Committee for Risk Assessment

RAC

Annex 1

Background document

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

Methylmercuric chloride

EC Number: 204-064-2
CAS Number: 115-09-3

CLH-O-0000001412-86-146/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 March 2017

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: METHYLMERCURIC CHLORIDE

EC Number: **204-064-2**

CAS Number: **115-09-3**

Index Number: **-**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Methylmercuric chloride**
EC number:	**204-064-2**
CAS number:	115-09-3
Annex VI Index number:	No specific entry but covered by the generic entry for organic compounds of mercury (index 080-004-00-7)
Degree of purity:	No data available in the technical dossier
Impurities:	No data available in the technical dossier

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation (generic entry for organic compounds of mercury)	Acute Tox. 2 * - H330 Acute Tox. 1 – H310 Acute Tox. 2 * - H300 STOT RE 2 * - H373 with SCL of 0.1% Aquatic Acute 1 – H400 Aquatic Chronic 1 H410 Note A Note 1 SCL: STOT RE 2 * - H373 ≥0.1%
Current proposal for consideration by RAC	Acute Tox. 2 – H330 Acute Tox. 1 – H310 Acute Tox. 2 – H300 STOT RE1 – H372 (nervous system, vision and kidneys) Carc. 2 – H351 Muta. 2 – H341 Repr. 1A – H360Df

	Lact. Effects – H362
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 2 – H330 Acute Tox. 1 – H310 Acute Tox. 2 – H300 STOT RE1 – H372 (nervous system, vision and kidneys) Carc. 2 – H351 Muta. 2 – H341 Repr. 1A – H360Df Lact. Effects – H362 Aquatic Acute 1 – H400 Aquatic Chronic 1 H410 Note 1

***minimum classification**

1.3 Proposed harmonised classification and labelling based on CLP Regulation

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 [1)]	Reason for no classification
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	Acute Tox. 2 – H300	Not applicable	Acute Tox. 2* – H300	
	Acute toxicity - dermal	Acute Tox. 1 – H310	Not applicable	Acute Tox. 1 – H310	
	Acute toxicity - inhalation	Acute Tox. 2 – H330	Not applicable	Acute Tox. 2* – H330	
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	None		None	Not evaluated
3.5.	Germ cell mutagenicity	Muta. 2 – H341	Not applicable	None	
3.6.	Carcinogenicity	Carc. 2 – H351	None	None	
3.7.	Reproductive toxicity	Repr. 1A – H360Df	None	None	

		Lact. Effects – H362			
3.8.	Specific target organ toxicity –single exposure	None		None	Not evaluated
3.9.	Specific target organ toxicity – repeated exposure	STOT RE1 – H372	None	STOT RE2* – H372 (SCL: 0.1%)	
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 – H400 Aquatic Chronic 1 H410		Aquatic Acute 1 – H400 Aquatic Chronic 1 H410	
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

[1)]. Existing classification from the generic entry for organic mercury
*minimum classification

Labelling: Signal word: *"danger"*

Pictogram: GHS06; GHS08; GHS09

Hazard statements: H300; H310; H330; H341; H351; H360Df; H362; H372; H410

Precautionary statements: not harmonised

Proposed notes assigned to the entry: Note 1.

Note 1 is included in the generic entry for organic mercury and is considered relevant for the specific entry for methylmercury compounds.

Note 1:

The concentration stated or, in the absence of such concentrations, the generic concentrations of this Regulation (Table 3.1) or the generic concentrations of Directive 1999/45/EC (Table 3.2), are the percentages by weight of the metallic element calculated with reference to the total weight of the mixture.

Note A is also included in the generic entry for organic mercury and is not considered relevant for the specific entry.

Note A:

Without prejudice to Article 17(2), the name of the substance must appear on the label in the form of one of the designations given in Part 3.

In Part 3, use is sometimes made of a general description such as '... compounds' or '... salts'. In this case, the supplier is required to state on the label the correct name, due account being taken of section 1.1.1.4.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Methylmercuric chloride has no specific harmonized classification but is covered by the generic entry for organic compounds of mercury (index 080-004-00-7). A classification proposal for human health was submitted at the TC C&L. It was discussed in November 2005 and concluded in October 2006 (see summary records of the meetings in Annex I).

Historically, methylmercuric chloride and methylmercury were proposed for classification within the same dossier. Indeed, studies on organic mercury compounds were carried out with these two substances. While submitting this hand-over dossier, it was first decided to split the dossier into two (one per substance) and then to withdraw the proposal for MeHg as this substance only exist naturally within our environment. Studies performed with MeHg are provided as supporting data.

Several new studies have been published on different endpoints since the TC C&L discussions and have been integrated in the present dossier. The following endpoints are concerned: acute toxicity, repeated toxicity, mutagenicity, carcinogenicity and toxicity for the reproduction.

The results of these new studies are consistent with the classification agreed by the TC C&L and proposed in the present dossier.

It is noted that no registration dossier is currently available for methylmercuric chloride.

2.2 Short summary of the scientific justification for the CLH proposal

A study (Yasutake, 1991) of **acute oral toxicity** on mouse showed a LD_{50} inferior to 20 mg/kg bw with decreased renal functions, so a classification Acute Tox 2, H300 was proposed. It was also proposed to maintain the existing classifications Acute Tox 1 – H310 and Acute Tox 2 - H330 based on data on the absorption of organic mercury compounds by dermal route and inhalation.

Several studies of **repeated toxicity** were performed on animals and humans, showing that the central nervous system was the mean target organ by oral route. So a classification STOT RE1 – H372 was proposed.

In vitro data show that methylmercuric chloride has a **genotoxic** potential. Numerical chromosome aberrations are also observed *in vivo*, so a classification on **mutagenicity** (Muta.2, H341) was proposed.

Three studies consistently report renal tumours in male mice at doses as low as 0.859 mg/kg bw/day. In humans, a study performed on the population of Minamata showed a positive association between MeHg exposure and leukemia. So a classification on **carcinogenicity** (Carc.2, H351) was proposed.

Based on animals' studies, **development** is severely impacted in several species (rats, mice, monkeys...). In humans, effects of methylmercury are described on neurodevelopment: very severe effects appear in children exposed *in utero* during periods of poisoning via food (via bread in Iraq, via fish in Japan). So a classification in reprotoxicity (Repr. 1A – H360Df) was proposed.

The intake of methylmercury by mothers could be toxic for the infants if they are strongly exposed via maternal milk, so a classification of the **lactation effects** is therefore required taking into account the possible poisoning of human populations. So a classification Lact. Effects – H362 was proposed.

2.3 Current harmonised classification and labelling

Both compounds are covered by the generic entry for organic compounds of mercury (index 080-004-00-7):	**CLP Regulation**
Generic entry for organic compounds of mercury	Acute Tox. 2 * - H330 Acute Tox. 1 – H310 Acute Tox. 2 * - H300 STOT RE 2 * - H373 with SCL of 0.1% Aquatic Acute 1 – H400 Aquatic Chronic 1 H410 Note A Note 1 SCL: STOT RE 2 * - H373 ≥0.1%

*Minimum classification

2.4 Current self-classification and labelling

No registration dossier is currently available on methylmercuric chloride. However, the classification notifications are presented in the confidential appendix I (separate file).

RAC general comment

Background to the proposal

Existing harmonised classification

Methylmercuric chloride (MeHgCl) is covered by the generic entry for organic compounds of mercury (index 080-004-00-7) in Annex VI of the CLP Regulation. This was based on data from both methylmercury and methylmercuric chloride. The harmonised entry is as follows:

- Acute Tox. 1; H310: Fatal in contact with skin;
- Acute Tox. 2*; H330: Fatal if inhaled;
- Acute Tox. 2*; H300: Fatal if swallowed;
- STOT RE 2**; H373: May cause damage to organs through prolonged or repeated exposure (SCL ≥ 0.1%);
- Aquatic Acute 1; H400: Very toxic to aquatic life;

- Aquatic Chronic 1; H410:Very toxic to aquatic life with long lasting effects.

* Minimal classification extrapolated by default from Annex I of the Dangerous Substances Directive.
** Extrapolation from labelling phrase R33 "Danger of Cumulative Risks".

The current entry also includes Note 1 and Note A.

Note 1 relates to concentration limits and is also considered applicable for the proposed entry. Note 1: The concentration stated or, in the absence of such concentrations, the generic concentrations of this regulation (Table 3.1) or the generic concentrations of directive 1999/45/EC (Table 3.2), are the percentages by weight of the metallic element calculated with reference to the total weight of the mixture.

Note A is relevant types of entry in Annex VI. Note A: Without prejudice to Article 17(2), the name of the substance must appear on the label in the form of one of the designations given in Part 3. In Part 3, use is sometimes made of a general description such as "…compounds" or "…salts". In this case, the supplier is required to state on the label the correct name, due account being taken of section 1.1.1.4.

First proposal to create a new harmonised classification

A new classification proposal for the monomethylmercury compounds methylmercuric chloride and methylmercury was previously submitted (by France) in accordance with the Dangerous Substances Directive to the Technical Committee on Classification and Labelling (TC C&L). Agreement was reached in October 2006:

- Carc. Cat. 3; R40
- Muta. Cat. 3; R68
- Repr. Cat. 1; R61
- Repr. Cat. 3; R62
- T+ ; R48/25 – R64
- N; R50-53

It was not stated whether Notes 1 and A would also be applied.

Given that this agreement was reached too late for inclusion in the final adaptation to technical progress of Annex I of the Dangerous Substances Directive, the dossier was handed over to ECHA by the European Chemicals Bureau.

New proposal for harmonised classification

France submitted a new classification proposal, applying specifically to methylmercuric chloride, in March 2016. This addressed the following classification hazard classes: acute toxicity, STOT SE, STOT RE, germ cell mutagenicity, carcinogenicity and reproductive toxicity.

Environmental hazards were not assessed in the CLH report. The DS proposed that the existing generic entry for the environmental hazards of mercury compounds in Annex VI should apply directly to methylmercuric chloride.

In line with the CLP Regulation, the DS further proposed retention of Note 1, and removal of Note A.

Application of data from other organic mercury compounds

Historically, methylmercuric chloride and methylmercury were proposed for classification within the same dossier. Studies into the toxicity of organic mercury compounds were carried out with these two substances. Whilst preparing the CLH report, the DS decided to split the original dossier into two (one per substance) and then to not submit a proposal for methylmercury as this substance only exists naturally within the environment. It is not supplied commercially. Studies performed with methylmercury were included in the proposal for classification of methylmercuric chloride as supporting data.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Methylmercuric chloride has CMR properties, i.e. Carc. 2 – H351, Muta. 2 – H341, Repr. 1A – H360Df, Lact. Effects – H362 that justify a harmonised classification and labelling.

French CA decided to focus the CLH report of hand-over substances on human health effects only and to propose their harmonization consistently with what was discussed at TC C&L. Therefore, the environment effects are not considered for their harmonization in this CLH report. However, we consider that the classification for the environment endpoints coming from the generic entry of the mercury compounds should apply.

It is noted that in the self-classification notified by manufacturers and importers, the classifications for these endpoints are the same of those specified in the generic entry for organic compounds of mercury, but differ with the proposed harmonized classification.

The notifications are presented in the confidential appendix I (separate file).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

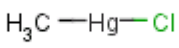
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity: Methylmercuric chloride

EC number:	204-064-2
EC name:	chloromethylmercury
CAS number (EC inventory):	115-09-3
CAS number:	115-09-3
CAS name:	-
IUPAC name:	chloro(methyl)mercury
CLP Annex VI Index number:	-
Molecular formula:	CH_3-Cl-Hg
Molecular weight range:	251.08

Structural formula:



1.2 Composition of the substance

No data

1.2.1 Composition of test material

See information given in the description of the studies.

