conditions at the maximum dose tested, thereby confirming bioavailability to the bacteria.

There are a variety of other bacterial studies available that examine the potential genotoxic effects of iodomethane, giving a mixture of positive and negative results. These data were generated in the 1970s and the early 1980s and contain substantial methodological and data reporting deficiencies, such that the data presented in these studies are considered somewhat unreliable. Therefore, in many of the older studies it is difficult to compare such findings with those of the modern, GLP/guideline compliant study.

In conclusion, although iodomethane has been reported to produce a mutagenic response in bacteria, the available evidence from historic, non-conventional protocols is of uncertain relevance when assessed against current standards. In a more recent study, regarded as the best and most reliable because it includes a conventional pre-incubation assay performed in accordance with regulatory guidelines, iodomethane gave a clear negative result

The potential of iodomethane for mutagenicity has also been studied in CHO and mouse lymphoma L5718Y cells. However, 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 due to the variable results obtained, the limited reporting and the non-standardised nature of the methods employed. As previously discussed the inclusion of *in vitro* mammalian gene mutation cell into the basic *in vitro* battery does not enhance either the specificity or sensitivity of the test battery. Therefore the lack of a definitive conclusion for this endpoint is not an area of concern as this has been adequately addressed by the test guideline compliant bacterial reverse gene mutation assay. In contrast, iodomethane has been demonstrated to induce chromosome aberrations in CHO cells in both the presence and absence of an exogenous metabolic activation system (S9).

In summary, the available data from studies in bacteria and cultured mammalian cells do not provide any reliable, reproducible indication of iodomethane being able to induce gene mutations in bacteria or cultured mammalian cells. Positive results have been reported, but they all have deficiencies in reporting and/or method design. However, iodomethane clearly has the potential to induce chromosome aberrations in cultured mammalian cells.

4.9.1.2 *In vivo* data

Table 17: Summary of the available *in vivo* genotoxicity studies

Method	Results	Remarks	Reference
Mouse (CD-1) bone marrow micronucleus assay (5 animals/sex/dose) Intraperitoneal injection	Negative (24 and 48 h exposure) A small, dose-related decrease in group mean P/N ratio was seen (most notably in females), providing evidence of bone marrow exposure.	Well conducted, GLP-compliant study. Purity: 99.7%	Gudi and Krsmanovic (2001)

Method	Results	Remarks	Reference
0, 25, 50 & 100 mg/kg EPA OPP 84-2, GLP	The doses were selected after doses of 200 mg/kg and above were found to increase mortality if mice in a range finding test.		
Rat (Fischer 344) DNA binding (5 animals/sex/dose) Inhalation (whole body): single 6h exposure. Termination immediately post exposure. 80 ppm equivalent to 4.1 and 6.3 mg/kg for males and females, respectively. Oral: singe oral gavage dose. Termination 24hrs post dosing.0.5 and 0.8 mg/kg for males and females, respectively. Non-GLP, non-guideline	DNA samples from the following tissues were analysed by HPLC: liver, lung, stomach, (including the forestomach) and kidneys. The highest amount of radioactivity ("binding") occurred in DNA from the stomach and forestomach for both routes of exposure.	The authors claimed that their results showed the formation of several types of alkylated base, but the study appears to have been compromised by the presence of radiolabel in the purine bases of the nucleotides measured, resulting from the transfer of the methyl group from methyl iodide into the one carbon pool for de novo synthesis of nucleotides. Regardless of the route of exposure, the greatest uptake of radiolabel was found in the stomach and forestomach, where significant de novo DNA sysnthesis occurs. Test material:[^{14}C] iodomethane Radiochemical purity: 92.5%; specific activity 56mCi/mmol	Gansewendt, *et al*. (1989, 1990, 1991)

Bone marrow micronucleus test

In a bone marrow micronucleus assay using CD-1 mice, a single intraperitoneal (*ip*) injection of iodomethane dissolved in distilled water was administered to groups of 5 male and 5 female animals, employing a dose volume of 20 mL/kg. Doses were selected from a pilot toxicity study where male mice were dosed at 100, 120, 180 and 225 mg/kg and at 280 mg/kg to male and female animals. As the maximum tolerated dose (MTD) was not determined, a further toxicity study was undertaken where 5 animals/sex were dosed at 50, 100, 200 or 280 mg/kg. Mortality was observed in both sexes at doses of ≥200 mg/kg. Doses selected for the micronucleus assay were 25, 50 and 100 mg/kg.

Negative control groups were treated with vehicle only (water), and positive control groups were treated with cyclophosphamide (CPA, 50 mg/kg). Bone marrow was sampled at 24 and 48 hours after dosing for the vehicle and iodomethane dosed groups. A single sampling time of 24 hours after dosing was used for the CPA positive control group. Slides of bone marrow cells were prepared from five animals/sex/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (MN PCE) and PCE/total erythrocyte ratios.

There were no statistically significant increases in the frequency of micronuclei in any treatment group. Individual animal and group mean MN PCE frequencies were consistent with

both the concurrent vehicle control values and the historical control. Positive control treatment induced the appropriate response.

No deaths or clinical signs of toxicity were observed in the iodomethane-dosed groups, vehicle or positive control groups. A reduction in mean PCE/total erythrocyte ratio for both male and females at the 24 h time point and to a lesser extent at the 48 h time point were observed when compared to the respective vehicle control groups. Although this did not reach statistical significance, the reductions in PCE ratio are indicative of iodomethane exposure to the bone marrow (Table 18).

Whilst there are no analytical data to confirm systemic exposure to iodomethane following *ip* injection, information from toxicokinetic studies confirm that following oral administration iodomethane is completely absorbed (refer to Section 4.1.3). 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 (see Probst, 1994, for a general discussion about target tissue exposure).

Table 18: Summary of micronucleus results in male and female mice

Treatment (mg/kg)	Harvest time (h)	Males (5/gp)		Females (5/gp)	
		PCE ratio ±SD	MN PCE ±SD	PCE ratio ±SD	MN PCE ±SD
0	24	0.451 ± 0.11	0.3 ± 0.27	0.470 ± 0.10	0.3 ± 0.27
25	24	0.518 ± 0.05	0.3 ± 0.27	0.484 ± 0.04	0.3 ± 0.27
50	24	0.443 ± 0.06	0.4 ± 0.22	0.406 ± 0.05	0.4 ± 0.22
100	24	0.384 ± 0.09	0.0 ± 0.00	0.374 ± 0.07	0.5 ± 0.35
CPA, 50	24	0.323 ± 0.04	24.7 ± 3.68*	0.313 ± 0.03	23.2 ± 5.66*
0	48	0.491 ± 0.05	0.1 ± 0.22	0.501 ± 0.05	0.4 ± 0.22
100	48	0.425 ± 0.02	0.5 ± 0.35	0.441 ± 0.02	0.3 ± 0.27
HCD	Mean ± SD	0.52 ± 0.07	0.52 ± 0.07	0.51 ± 0.61	0.51 ± 0.66

HCD historical control data
*p ≤0.05 (Kastenbaum-Bowman Tables)
CPA cyclophosphamide

In conclusion, iodomethane was not genotoxic in this study, in which evidence was provided to demonstrate target organ exposure.

Overall summary of *in vivo* genotoxicity data

Of the two *in vivo* studies available, only one study was performed to GLP and considered guideline compliant. This study (Gudi and Krsmanovic, 2001) showed that iodomethane did not induce micronuclei in the bone marrow polychromatic erythrocytes of mice when tested up to a dose of 100 mg/kg (maximum tolerated dose - MTD). The DNA binding study (Gansewendt *et al.*, 1989, 1990, 1991) measured radioactivity in the DNA obtained from several tissues following oral and inhalatory routes of exposure and was reported to show evidence of DNA interaction. However, the degraded radiolabelled ^{14}C of iodomethane was added to the carbon pool, which was then incorporated into DNA during synthesis, with no reliable evidence of DNA adduct formation.

4.9.2 Human information

None.

4.9.3 Other relevant information

The alkylating activity of iodomethane has been investigated by Hemminki *et al* (1980). who reported alkylation of both 4-(p-nitrobenzyl)-pyridine and the nucleic acid base, deoxyguanosine. The deoxyguanosine alkylation was performed at 37°C at a concentration of 0.1M iodomethane. This study demonstrated the direct alkylating potential of iodomethane in a simple, non-biological, *in vitro* test system.

Coultier *et al* (2001) investigated the pattern of DNA alkylation following treatment of purified DNA and human lymphoblastoid (TK6) cells by a variety of alkylating agents, including iodomethane. DNA was isolated from the exposed cells and piperidine used to introduce single strand nicks at sites of N-methylpurine damage. Evidence of methylation was found by electrophoresis of samples on agarose gels. The authors were interested in characterising whether different agents targeted specific residues (primarily guanine) believed to be 'hot spots' for adduction. They demonstrated that the intact chromatin structure and the presence of other non-reactive molecules significantly protect DNA from alkylation. Data were presented to show that iodomethane could methylate DNA, but there were no details of the exposure conditions or concentrations of iodomethane used, or of the viability of the exposed cells. The study confirms the inherent potential (*in vitro*) of iodomethane to act as an alkylating agent.

4.9.4 Summary and discussion of mutagenicity

The data on the potential mutagenicity of iodomethane have been presented in detail to support the view expressed in the following section (4.10: Carcinogenicity) that any increased tumour frequencies arising in rats and mice treated with iodomethane occur by a non-genotoxic mode of action. This will be discussed further in Section 4.10.