1.3 Physico-chemical properties

Table 5: Summary of physico - chemical properties

Property	**Value** **Methylmercuric chloride**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Crystals, white with disagreeable odour	ATSDR, 1999	
Melting/freezing point	170°C	ATSDR, 1999	
Boiling point	Volatilizes at 100°C	ATSDR, 1999	
Relative density	4.06 g/mL at 25°C	ATSDR, 1999	
Vapour pressure	1.12 Pa at 25°C (0.0085 mm Hg)	ATSDR, 1999	
Surface tension	No data		
Water solubility	High solubility in water DMSO: >100 mg/L at 27°C Acetone: >100 mg/L at 27°C	ATSDR, 1999	
Partition coefficient n-octanol/water	: log Kow = 0.4	ATSDR, 1999	Calculated
Flash point	No data		
Flammability	Probably not flammable	ATSDR, 1999	
Explosive properties	No data		
Self-ignition temperature	No data		
Oxidising properties	No data		
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No data		
Viscosity	No data		

2 MANUFACTURE AND USES

2.1 Manufacture

No data.

2.2 Identified uses

Laboratory chemical.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics of mercury compounds

Justification of the read-across between methylmercuric chloride and mercury compounds:

Study shows that MeHgCl presents a different bioavailability than MeHg as it is more hydrophobic than MeHg that systematically bind to thiols (Harris et al.; 2003). MeHgCl displays therefore a toxicity, which is more important compared to MeHg (Glover CN; 2009; Berntssen et al., 2004; Oyama et al., 2000). Studies performed with MeHg are presented as supportive data as MeHgCl is the most toxic form of organic mercury compounds.

4.1.1 Mercury in water and food

First, the mercury cycle in the environment is explained in order to better understand the formation of methylated forms in the environment and then the exposition to the human.

The change in mercury speciation from inorganic to methylated forms is the first step in the aquatic bioaccumulation process. Methylation can occur non-enzymatically or through microbial action. Once methylmercury is released, it enters the food chain by rapid diffusion and tight binding to proteins. It attains its highest levels, through food-chain biomagnification, in the tissues of fish predatory species (EHC 1991). Moreover, the greatest source of human exposure to methylmercury (MeHg) is the diet, in particular the consumption of seafood (Glover CN; 2009). Then, the cation MeHg2+ can be bound by ionic liaisons with mineral ligands like chloride to form MeHgCl (Picot; Proust; 1998).

Monoalkyl mercury compounds (e.g., methylmercuric chloride) are relatively soluble in water; however, the solubility of methylmercury is decreased with increasing dissolved organic carbon content, indicating that it is bound by organic matter in water (Miskimmin 1991). Dialkyl mercury compounds (e.g., dimethylmercury) are relatively insoluble in water (Callahan et al. 1979; EPA 1984b) (ATSDR 1999).Metabolism

Distribution (ATSDR 1999)

Organic mercury compounds distribute throughout the body following oral exposure and have the highest accumulation in the kidneys. As with metallic mercury, the ability of methyl- and phenyl mercury compounds to cross the blood-brain and placental barriers allows distribution, and subsequent accumulation, in the brain and foetus.

4.1.2 Mercury in breast milk

4.1.2.1 Non-human information

Organic mercury is excreted in breast milk, less efficiently than inorganic mercury. However, Sundberg et al. (1998) conducted a study which was designed to provide additional information on the speciation of mercury in breast milk and the differences between methylmercury and inorganic mercury migration into milk.
The values for the methylmercury kinetic parameters were significantly higher in lactating than nonlactating mice: plasma clearance (93.5 and 47.1 mL/hour/kg, respectively) and volume of distribution (18,500 and 9,400 mL/kg, respectively). The milk-to-plasma concentration ratios for total mercury after methylmercury administration were lower than those seen with inorganic mercury, and varied between 0.1 and 0.7 with a mean of 0.20.

Methylmercury is also excreted in the breast milk of rats, humans, and guinea pigs (Sundberg and Oskarsson 1992; Yoshida et al. 1992).

4.1.2.2 Human information

Concentrations of mercury have also been measured in breast milk from several populations. Breast milk concentrations have been reported for two U.S. populations; one in rural Iowa (Pitkin et al. 1976) and the other from Alaska (Galster 1976). Pitkin et al. (1976) reported a total mean mercury concentration in breast milk of 0.9 ± 0.23 ng/g (range, 0.8–1.6 ng/g). The mean total mercury concentrations in the Alaskan populations were 3.3 ± 0.5 ng/ml for the urban population, 3.2 ± 0.8 ng/ml for the interior population, and 7.6 ± 2.7 ng/ml for the coastal population that consumed fish and marine mammals. Total mercury concentrations in breast milk from other countries and exposure scenarios were 3.6 ± 2.2 ng/g for an urban population in Tokyo, Japan (Fujita and Takabatake 1977), 0.6 ± 0.4 ng/g for Swedish women that were fish consumers with 12 dental amalgams (Oskarsson et al. 1996), 0.2–6.3 ng/g (range) for Swedish women that consumed fish (Skerfving 1988), and 9.5 ± 5.5 ppb for an urban population of women in Madrid, Spain (Baluja et al. 1982).
Some of the highest levels were reported in fish eaters, and about 20% of the total mercury content of the milk was methylmercury. The median and maximum mercury concentrations in breast milk from women in the Faroe Islands, a population that consumes large quantities of fish and marine mammal tissue, were 2.45 and 8.7 ng/ml, respectively (Grandjean *et al.* 1995). Breast milk mercury concentrations reported by these authors were significantly associated with mercury concentrations in cord blood and with the frequency of pilot whale dinners during pregnancy.
These are relatively low values in contrast to the values reported in Minamata, Japan, for women who ate contaminated seafood in the Minamata episode, which resulted in total mercury concentrations in breast milk of 63 ppb (Fujita and Takabatake 1977), and in Iraq, where consumption of homemade bread prepared from methylmercury-contaminated wheat occurred, resulted in breast milk concentrations of up to 200 ng/g (about 60%) methylmercury (Amin-Zaki et al. 1976; Bakir et al. 1973) (ATSDR 1999). These two episodes were poisoning contexts.
Amin-Zaki *et al* (1979, 1981), studied children that were born just before the poisoning of farmers (see Chapter 3.2.). They were breast-fed via their mother’s milk, so they had a relatively large postnatal intake of methylmercury from maternal milk. The effect observed during five years was considered evidence of damage for the central nervous system. The

concentration in breast-milk was up to 200 ng/g (the value is about 100 fold larger than the concentrations in women from several various populations).

So, methylmercury could migrate in breast milk of females exposed to a large quantity of methylmercury (poisoning), and large intake of methylmercury by mothers could be toxic for the infants if they are breast-fed.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Studies presented in the TC C&L dossier:

Species	LD50 (mg/kg)	Observations and Remarks	Ref.
Mouse (C57BL/6N Jcl)	LD_{50} < 20 mg MeHgCl/kg for the males LD_{50} > 50 mg MeHgCl/kg for the females	Substance tested: Methylmercuric chloride. Study on the nephrotoxicity of the MeHgCl (and the sex-related difference of the renal effects). The animals were exposed at a single administration of 5, 10, 20, 30, 40, 50 mg/kg of Methyl Mercury Chloride. For 7 days following the administration, survival rates of the mice (6 for each dosing group) were examined: 20 mg MeHgCl/kg (= 16 mg Hg/kg): 4/6 males mice died and 0/6 females mice died 50 mg MeHgCl/kg (= 40 mg Hg/kg): 6/6 males mice died and 2/6 females mice died. Decreased renal function (decreased phenolsulfonphthalein excretion), increased plasma creatinine, and swelling of tubular epithelial cells, with exfoliation of the cells into the tubular lumen. The renal function was disturbed as early as 24 h after MeHgCl treatment with dose levels of 20 mg/kg and 50 mg/kg for males and females respectively.	Yasutake 1991

Male Sprague-Dawley Rats	LD_{50} = 25 mg Hg/kg bw (= 31.3 mg MeHgCl/kg) in adult rats LD_{50} = 40 mg Hg/kg bw (= 50 mg MeHgCl/kg) in young rats	Substance tested: Methylmercuric chloride. Methods: Analytical grade MeHgCl was dissolved in corn oil (Mazola) at suitable concentrations and each animal was dosed with 1ml/100g of its body weight. The LD_{50} was determined by the least square method. Rats were observed for 10 days to determine the LD50, but the groups of 200g, 350g and 450g rats were observed for a further period of 20 days to assess the onset of neurological signs by suspending the animals by their tails, when either of the hind limbs began to show flexion. Acute toxic stages were denoted by crossing of hind limbs and flailing movement during suspension. 6 groups according to the body weights: 200g, 300g, 350g, 400g, 450g, and 500g. Each group was divided into 4 sub-groups of 20 rats. Each sub-group orally received a single dose of a solution of MeHgCl. Sub-groups 1 and 2 were dosed with 25, 30, 35 and 40mg Hg/kg; Sub-groups 3, 4 and 5 were dosed with 20, 25, 30 and 35 mg Hg/kg; Sub-group 6 were dosed with 15, 20, 25 and 30 mg Hg/kg. Results: This study shows the inversely proportional relationship between the LD_{50} of MeHgCl and the ages of the rats. As the age increases, the LD_{50} decreases, the younger rats could tolerate higher doses of MeHgCl than the older ones. The onset of neurological symptoms after receiving 25mg Hg/kg of MeHgCl occurred between 8 to 15 days post dosing in the surviving rats.	Lin *et al*, 1975

New study added in the present dossier:

6 adult males cats	6.4 mg/kg dissolved in milk Of Methyl mercury chloride	Six adult males cats received a single dose of methyl mercury chloride (MeHgCl) (6.4 mg/kg) dissolved in milk. After 30 days, we observed changes of the axon terminal morphologies in a large extent and alterations of different morphometric features of fragments in different proportions. The synthetic enzyme of nitric oxide of white matter cells of the cat striate cortex, after MeHg intoxication, presents higher decrease of its histochemical activity in the distal branches of the dendritic trees of type I NADPH-diaphorase neurons. These results suggest that cellular changes start to become first evident in the distal portions of axon terminals and dendrites in the **cat visual cortex**. Dramatical morphological changes in both types I and II axon fragments, 30 days after MeHg intoxication, were observed. The results may suggest a general impairment of synaptic transmission in the intrinsic circuits of the visual cortex affecting both GABA-ergic and glutamatergic connections near the border of areas 17 and 18. The visual functions were not tested after MeHgCl intoxication.	Oliveira et al., 2008

4.2.1.2 Acute toxicity: inhalation

Human data:

Following acute inhalation exposure of dust containing **methylmercury**, four men had initial symptoms including numbness and tingling of limbs, unsteadiness in gait, difficulty in performing fine movements (e.g., buttoning a shirt), irritability, and constricted vision (Hunter et al. 1940). At least 2 years after these occupational exposures, the subjects had not fully recovered from their symptoms.

Animal data:

No data.

4.2.1.3 Acute toxicity: dermal

No data.

4.2.1.4 Acute toxicity: other routes

Studies presented in the TC C&L dossier:

Species	Substance tested	LD50 (mg/kg)	Observations and Remarks	Ref.

Male Sprague Dawley Rats	Methylmercuric chloride dissolved in sterile isotonic saline administered intraperitoneally in 1 or 2 ml of saline (one single injection) Controls: like amount of isotonic saline	intraperitoneal injection : 9.5 mg MeHgCl /kg bw	15 min after intraperitoneal injection of doses > 3 mg/kg bw: the animals became lethargic, with drooping heads and dulled eyes; some died after developing dyspnea, spasticity, and loss of the ability to walk. Animals given < 3 mg/kg bw became drowsy but survived 50% of mortality after 24 h	Hoskins *et al*, 1978
		intraperitoneal injection : 8.1 mg MeHgCl /kg bw	50% of mortality after 30 days.	
Male Syrian Hamsters		intraperitoneal injection : 20 mg MeHgCl /kg bw	50% of mortality after 24 h	
		intraperitoneal injection : 12 mg MeHgCl /kg bw	50% of mortality after 30 days	
Adults males and females Squirrel monkeys		intraperitoneal injection : 9.5 mg MeHgCl /kg bw	50% of mortality after > 14 h	
		intraperitoneal injection : 3.8-5.1 mg MeHgCl /kg bw	50% of mortality after 30 days	

4.2.2 Summary and discussion of acute toxicity

Animal data on MeHgCl are available for acute toxicity by oral route and human data on MeHg dust by inhalation. However, no studies were located regarding death in humans or animals after dermal exposure to methylmercury (ATSDR, 1999) and regarding death in animals after inhalation.

The critical value for classification by oral route is the LD_{50} inferior to 20 mg MeHgCl/kg for the male mice.

Modification of axon morphology but no mortality was observed in cats at 6.4 mg/kg 30 days after a single oral administration of MeHgCl but the visual functions were not tested (Oliveira 2008).

Case studies in humans report deaths by inhalation of alkylmercury compounds but with prolonged exposure of several months or years. These data are therefore not relevant to classify MeHgCl for acute toxicity by inhalation.

4.2.3 Comparison with criteria

The acute oral LD_{50} value for MeHgCl is less than 50 mg/kg bw and a classification "**Acute Tox. 2, H300**" is proposed according to CLP criteria.

4.2.4 Conclusions on classification and labelling

Animal data support the existing classification **Acute Tox. 2 - H300** according to the CLP by oral route.

No acute toxicity relevant information is available on methylmercury compounds by dermal and inhalation routes. However, information on acute toxicity and absorption by the different routes of organic mercury compounds in general can assist in the assessment of acute toxicity of methylmercury compounds by the different routes of exposure.

No information was identified on absorption of methylmercury via **dermal route**. It was however reported that dermal absorption of phenylmercuric acetate from the vaginal tract was 75% of the dose within 8 hours after administration in rats. In humans, a case history indicates nearly complete absorption of dimethylmercury through the skin: a 48-year old woman died 9 months after she inadvertently spilled several drops (estimated at 0.4-0.5ml) of dimethylmercury on her disposal latex gloves. Gloves were shown to be penetrated completely by dimethylmercury in 15 seconds or less. Dimethylmercury is also able to volatilise and inhalation exposure might also have occurred.

No animal studies on absorption of organic mercury **by inhalation** are available. Indirect evidence shows that organic mercury is absorbed readily through the lung. Indeed, case studies of occupational exposure to unspecified alkyl mercury compounds have reported deaths in humans following inhalation. Most subjects died after developing profound neurotoxicity. Exposure to diethylmercury vapour (estimated exposure level: 1-1.1 mg/m^3) for 4-5 months resulted in the death of two women. A 41-year-old man with to 3-4 years of exposure to alkyl mercury compounds used in seed dressing died within approximately 3 months after cleaning up a spill of liquid containing alkyl mercury.

By the **oral route**, no quantitative data are available in humans with methylmercury or methylmercuric chloride but 95% of an oral dose of methylmercuric nitrate was absorbed. In animals, absorption was nearly complete within 6 hours after female Cynomolgus monkeys were given 0.5 mg/kg as methylmercuric chloride by gavage.

Overall, no direct quantitative data are available on absorption of methylmercury or methylmercuric chloride via inhalation or dermal route. However, data on other organic mercury compounds provides evidence of a massive percutaneous absorption of organic mercury compounds. On inhalation, data on organic mercury compounds are not quantitative but indicate that some organic mercury compounds are significantly absorbed in the lung.

It should also be noted that the current classification of these substances from the general entry "Organic compounds of mercury" is **Acute Tox. 2* – H330** (*minimal classification) by **inhalation** and **Acute Tox. 1 – H310** by **dermal route**.

The data above provide evidence for significant exposure to methylmercury by inhalation or dimethylmercury by skin contact. As detailed previously, MeHgCl is the most toxic form of organic mercury compounds and it is therefore considered that the existing general classification for acute toxicity for these routes should not be removed.

So, based on this consideration, the existing classification **Acute Tox 1; H310** and **Acute Tox 2; H330** according to the CLP regulation criteria is supported.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral

The critical value for classification by the oral route is the LD_{50} (LD_{50} < 20 mg/kg methylmercuric chloride) in male mice. Since this value is less than 50 mg/kg bw, the DS proposed a classification Acute Tox. 2; H300 (Fatal if swallowed).

Dermal and inhalation

No data are available on the toxicity of methylmercuric chloride by either the dermal or inhalation route.

The DS concluded that data from studies with other organic mercury compounds provide evidence of massive percutaneous absorption following dermal exposure.

Regarding inhalation exposure, the DS included information on 4 men who were occupationally exposed to dust containing methylmercury. Initial symptoms included numbness and tingling of limbs, unsteadiness in gait, difficulty in performing fine movements, irritability and constricted vision. The men had not fully recovered from their symptoms 2 years post-exposure.

The DS concluded that further data on organic mercury compounds indicate that at least some organic mercury compounds are significantly absorbed in the lung. Case reports of deaths among workers occupationally exposed by inhalation to alkyl mercury compounds support this view.

According to the DS [although comparative data were not presented], methylmercuric chloride is the most toxic form of organic mercury compound and the evidence suggested significant exposure to methylmercury by inhalation or to dimethylmercury by skin contact, the DS supported retaining the classifications of Acute Tox. 1; H310 (Fatal in contact with skin) and Acute Tox. 2; H330 (Fatal if inhaled).

Comments received during public consultation

Three MSCA supported the proposal.

Assessment and comparison with the classification criteria

Oral

Three acute oral toxicity studies in three species (rats, mice and cats) were available.

In mice, the LD_{50} value for methylmercuric chloride was < 20 mg/kg in males and > 50 mg/kg in females. In rats, the LD_{50} values found were 31.3 mg/kg and 50 mg/kg in adult rats and young rats, respectively.

Male cats were exposed to a single dose (6.4 mg/kg) of methylmercuric chloride dissolved in milk. No deaths were reported in the CLH proposal.

The most sensitive species was the mouse, with an LD_{50} value of < 20 mg/kg in males. Since no deaths were reported at 10 mg/kg in the CLH proposal, it can be presumed that $10 < LD_{50} < 20$ mg MeHgCl/kg.

Methylmercuric chloride therefore meets the criteria ($5 < ATE \leq 50$ mg/kg bw) for classification as Acute Tox. 2; H300 (Fatal if swallowed).

Dermal

No data on acute toxicity following dermal exposure are available for methylmercuric chloride.

RAC agrees with the DS that the available toxicokinetic data indicate that some absorption occurs following dermal exposure to methylmercury. A human case study also provides evidence that dimethylmercury can be absorbed following dermal exposure. The case study describes a woman who died 9 months after spilling 0.4-0.5 mL of dimethylmercury on her disposal latex gloves.

However, the original basis for classification in Category 1 for acute dermal toxicity is unclear to RAC. There appears to be no reason to assume that methylmercuric chloride is more toxic via the dermal route than the oral route. While the oral absorption of methylmercury is almost 100%, the dermal absorption of this compound is considered to be similar to that of inorganic mercury salts, i.e. around 5% (EPA Mercury Study Report to Congress Vol. V). On the basis of these absorption values and the oral LD_{50} value (between 10 and 20 mg MeHgCl/kg), an expected dermal LD_{50} value can be calculated. This calculation estimates a dermal LD_{50} value of approximately 200 mg/kg bw. A dose of 200 mg/kg bw lies on the borderline between Category 2 and Category 3. Therefore, in contrast to the DS, RAC considers that the most appropriate classification for acute dermal toxicity is Category 2; H310 (Fatal in contact with skin).

Inhalation

No animal data were available. The DS cited a study from over 70 years ago in which it was claimed 4 workers had symptoms of toxicity following exposure by inhalation to a dust containing methylmercury. The symptoms included numbness, tingling of limbs, unsteadiness in gait, difficulty in performing specific movements, irritability and constricted vision. These symptoms had not resolved after 2 years. Unfortunately, from the data provided, it is not possible to confirm the nature of the exposure incurred by these workers; for example, there is a possibility that uptake may also have occurred via the skin and other substances present in the dust may have contributed to the toxicity observed.

Additionally, the DS commented briefly on several additional case studies of occupational exposure to alkylmercury compounds. Very few details were provided but, most significantly, the DS indicated that most subjects "died after developing profound neurotoxicity". In one report, 2 women died following exposure to diethylmercury vapour (estimated exposure level 1-1.1 mg/m^3). Overall, the weight of evidence at least strongly suggests the potential for toxicity of organic mercury compounds (including methylmercuric chloride) following inhalation exposure.

The generic entry for organic compounds of mercury (index 080-004-00-7) in Annex VI to CLP currently includes Acute Tox. 2*; H330 (Fatal if inhaled). Reports of deaths following occupational exposure to unspecified alkylmercury compounds appear to support the potential for acute toxicity via this exposure route. On this basis, and in the absence of evidence to suggest that the current group entry is not appropriate, RAC agrees that the existing classification should be retained: **Acute Tox. 2; H330 (Fatal if inhaled)**.

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

Human data by inhalation reported in section 4.2.1.2 provide evidence of neurotoxicity after single acute exposure. However, it is not known whether co-exposure to other substances may exit for these cases. The dose of exposure is also not known.

Besides, a classification for acute toxicity by inhalation is proposed in relationship with lethal effects and a classification as STOT SE would be redundant for consideration of acute toxicity on methylmercury compounds.

No classification for STOT SE is proposed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Given that the DS proposed classification for acute lethal toxicity following inhalation exposure, they considered that STOT SE would be redundant and therefore proposed no classification for this endpoint.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Following acute inhalation exposure of dust containing methylmercury, 4 men had initial symptoms including numbness, tingling of limbs, unsteady gait, difficulty in performing specific movements (e.g. buttoning a shirt), irritability and constricted vision. After 2 years, the subjects had not recovered fully.

It is not known whether co-exposure to other toxic substances also occurred on this occasion and there were no details about amount of methylmercury involved. Given these limitations, these cases provide limited evidence of neurotoxicity after single exposure to non-lethal concentrations of methylmercury.

These limited findings are insufficient to support classification of methylmercuric chloride with STOT SE. **No classification is proposed**.

4.4 Irritation

Not evaluated.

4.5 Corrosivity

Not evaluated.

4.6 Sensitisation

Not evaluated.