Iodomethane appears to have an inherent capacity to alkylate DNA. However, in spite of numerous tests being conducted, it has not been found to yield clear, unambiguous positive results in bacterial and *in vitro* mammalian cell gene mutation assays. In bacteria the best available test (showing a reduced background lawn confirming exposure) gave a negative result. In mammalian cells, both negative and positive results have been reported, but the available studies were conducted prior to recent developments in study design, with the publicly available studies being conducted during the infancy of this assay type, and it is not possible to reach any definitive conclusions about the mutagenicity of iodomethane from them. Although positive results were observed with and without exogenous metabolic activation in an *in vitro* chromosome aberration assay with CHO cells, this genotoxic activity was not confirmed in a well-conducted *in vivo* mouse bone marrow micronucleus assay (with target organ exposure). Given this profile, there is no proposal to classify iodomethane for mutagenicity.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

The results of experimental studies are summarised in the following table:

Table 19: Overview of experimental studies on carcinogenicity after oral administration

Method	Results	Remarks	Reference
78wk, mouse (CD-1) (50 animals/sex/group) oral: feed 0, 60, 200, 600ppm equivalent to 0, 8, 28, 84 mg/kg/day in males and 0, 10, 35, 100 mg/kg/day in females. OECD 451, GLP	<u>Neoplastic effects:</u> Males: A NOAEL of 200 ppm was derived Based on a slightly increased incidence of thyroid follicular tumours at the next highest dose. Females: A NOAEL of. 600 ppm was derived (No treatment related neoplastic findings observed in females dosed up to 600 ppm) <u>Non-neoplastic effects:</u> Males and Females: A NOAEL of 600 ppm was derived based on adaptive changes in the thyroid in response to chronically elevated TSH and local irritant rather than systemic changes in the upper gastrointestinal tract. However, a more conservative NOAEL of 200 ppm could be derived	Purity: 99.7%	Harriman (2005) Kirkpatrick (2008a)

Carcinogenicity study in the mouse

Microencapsulated iodomethane was administered on a continuous basis in the diet for a minimum of 78 weeks to 50 male and 50 female CD-1 mice/group. Dosage levels were 60, 200 and 600 ppm (equivalent to a maximum dose of 84 or 100 mg/kg/day for males and females respectively).

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. This was reflected in lower cumulative body weight gains. By week 78 group mean body weight gain of males at 600 ppm was 27% lower than control and females showed a similar though slightly less marked effect with mean body weight gain 24% lower than control. The degree of bodyweight gain reduction at 600 ppm is considered to exceed a MTD.

Elevated plasma TSH concentrations were observed in the 200 and 600 ppm group males compared to the control group when measured in animals at scheduled termination (Table 23). Plasma TSH levels in the test material-treated females tended to be higher than the control group, but the increases were not statistically significant. Correspondingly, lower T_4 concentrations were noted in the 600 ppm group males. No differences in total T_3 levels were observed between the test material-treated and control groups.

Neoplastic changes were observed in the thyroid glands. A statistically significant ($p \leq 0.025$) dose related trend in the incidence of follicular cell adenoma/carcinoma in the thyroid glands

was noted in the test material treated males (Table 20). Pairwise comparisons with the control group did not reveal statistical significance for the combined incidence of follicular cell adenoma/carcinoma of the thyroid gland in males. This finding was considered test material-related in the 600 ppm group males only, however, the incidence was low. A single occurrence in the 200 ppm group males was consistent with normal background lesions in clinically normal mice of this age and strain.

A potential test material related effect on the incidence of proliferative mesenchymal cell lesions in the cervix and uterus was noted. This was investigated further by preparing additional sections and employing special stains in attempt to further characterise the lesions. Differences of opinion between the study and reviewing pathologists were resolved by agreement on the final diagnoses, with additional review of selected undifferentiated proliferative lesions of the uterus and cervix by a pathology working group (PWG) which prepared an independent report (Hardisty, 2005). The consensus diagnosis of the PWG of these lesions and their interpretation were presented in the final report of the study. Among the lesions diagnosed the incidence of fibroma of the cervix and uterus was slightly increased as summarised below.

A statistically significant ($p \leq 0.025$) dose-related trend in the incidence of fibroma in the cervix was noted in the test material treated females (Table 20). However, the number of mice with uterine and/or cervical fibromas was low. All of the fibromas were observed at the terminal sacrifice and all were microscopic in size. The tumours were benign, growing slowly by local expansion and there was no indication that any of these tumours were potentially malignant since there was no increase in mitosis, local invasion, cellular pleomorphism or cellular anaplasia. The fibromas were considered not to be associated with treatment due to their low incidence, appearance only at the terminal sacrifice, microscopic size, absence of precursor lesions or other evidence of a treatment-related response involving the uterus or cervix and their benign appearance with complete lack of any evidence of progression. Furthermore, similar changes were not observed in the carcinogenicity study conducted in rats. Fibroma of the uterus and cervix has no known clinical or biological significance in animals or humans. The apparent increase in the high-dose group as compared to the control group was considered to be due to the absence of fibromas in the control group in this study rather than a treatment-related increase. However, the detailed sampling and histological examination of the proximal uterine horns, body of the uterus and distal cervix conducted for this study was atypical for routine evaluations of the female reproductive tracts in carcinogenicity studies and resulted in finding additional tumours that otherwise would have gone undetected. Consequently it was not possible to make meaningful comparisons with published or laboratory historical control data.

Non-neoplastic changes in the thyroid glands characterized by increased colloid, cytoplasmic vacuolation and hyperplasia of follicular epithelial cells were noted from treated male and female groups. The incidence and/or severity tended to increase in a dose related manner (Table 20).

A higher incidence of basophil hypertrophy was observed in the pituitary gland of female treated groups compared with controls although there was no similar trend in males where the incidence was high across all treated and control groups (Table 21).

In the upper gastrointestinal tract, test material related hyperkeratosis was noted in the 200 and 600 ppm group males and females (Table 21). Since the route of exposure was oral (*via* the diet) and the hyperkeratosis was limited to the squamous regions of the upper gastrointestinal tract the effect was considered most likely to be an irritant effect of iodomethane and not a manifestation of systemic toxicity.

The NOAEL for neoplastic findings was established at 200 ppm for males and 600 ppm for females based on a slightly increased incidence of thyroid follicular tumours in males at 600 ppm. A NOAEL for non-neoplastic findings was concluded to be 600 ppm based on adaptive changes in the thyroid in response to chronically elevated TSH and local irritant rather than systemic changes in the upper gastrointestinal tract. The study was not designed to establish a NOAEL for systemic toxicity.

Table 20: Neoplastic and non neoplastic thyroid gland findings and neoplastic findings in the cervix and uterus

Parameter	Male (50/gp)				Female (50/gp)			
Dose level (ppm)	0	60	200	600	0	60	200	600
Thyroid gland								
Increased colloid	3(50)	28(50)	37(50)	44(50)	8(50)	35(50)	31(50)	36(50)
Vacuolation, cytoplasmic	0(50)	12(50)	22(50)	15(50)	0(50)	15(50)	14(50)	13(50)
Hyperplasia, follicular cell	0(50)	1(50)	3(50)	6(50)	1(50)	25(50)	22(50)	26(50)
Adenoma/carcinoma, follicular cell tumour (R)	0(50)	0(50)	1(50)	3(49)#	0(42)	0(39)	0(42)	0(44)
{Incidence (%)					0	2	0	0}
	[for males no incidence reported¥				1/474 = 0.21%¥			]
	[adenoma 1.11-2.00%‡				0.77-2.08%‡			]
	[carcinoma 2.00-2.00%‡				1.56-1.56%‡			]
Cervix								
Fibroma (R)					0(49)	1(50)	0(47)	3(50)#
{Incidence (%)	-	-	-	-	0	2	0	6}
	[-	-	-	-	no incidence reported¥			]
	[-	-	-	-	none‡			]
Uterus								
Fibroma (R)	-	-	-	-	0(50)	1(50)	0(50)	1(50)
{Incidence (%)	-	-	-	-	0	2	0	2}
	[-	-	-	-	no incidence reported¥			]
	[-	-	-	-	1.67-2.0%			]

Significant (p≤0.025) linear dose response
(R – rare tumor, based on concurrent and laboratory's historical control data – spontaneous incidence rat <1%)
Values in square parenthesis refer to historical control (laboratory¥ and/or animal supplier‡) control data. Total tumours / total tissues examined and the % incidence are reported for the laboratory, where as the %incidence range (min-max) has been reported from the animal supplier)

Table 21: Selected clinical chemistry and histological non-neoplastic pathology findings among mice

Parameter	Male (50/gp)				Female (50/gp)			
Dose level (ppm)	0	60	200	600	0	60	200	600
Clinical chemistry (mean values)								
TSH (ug/mL)	0.45	0.54	0.69*	0.86**	0.28	0.45	0.47	0.39
T_4 (ng/dL)	2.68	2.60	2.55	1.87**	1.82	1.91	1.87	1.76
Oesophagus								
Hyperkeratosis	3(50)	4(50)	28(50)	38(50)	0(50)	5(50)	27(50)	45(50)
Pharynx								
Hyperkeratosis	1(50)	3(50)	11(50)	26(50)	1(50)	5(50)	16(50)	31(50)
Pituitary								
Hypertrophy	37(50)	11(13)[a]	5(8)[a]	44(50)	13(50)	30(50)	28(50)	35(50)
Stomach, non glandular								
Hyperkeratosis	5(50)	11(50)	32(50)	38(50)	19(50)	20(50)	34(50)	36(50)

* Significant ($p \leq 0.05$) vs control, ** Significant ($p \leq 0.01$) vs control
(Values in parenthesis represent the total of animals examined for that particular histopathological endpoint)
a - only examined in unscheduled death animals

4.10.1.2 Carcinogenicity: inhalation

The results of experimental studies are summarised in the following table:

Table 22: Overview of experimental studies on carcinogenicity after inhalation exposure

Method	Results	Remarks	Reference
52 or 104 wk, rat (Sprague-Dawley) 0, 5 and 20 ppm/6h/d (60 animals/sex/group) 60 ppm/6h/d (70 animals/sex/group) inhalation: vapour (whole body) 0, 5, 20, 60 ppm (nominal conc.) 0, 5.1, 20, 59.5 ppm (analytical conc.) OECD 453, GLP Note: 1 ppm = 5.81 mg/m^3 iodomethane	<u>Neoplastic effects:</u> Males and Females: A NOAEC of 20 ppm was derived based on increased thyroid follicular tumours in males at 60 ppm <u>Non-neoplastic effects:</u> Males and Females: A NOAEC of 5.1 ppm was derived based on squamous metaplasia of the salivary glands observed at 20 ppm	Purity: 99.6 – 99.8%	Kirkpatrick (2005) Kirkpatrick (2008b)

Combined chronic toxicity / carcinogenicity study in the rat

During a 24 month combined toxicity/carcinogenicity study male and female Sprague-Dawley rats were exposed to iodomethane for 5 days/week (for a total of 104 weeks) *via* whole body

inhalation as a vapour at target exposure concentrations of 5, 20 and 60 ppm. An interim necropsy took place during week 52 of the study, with selected tissues examined microscopically.