4.7 Repeated dose toxicity

4.7.1 *Non-human information*

4.7.1.1 Repeated dose toxicity: oral

Studies presented in the TC C&L dossier:

Species	Dose (mg/kg/body weight)	Duration of treatment	Observations and Remarks	Ref.
Male Rat (Wistar)	0.5 mg MeHgCl/kg bw every day by gavage (group 1) 1.5 mg MeHgCl/kg bw every 3 days by gavage (group 2) Substance tested:	3-4 wk	This study (not in compliance with a guideline) focuses on the effects of acute and chronic methyl mercury chloride treatment on rat **blood pressure**. Increased **systolic blood pressure (SBP)** in male rats (group 1). The effect began 60 days after initiation of exposure and	Wakita 1987

	methyl mercury chloride		reached levels higher than those of the control by 10-25 mm Hg, 84 days after the cessation of treatment. In the group 2, significant increases of SBP were found to occur after cessation of the treatment and to persist for at least 9 months.	
Female Mouse (BALB/c)	0 and 0.625 mg MeHg/kg bw (diet) Substance tested: methyl mercury	12 wk	The study was performed to determine whether a MeHg-enriched diet during 12 weeks affects **immune cell activities** or cellular counts in the thymus, spleen and blood. (This study is not in compliance with a guideline). 22% decrease in **thymus** weight and 50% decrease of **cell number** compared to controls. The **natural killer** cell activity was reduced by 44 and 75% in the spleen and blood, respectively. However, the **lymphoproliferative** response in the spleen increased at this dose of mercury.	Ilbäck 1991
Rat	0.2 mg MeHgCl/kg bw or 2 ppm MeHgCl (diet) Substance tested: methyl mercury chloride	12 wk	This study focuses on the **nephropathy** induced by long-term exposure to small amounts of MeHgCl. This is not in compliance with a regulatory guideline. **Renal effects**: ultrastructural changes (cytoplasmic masses containing ribosomes and bundles of smooth endoplasmic reticulum) in kidney proximal tubule cells of female rats, despite the normal appearance of the glomeruli at light microscope.	Fowler 1972
Mouse (ICR)	0; 0.05 (approx); 0.2 (approx); 0.906 mg MeHgCl/kg bw 0, 0.4, 2, 10 ppm of methylmercuric chloride (diet)	26 wk	This study (not in compliance with regulatory guideline) was performed to evaluate **renal carcinogenic** potentiality at low level. **Renal effect at 10 ppm (0.906 MeHgCl mg/kg/day)**: degeneration of the proximal tubules characterised by nuclear swelling and vacuolation of the cytoplasm.	Hirano 1986
Mouse (ICR)	0; 0.05 (approx); 0.2 (approx); 0.906 (mean daily intake) mg MeHgCl/kg bw 0, 0.4, 2, 10 ppm	104 wk	A second study was performed by Hirano *et al.*, during a longer period (not in compliance with regulatory guidelines). At 2 ppm: Epithelial cell degeneration and	Hirano 1986

	(diet)		interstitial fibrosis in **kidney.** Renal epithelial tumors, mostly adenocarcinomas, were found, in 13 of 59 male at 10 ppm. No renal tumors were induced in other groups including females.	
Mouse (B6C3F1)	0.4, 2 and 10 ppm MeHgCl (in feeding) 0.0382; 0.174; 0.859 mg/kg/d for males 0.0332; 0.166; 0.752 mg/kg/d for females (diet)	104 wk	This study was conducted in B6C3F1 mice with the same dosages used in previous study (Hirano *et al.*, 1986) in ICR mice, to compare the results of both studies. (In compliance with regulatory guidelines of carcinogenicity TG 451 of OECD). At 10 ppm of MeHgCl: increased mortality in males but not in females. Renal epithelial tumors were observed in 16 of 60 males: 13 were diagnosed as carcinomas and 5 as adenomas (as quoted in the publication; the discrepancy was not explained). One female exhibited an adenoma at 10 ppm but there was not any carcinoma in females at any doses level. At 2 ppm: one male exhibited an adenoma but there was not any carcinoma at this dose level. At 0.4 ppm of MeHgCl: no renal tumors were observed neither in males neither in females. Epithelial cell degeneration and interstitial **fibrosis** in **kidney**, with on-going regeneration of the tubules present were observed in 59 of 60 males and 56 of 60 females at 10 ppm but the renal damage was more prominent in males than in females. Similar nephropathy was also observed in males of the 2 ppm group. The morphological features of the renal epithelial tumors observed in male M6C3F1 mice of the 10-ppm group in the present study were similar to those induced in male ICR mice treated with 10 ppm MeHgCl (Hirano *et al.*, 1986). General toxicology: neurological signs from posterior paresis to paralysis were first observed at week 59 in males (33 of	Mitsumori 1990

			60) and at week 80 in females (3 of 60) of the 10 ppm group. A marked increase in mortality was observed for males of the 10 ppm group after 60 weeks. The final survival rate of males in this group was 17%, in contrast to 48% in the male's controls.	
Rat	0, 0.01, 0.05 and 0.25 mg/kg bw (approx) 0, 0.1, 0.5 and 2.5 ppm MeHgCl (diet) Substance tested: methylmercuric chloride	104 wk	A long term toxicity study was performed in rats to evaluate histology of several organs (kidneys...). (Not in compliance with regulatory guidelines). No treatment-related histopathological lesions of the heart, lung, stomach or jejunum. No change in haematological or hepatic parameters. Increased **kidney** weights and decreased **enzymes** (alkaline phosphatase, ATPase, NADH- and NADPH-oxidoreductase, and AMPase) in the proximal convoluted tubules. No histological lesions.	Verschuuren 1976

Neurotoxicity of methylmercury was also studied extensively. As described in the WHO food additives series (2000) the role of the granular layer of the cerebellum and the posterior root fibres as a target of methylmercury was identified in rats 60 years ago (Hunter et al., 1940). This study also described the clinical course of severe poisoning as weight loss, ataxia, paralysis, and death in rats given 2.4 mg Hg/kg/d **for 29 days**. Axoplasmic and myelin degeneration of posterior root fibres was produced by **daily doses of 0.8 mg/kg bw** as methylmercuric chloride (Chang & Hartmann, 1972), while the ventricular root fibres and the dorsal root nerves remained intact after administration of **1.6 mg/kg bw per day** in rats exposed up to **11 weeks** (Yip & Chang, 1981).

New studies added in the present dossier:

Sprague-Dawley Rats	5 or 500 µg/kg of MeHgCl	8 weeks prior to matting	This study (not in compliance with a guideline) focuses on the effects of methyl mercury chloride treatment on rat **immune system.** The **Lymphocyte Proliferative Response** of splenocytes to both mitogens (PWM and Con A) was enhanced in the **both** MeHgCl-exposed groups at 6 weeks of age. At 12 weeks of age, the LPR of splenocytes to PWM remained enhanced in the both groups. The response to Con A and PWM was increased ($p < 0.05$). **Natural Killer** (NK) cell activity was markedly depressed (56%) at 12 weeks of age in **both** groups	Wild et al., 1997

			compared to the controls ($P < 0.05$).	
Male Wistar Rats 2 groups of 7 rats each one: one control and one exposed group	0 and 100 µg/kg/day (drinking water by gavage) Low level of methyl mercury chloride	100 days Sub-chronic exposure	This study (not in compliance with a guideline) focuses on the effects of methyl mercury chloride treatment on rat **blood pressure**. At the beginning of the experiment and during the following three weeks: the **Systolic Blood Pressure** (SBP) did not change. After 4 weeks of treatment, SBP significantly increased in MeHg-treated rats compared with the control (respectively for the exposed then the control group, for the weeks 6, 10 and 14: 150 mmHg/125 mmHg, 170 mmHg/125 mmHg, 175 mmHg/125 mmHg, $p < 0.01$).	Grotto *et al.*, 2009

4.7.1.2 Repeated dose toxicity: inhalation

No data

4.7.1.3 Repeated dose toxicity: dermal

No data

4.7.1.4 Repeated dose toxicity: other routes

New studies added in the present dossier:

Adult Swiss Albino mice	**Subcutaneous** exposure to methylmercuric chloride MeHgCl (7 mg/kg bw)	N=5 pups for each group; one control group (daily subcutaneous treatment of a 150 mM NaCl solution) and 4 groups: PND 1-5, PND 6-10, PND 11-15, PND 16-20	MeHg treatment caused a significant increase in the **locomotor activity** (**open field task)** in a time period dependent way ($F_{4.34}$=3.33, P=0.021). However, the motor performance of animals in the **rotarod** task was not affected by MeHg exposure ($F_{4.34}$=1.09; P=0.376). MeHg exposure led to a diminished activity of cerebellar antioxidant enzymes (glutathione peroxidase GPx and glutathione reductase GR), ($F_{4.35}$=2.99, P=0.032) during PND 16-20, causing oxidative stress and behavioral alterations and increased the cerebellar lipid peroxidation during PND 11-15 and PND 16-20 (measured by TBARS levels, thiobarbituric acid reactive substances), ($F_{4.34}$=4.03; P=0.009). So, MeHg caused significant neurotoxic effects related to oxidative stress in mouse cerebellum (a vulnerable target for the neurotoxic effects) mainly from the second half of the suckling period (PND 11-20), resulting in behavioural changes (hyperlocomotor activity).	(Stringari *et al.* 2006)

		During five consecutive days in five different post natal periods.		

4.7.2 Human information:

New studies added in the present dossier:

52 Chinese children with ADHD (Attention Deficit Hyperactitiv y Disorder) and 59 normal controls	High fish consumptio n in this population over the previous six months before the beginning of the study.	The relationship between fish consumption and mercury toxicity is still controversial, as the toxicity of methylmercury, which is bound to cysteine in fish, seems to be far lower than Me-Hg-Cl or Me-Hg-I usually used in experiments. The mean ages of cases and controls were 7.06 years and 7.81 years respectively. Children with ADHD (attention deficit hyperactivity disorder) had a significantly higher mean blood level than control. The geometric mean blood mercury levels were 18.2 nmol/L (95% CI 15.4 – 21.5 nmol/L) in the ADHD group and 11.6 nmol/L (95% CI 9.9 – 13.7 nmol/L) in the control group, with a difference of 6.6 nmol/L ($p < 0.001$). 35 children (67.3%) with ADHD were classified to the combined subtype while 13 (25%) and 4 (7.7%) patients belonged to the predominantly inattentive and predominantly hyperactive-impulsive subtypes respectively. The geometric means of blood mercury levels of children with the inattentive (19.4 nmol/L, 95% CI 13.3 – 28.5 nmol/L) and the combined (18.0 nmol/L, 95% CI 14.9 – 21.8 nmol/L) subtypes were also significantly different from those of controls ($p = 0.03$ and $p = 0.01$, respectively). However, although the geometric mean blood mercury level of children with the hyperactive-impulsive subtype of ADHD (16.1 nmol/L, 95% CI 7.3 – 35.5 nmol/L) was higher than that of controls, the difference was not statistically significant. After adjusting for age, gender and parental occupational status using the multiple linear regression method, the mean blood mercury level was 75% higher in children with ADHD ($p < 0.001$). Children with ADHD were more likely to have blood mercury levels greater than 29 nmol/L (5.8 µg/L, the threshold of possible adverse effects considered by the US Environmental Protection Agency and the National Academy of Sciences) compared to controls (26.9% vs. 10.2%, $p=0.022$).	(Cheuk and Wong 2006)

22 adult male subjects	22 adult controls	A study was performed on humans, in order to assess early **neurotoxic** effects following **low levels** of organic mercury (methylmercury) absorbed through fish eating. In this purpose, two groups of 22 adult male subjects, habitual consumers of tuna fish, and 22 controls were examined using neurobehavioral tests of vigilance and psychomotor function, hand tremor measurements and serum prolactin assessment. Mercury in urine (U-Hg) was significantly higher among exposed subjects (median 6.5 µg/g of creatinine, range 1.8-21.5) than controls (median 1.5 µg/g of creatinine, range 0.5-5.3). The organic component of mercury in blood (O-Hg) was 41.5 µg/l among the tuna fish eaters and 2.6 µg/l in the control group. Both U-Hg and O-Hg were significantly correlated with the quantity of fish consumed per week. Significant differences in serum prolactin (sPRL) were found between exposed (12.6 ng/ml) and controls (9.1 ng/ml). The neurobehavioral performance of subjects who consumed tuna fish regularly was significantly worse on color word reaction time, digit symbol reaction time and finger tapping speed (FT) in comparison with the control group. So, long-term increased MeHg intake can be associated with impairment of psychomotor performance.	Carta *et al.*, 2003
684 men examined	724 men for the control group	A clinical study was performed on 684 men with a first diagnosis of **myocardial infarction** and 724 men for the control group (Guallar *et al*., 2002). The mercury levels in the patients with cardio-vascular risk were 15 percent higher than those in controls. Moreover, analysis with adjustment for age and center, showed an increased **risk of myocardial infarction** at **high mercury** levels.	Guallar *et al*., 2002

New studies added in the present dossier:

Visual system manifestations after methylmercury exposure in human:

In the study of Hunter (1954), four cases of workers inhaling methylmercury vapour discharged from a fungicide plant were reported. Constriction of visual fields occurred in all of the workers, and it was localized in central visual areas in three cases. One patient who was monitored for 15 years until his death, continued to have constricted visual fields, ataxia, and other symptoms. After death, the patient was found to have atrophy of the visual cortex.