In general, adverse clinical signs were observed with a higher incidence in the 60 ppm group (during the chronic (1-52 weeks) phase of the study) and appeared to be secondary to compromised health status and/or low weight gain among a proportion of animals. These signs included (but were not limited to) hypoactivity, impaired muscle coordination or equilibrium, prostration, twitching or tremors, body and/or extremities pale or cool to touch, gasping, rales, emaciation and dermal atonia.

There were no adverse treatment related effects on survival after 2 years and group survival rates ranged from 34% to 48%.

Lower mean body weights and food consumption were noted for both male and females in the 60 ppm group throughout the first and second years of the study. Mean body weight gains were reduced in the 60 ppm group males and females generally through study week 51 (often significant at $p \leq 0.05$ or $p \leq 0.01$). Body weights of males and females at 60 ppm were 18% and 15% lower than control, respectively, at week 51 and both sexes were 20 % lower than control by week 103. There were corresponding reductions in weight gain relative to controls of 24% and 26% for males and females at 60 ppm, respectively, by week 51. Thereafter, sporadic, occasionally statistically significant body weight losses were observed. The magnitude of the reductions in body weight gain combined with the increased incidence of compromised health status among animals of this group demonstrated that the MTD was exceeded at 60 ppm.

Serum pituitary and thyroid hormone data are summarised in Table 23. Serum TSH levels were elevated and statistically significant ($p \leq 0.01$) at study week 26 in the 60 ppm group males and females. At study weeks 52 and 104, these values were still elevated when compared to the control group, but only statistical significance ($p \leq 0.01$) was obtained for males at study week 104. Although not statistically significant, mean serum T_3 values were numerically lower when compared to the control group in the 60 ppm males and females at study weeks 26, 52 and 104. In addition, serum reverse T_3 values in the 60 ppm males and female were elevated at study weeks 26, 52 and 104 and reaching statistical significance at the latter two time points. T_4 levels in the 60 ppm mean group were significantly decreased ($p \leq 0.01$) in males at study week 26 and were elevated in males ($p \leq 0.05$) and females at study week 52 and in females at study week 104 ($p \leq 0.01$). These changes may be correlated with the elevated TSH levels.

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

Male data: Note: Weeks 26 and 52 total T3 and reverse T3 compared using the Kruskal-Wallis test. All total T4 and TSH and week 104 total T3 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.

The only treatment related oncogenic effect was a significantly higher incidence of thyroid follicular cell adenomas in males at 60 ppm and a marginal but not statistically significant increase in follicular cell adenomas in females at 60 ppm. Although the combined incidence of thyroid follicular cell adenoma and carcinoma in males was also significantly increased there was no significant increase in the incidence of follicular cell carcinoma alone. The thyroid follicular tumour incidence data for all animals are summarised in Table 24. There was an increased incidence of thyroid follicular adenomas in male rats at 60 ppm after 52 weeks of exposure. Three out of 20 animals had tumours compared with none in the controls. This contrasted with a higher proportion of males with tumours (7/17) at the terminal sacrifice after 104 weeks.

There was a corresponding increase in thyroid follicular hyperplasia among males at 60 ppm after 52 and 104 weeks of exposure and among females after 104 weeks of exposure, including all deaths combined (Table 25). Other treatment related non neoplastic thyroid findings were confined to males at 60 ppm and consisted of increased incidences of cytoplasmic vacuolation of follicular cells (indicative of follicular degeneration) and follicular cysts and cystic

hyperplasia. These changes were correlated with macroscopic findings of enlarged thyroid gland and increased mean absolute and relative thyroid weights in males only.

Table 24: Thyroid follicular tumour incidences

Parameter	Males (total animals examined)				Females (total animals examined)			
Dose level (ppm)	0	5	20	60	0	5	20	60
Ben. adenoma, follicular cell	2(60)	2(60)	4(60)	13(70)#	1(60)	1(59)	0(60)	3(70)
Met. carcinoma, follicular cell	2(60)	0(60)	0(60)	4(70) **,##	1(60)	0(59)	1(60)	1(70)
Combined Adenoma / carcinoma, Folliclaur cell	4(60)	2(60)	4(60)	15(70) **,##	2(60)	1(59)	1(60)	4(70)

(Values in rounded parenthesis represent the total of animals examined for that particular histopathological endpoint)
Ben – benign; Met. – metastatic
** Significant (p≤0.01) vs control; # Significant linear dose response (p≤ 0.05); ## Significant linear dose response (p≤ 0.0001)

Table 25: Selected non neoplastic thyroid findings

Parameter	Males (total animals examined)				Females (total animals examined)			
Dose level (ppm)	0	5	20	60	0	5	20	60
Cyst, follicular	1(60)	4(60)	4(60)	8(60)*,#	1(60)	2(59)	1(60)	1(70)
Hyperplasia, follicular cell	0(60)	2(60)	1(60)	21(60)*,#	1(60)	2(59)	1(60)	12(70)*, #
Follicular cell cytoplasmic vaculoation	0(60)	1(60)	0(60)	16(60)*,#	0(60)	1(59)	0(60)	1(70)
Hyperplasia, follicular cystic	1(60)	5(60)	3(60)	8 (60)*,#	1(60)	2(59)	1(60)	12(70)

(Values in rounded parenthesis represent the total of animals examined for that particular histopathological endpoint)
* Statistically significant (p≤0.05) when compared to control group using Mann-Whitney U Test
# Statistically significant (p≤0.05) when compared to control group using Fishers Exact Test

There were no other statistically significant tumour incidences noted in the report or incidences considered related to iodomethane exposure. However, the incidence of astrocytomas in the brain is worthy of note. A marginal non statistically significant increase in astrocytomas was observed in high dose male rats compared with controls. There was a single incidence in the female high dose group. The data are summarised in Table 27. The incidences of malignant, benign or combined astrocytomas in the male high dose group were within or very close to the historic control incidences (comparable data excluding interim kill animals). There was a single incidence of malignant astrocytoma in the low dose group. It is not possible to ascertain whether there was a dose response because the low and mid dose group animals from the scheduled terminal sacrifice were not examined. The single benign astrocytoma in the female high dose group corresponded with the historic control incidence. Toxicokinetic data show that radioactivity from [^{14}C] iodomethane was detected in the blood, brain and other tissues at all times after exposure, but as radioactivity entered the one carbon pool it was incorporated into endogenous metabolites and residues would be expected (see Section 4.1). There is no toxicokinetic or mechanistic explanation to account for a marginal increase in astrocytomas as a result of iodomethane exposure. It is concluded that the data do not provide convincing

evidence of a treatment related increase in astrocytomas and it is likely that they arose by chance. This is supported by the historic control data. Furthermore, the high dose group exceeded a MTD and this reduces the level of potential concern.

Table 26: Brain astrocytoma incidences

Parameter	Males (total animals examined)[1]				Females (total animals examined)[1]			
Dose level (ppm)	0	5[2]	20[2]	60	0	5[2]	20[2]	60
Malignant astrocytoma	0(50)	1(27)	0(26)	2(49)	0(50)	0(27)	0(28)	0(50)
Benign astrocytoma	0(50)	0(27)	0(26)	1(49)	0(50)	0(27)	0(28)	1(50)
Combined malignant and benign astrocytoma	0(50)	1(27)	0(26)	3(49)	0(50)	0(27)	0(28)	1(50)
Historic control incidence[3]	0.00 – 4.35% (3/69) Malignant astrocytoma				0.00 – 3.33% (2/60) Malignant astrocytoma			
	0.00 – 3.39% (2/59) Benign astrocytoma				0.00 – 1.96% (1/51) Benign astrocytoma			
	0.00 – 4.35% (3/69) Combined benign and malignant astrocytoma				0.00 – 3.33% (2/60) Combined benign and malignant astrocytoma			

[1] Unscheduled deaths and scheduled sacrifice week 104. Week 52 interim kill excluded - no astrocytomas found
[2] Low and intermediate dose groups not examined at scheduled sacrifice week104 because the findings in the high dose group were considered unrelated to treatment
[3] Laboratory historic control groups (Sprague-Dawley rats); min. – max. % incidence and (max. incidence/animals examined)

Other treatment related non neoplastic changes occurred in the nasal olfactory epithelium (60 ppm group, both sexes) and salivary glands (20 and 60 ppm, both sexes). Degenerative changes and/or regenerative cyst-like formation were observed in the olfactory epithelium at nasal levels II-VI in animals at 60 ppm. At nasal level V, 63/70 males and 60/70 females in the 60 ppm group had degeneration of the olfactory epithelium. At this same level, cysts in the olfactory epithelium were observed for 35/70 males and 35/70 females at 60 ppm. There was a slight increase in epithelial degeneration without cystic regeneration in animals at 20 ppm. The very low incidence of minimal changes at 5 ppm was not considered adverse as similar findings were noted in control animals. Squamous metaplasia of the salivary ductular epithelium occurred at a very high incidence at the 60 ppm exposure level for both males and females; and somewhat at lower incidences for both genders at the 20 ppm exposure level. An apparent associated finding was atrophy of the salivary acinar structures that was observed in both genders at the 20 and 60 ppm exposure level. These salivary gland findings were mainly graded as minimal or mild at both the study week 52 interim and study week 104 necropsies, thus the severity did not progress with time. Although there was a slightly increased incidence of salivary gland changes at 5 ppm this was considered not to be adverse based on their low incidence and lack of progression in severity

Based on the results of this study the NOAEL for non-neoplastic findings was 5 ppm based on squamous metaplasia of the salivary glands at 20 ppm. The NOEL for neoplastic findings was 20 ppm. The MTD was considered to have been exceeded at 60 ppm.

4.10.1.3 Carcinogenicity: dermal

The Druckrey *et al*. (1970) study summarised below has been assigned a Klimisch reliability score of 4 (not assignable) based on the criteria in Klimisch, *et al*., (1997). This is based on several factors including: non-GLP / non-guideline compliant, insufficient /conflicting reporting of data, absence of historical data and untreated and vehicle control groups.