Another case involves poisoning due to fungicides using a dimethyl compound of mercury; however, only the central nervous system was affected, with visual manifestations including immediate constriction of vision accompanied with tremor and ataxia (Stein, 1992).

A further incident occurred in the 1940s in which two workers were poisoned by spraying wood with a preservative containing methylmercury. Bilateral concentric narrowing of visual fields occurred in both workers, causing them to develop total blindness, followed by death (Ahlmar A., 1948; Merigan; 1980).

Methylmercury primarily causes neuro-ophtalmological effects as experienced in Minamata disease and in Iraq. Visual effects include the degeneration of peripheral vision (a unique sign in all of these cases) while patients claim that their central vision is unharmed.

Other vision-related effects of methylmercury include the visually evoked response (VER) (Jalili MA, 1961) and contrast sensitivity (NIOSH 94-116, 1994). In usual brightness conditions, VERs were degraded only when visual fields became constricted. However, in scotopic brightness the VERs decreased even when visual fields were normal.

Other neuro-ophtalmological effects of methylmercury involve eye movements. Superficial ptosis, as well as uneven nystagmus occurs infrequently. Methylmercury also causes degenerative effects on the occipital visual cortex and cerebellum.

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

4.8.1 Summary and discussion of repeated dose toxicity findings

Two studies (Wakita 1987; Grotto 2009) show the increase of the **blood systolic pressure** on rats with MeHgCl treatment: the lowest dose of 100 µg/kg/day can increase the pressure more than 150 mm Hg/ 125 mm Hg after 4 weeks of MeHgCl treatment.

Wild and Ilbäck studied the impact of the MeHgCl on the **immune system**. The same results were found: Lympho Proliferative Response enhanced, Natural Killers decreased at the lowest dose of 5µg/kg (Wild 1997).

Several studies show the **renal toxicity** after MeHgCl oral treatment: at the lower dose of 0.2 mg/kg MeHgCl bw (2 ppm), ultrastructural changes were observed in kidney proximal tubule cells of female rats (Fowler 1972); Epithelial cell degeneration and interstitial fibrosis in kidney was observed at 2 ppm too (Mitsumori, 1990).

Methyl mercury mainly targets the **CNS** as evidenced by data on rodents and non-human primates. In humans, neurotoxicity following oral exposure was also characterised further the poisoning in Minamata and Iraq. Cheuk performed clinical studies on children of 7 and 14 years, showing adverse effects in regard to motor speed, attention and language. The study shows that children with ADHD (attention deficit hyperactivity disorder) had a significantly higher mean blood MeHg level than control. In addition, Carta shows that long-term increased MeHg intake can be associated with impairment of psychomotor performance.

No studies were located regarding effects in humans or animals after dermal exposure (ATSDR, 1999).

Via inhalation, neurotoxicity and death were reported further occupational exposure to alkylmercury compounds. Besides, several occupational exposures by inhalation to methylmercury vapor were reported, showing constriction of visual fields, localized in central visual areas. In some cases, consequences were total blindness, followed by death (Ahlmar, 1948; Merigan, 1980). However, duration of exposure was not specified in many case reports and it is not possible to know whether effects are due to chronic or acute (poisoning) exposures and no classification is proposed by inhalation.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Under CLP, the following effects are considered as relevant:

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

In the available studies, the adverse effects induced by methylmercury are:

- (a): substance-related deaths after oral exposure in animals (2.4 mg Hg/kg for 29 days; Chang 1972), (4.3 mg/kg bw for 26 weeks Mitsumori 1981), 10 ppm (0.859 mg/kg in males) for 2 years (Mitsumori 1990).
- (e): histological effects in the kidney including fibrosis by oral route in animals (2 ppm = approx 0.1 mg/kg bw).
- (b): Changes in the central nervous system, which affects the visual cortex, evaluated by clinical signs and brain necropsy (atrophy of the visual cortex) in animals (doses not known).

By oral route, substances shall be classified under CLP in category 1 when they cause significant and/or severe toxic effects of relevance to human health at levels ≤ 10 mg/kg in a 90-day study.

Besides, adverse effects observed in humans trigger classification in **category 1** without consideration of the dose inducing the effects.

Methylmercury is responsible for neurotoxical effects by oral route (causing visual constrictions). The doses at which these effects appear in humans are not exactly known; however, the long term exposure studies and human cases experiences support the conclusion that a classification of the substance is necessary (cf. the paragraph 3.9.2.9.9. of the CLP).

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

A classification STOT RE 1 - H372 is proposed according to CLP.

The central nervous system (and the visual cortex in particular) and kidneys are the target organs and should be identified in the hazard statement. H372 (nervous system, vision and kidneys).

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Oral

The following short summary is copied directly from the CLH report.

"In the available studies, the adverse effects induced by methylmercury:

- Substance-related deaths after oral exposure in animals (2.4mg Hg/kg for 29 days), (4.3mg/kg bw for 26 weeks), 10ppm (0.859 mg/kg in males for 2 years)
- Histological effects in the kidney including fibrosis by oral route in animals (2ppm = approx. 0.1 mg/kg bw)
- Changes in the central nervous system, which affects the visual cortex, evaluated by clinical signs and brain necropsy (atrophy of the visual cortex) in animals (doses not known)"

The DS also drew attention to the repeated dose effects of methylmercury in humans, noting that classification in Category 1 is possible without consideration of the dose inducing the effects when these are observed in exposed humans. The DS summarised: "methylmercury is responsible for neurotoxic effects by the oral route (causing visual constrictions). The doses at which these effects appear in humans are not exactly known; however, the long term exposure studies and human cases experiences support the conclusion that a classification of the substance is necessary."

The DS proposed the classification STOT RE 1; H372, indicating that the central nervous system (CNS), and the visual cortex in particular, and kidneys are the target organs and should be identified in the hazard statement: H372 (nervous system, vision and kidneys).

The existing harmonised classification of STOT RE 2 was extrapolated directly from the previous coding with R33 (Label: Danger of cumulative risks). A specific concentration limit (SCL) of 0.1% was assigned for STOT RE 2. The basis for this is obscure. The DS proposed to remove this SCL, but did not provide a justification. If methylmercuric chloride were to be classified as STOT RE 1, the generic concentration limits for methylmercuric chloride would be ≥ 10% (resulting in classification of a mixture in Category 1) and then ≥ 1% (resulting in classification of a mixture in Category 2).

Dermal

No studies were available regarding effects in humans or animals after dermal exposure.

Inhalation

Via inhalation, neurotoxicity and death were reported following occupational exposure to alkylmercury compounds. Additionally, several occupational exposures via inhalation to methylmercury vapour were reported, showing constriction of visual fields, localised in

central visual areas. In some cases, consequences were total blindness, followed by death. However, duration of exposure was not specified in many case reports and it is not possible to know whether effects are due to chronic or acute (poisoning) exposures and no classification is proposed by inhalation.

Comments received during public consultation

Three MSCA supported the proposal for STOT RE 1.

However, one of the MSCAs considered that the effect on vision is covered by the CNS since the effects arise from the visual cortex in the brain and therefore considered "vision" should not be mentioned as a target organ. The DS agreed.

Assessment and comparison with the classification criteria

It is difficult from the CLH report to relate the tabulated information to the text summary for this hazard class. Notably, it is unclear which findings were from studies with methylmercuric chloride and which from animals dosed with methylmercury.

Unfortunately, relatively few methodological details and only limited summaries of the results were provided for the relevant studies. However, it is clear that the repeated dose toxicity of methylmercuric chloride has been adequately investigated following oral administration to rats and mice to enable the need for classification to be assessed. No studies by the dermal or inhalation route were available. Additional information is available from studies with humans (reports of human poisonings; comparisons of various parameters with blood mercury levels in general populations). Some studies with methylmercury were also provided. The results did not contradict the results of studies on methylmercuric chloride.

Studies in animals

The following table illustrates those findings that occurred in animal studies at sufficiently low dose levels to support classification. Although the individual data points cannot be scrutinised, the consistent nature of the findings provides a clear profile of repeated dose toxicity to the kidneys and CNS. The findings related to blood pressure changes and markers of immune activity are more limited in nature.

Species, dosing	Oral exposure	
	Below guidance value for Category 1 **28 day study:** C ≤ 30 mg/kg bw/d **90 day study:** C ≤ 10 mg/kg bw/d **104 weeks:** C ≤ 1.25 mg/kg bw/d	**Below guidance value for Category 2** **28 day study:** 30 < C ≤ 300 mg/kg bw/d **90 day study:** 10 < C ≤ 100 mg/kg bw/d **104 weeks:** 1.25 < C ≤ 12.5 mg/kg bw/d
Kidney		
Rats 0, 0.01, 0.05, 0.25 mg MeHgCl/kg bw for 104 weeks	↑ Kidney weights and ↓ enzymes (alkaline phosphatase, ATPase, NADH- and NADPH-oxidoreductase and AMPase) in the proximal convoluted tubules.	N/A
Mice 0, 0.05, 0.2 or 0.906 mg MeHgCl/kg bw 26 weeks	Degeneration of the proximal tubules characterised by nuclear swelling and vacuolation of the cytoplasm at 0.906 mg/kg bw/d	N/A
Mice 0, 0.05, 0.2 or 0.906 mg MeHgCl/kg bw 104 weeks	Epithelial cell degeneration and interstitial fibrosis in the kidney at the mid dose. At the top dose, renal epithelial tumours (mainly adenocarcinomas) in 13/59 males.	N/A
Mice 0.04, 0.2 and 0.9 mg/kg/d (males) 0.03, 0.2 and 0.8 mg/kg/d (females) 104 weeks OECD TG 451	Top dose, increased mortality in males only (survival rate was 17% compared to 48% in control males) Tumours (reported in carcinogenicity section) Renal nephropathy in males at the mid dose and in both sexes at the top dose (more prominently in males). At the top dose, epithelial cell degeneration and interstitial cell fibrosis in the kidney, with ongoing regeneration of the tubules in 59/60 males and 56/60 females.	N/A
Central Nervous System		
Rats 0.8 mg MeHgCl/kg bw/d	Axoplasmic and myelin degeneration of posterior root fibres	N/A
Mice 0.04, 0.2 and 0.9 mg/kg/d (males) 0.03, 0.2 and 0.8 mg/kg/d (females) 104 weeks, OECD TG 451	At the top dose, neurological signs from posterior paresis to paralysis in 33/60 males and 3/60 females from weeks 59 and 80, respectively.	N/A
Blood pressure		
Male Wistar rats 0.5 mg MeHgCl/kg bw/d for 3-4 weeks --- Or 1.5 mg/kg bw/d every 3 days	↑ Systolic blood pressure (SBP) began 60 days after initial exposure --- Significantly ↑ SBP when treatment ceased. The effect persisted for at least 9 months.	N/A
Male Wistar rats 0 and 100 µg MeHgCl/kg/day	↑SBP after 4 weeks of exposure	N/A

100 days		
Immune System		
SD rats 5 or 500 µg MeHgCl /kg/day 8 weeks	Lymphocyte proliferative response of splenocytes to mitogens (PWM and Con A) was enhanced at 6 and 12 weeks. At 12 weeks of age, natural killer cell activity was 56% lower in both treated groups than in controls.	N/A

The adverse effects found on the kidney and the CNS of animals given repeated, low, oral doses of methylmercuric chloride occurred at doses below the guidance values for classification with STOT RE 1.