The potential carcinogenicity of iodomethane following subcutaneous injection to rats was reported in a scientific publication in the open literature (Druckrey *et al*., 1970). This was a non GLP and non guideline study. The study is published in German with an English abstract. Although a limited translation has been obtained the methodology and the results are poorly reported (conflicting data are reported in the text of the report and in tables), and it is not possible to provide a meaningful summary of the study.

It appears that small numbers of animals (between 8 to 12/group) were administered doses of 10 or 20 mg/kg by weekly subcutaneous injection for an indeterminate period until necrosis was observed at the injection sites. A further group of 14 animals received a single dose of iodomethane *via* subcutaneous injection at 50 mg/kg. Again the post dose exposure period was not specified although it appeared to be until animals died.

Most animals in the 10 and 20 mg/kg groups developed local site sarcomas whereas 4/14 rats developed local sarcomas after receiving a single dose.

There were no vehicle control or untreated control groups or historical control data for comparison. Whilst the conclusion of the study provided by the author was that iodomethane caused local site sarcomas at the site of injection following subcutaneous injection, due to the poor construct of the study design and limited/conflicting reporting of the data, it is deemed that a definitive conclusion cannot be drawn.

Comment
The study does not conform to the standards required for an acceptable evaluation of the carcinogenicity of iodomethane. Iodomethane is a skin irritant and 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 (IPCS, 1982). It is noteworthy that lifetime cancer studies of iodomethane by standard routes of exposure did not cause tumours at the portal of entry. Specifically, the two-year inhalation study of iodomethane in rats did not cause nasal or respiratory tract tumours, and the 18 month dietary study in mice did not result in gastrointestinal tumours.

4.10.2 Human information

None.

4.10.3 Other relevant information

The Poirier *et al*. (1975) study summarised below has been assigned a Klimisch reliability score of 3 (not reliable). This is based on several factors including: non-GLP / non-guideline compliant, insufficient reporting of data, use of a mouse strain susceptible to the development of lung adenomas by a very young age.

The potential carcinogenicity of iodomethane following intra-peritoneal (i.p.) injection to mice was reported in a scientific publication in the open literature (Poirier *et al*., 1975). This was a non GLP and non guideline study.

Three groups of mice (10 male and 10 female mice/group) were dosed *via* i.p. injection with iodomethane formulated in tricaprylin and dosed 3 times/week for 24 weeks to give total doses of 0.06, 0.15 and 0.31 mmol/kg. A further group of mice were dosed with the vehicle (tricaprylin), and there was an untreated group. The objective of this study was to evaluate the number and production of lung adenomas in strain A/Heston (A/He) mice following multiple injections of iodomethane (other alkyl halides were also dosed, but these data are not of interest in context of the iodomethane data discussion and therefore these data have been omitted). This mouse strain was susceptible to lung adenoma formation by alkylating agents.

Following 24 weeks of dosing, a slight but significant increase ($p \leq 0.05$) in the average number of lung tumours/mouse was noted in mice dosed at 0.31 mmol/kg. For mice in this group the average number of lung tumours/mice was 0.55 compared to 0.21 and 0.22 in untreated and vehicle treated mice. However, the slight increase in lung adenomas was only seen at the highest dose which appeared to exceed a MTD based on only 55% of animals surviving *vs*. almost 100% in controls and the lower dose groups. Positive controls (urethane) developed a clear tumour response in this study, unlike the response observed for iodomethane.

Comment
These data indicate that iodomethane induces a slight increase in the numbers of lung adenomas at a dose level which exceeded a MTD in a strain susceptible to lung adenoma formation. However, the study does not conform to the standards required for an acceptable evaluation of the carcinogenicity of iodomethane.

4.10.4 Summary and discussion of carcinogenicity

Overall, chronic iodomethane exposure *via* inhalation in the rat or *via* dietary administration in the mouse is associated with increased incidences of thyroid follicular adenomas. The effect was confined to male rats and male mice at the highest dose levels, which were considered to exceed a MTD. The rat was more susceptible than the mouse. Thyroid follicular hyperplasia, which is indicative of cell proliferation was observed in the rat at the highest dose level and in the mouse at all dose levels. There was evidence of perturbation of thyroid hormone homeostasis in the rat and to some degree in the male mouse.

It is recognised that iodomethane is a methylating agent that readily interacts with macromolecules, SH groups of proteins and causes time and concentration dependent reductions in tissue glutathione concentrations (including olfactory and respiratory epithelia and to a lesser extent blood, kidney and liver – Himmelstein, 2004 [Section 4.12.1.3]). Both the rat and mouse carcinogenicity studies failed to increase tumour incidence at the site of first contact (for the rat olfactory tissue / lung, or digestive tract associated organs in the mouse), glutathione rich tissues (blood, liver or kidney) or in the haematopoietic system. Furthermore, whilst S-methyl cysteine adducts were also detected in haemoglobin showing that iodomethane had also methylated thiol groups in globin, haematological parameters from sub-chronic and chronic studies failed to show evidence of haematotoxicity. Therefore, whilst iodomethane may have propensity to interact with haemoglobin, partial evidence of haematotoxicity was only present in the mouse bone marrow micronucleus study where a decrease in the PCE population was observed following dosing over an acute period (2 days). This observation was not carried forward in the sub-chronic or chronic studies conducted in a variety of species. The *in vitro* and *in vivo* genotoxicity data confirm that iodomethane does not interact directly with DNA, with adequate exposure to the organisms under investigation demonstrated by various measures of toxicity. There were no other treatment related oncogenic effects in either species.

A number of policies have been developed by regulatory agencies and other authoritative bodies on the relevance of thyroid tumours in rodents produced by perturbations of thyroid hormone homeostasis to hazard and risk assessment in humans. For example, the US EPA noted that although the rodent model provides a qualitative indicator of a potential human thyroid cancer hazard, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitary status (EPA, 1998). IARC stated that agents which induce thyroid follicular-cell tumours in rodents by interfering with thyroid hormone homeostasis can be assumed not to be carcinogenic in humans at concentrations that do not lead to alterations in thyroid hormone homeostasis (IARC, 2001). A group of Specialised Experts in the EU agreed that there is convincing scientific evidence that humans are considerably less sensitive than rodents (especially rats) to perturbation of thyroid hormone homeostasis and the subsequent development of thyroid follicular tumours induced by non genotoxic xenobiotics (ECBI, 1999).

In Annex I to this report, the evidence for a proposed non genotoxic mode of action of thyroid follicular tumours in rodents chronically exposed to iodomethane is presented. The MOA is systematically evaluated using the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action to Humans (IPCS, 2007).

In summary, the primary MOA for iodomethane induced thyroid follicular tumours in male rats involves the perturbation of homeostasis of the pituitary-thyroid axis caused by excess circulating iodide derived from the metabolism of iodomethane. Excess iodide acts centrally on the thyroid by inhibiting production and release of T_4. Reduced circulating levels of T_4 and T_3 cause a compensatory increase in circulating TSH from the pituitary resulting in sustained stimulation of the thyroid to produce thyroid hormone. This leads to enlargement of the thyroid and proliferation of thyroid follicular cells (hyperplasia). Thyroid hyperplasia can eventually progress to neoplasia; in this case a significantly increased incidence of benign adenomas. Prolonged inhibition of type I and type II deiodinase activities by excess iodide from chronic iodomethane exposure would also lead to reductions in T_3 and T_4 and compensatory sustained increases in TSH, and may contribute to the primary centrally acting effects of excess iodide.

It has been demonstrated from the open published literature that humans are far less sensitive than rats to perturbations of thyroid hormone homeostasis and consequent effects on the thyroid. Humans would be less sensitive to the effects of excess iodide on thyroid function and any prolonged increase in TSH is unlikely to lead to thyroid tumour development. Thyroid tumours in male rats were only induced at a dose level which resulted in general toxicity and which exceeded a MTD. Thus it is extremely unlikely that exposure of humans to iodomethane would lead to sustained elevation of TSH since exposures would have to exceed tolerable levels of iodomethane exposure. In conclusion, the exposure necessary for an effect in humans would not be achievable through any plausible scenario.

4.10.5 Comparison with criteria

Iodomethane is currently classified Category 3 R40 according to EU Directive 67/548/EEC. This is presumed to have been based on the early studies of carcinogenesis summarised in Sections 4.10.1.3 and 4.10.3. These studies are considered inadequate for determining the carcinogenicity classification of iodomethane.

The implications of the increased incidence of thyroid follicular tumours in male rats for hazard classification is evaluated using the ECHA Guidance on the Application of the CLP Criteria in Regulation (EC) No. 1272/2008 (ECHA, 2009), the criteria in Directive 67/548/EEC and Specialised Experts guidance on non-genotoxic thyroid carcinogens (ECBI, 1999).

4.10.5.1 Specialised Experts (EC, 1999)

The main conclusion from the Specialised Experts' report was:

Essentially, it was agreed that non-genotoxic carcinogenic 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.

Inhibition of T_4 release was listed as one of the clearly established mechanisms for perturbation of the pituitary-thyroid axis and the MOA for iodomethane is consistent with this mechanism. The key events critical for the induction of tumours have been identified and provide sufficient evidence for the postulated MOA.

The oncogenic potency of iodomethane is medium to low based on the following calculations.

The T_{25} approach is used as a crude index of carcinogenic potency in setting concentration limits for carcinogens in Annex 1 of Directive 67/548/EEC (EU, 1999). The T_{25} is the daily dose inducing a tumour incidence of 25% upon lifetime exposure assuming a linear dose response between and above the experimental doses. According to the default parameters for dose calculations given in the EU guidance, the lifetime average daily dose at the oncogenic iodomethane concentration of 60 ppm is:

Dose (mg/kg/day) = 350 mg/m^3 (60 ppm) / 19.4 (mg/m^3)*
= **18 mg/kg/day**

* giving a dose of 1 mg/kg/day based on 6 hour exposures, 5 days/ week

An alternative calculation based on the default respiration rate for rats given in the AOEL guidance document (EU, 2001) is as follows:

Dose (mg/kg/day) = 0.35 mg/l x 45 l/kg/hour x 6 hours
= 94.5 mg/kg/day
= 94.5 x 5/7 to adjust for 5 day per week exposure
= **68 mg/kg/day**

The potency classifications are:

Carcinogens of high potency: T_{25} value < 1 mg/kg bw/day

Carcinogens of medium potency: 1 mg/kg bw/day < T25 value < 100 mg/kg bw/day

Carcinogens of low potency: T_{25} value > 100 mg/kg bw/day.