These animal data provide some indication that blood pressure and the immune system may have been affected in rats. However, there is no definitive evidence to conclude that these are specific targets of methylmercuric chloride. The data are considered insufficient to highlight concerns about the cardiovascular and immune systems alongside the classification.

Adverse effects on the CNS were observed in rats and mice. Axoplasmic and myelin degeneration of posterior root fibres was observed at 0.8 mg/kg methylmercuric chloride in rats. However, the DS reported that exposure of rats to 1.6 mg/kg bw/d for 11 weeks in another study did not result in degeneration of the ventricular root fibres and the dorsal root nerves.

Findings in humans

Further evidence to show that repeated exposure to methylmercuric chloride can produce adverse effects on the CNS system is available from studies reporting signs of toxicity in humans.

No data on human exposure specifically to methylmercuric chloride were presented in the CLH report. However, the DS observed that toxicological findings following methylmercury exposure in animals did not contradict the findings of methylmercuric chloride exposure. Data on methylmercury were therefore considered relevant to evaluate the toxicity of methylmercuric chloride in humans. RAC agrees with this assessment.

The following table summarises the data presented by the DS. Although the nature of the exposures, their intensity and duration, are unclear from the information provided the findings support the view that the CNS is a target organ for methylmercuric chloride toxicity.

Reports of 4 workers exposed to MeHg vapour by inhalation following discharge from a fungicide plant	The visual fields were constricted in all workers (localised in central visual areas in 3 of these). One of the men was monitored for 15 years until his death. Constricted visual fields, ataxia and other symptoms persisted. Following his death, examination revealed atrophy of the visual cortex.
Report of poisoning due to fungicides using a dimethyl compound of mercury	The CNS was affected. Immediate constriction of vision, alongside tremor and ataxia, was reported.

Exposure after spraying wood with a preservative containing methylmercury	2 workers were reported to have bilateral concentric narrowing of the visual fields (causing total blindness), followed by death.
Minamata disease and poisoning in Iraq	Degeneration of peripheral vision, but not central vision, has been reported in these cases.
Human study 52 Chinese children with Attention Deficit Hyperactivity Disorder (ADHD) mean age: 7.06 years	Children with ADHD had significantly higher mean blood mercury level (18.2 nmol/L) than controls (mean blood mercury level of 11.6 nmol/L; 59 children; mean age 7.81 years). After adjustments for age, gender and parental occupational status, the mean blood mercury level of children with ADHD was 75% higher than controls. The US Environmental Protection Agency and the National Academy of Sciences consider blood mercury levels of > 29 nmol/L to be the threshold of possible adverse effects. This study found that children with ADHD were more likely to have blood levels exceeding this threshold (26.9%) compared to controls (10.2%).
Human study 22 adult male subjects and 22 adult controls To assess neurotoxic effects following low level of MeHg, absorbed by eating fish	Mercury in urine was significantly higher among exposed subjects (frequent consumers of tuna fish). Levels in urine and the organic component of mercury in blood correlated significantly with the quantity of fish consumed per week. The neurobiological performance of subjects was significantly worse than controls on colour word reaction time, digit symbol reaction time and finger tapping speed.

Although provided with limited details, RAC concludes that the consistency of the human data indicates that methylmercury may affect the CNS, thereby supporting the findings reported in rats and mice. Of particular note are the adverse effects on vision, reported in 4/6 of the studies above. Since atrophy of the visual cortex was observed in one worker exposed to methylmercury vapour, the effects on vision are considered to be a result of damage to the CNS rather than to the eye itself.

Conclusion

The observed adverse effects in the kidney (in rats and mice) and CNS (in humans, rats and mice) justify classification of methylmercuric chloride for STOT RE. The visual effects are considered to be covered by the inclusion of "central nervous system" in the hazard statement. All doses in the animal studies were below the guidance value for Category 1. Therefore, RAC supports the proposal for **STOT RE 1; H372: Causes damage to nervous system and kidneys through prolonged or repeated exposure**.

As the possibility of adverse effects on the kidneys and CNS occurring after dermal and inhalation exposure cannot be discounted, in accordance with the criteria for this hazard class, **no exposure route should be specified**.

As the CLH report did not make a proposal for a specific concentration limit and the reason for the pre-existing limit is obscure, RAC has no basis to comment further. **No specific concentration limit would seem to be appropriate**.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Studies presented in the TC C&L dossier:

Tissue	Dose	Metabolic activation	Observations and Remarks	Ref.
Saccharomyces cerevisiae	Organic mercury	Without	Neither mutagenic nor caused recombination in, but it did produce a slight increase in the frequency of chromosomal nondisjunction	Nakai and Machida 1973
Bacillus subtilis	Methyl mercuric chloride at 0.005 M and methylmercury at 0.05 M	Without	Induction of primary DNA damage in the rec-assay	Kanematsu et al. 1980
Human lymphocytes	Methylmercuric chloride at 0.12, 0.6, 1, 3, 5, 15 and 25 * 10^{-6} M	Without	Significant increase in chromosomal aberration, mainly chromatid breaks at 0.6 * 10^{-6} M Structural and numerical chromosomal aberrations at higher doses (symmetrical and asymmetrical exchanges, more than 10 aberrations per cell). Hyperdiploid cells increased linearly from the lowest dose (but significance was obtained at higher dose). Polyploid cells appeared in all treated culture but without a clear dose-response relationship (significant at only 15*10^{-6} M).	Betti 1992
Human lymphocytes	Methylmercuric chloride at 0, 3, 5, 15 and 25 * 10^{-6} M	Without	Chromosome and chromatid aberrations with dose-effect relationship ($p<0.05$). Weak effect on sister chromatid exchange but not dose-related (at 3 and 5 * 10^{-6} M). Production of aneuploidy (particularly hyperdiploidy) ($p<0.001$, linear and significant	Betti 1993

			increase up to a dose of $15*10^{-6}$ M).	
Human peripheral lymphocytes	Methylmercuric chloride at 10^{-5}, 10^{-6}, and 10^{-7} M	Without	Induction of aberrant metaphases (including gaps) in a dose-dependent manner ($p<0.05$). At the higher concentrations, also induction of a significant number of breaks. Induction of a significant number of SCEs per cell in a dose-dependent manner, which was reduced by treatment with gamma linolenic acid.	Bala 1993
Indian muntjac fibroblasts and human lymphocytes	Methylmercuric chloride at 5, 15, $30*10^{-6}$ M	Without	Clear increase in C-mitotic figures in human lymphocytes up to the final dose of $30*10^{-6}$ M. No C-mitosis was observed in muntjac fibroblasts because of a lethal toxic effect preceding any increase of C-mitosis effect.	Verschaeve 1984b
Blastocysts of early ICR mouse embryos	1 or 2 µM Substance tested: methylmercuric chloride	Without	Substance tested: methylmercuric chloride prepared in absolute ethanol at a concentration of 3.5 mM. Then a series of dilutions in ethanol was made from this solution to obtain desired final concentrations. Method: Sister Chromatid Exchange (SCE) assay: blastocysts were incubated with various concentrations of MeHgCl to find out if the severity of delayed effects (interference with trophoblast outgrowth and ICM growth and differentiation at 0.5 µM) was related to the number of SCEs. Results: At concentrations of 0.25 µM or greater, metaphase plates were too few and it was difficult to make good spreads. In blastocysts treated with 0.125 µM MeHgCl, chromosomes were well spread but the number of SCEs (7.3 ± 0.94, n = 8) was not significantly different ($p>0.05$, Student's t test) from that for control blastocysts (7.9 ± 0.6, n = 43). Conclusion; No increase in the frequency of sister chromatid	Matsumoto and Spindle 1982

			exchanges.	
Human whole blood	Methylmercuric chloride at doses ranging from 8 * 10^{-8} to 2.5 * 10^{-4} M	Without	Dose-dependent increase in sister chromatide exchanges. SCEs per cell at the dose of 2*10-6 M: (mean ± SEM) 12.69 ± 0.60 ($p<0.001$).	Morimoto 1982
Rat glioblastoma cells, Chinese hamster V79 cells, human lung cells, and human nerve cells		Without	Induction of single-strand breaks	Costa 1991
Chinese hamster V-79 cells	0.08–0.4 µg Hg/mL	Without	Weak but dose-related mutagenic responses near the cytotoxic threshold.	Fiskesjo 1979

4.9.1.2 In vivo data

Studies presented in the TC C&L dossier:

Species	Dose (mg/kg body weight)	Duration of treatment	Observations and Remarks	Ref.
Cat (n=3/group)	0.0084, 0.02, or 0.046 mg Hg/kg bw as methylmercury in diet	39 months	No clear evidence of UDS in lymphocytes. Significant increases in nuclear abnormalities in bone marrow cells from the 3 treatment groups but response was not dose-related. Number of cats scored positive on the total number of cats for the cats treated by respectively 8.4; 20 and 46 µg Hg/kg/day: • for multinucleated in myeloid cells: 0/5; 0/8; 0/5; • for nuclear abnormalities in myeloid cells: 4/6*; 7/8*; 3/5*; *Significantly different from control ($P < 0.05$). • for multinucleated in	Miller 1979

			erythroid cells: 2/6; 2/8; 3/5; • for nuclear abnormalities in erythroid cells: 0/6; 0/8; 0/5;	
Syrian hamster (females)	7.4 mg Hg/kg by intraperitoneal injection Methyl mercury chloride	Single IP injection	No chromosomal aberrations produced in metaphase II oocytes. However, the frequency of hyperploid cells in the treated animals was significantly ($p<0.01$) increased compared to the control. A borderline significant increase in hypoploid cells was also seen.	Mailhes 1983
Mouse (BALB/c) (females)	0, 2.5, 5.0 or 7.5 mg/kg of methyl mercury chloride bw by intraperitoneal injection	Single IP injection	Dominant lethal assay: significant increase in especially pre- and early post-implantation foetal loss. No data on maternal toxicity.	Verschaeve 1984a

4.9.2 Human information

Studies presented in the TC C&L dossier:

Tissue	Population	Exposure	Observations and remarks	Ref
Lymphocytes	N=23 people who consumed mercury-contaminated fish.	Not specified	Positive correlation between blood mercury levels and structural or numerical chromosomes aberrations. No data on smoking status. Effects were significant only when lymphocytes cultures were initiated several days after collection and not on the day of collection.	Skerfving 1974
Lymphocytes	N=9 people who consumed methylmercury-contaminated fish.	Not specified	Significant ($p<0.05$) correlation between mercury levels and chromosomes breaks. No data on smoking status. Effects were significant only when lymphocytes cultures were initiated several days after collection and not on the day of collection.	Skerfving 1970
	Humans who ate seal contaminated meal.	Not specified	Increased incidence of sister chromatide exchange. No data on smoking and consumption of other heavy metals.	Wulf 1986