On this basis iodomethane would be considered a carcinogen of medium potency. However, an ECETOC Task Force (TF) charged to develop guidance for the application of the existing GHS criteria on carcinogenicity, proposed that the T_{25} approach is in general insufficiently robust to serve as a basis for potency considerations for the purposes of classification (McGregor *et al*., 2010). The TF proposed an alternative approach using the guidance values for Category 1 and 2 classification for 90-day repeat dose target organ toxicity in the GHS guidelines (reproduced in the ECHA CLP guidance (EU, 2009)). The TF defined cut-off values for carcinogens of high and low potency are as follows:

For inhalation exposure to vapour: High potency: ≤ 0.02 mg/L
Low potency: > 0.02 mg/L

On this basis iodomethane would be considered a carcinogen of low potency (i.e. oncogenic dose = 0.35 mg/L).

Overall, it is concluded that iodomethane does not need to be classified for carcinogenicity based on this guidance, taking into account also that the significantly increased tumour incidence in male rats only was attributed to benign follicular adenomas at a dose level which exceeded the MTD.

4.10.5.2 CLP Regulation (EC) No 1272/2008 and guidance (ECHA, 2009)

Classification of a substance as a carcinogen is based on a weight of evidence approach and expert judgment. The IPCS framework applied in Annex 1 to this report is quoted as a basis for systematic assessments.

Consideration of the animal carcinogenicity data on iodomethane indicates that there is "limited evidence of carcinogenicity" based on:

- "The agent increases the incidence only of benign neoplasms" (Note: there is only a marginal non statistically significant increase in malignant thyroid neoplasms with iodomethane)
- "The evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs" (ie. only the thyroid gland).

Additional considerations for classification in the case of iodomethane include:

- "Tumour type and background incidence". Rodents, particularly the rat, are known to be susceptible to the induction of thyroid tumours associated with perturbations of thyroid hormone homeostasis. This tumour type is less relevant for humans.
- "The possibility of a confounding effect of excessive toxicity at test doses", ie, more doubtful potential for carcinogenicity in humans. The MTD was exceeded in both the rat and mouse studies at the oncogenic dose based on the degree of body weight reduction.
- "Mode of action and its relevance for humans, such as mutagenicity, cytoxicity with growth stimulation, mitogenesis, immunosuppression". A robust assessment of the carcinogenicity data and the postulated MOA was undertaken by applying the IPCS framework (refer to Annex I). There is convincing evidence for a non genotoxic MOA for thyroid follicular tumours in the rat and mouse. Humans are shown to be far less sensitive to this MOA.
- The ECHA CLP guidance specifically lists some mechanisms of tumour formation considered not relevant for humans, one of which is:

  "Certain thyroid tumours in rodents mediated by UDPGT induction (IARC, 1999; EU Specialised Experts, 1999)". This is very specific to only one thyroid mechanism involved in sustained elevation of TSH and its *sequelae*, whereas the Specialised Experts listed a number of mechanisms, including inhibition of T_4 release which is relevant to iodomethane.

- Mutagenicity: "Evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects". In the case of iodomethane the weight of evidence supports the absence of genotoxicity *in vivo*

It was shown earlier and in Annex I 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

because tolerable levels of iodomethane exposure would have to be exceeded. Therefore, overall it is concluded that iodomethane does not meet the criteria for carcinogenicity classification according to Regulation (EC) No. 1272/2008.

4.10.5.3 Directive 67/548/EEC

The considerations noted above also apply to the criteria in Directive 67/548/EEC. Iodomethane does not meet the criteria for Category 1 or 2; i.e. it is not a known human carcinogen and there is not sufficient evidence to provide a strong presumption that human exposure to iodomethane may result in the development of cancer. Furthermore, it is considered that iodomethane does not meet the criteria for Category 3 on the basis of the arguments presented above with regard to the EU Specialised Experts criteria and the considerations against the criteria in Regulation (EC) No. 1272/2008 which are equally applicable to Directive 67/548/EEC. A key argument for no classification rather than classification in Category 3 is based on the following criterion:

"A substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to humans".

It was shown earlier and in Annex I 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 because tolerable levels of iodomethane exposure would have to be exceeded; i.e. the mode of action cannot be extrapolated to humans on this basis.

4.10.6 Conclusions on classification and labelling

There is a convincing weight of evidence to conclude that iodomethane does not present a carcinogenic hazard to humans and consequently it should not be classified for carcinogenicity. For reasons stated earlier, there are no plausible scenarios whereby iodomethane exposure would lead to sustained elevation of TSH in humans and potential thyroid tumour formation. Therefore, it is proposed that the current classifications for carcinogenicity of iodomethane in Annex VI of Regulation (EC) No. 1272/2008 and Annex I of Directive 67/548/EEC are removed.

4.11 Toxicity for reproduction

Not relevant to this proposal.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Not relevant to this proposal.

4.12.1.2 Immunotoxicity

Not relevant to this proposal.

4.12.1.3 Specific investigations: other studies

A supplementary 2-day inhalation mechanistic study was conducted in rats. Measurements of 5'-deiodinase activity in tissue samples taken from animals in this study were reported separately.

2-day inhalation mechanistic study in rats (Himmelstein, 2004)

The objective of this study was to evaluate the toxicokinetic behaviour of iodomethane in rats exposed by inhalation. Male rats (10/group) were exposed to iodomethane (*via* whole body inhalation) for 6 hours/day over two days, with scheduled necropsy the following day post the end of exposure. Intended exposure concentrations were 0, 25 and 100 ppm. Key study endpoints included evaluation of glutathione status in selected target tissues, inorganic serum iodide and haemoglobin adducts as measures of internal dose and clinical chemistry, haematology, thyroid hormone status, liver UDP-glucuronyltransferase (UDPGT) activity and pulmonary function as measures of exposure or toxicity. Glutathione and serum iodide measurements were performed at intervals during the 2-days. Other measurements were performed on the morning after the last exposure.

Significant treatment related minimal to mild increases in serum total cholesterol concentrations and minimal to mild decreases in triglyceride concentrations were observed in both groups. Dose related decreases in both serum T_4 and T_3 and dose related increases in serum TSH were observed, the change being statistically significant at 25 and/or 100 ppm compared with the control (Table 27). There was no treatment related effect on serum reverse T_3 (rT_3), the inactive form of T_3.

Table 27: Group mean serum thyroid and pituitary hormone levels

Dose level (ppm)	0	25	100
Total T_3 (ng/dL)[a]	74.1 ± 11.4	65.9 ± 9.2	50.8 ± 14.4*
Total T_4 (µg/dL)[a]	3.4 ± 0.5	3.1 ± 0.8	2.1 ± 0.9*
Reverse T_3 (ng/ml)[a]	0.067 ± 0.049	0.119 ± 0.024	0.039 ± 0.037
TSH (ng/ml)	5.9 ± 1.4	10.9 ± 7.7*	21.1 ± 11.2*

[a] Mean ± standard deviation, n = 10 (n = 9 for serum rT3 100 ppm)
* p ≤0.05 compared with control

Hepatic UDPGT activity was not altered under the conditions of the study (Table 28).

Table 28: Group mean hepatic T_4-UDPGT levels

Dose level (ppm)	0	25	100
T_4-UDPGT (nmol/min/mg)[a]	16.1 ± 3.1	17.5 ± 2.0	17.8 ± 4.8

[a] n = 5 per group

Iodomethane exposure caused time and concentration dependent reductions in tissue GSH concentrations. Depletion was less pronounced in blood, kidney and liver than in olfactory and respiratory epithelia. Substantially increased inorganic serum iodide levels were observed in animals exposed to iodomethane in a concentration and time dependent manner (Table 29). During the 18-hour recovery periods serum iodide concentrations decreased dramatically and the levels after the second recovery period were lower than those after the first recovery period.

Table 29: Summary of serum iodide data (± standard deviation)

Parameter	Males (10/gp)		
Dose level (ppm)	0	25	100
Collection time (h)			
0	17±NA	NA±NA	NA±NA
1	17±NA	5070±721	22900±1620
3	19±NA	9510±3800	60300±2860
6	22±NA	25600±1940	53800±4480
9	39±NA	18400±1550	52500±8230
24	19±NA	1260±83.9	8170±1850
25	14±NA	5960±576	27200±13700
27	14±NA	10800±1100	55200±3050
30	4.1±NA	34100±8170	83200±7840
33	13±NA	24700±1310	58300±6520
48	14±NA	742±141	4500±396
0 - 48	17±9	NA±NA	NA±NA

NA = not applicable

Inhalation exposures to 25 and 100 ppm iodomethane for 6 hour did not alter the overall pattern of breathing frequency compared to the control rats.

The objective of this study was not to identify a NOAEL, but provide toxicity and dosimetry endpoints which could be used in support of physiologically-based pharmacokinetic modelling and product safety assessment.

Assessment of 5'-deiodinase activity in tissue samples from 2-day inhalation study (Farwell, 2004)

The objective of this study was to characterise the effect of iodomethane on the deiodinase enzymes with *in vitro* studies and analysis of deiodinase activity in tissues obtained from studies including the 2-day inhalation mechanistic study. The investigations from studies in the rabbit are not summarised here as they are not relevant to this classification proposal.

Liver, kidney and brain samples from the 2-day inhalation study were analysed as detailed below. Furthermore, microsomal preparations of liver and kidney were obtained from pregnant (16 -17day gestation) rats and primary astrocyte cell cultures were prepared from neonatal rat brains for the *in vitro* experiments.

D1 (type I 5'deiodinase) which catalyses phenolic (outer) ring deiodination of T_4 and rT_3 is found predominantly in the liver, kidney, thyroid and brain.

D2 (type II 5'-deiodinase) catalyses phenolic ring deiodination and regulates tissue-specific intracellular T_3 production, particularly in the brain and the pituitary and is limited to the brain, pituitary and brown adipose tissue in rodents.

D3 (type III 5'-deiodinase catalyses tyrosyl (inner) ring deiodination of T_4 and T_3 and is found in most tissues, with the highest levels found in the placenta.

Table 30: Summary of assessment of deiodinase activity from the Farwell and Himmelstein studies

Study	D1 Activity	D2 Activity	D3 Activity
Present study	Microsomal preparations from liver and kidney	Astrocyte cultures prepared from neonatal brains	-
2-day rat	Homogenised liver and kidney	Homogenised brain	Homogenised brain

All homogenised tissue samples and primary cell cultures were assayed in triplicate for D1 and D2 activity by the iodide release method. HPLC was used to analyse D3 activity.

For the homogenised liver and kidney samples and astrocyte cultures prepared from neonatal rat brains were treated with iodomethane at ranges of 100 nM to 100 mM.

Astrocytes from rats were used as a marker of cell toxicity. Cells were incubated overnight with increasing concentrations of iodomethane (100 nM to 100 mM), and trypan blue was used to determine cell viability *via* both visual inspection and spectrophotometric analysis.

D1 activity:

In vitro
Microsomal preparations of liver and kidney were incubated with increasing concentrations of iodomethane (100 nM to 100 mM) then assayed for D1 activity. There was no effect of iodomethane on D1 activity at concentrations up to 10 mM in either the liver or the kidney. At concentrations of 50 mM and greater there was a loss of D1 of ~50% in both tissues.

Kinetic analysis suggested that the loss of D1 activity at high concentrations was a result of enzyme inactivation rather than inhibition. This was confirmed with incubated 10x kidney microsomes with 10, 50 and 100 mM iodomethane for 15 minutes, followed by dilution of the samples 10 -fold before assaying of for D1 activity.

In vivo
In the 2-day inhalation mechanistic study rats were exposed to iodomethane (*via* inhalation) at exposure levels of 0, 25 and 100 ppm iodomethane.

In unexposed tissue homogenates D1 activity ranged from 95 to 125 pmol iodide released/mg protein/min. In kidney there was a 15 -20% ($p \leq 0.05$) decrease in activity and 10 -15% decrease (not significant) in liver from rats exposed to 25 ppm iodomethane. At 100 ppm a significant ($p \leq 0.05$) decrease of 40% in both kidney and liver D1 activity was observed.

D2 activity:

In vitro
Astrocyte cultures were incubated with increasing concentrations of iodomethane (100 nM to 100 mM) during overnight stimulation with cAMP and hydrocortisone. A decrease in D2 activity at concentrations of conc. >100 μM was observed. At high concentrations (>1 mM) of iodomethane cell death was apparent, with 30-40% loss of cells. At conc. >10 mM widespread cell death was apparent. With further experiments (in the serum free media with cAMP and hydrocortisone) it was confirmed that the decrease in D2 activity was due to enzyme inactivation rather than due to enzyme inhibition >10 mM.

In vivo
Brain homogenates were analysed for D2 activity in rats exposed iodomethane. D2 was present at 15 -20 fmol iodide released/mg protein/h in unexposed brains. In rats exposed at 25 ppm a

significant decrease ($p \leq 0.05$) in activity of ~35% was observed. In rats exposed to 100 ppm a decrease of 50-55% was observed.

D3 activity:

In vivo

No effect of iodomethane on brain D3 activity in exposed rats.

It is clear that there is no direct effect of iodomethane on deiodinase activity at levels that may be reasonably expected during inhalation exposure. In the *in vitro* studies, there was a significant decrease in deiodinase activity only at high concentrations of iodomethane (>10 mM for D1, >1 mM for D2). These levels are equivalent to >1000 ppm for 1 mM and 10000 ppm for 10 mM, some 50 to 100 fold greater than what animals were exposed to during the *in vivo* studies. The decrease in deiodinase activity at high iodomethane concentrations appeared to be a result of non-specific inactivation rather than by reversible inhibition of iodomethane.

The changes observed in thyroid hormone parameters in rats exposed to iodomethane in the 2-day inhalation study show that rats became progressively more hypothyroid the higher the exposure to iodomethane. In fact, there was a ~40% decrease in serum T_4, ~30% decrease in serum T_3 and ~3 fold increase in serum TSH values. Thus it is likely that the drop in serum T_4 concentrations is usually a powerful stimulus to increase brain D2 activity, however in the case of this study D2 activity fell between 35 -50%. D2 was slightly more sensitive to iodomethane inactivation than D1 in the *in vitro* studies; however it still required significantly higher (i.e. mM) concentrations than could be achieved in this *in vivo* study to effect any change in D2 activity.

4.12.1.4 Human information

None.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant to this proposal.

6 OTHER INFORMATION

None.

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ANNEXES

ANNEX I – IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION TO HUMANS.

1 IPCS FRAMEWORK ANALYSIS

The IPCS Framework for Analysing the Relevance of a Cancer Mode of Action to Humans (IPCS, 2007) provides a structured framework for the evaluation and establishment of a cancer MOA in experimental animal studies followed by an assessment of its relevance to human hazard and risk assessment.

The MOA framework analysis comprises the following steps:

Postulated MOA action - theory of the case

Key events - measurable events that are critical to the induction of tumours in the postulated MOA

Concordance of dose-response relationships – dose-effect/response relationship for each of the key events and for the tumour response and their interrelationships, in the context of the Bradford Hill criteria

Temporal association – characterisation of the temporal relationships for each of the key events and for the tumour response

Strength, consistency, and specificity of associations of tumour responses with key events – the weight of evidence linking the key events, precursor lesions, and the tumour response

Biological plausibility and coherence – consistency of MOA with what is known about carcinogenesis in general (biological plausibility) and also in relation to what is known about the substance specifically (coherence)

Other modes of action – evaluation of other possible MOAs

Uncertainties, consistencies and data gaps – evaluation and impact on proposed MOA

Assessment of postulated MOA – clear statement of the outcome of the analysis and level of confidence in the postulated MOA

The assessment of human relevance requires 3 fundamental questions to be addressed:

1 Is the weight of evidence sufficient to establish a mode of action in animals?

2 Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

3 Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

This is followed by an overall statement of confidence, analysis and implications.

1.1 Postulated MOA for the induction of thyroid follicular cell tumours in rats and mice

The postulated primary MOA for iodomethane induced thyroid follicular tumours in rodents involves the perturbation of homeostasis of the pituitary-thyroid axis caused by excess circulating

iodide derived from the metabolism of iodomethane. Excess iodide acts centrally on the thyroid by inhibiting production and release of T_4. Reduced circulating levels of T_4 and T_3 cause a compensatory increase in circulating TSH from the pituitary resulting in sustained stimulation of the thyroid to produce thyroid hormone. This leads to enlargement of the thyroid and proliferation of thyroid follicular cells (hyperplasia). Thyroid hyperplasia can eventually progress to neoplasia.

The postulated MOA for iodomethane has been summarised in the scientific literature (Mileson *et al*., 2009).

1.2 Key events in experimental animals

The sequence of key events in the proposed oncogenic MOA of iodomethane includes;

- Excess circulating iodide
- Decreased serum T_4 and T_3
- Increased serum TSH
- Thyroid enlargement with thyroid follicular cell hyperplasia

The evidence for each of these key events is summarised below. Mechanistic investigations were focused on the rat as this was the most sensitive species. There was only a marginal increase in thyroid tumours in the male mouse.

1.2.1 Excess circulating iodide

The effects of exposure to iodomethane on serum iodide levels in the rat were determined in a 2-day inhalation study (Section 4.12.1.3). There was a marked dose dependent and exposure time related increase in serum iodide levels among rats exposed to 25 or 100 ppm iodomethane (Table 29). During the 18-hour recovery periods serum iodide concentrations decreased dramatically and the levels after the second recovery period were lower than those after the first recovery period.

Although iodide is essential for normal thyroid function iodide deficiency and iodide excess both inhibit thyroid function (Ganong, 2003). The primary action of high doses of iodide is inhibition of thyroid hormone synthesis (the Wolff-Chaikoff effect) and release (Ganong, 2003; Greenspan and Dong, 2004). Excess iodide in the thyroid transiently blocks thyroid peroxidase and thereby inhibits oxidation of iodide and binding of iodine to thyroglobulin, and ultimately blocks synthesis of thyroid hormone. Elevated iodide also inhibits thyroid hormone release (T_3 and T_4) possibly through the proteolysis of thyroglobulin. Excess iodide may also reduce the effect of TSH stimulation by reducing the cAMP response to TSH receptor binding.

1.2.2 Decreased serum T_4 and T_3

Changes in serum T_4 and T_3 levels measured at intervals during the 2-year inhalation study in rats were summarised in Section 4.10.1.2 (Table 23). Serum levels of both hormones 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, suggests that compensatory mechanisms were operating after prolonged exposure to iodomethane.

Additional thyroid hormone data were generated in the 2-day inhalation study in male rats referred to in Section 4.12.1.3. Dose related decreases in both serum T_4 and T_3 were observed after 18 hours following the second exposure, the change being statistically significant at 100 ppm compared with the control (Table 27). There was no treatment related effect on serum reverse T_3 (rT_3), the inactive form of T_3.

The results from these studies demonstrate that circulating levels of both T_3 and T_4 are reduced in the rat following short and longer term inhalation exposure to iodomethane.

1.2.3 Increased serum TSH

Serum levels of the pituitary hormone TSH were markedly increased at week 26 in male rats and to a lesser extent in female rats at 60 ppm in the 2-year inhalation study (Table 23). Serum TSH levels remained elevated at weeks 52 and 104 but the magnitude of the difference from control was lower. Dose related significant increases in TSH were also observed in the 2-day inhalation study in male rats at 25 and 100 ppm (Table 27).

These results demonstrate that circulating levels of TSH are increased significantly after short term and longer term inhalation exposure to iodomethane. The increased TSH is considered to be a compensatory response to lower circulating thyroid hormone levels causing a perturbation of homeostasis of the pituitary-thyroid axis.

1.2.4 Thyroid enlargement with thyroid follicular cell hyperplasia

Thyroid growth was evident in male rats at 60 ppm in the chronic study characterised by significantly increased thyroid weights after 52 weeks of exposure and increases in follicular cell hyperplasia in 8/20 and 5/17 males after 52 and 104 weeks of exposure, respectively. The mean absolute thyroid weight of the 60 ppm group was 83% higher than that of the control group. These findings correlated with enlarged thyroids noted macroscopically. There was a very low incidence of thyroid follicular hyperplasia in females at 60 ppm after 52 weeks of exposure and there were no significant increases in thyroid weight.