Blood peripheral lymphocytes	51 fishermen who had eaten mercury contaminated seafood	The first year, blood mercury levels ranged from 10,08 ng/g to 252,25 ng/g with a mean of 81,97 ng/g (±49,96 ng/g). In 1991, the average mercury concentration in blood was 97,72 ng/g (SD=58,57 ng/g).	A statistical correlation between micronucleus frequency in peripheral blood lymphocytes and total mercury concentration in blood (p=0.00041), as well as between micronucleus frequency and age (p=0.017). Peripheral venous blood from 51 fishermen was collected during two years of analysis (1990-1991). The blood was used for micronucleus analysis and mercury detection. The first year, the average frequency of micronucleated lymphocytes was 8,7 ‰ with a SD of 2,47. When data were analysed with linear regression analysis, a significant correlation was found between micronucleus frequency and blood mercury levels (p=0,0118, r=0,786). In 1991, there was a significant correlation between micronucleus frequency and blood mercury concentration (p=0,017; r=0,706).	Franchi 1994

New studies added in the present dossier:

Blood peripheral lymphocytes	98 adults whom one third (33.3%) of the men and two of the women had lived in the gold-mining region, exposed to **mercury vapours,** in a region contaminated by **methyl mercury**.	Not specified exactly	The median level of total hair mercury for this population was 13.50 µg/g, ranged from 0.57 µg/g to 153.8 µg/g. The first apparent biological effect with increasing MeHg hair level was the impairment of lymphocyte proliferation measured as mitotic index (MI), the proportion of cells in M-phase of the cell cycle. So, decreased MI reflects inhibition of cell-cycle progression. The mitotic index ranged from 8 to 36 per 1000 cells, with a mean of 25.20 ± 7.8. No significant differences were observed with having lived in the gold mining area. The frequency of polyploides per 1000 lymphocyte cells ranged from 0 to 16. The majority of participants (63.9%) did not present this aberration. The lowest MeHg-level at which polyploidal aberrations (PA) are observed is 7.25 µg/g. At MeHg ≥ 20 µg/g, the prevalence of persons with polyploidal aberrations is 86.7% compared to 18.8% for those with levels comprised between 10 µg/g and 20 µg/g of MeHg. The differences are highly significant (Chi square: 48.9, df=2; $p < 0.001$). Between 1-3 breaks were observed in lymphocytes for 14 persons (14.6%); for 11 there was only one break, for two there were 2 breaks and 1 person presented 3 breaks. The persons with chromatid breaks have significantly higher levels of MeHg as compared to those without (30.46 µg/g ± 10.7 vs 14.5 µg/g ± 11.6; ANOVA F=23.3; $p<0.001$). Among those with MeHg ≥ 20 µg/g, the prevalence of persons with breaks is 37.9%, as compared to 9.4% for those with 10 µg/g ≤ MeHg ≤ 20 µg/g; none of the persons with MeHg levels below 10 µg/g presented breaks. The differences are highly significant (Chi square: 19.4, df=2; $p<0.001$).	Amorim *et al.*, 2000.

Glioblastoma (U373) and Neuroblastoma (B103), human brain cell lines, incubated for 24h at 37°C with MeHgCl	**Methylmercuric chloride** at the concentrations of 0-1 µM		Exposure of cells to MeHgCl for 24h affected the binucleation index (BI, cells divided once) in a concentration dependent manner. The BI of neuroblastoma decreased significantly ($p<0.05$) even at the dose of 0.1 µM of MeHgCl. Results of frequencies of micronucleated cells demonstrated that MeHgCl increase the number of these cells found in neuroblastoma samples. No significant differences were detected in glioblastoma cells, although it seems to be a tendency to increase the number of micronucleated cells at higher concentrations of MeHgCl. In addition, an increased number of **micronuclei** in each micronucleated cell were detected in glioblastoma cells, in a statistically significant manner at the highest dose of 1 µM MeHgCl. The increased number of cells in metaphase, calculated by the metaphasic index, was significantly higher ($p<0.05$) in neuroblastoma (a six-fold increase) than in glioblastoma cells (a two-fold increase) at the highest concentration (1µM) when compared with the control groups. A higher proportion of cells containing nucleoplasmic bridges (index of nucleoplasmic bridges) was also found for both cell lines exposed to 1 µM MeHgCl.	(Crespo-Lopez *et al.* 2007)

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

In vitro data shows that methylmercury has a genotoxic potential. Crespo-Lopez *et al* shows that the human brain cell lines are also affected by MeHgCl: binucleation and metaphasic index are affected, cells containing nucleoplasmic bridges appear. *In vivo*, two studies show effects on germinal cells. However, according to the CLP guidance 3.5.2.3.9, the intraperitoneal route tested in animals (hamster and mouse, respectively Mailhes 1983 and Verschaeve 1984) is not considered as relevant compared to the expected route of human exposure (mainly by oral route, which is the most common route of exposure in human), to evaluate potential effects on

germinal cells. Besides, in the dominant lethal assay, treatment was administered to females so that the foetal loss may also be induced by maternal toxicity. These results nevertheless indicate a genotoxic potential *in vivo*, which is supported by induction of nuclear abnormalities in bone marrow in cats chronically exposed through the diet.

A statistical correlation between micronucleus frequency in peripheral blood lymphocytes and total mercury concentration in blood (p=0.00041), as well as between micronucleus frequency and age (p=0.017), was found in a population of fishers who had eaten mercury contaminated seafood (Franchi *et al.* 1994).

Four studies involving subjects exposed to methylmercury compounds from contaminated seal or fish meal were either inconclusive or indicated some chromosomal effects (Franchi 1994). Considering potential confounding factors such as smoking, age or consumption of other heavy metals, human data are however not sufficient to establish a link between chromosomal effects and exposure to methylmercury. One study on the population living in a gold-mining region contaminated by methylmercury (Amorim *et al.*, 2000) showed impairment of lymphocytes proliferation and inhibition of the cell cycle progression and increased polyploidal aberrations when MeHg concentration was higher than 20 µg/g.

4.9.5 Comparison with criteria

According to the CLP criteria for classification, *the classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.*

Classification in category 1A is not appropriate because no study clearly shows that MeHgCl induce heritable mutations in the germ cells of humans.

The classification in Category 1B is based on:

– *positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or*

– *positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or*

– *positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.*

Classification in category 1B is not appropriate because the intraperitoneal route of exposure is not the most common route of exposure for humans (which is the oral route) (according to the CLP guidance 3.5.2.3.9), although there are positive results from *in vivo* heritable germ cell mutagenicity tests in mammals (hamster and mouse).

The CLP criteria for classification in Muta.2 are as follow:

"Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on:

— Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

— Somatic cell mutagenicity tests in vivo, in mammals; or

—Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."

Classification in category 2 is appropriate, based on positive results in mammals.

So a classification Muta. 2 – H341 is supported in CLP regulation.

4.9.6 Conclusions on classification and labelling

A classification Muta. 2 - H341 according to the CLP regulation is therefore proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro data showed that methylmercury has genotoxic potential. *In vivo*, two studies showed effects on germinal cells. However, according to the CLP guidance 3.5.2.3.9, the intraperitoneal route tested in animals (hamster and mouse) is not considered as relevant compared to the expected route of human exposure (mainly by oral route, which is the most common route of exposure in human), to evaluate potential effects on germinal cells. Besides, in the dominant lethal assay, treatment was administered to females so that the foetal loss may also be induced by maternal toxicity. These results nevertheless indicate a genotoxic potential *in vivo*, which is supported by induction of nuclear abnormalities in bone marrow in cats chronically exposed through the diet.

A statistical correlation between micronucleus frequency in peripheral blood lymphocytes and total mercury concentration in blood ($p = 0.00041$), as well as between micronucleus frequency and age ($p = 0.017$), was found in a population of fishers who had eaten mercury contaminated seafood. Four studies involving subjects exposed to methylmercury compounds from contaminated seal or fish meal were either inconclusive or indicated some chromosomal effects. Considering potential confounding factors such as smoking, age or exposure to other heavy metals, human data are however not sufficient to establish a link between chromosomal effects and exposure to methylmercury. One study on the population living in a gold-mining region contaminated by methylmercury showed impairment of lymphocytes proliferation and inhibition of the cell cycle progression and increased polyploidy when the methylmercury concentration was higher than 20 µg/g.

Classification in Category 1A is not appropriate because no study has shown clearly that methylmercuric chloride can induce heritable mutations in the germ cells of humans.

Category 1B classification is not appropriate because the intraperitoneal route of exposure is not the most common route of exposure for humans (which is the oral route) (according to the CLP guidance 3.5.2.3.9), although there are positive results from *in vivo* heritable

germ cell mutagenicity tests in mammals (hamster and mouse).

Classification in Category 2 is appropriate, based on positive results in mammals.

Comments received during public consultation

Three MSCA supported the proposal to classify methylmercuric chloride in Category 2.

One MSCA agreed that this substance has genotoxic potential, but that Category 1B could be considered rather than Category 2. This suggestion was made on the basis of the positive results for mutagenicity following both intraperitoneal (germ cells) and oral (somatic cells) exposure, where the oral study demonstrated systemic availability for the most likely route of human exposure. Furthermore, they commented, an oral study in Pekin ducks showed that methylmercuric chloride causes disruption of cellular microtubules, degenerative changes in primary spermatocytes and abnormal spindle formation during metaphase. This MSCA considered that the data demonstrated that the substance (which is systemically available after oral exposure and interacts with spindle formation) has an intrinsic mutagenic property expressed in germ cells as hyperploidy.

In response, the DS clarified that the study in cats was only considered supportive due to limitations of the study:

- Unusual test species;
- Low number of animals;
- Unusual measurement of positive animals (cats characterised as with or without the presence of 2 or more micronuclei);
- No dose-relation observed;
- No positive and negative controls.

Therefore, the DS maintained its position; i.e. that classification Mut. Cat. 2; H341 is most appropriate.

Assessment and comparison with the classification criteria

In vitro studies

Multiple *in vitro* studies were presented by the DS. However, no recent, regulatory standard mutagenicity/genotoxicity tests are available for methylmercuric chloride.

The following table summarises the results of three non-standard studies to investigate the potential of methylmercuric chloride to induce chromosome aberrations in mammalian cells and one test for gene mutations, all without metabolic activation. The data are presented to the extent reported by the DS.

Cell type	*Methylmercuric chloride concentration*	*Results*
Human lymphocytes (1992)	0.12, 0.6, 1, 3, 5, 15 and 25 x 10^{-6} M	*Positive* Significant increase in chromosomal aberrations (mainly chromatid breaks) at 0.6 x 10^{-6} M. Increased frequency of structural and numerical chromosomal aberrations at higher

		doses (symmetrical and asymmetrical exchanges, > 10 per cell). Hyperdiploid cells increased linearly from the lowest dose (significant at higher dose) Polyploid cells were observed in all treated cultures but without a clear dose-response relationship (significant only at 15 x 10^{-6}M).
Human lymphocytes (1993)	0, 3, 5, 15 and 25 x 10^{-6} M	*Positive* Dose-related increase of chromatid aberrations ($p<0.05$). Dose-related increase in aneuploidy (particularly hyperploidy) – linear and significant increase up to 15 x 10^{-6} M.
Human peripheral lymphocytes (1993)	10^{-5}, 10^{-6} and 10^{-7} M	*Positive* Dose-related increase of aberrant metaphases (including gaps). At the higher concentrations, also induction of a significant number of breaks.
Chinese hamster V79 cells (1979)	0.08-0.4 µg Hg/mL	*Positive* Dose related increase in mutant fraction "near the cytotoxic threshold"

Although the DS provided limited information about the conduct of these studies and presented no quantitative data, the apparent consistency between them provides evidence that methylmercuric chloride has potential to damage the genetic material of mammalian cells, *in vitro*.