The changes in thyroid weight and morphology demonstrate that chronic exposure to iodomethane has a specific effect on the rat thyroid, increasing thyroid size primarily through stimulation of cellular hyperplasia.

1.2.5 Concordance of dose response relationships

A summary of the no-observed-adverse-effect-levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) for the key effects in the proposed oncogenic MOA of iodomethane is provided in Table 31. The data are confined to male rats since males were more sensitive than females to the effects of iodomethane on perturbation of thyroid hormone homeostasis and no significant increase in thyroid follicular cell tumours was observed in females.

Table 31: Summary of key effects and NOAELs / LOAELs in male rats

Effect	Study (inhalation)	NOAEL ppm	LOAEL ppm
Iodide			
Increase in serum iodide	2-day	-	25
Hormones			
Decrease in serum T_3 and T_4	2-day	-	25
	2-year	20	60
Increase in serum TSH	2-day	-	25
	2-year	5	20
Thyroid			
Increase in thyroid weight	2-year	20	60
Increase in thyroid hyperplasia		20	60
Increase in thyroid tumours		20	60

The data show that there is a good dose correlation for the key effects in the proposed MOA and the occurrence of thyroid tumours. The increase in circulating TSH was the most sensitive indicator of altered thyroid hormone homeostasis.

1.3 Temporal relationships

In the 2-year rat study the first thyroid and pituitary hormone measurements were made in week 26. The data showed a clear pattern of decreased circulating T_3 and T_4 levels and increased TSH levels at 60 ppm, particularly in males. However, by weeks 52 and 104 there were no clear dose related differences in circulating thyroid hormone levels at 60 ppm compared with controls. Serum TSH remained elevated above control (predominantly in males), although to a smaller degree compared with week 26. At the interim sacrifice after 52 weeks of exposure there was clear evidence of thyroid growth (thyroid weight increase and follicular cell hyperplasia) in males at 60 ppm and an early onset of thyroid follicular tumours (3/20 males compared with none in controls). The proportion of males with tumours at 60 ppm was significantly higher in animals killed at termination after 104 weeks (7/17 males affected), demonstrating the progression from hyperplasia to neoplasia.

The results of the 2-day study in male rats showed that circulating iodide levels were markedly increased after the first and second exposures to iodomethane at 25 and 100 ppm. Altered serum thyroid and pituitary hormone levels consistent with the pattern of effects noted in the 2-year study in week 26 were also observed at both concentrations. Thus, there is a rapid onset and probable continued perturbation of thyroid hormone homeostasis for at least 26 weeks of exposure.

The data show that there is a logical temporal response for the key events in the proposed MOA in which the key events characterising altered thyroid function precede thyroid tumour formation.

1.4 Strength, consistency and specificity of associations of tumour response with key events

Strength, consistency and specificity of associations can be established from the studies described above and from consideration of other relevant studies in the database on iodomethane. It has been shown that the initial key event, markedly increased circulating iodide levels is associated with concomitant alterations in circulating thyroid and pituitary hormone levels. It is probable that there is a sustained perturbation of thyroid hormone homeostasis for at least 26 weeks based on hormone data from the 2-year rat study. The results of the 28-day inhalation range-finding study in

the rat showed increased thyroid weights, thyroid follicular hypertrophy and hyperplasia, and hypertrophy of the pituitary gland in rats exposed to 100 ppm iodomethane for 6 hours per day, 5 days/week for 4 weeks. Thyroid weights were also slightly increased in males at 75 ppm. The pituitary gland hypertrophy was located in the *pars distalis*, the site of TSH synthesis, and was therefore indicative of increased TSH synthesis and perturbation of the pituitary-thyroid axis. Histopathological examination was not conducted on animals exposed to 25 or 75 ppm iodomethane, thus it is not possible to ascertain whether thyroid and pituitary gland morphology was affected at these exposure levels. However, it is clear that exposure to a high concentration of iodomethane for 4 weeks results in thyroid growth consistent with that observed after 52 weeks in males at 60 ppm in the 2-year study. The study of up to 13 weeks duration revealed no evidence of thyroid weight or histopathological changes at the highest exposure level of 70 ppm. These results suggest that induction of thyroid changes is both dose and exposure duration related.

The thyroid hormone data at weeks 52 and 104 in the 2-year study suggest that compensatory homeostatic mechanisms were activated and the sustained elevation of serum TSH indicated continuing perturbation of thyroid function. The presence of thyroid growth and tumours after 52 weeks of exposure is consistent with stimulation of the thyroid resulting from the sustained effects of excess iodide (from daily iodomethane exposure) on altered pituitary-thyroid function.

In the 90-day dietary dose range finding study in the mouse, increased thyroid weights and histopathological changes characterised by accumulations of follicular colloid with corresponding attenuation of follicular lining cells, were observed in all dose groups. The histopathological changes were broadly consistent with those observed in the dietary carcinogenicity study at ≥60 ppm although in this study there was no evidence of follicular cell hyperplasia. However, the tumour response in the mouse was marginal compared with the clear effect in the male rat.

Reversibility of effects of iodomethane exposure was demonstrated in the 2-day inhalation study where circulating levels of iodide were shown to decrease rapidly after each exposure. Iodide is the putative metabolite of iodomethane responsible for alterations in thyroid-pituitary function and the effects of iodide are known to be reversible after cessation of treatment in humans (ATSDR, 2004; Greenspan and Dong, 2004). Although there are no iodomethane specific studies on the reversibility of thyroid changes in the rat, the proposed non genotoxic MOA involving perturbation of the thyroid-pituitary axis has been reported for a wide range of pesticides for which there is considerable evidence of reversibility following cessation of exposure (Hurley *et al*., 1998).

1.5 Biological plausibility and coherence

There are many studies in rodents demonstrating the relationship between sustained perturbation of the hypothalamic-pituitary-thyroid axis, prolonged stimulation of the thyroid gland by TSH, and the progression of thyroid follicular cells to hyperplasia and eventually neoplasia (Hurley *et al*., 1998; Capen *et al*., 1999; IARC, 2001). Increased secretion of TSH may result via several mechanisms, including the inhibition of production and release of thyroid hormones as postulated for iodomethane via iodide. Iodide treatment in humans is associated with decreases in thyroid hormones and increases in circulating TSH (ATSDR, 2004).

The increased incidence of thyroid tumours in male rats compared with the equivocal or marginal increases in female rats and mice is typical of rodent thyroid oncogens (Hurley *et al*., 1998). Adult male rats have higher serum TSH levels than females and are often more sensitive to stimulation of the thyroid gland and oncogenesis (Hill *et al*., 1998). This is consistent with the 2-year study on iodomethane in which the basal levels of serum TSH and the magnitude of the increases in TSH were both higher in male rats than in females.

Evidence of toxicity attributed to excess iodide was noted in the 1-year oral toxicity study in dogs (Section 4.7.1.1). Increased serum TSH and histopathological changes in the pituitary and thyroid glands were observed in 2 dogs receiving the highest dose of 12 mg/kg/day. The thyroid changes were characterised by colloid depletion and moderate follicular cell hypertrophy. Mild hyperplasia of basophilic cells within the *pars distalis* of the pituitary gland was probably associated with the increases in serum TSH.

Carcinogenicity studies with other iodinated compounds have been reported. In a 2-year study in which rats were given potassium iodide (KI) in drinking water there was evidence of thyroid dilatation characterised by increased colloid and flattened epithelia at all dose levels (up to an average intake of 53 – 67 mg KI/kg/day), however, there was no evidence of an increased incidence of thyroid tumours (Takegawa *et al*., 2000). Pituitary and thyroid hormone measurements were not undertaken, however, short term studies have shown that KI in drinking water significantly increased the thyroid weight, thyroid follicular height and colloid accumulation, and serum TSH and serum T_4 in male rats (Kanno *et al*., 1994).

In another study iodinated glycerol was administered by oral gavage to rats for 2 years (NTP, 1990). There was an increased incidence of thyroid follicular cell carcinomas in male rats. No pituitary or thyroid hormone measurements were undertaken and consequently the mode of action of iodinated glycerol is not known.

Among other mono-methyl halides, methyl bromide (MeBr) is considered not to be carcinogenic and it does not target the thyroid (MeBr DAR, 2006). An equivocal increase in thyroid follicular adenocarcinomas (male) in one of two chronic inhalation studies with MeBr in the rat was unlikely to be of biological significance (supplemental study: non GLP). Methyl chloride (MeCl) does not affect the thyroid. It is reported to increase the incidence of renal tumours in male mice but there is no other evidence of carcinogenicity (IPCS, 2000 and IARC 1999). Overall, iodomethane appears to be unique among the mono-methyl halides for its effects on the thyroid, which supports the proposal that altered thyroid function is attributed to the effects of excess iodide rather than a direct effect of iodomethane.

1.6 Other modes of action

The possibility of a genotoxic MOA is considered. A comprehensive range of *in vitro* and *in vivo* guideline compliant genotoxicity studies has been conducted on iodomethane (Section 4.9)

In conclusion iodomethane is a well studied alkylating agent belonging to a class that does not efficiently induce genetic damage or are not potent genotoxic carcinogens (Section 4.9.3). This may be because the damage it induces is efficiently repaired by a battery of DNA repair systems. The lack of clastogenicity in the *in vivo* mouse bone marrow micronucleus test, accompanied by a reduction in PCE ratio thus providing evidence of bone marrow exposure, is a key finding regarding potential to induce genotoxic damage *in vivo*. Thus, overall iodomethane is unlikely to be genotoxic in the whole animal and as such a genotoxic MOA is unlikely to be a plausible MOA for the target organ specificity of thyroid follicular cell tumours in the long term rodent bioassays. There are many precedents for the proposed non-genotoxic MOA and the profile of the toxic and histological effects seen do not fit with a genotoxic MOA as a contributor. If iodomethane acted as a genotoxic carcinogen the incidence of tumours at the site of first contact would be expected to have increased. Both the rat and mouse carcinogenicity studies failed to increase tumour incidence at the site of first contact, for the rat olfactory tissue / lung following exposure *via* inhalation (as is the case for inhaled formaldehyde) and the mouse digestive tract associated organs following exposure *via* the diet. Although toxic 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. Furthermore , iodomethane is known to deplete glutathione rich tissues and has a propensity to methylate thiol groups in globin. The carcinogenicity studies failed to show increased tumour incidence in glutathione rich tissues (blood, liver or kidney – along with the olfactory epithelium) or in the haematopoietic system. Only the mouse bone marrow micronucleus study showed partial evidence of haematotoxicity (reduction in %PCE population) following dosing over an acute period (2 days). This observation was not carried forward in the sub-chronic or chronic studies conducted on a variety of species.

Other non genotoxic modes of action which perturb thyroid hormone homeostasis and are known to produce thyroid follicular cell tumours were also investigated as summarised below.

The potential induction of UDP-glucuronyltransferase (UDPGT) in the liver of male rats exposed to iodomethane was investigated in the 2-day inhalation study. UDPGT is involved in the normal metabolic conjugation of T_4. There was no evidence of hepatic UDPGT induction in male rats after two 6-hour exposures to 25 or 100 ppm (Table 28).

These results indicated that there was no enhanced metabolism and clearance of T_4 which would account for the observed decrease of serum T_4 and increased serum TSH. Serum rT_3 levels were also measured and as shown in Table 8 there were no treatment related effects after exposure to iodomethane. Serum rT_3 can be increased in response to inhibition of 5'-deiodinase activity because T_4 is preferentially converted to rT_3 rather than to T_3 (Capen, 1997). Potential effects of iodomethane on 5'-deiodinase enzyme activities in brain, liver and kidney samples collected from rats in the 2-day inhalation study were reported separately (Section 4.12.1.3). In general there were dose related reductions in 5'-deiodinase enzyme activities which were attributed to the hypothyroid state, however, as noted above there was no corresponding increase in serum rT_3. *In vitro* experiments in the same study suggested that 5'-deiodinase enzyme activities would not be inhibited at the relevant *in vivo* concentrations of iodomethane. Overall, these results indicate that T4 metabolism is not the primary site of iodomethane action on the pituitary-thyroid axis following acute exposure.

1.7 Uncertainties, inconsistencies and data gaps

Whilst the 2-day inhalation study did not specifically investigate the potential effects of iodomethane exposure on the inhibition of thyroid hormone synthesis and release from thyroglobulin, this is the primary action of high doses of iodide as discussed in Annex I, Section 1.2.1.The weight of evidence would suggest that this is the main mode of action of iodomethane although as discussed below excess circulating iodide also perturbs deiodinase enzyme activities.

There was no increase in serum rT_3 in the 2-day inhalation study but it was increased in high dose animals at 52 and 104 weeks in the chronic rat study (Table 23), suggesting perturbation of the 5-deiodinase enzymes involved in thyroid hormone metabolism, induced either by the hypothyroid state and/or by a direct effect of iodomethane (see discussion above). Prolonged inhibition of type I (D1) and type II (D2) deiodinase enzymes would result in decreased T_3 and T_4 levels, a decreased ability of the pituitary to monitor the circulating thyroid hormone levels, and therefore elevated TSH output. Serum levels of rT_3 would also be elevated which is consistent with the findings in the 2-year rat study. This could be the result of feedback inhibition of iodide, rather than a specific inhibition of iodomethane on deiodinase activities as indicated in the study in Section 4.12.1.3. Prolonged effects of excess iodide on peripheral metabolism of thyroid hormones via perturbations of 5'-deiodinase enzyme activities would contribute to the effects of excess iodide acting centrally on the thyroid. Iodinated compounds such as erythrosine and amiodarone are known to inhibit 5'-deiodinase activity, and in the case of erythrosine, produce

thyroid follicular tumours in rats via increased TSH (Capen, 1997; Martino *et al*., 2001). Therefore, possible effects of iodomethane exposure on 5'-deiodinase activity are probably attributed to excess iodide.

1.8 Assessment of postulated MOA

A non genotoxic MOA for the induction of thyroid follicular tumours in the rat following long term inhalation exposure to iodomethane has been identified with a high degree of confidence. The key events for the primary MOA are consistent with perturbation of thyroid hormone homeostatis. Excess iodide from metabolism of iodomethane acts directly on the thyroid to reduce T_4 and T_3 levels with a compensatory increase in circulating TSH. This in turn leads to thyroid stimulation and growth, and eventually tumours. Possible perturbation of peripheral metabolism of thyroid hormones via inhibition of 5'-deiodinase activity by excess iodide after prolonged iodomethane exposure may contribute to the primary centrally acting effects of excess iodide.

1.9 Human relevance of the proposed MOA

The human relevance of the MOA identified for the thyroid tumours in the rat is assessed as follows:

Is the weight of evidence sufficient to establish a mode of action in animals?

The application of the IPCS framework to the postulated MOA provides sufficient support to conclude that there is a causal relationship between the proposed key events in the rat and the formation of thyroid tumours in male rats.

Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

Thyroid cancer in humans is rare and the only known human thyroid carcinogen is ionizing radiation (Capen, 1999; IARC, 2001). The evidence for other factors including iodine deficiency and iodine excess is less consistent. Some epidemiological studies suggest that increased iodine intake may be a risk factor for thyroid cancer in certain populations, particularly those with endemic iodine deficiency, but equally there are data to show that it is not a risk factor (ATSDR, 2004). Although both iodine deficiency and iodine excess may cause an increase in circulating TSH there are few epidemiological data available on the relationship between thyroid cancer (follicular carcinomas) and TSH concentrations (IARC, 2001). Data in humans suggest that prolonged TSH stimulation of the thyroid is unlikely to induce malignant neoplasias. Follicular adenomas are relatively common in humans with congenital defects in thyroid hormone synthesis and consequently elevated levels of TSH. Follicular carcinomas appear to arise by progression from follicular adenomas in humans and a role for prolonged elevation of TSH is possible but the effect is considered to be small (Capen, 1999). Most thyroid disorders including cancer are several times more prevalent in women than in men suggesting a possible role of female hormones (IARC, 2001). Thus, overall humans appear to have a low susceptibility to thyroid cancer and the risks arising from disturbances to thyroid hormone homeostasis are low.

Fundamental mechanisms in regulation of hypothalamic-pituitary-thyroid axis in rodents are qualitatively similar to those in humans and a decrease in T_3 and T_4 levels will increase TSH levels in humans (Hill *et al*., 1998; Meek, 2003). In general the goitrogenic effects of chemicals do not operate via a species specific mechanism (Capen, 1999). Thus, there are no fundamental qualitative differences in key events between rodents and humans and the MOA cannot be

reasonably excluded on this basis. However, there appear to be important quantitative species differences and these are discussed below.

Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

There are several species differences in thyroid physiology and biochemistry between rodents and humans which have been summarised in various reviews (e.g. Capen, 1999; Hill *et al*., 1998). Factors considered important in the apparent differences in interspecies sensitivity to altered thyroid function and stimulation are taken from these reviews and presented below.

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 T_4 and to a lesser extent T_3 in humans. As a result T_4 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 T_4 and T_3 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 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.

Overall, the weight of evidence suggests that rodents are quantitatively much more sensitive than humans to thyroid-pituitary disruption and hormonal imbalances leading to thyroid growth and tumour formation. Therefore, thyroid tumours induced in male rats as a result of altered thyroid hormone homeostasis following long term exposure to iodomethane are considered not relevant to humans based on quantitative dynamic differences.

Conclusion: statement of confidence, analysis and implications

There is sufficient robust data to establish that the primary MOA for iodomethane induced thyroid follicular tumours in male rats involves the perturbation of homeostasis of the pituitary-thyroid axis caused by excess circulating iodide derived from the metabolism of iodomethane. Excess iodide acts centrally on the thyroid by inhibiting production and release of T_4. Reduced circulating levels of T_4 and T_3 cause a compensatory increase in circulating TSH from the pituitary resulting in sustained stimulation of the thyroid to produce thyroid hormone. This leads to enlargement of the thyroid and proliferation of thyroid follicular cells (hyperplasia). Thyroid hyperplasia can eventually progress to neoplasia; in this case a significantly increased incidence of benign adenomas. Prolonged inhibition of type I and type II deiodinase activities by excess iodide from chronic iodomethane exposure would also lead to reductions in T_3 and T_4 and compensatory sustained increases in TSH, and may contribute to the primary centrally acting effects of excess iodide.

It has been demonstrated from the open published literature that humans are far less sensitive than rats to perturbations of thyroid hormone homeostasis and consequent effects on the thyroid. Humans would be less sensitive to the effects of excess iodide on thyroid function and any prolonged increase in TSH is unlikely to lead to thyroid tumour development. Thyroid tumours in male rats were only induced at a dose level which resulted in general toxicity and which exceeded a MTD. Thus it is extremely unlikely that exposure of humans to iodomethane would lead to

sustained elevation of TSH since exposures would have to exceed tolerable levels of iodomethane exposure. In conclusion, the exposure necessary for an effect in humans would not be achievable through any plausible scenario.

ANNEX II - ABBREVIATIONS

CD	Caesarean Derived
CHO	Chinese Hamster Ovary
CPA	Cyclophosphamide
D1	type I 5'deiodinase
D2	type II 5'-deiodinase
D3	type III 5'-deiodinase
DNA	Deoxyribose Nucleic Acid
GLP	Good Laboratory Practice
HCD	Historical Control Data
hprt	Hypoxanthine guanine Phosphoribosyl Transferase
HSE	Health Safety Executive
Ip	Intraperitoneal
MOA	Mode of Action
MI	Mitotic Index
MLA	Mouse Lymphoma Assay
MN PCE	Micronucleated Polychromatic Erythrocytes
MTD	Maximum Tolerated Dose
NOAEC	No Adverse Effect Concentration
NOAEL	No Adverse Effect Level
NOEL	No Observable Effect Level
NTP	National Toxicology Programme
PCE	Polychromatic Erythrocyte
ppm	parts per million
PWG	Pathology Working Group
RCE	Relative Cloning Efficiency
rT_3	Reverse Triiodothyronine
-S9	Absence of rat liver enzyme homogenate
+S9	Presence of rat liver enzyme homogenate obtained following centrifugation at 9000*g*
sd	Standard Deviation
SD	Sprague Dawley
STOT SE	Specific Target Organ Toxicity Single Exposure
T_3	Triiodothyronine
T_4	Thyroxine
TF	Task Force
Tk	Thymidine Kinase
TSH	Thyroid-Stimulating Hormone
UDPGT	UDP-glucuronyltransferase