The results of a variety of additional, non-standard *in vitro* tests were also presented briefly by the DS. These included a *Bacillus subtilis* rec-assay for gene mutations, a *Saccharomyces cerevisiae* assay for chromosomal non-disjunction and a single strand break assay in rat glioblastoma cells, Chinese hamster V79 cells, human lung cells and human nerve cells. Positive results were claimed for all of these tests.

In vivo studies

No standard *in vivo* study was available for methylmercuric chloride. The DS provided a brief summary of three non-conventional studies; 2 of which targeted the germ cells, the other targeting somatic cells. The test substance was either methylmercuric chloride or methylmercury (see below).

Species/test systems	*Test Substance*	*Dose*	*Results*
Cat (unclear how many animals /group) "Nuclear abnormalities" in bone marrow cells (1979)	Methylmercuric chloride	In diet 0.0084, 0.02 or 0.046 mg Hg/kg bw for 39 months. The cats received a control fish diet (not contaminated with alkylmercury compounds) supplemented with methylmercuric chloride. A control group received the fish diet only: this resulted in a dose of 0.003 mg Hg/kg day.	This was a non-standard, poorly reported study and the results were collated in an unconventional way. No. of cats with any "bi- or multinucleated nuclear abnormalities" among 500 cells scored/total No. of cats: *Myeloid cells:* Males – 0/10, 0/5, 0/8, 0/5 Females – 0/10, 4/6, 7/8, 3/5 *Erythroid cells*: Males – 4/10, 2/6, 2/8, 3/5 Females – 0/10, 0/6, 0/8, 0/5 The authors claimed that there was a non dose-related increase in nuclear abnormalities in bone marrow cells from treated animals.
Syrian Hamster (female) Study of metaphase II chromosomes Oocytes were liberated from oviducts 23 h post –dosing. 150 oocytes/group analysed (1983)	Methymercuric chloride 21 negative controls 13 positive controls (0.2 5mg Trenimon/kg) 15 in treated group	Intraperitoneal injection Single dose of 10 mg/kg	150 oocytes were analysed in both the methylmercuric chloride and negative control groups; 281 were analysed from the Trenimon group. Significant increase in frequency of hyperploid cells in treated animals (6/150 compared to 0/150 in negative controls). A small increase in hypoploid cells (21/150 in treated group compared to 12/150 in negative controls) was also evident. Trenimon (an alkylating agent) produced a clear increase in the frequency of chromatid acentric fragments and fragmented chromosomes. No such structural lesions were seen in the negative control and test groups. Aneuploidy could not be assessed in the Trenimon group because of the high frequency of structural aberrations.

Mouse (BALB/c) (20 females/ group) Dominant lethal assay (1984)	Methylmercuric chloride Positive control: cyclophosphamide (210 mg/kg bw)	Intraperitoneal injection Single dose of 0, 2.5, 5.0 or 7.5 mg/kg bw Females dosed at 12 weeks of age, before mating Males were not treated Similar to OECD 478	Significant increase in pre- and early post-implantation foetal loss. No data on maternal toxicity. The study authors concluded that the substance is harmful to the female reproduction system, but the results were inconclusive as to whether the effect was genetic or physiological. The author suggested that a non-genetic effect was the more probable explanation for the results observed.

These 3 studies were non-conventional and clearly not compatible with current regulatory standards. There is no indication whether the laboratories who undertook them had previous experience of the tests they performed or whether their work was subject to Quality Assurance. They were not GLP-compliant.

The authors of the study in cats claimed that repeated exposure to methylmercuric chloride produced an increase in "nuclear abnormalities" in myeloid cells derived from the bone marrow. However, in addition to the limitations described in the previous paragraph, the cat is not a common species for mutagenicity testing of chemicals and the methodology employed and the formulation of the results were very difficult to follow from the brief report published in the open literature. It appears that an effect was seen in female cats and not male, but there was no dose-response, no positive control and no historical data to help set the results into context. Therefore, no firm conclusion can be drawn from these data and little weight can be placed on this study.

In this study, it was also found that dosing with methylmercuric chloride had no inhibitory effect on the capacity of leucocytes to repair DNA damage induced *ex vivo* by methylmethanesulphonate. In the absence of this alkylating agent, there was no evidence of DNA repair being induced by methylmercuric chloride itself. These findings do not support classification of methylmercuric chloride as a mutagen.

In oocytes derived from Syrian hamsters treated with methylmercuric chloride, there was a significant increase in the rate of aneuploidy (especially hyperploidy) in phase II metaphases. The animals had received a single intra-peritoneal dose of methylmercuric chloride during the preovulatory period. This study included positive and negative controls and appears to have been conducted well. However, it is not possible to conclude from this study whether exposure to methylmercuric chloride by a physiological route would have produced a similar positive result, but it does seem to support the results seen in the *in vitro* studies, showing that methylmercuric chloride has the potential to damage mammalian chromosomes.

The study in mice is described by the DS as a dominant lethal assay. However, the test substance was administered to females only, and not to males only, and therefore is not a conventional dominant lethal assay. A significant increase in pre- and early post-implantation loss was observed. However, given that the females had been dosed, the study authors were unable to conclude whether the observed effects were indicative of a genotoxic response. The possibility of an effect of the test substance on the female reproductive system and/or the developing foetuses cannot be excluded given this study design.

Summary

Overall, although there are no well-conduced, standard tests available, it appears that methylmercuric chloride has the potential to produce structural and/or numerical damage to chromosomes. Given that this substance is readily taken up and distributed in the body, as seen from the studies of other toxicological endpoints, it is possible that these effects could also occur *in vivo*. However, definitive, reliable positive *in vivo* data are lacking. On this basis, the case for classification does not seem to have been sufficiently well made by the DS.

Findings in humans

Five studies were summarised by the DS (see table below). Positive correlations were found between mercury levels and structural/numerical chromosomal aberrations in humans. However, in all these studies, data on smoking status of the subjects were either not available or not mentioned and there was no mention of controls, health status, age, medical history or occupation for any of the test groups. Information on individual dietary fish intake was not available and it is unclear whether the forms of mercury to which the subjects had been exposed were adequately representative of methylmercuric chloride. In the absence of such information, no firm conclusion can be drawn about the mutagenic potential of methylmercuric chloride from any of the human studies.

Cell type	**Number of people**	**Exposure**	**Observations/conclusions**	**Notes/data**
Lymphocytes (1974)	23	Consumption of mercury-contaminated fish	Positive correlation between blood mercury levels and structural or numerical aberrations.	Effects were significant only when lymphocyte cultures were initiated several days after collection and not on the day of collection.
Lymphocytes (1970)	9	Consumption of mercury-contaminated fish	Significant correlation between mercury levels and chromosome breaks.	Effects were significant only when lymphocyte cultures were initiated several days after collection and not on the day of collection.
Not specified (1986)	Not specified	Consumption of seal contaminated meal	Increased incidence of sister chromatid exchange.	Too little data available to assess this study.

Blood peripheral lymphocytes (1994)	51 fishermen Sampling took place in 2 consecutive years (1990 and 1991)	Consumption of mercury contaminated seafood	When data analysed by linear regression, correlation found between micronucleus frequency in peripheral blood lymphocytes and total blood mercury levels. Correlation between age and micronucleus frequency	1990: Mean blood mercury level = 81.97 ng/g (range: 10.08 ng/g – 252.25ng/g) Mean frequency of micronuclei = 8.7/1000 (standard deviation 2.47). 1991: Mean blood mercury level = 97.72 ng/g.
Blood peripheral lymphocytes (2000)	98 adults	One third of the men and two of the women had lived in in a region contaminated by methylmercury (exposure to mercury vapours)	Increased methylmercury hair levels correlated with impaired lymphocyte proliferation. At ≥ 20 µg/g hair mercury, polyploidy was found in 86.7% of subjects, compared to 18.8% of subjects at levels between 10 and 20 µg/g. 1-3 chromatid breaks were observed in 14.6% of subjects. At ≥ 20 µg/g, 37.9% had chromatid breaks (compared to 9.4% at levels between 10 and 20 µg/g and 0 chromatid breaks in people with methylmercury levels < 10 µg/g)	Total hair mercury: 0.57 – 153.8 µg/g (mean: 13.5 µg/g)

Conclusion

The data presented by the DS show that methylmercuric chloride has mutagenic potential *in vitro*. As discussed above, it is possible that this substance could be mutagenic *in vivo* but this is not shown definitively by the available data. Strictly, the classification criteria requiring positive evidence from mutagenicity or other genotoxicity experiments in mammals do not appear to have been met. No firm conclusion can be drawn from the available information in humans. Therefore, in contrast to the DS's proposal, although RAC recognises the genotoxic potential of methylmercuric chloride exhibited *in vitro*, it is concluded that the available data are insufficient to demonstrate activity *in vivo* and that they **do not support classification for germ cell mutagenicity**.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Studies presented in the TC C&L dossier:

Species	Dose (mg/kg body weight)	Duration of treatment	Observations and Remarks	Ref.
Rat	Four groups (25 males and 25 females each one) of 0, 0.1, 0.5 and 2.5 ppm MeHgCl (diet) 0.01, 0.05 and 0.25 mg/kg bw	2 years	This study deals with the **renal carcinogenicity** of the methylmercuric chloride (not in compliance with a regulatory guideline because of the insufficient number of animals used in the experiment). No increase in tumour incidence at any level.	Verschuuren 1976
Mouse (ICR) (60/group)	0, 2.14, 4.3 mg/kg bw MeHgCl 0, 15 and 30 ppm MeHgCl (diet) Substance tested: methyl mercury chloride	78 wk	This study deals with the **renal carcinogenicity** of the methylmercuric chloride (equivalent to the TG 451 on the carcinogenicity (OECD)). Majority of mice (51 males mice on 60 and 59 females mice on 60) in the 30 ppm group died due to the neurotoxicity by week 26. First renal mass was grossly seen in a male of the 15 ppm group at week 58. Renal tumours were revealed in 13 of 16 males of the 15 ppm group (1 of 37 males of the control). No renal tumours were seen in the female treated groups and/or control groups.	Mitsumori 1981
Mouse (ICR) 60 / sex / group	Four groups were tested at the doses of 0, 0.4, 2 and 10 ppm MeHgCl. 0, 0.06, 0.3, 1.4 mg/kg bw/day MeHgCl (approx)	104 wk	This study deals with the **renal carcinogenicity** of the methylmercuric chloride (equivalent to the TG 451 on the carcinogenicity (OECD)). Increased incidence of renal epithelial cell adenocarcinomas in males: overall, tumor incidence increased of	Hirano 1986

	(diet) of Methyl Mercury Chloride (purity = 99.3%)		50% (13/26) in the 10 ppm group (0.906 mg/kg/day), after 58 weeks. No general toxicity was described at 10 ppm, in the study. No neurotoxical signs were observed. Although the dead of male mice in the 10 ppm group by 98 weeks, there were no marked differences in mortality between treated groups and the control group. The body weight and food consumption in both sexes in the treated groups were comparable to those of the controls throughout the study. Except for the lesions and neoplastic masses in the kidney, there were no significant increases in the incidence of macroscopic findings in the treated groups as compared to the control. In the 10 ppm group, the incidences of decreased spermatogenesis in males and degeneration or fibrosis of the sciatic nerve in females were 11/59 and 23/60, respectively, and increased significantly as compared to the control group (1/58 and 9/19, respectively). Besides the above lesions, various kinds of spontaneous lesions were observed in animals of both treated and control groups. However, the incidences of spontaneous lesions were comparable to the controls.	
Mouse (B6C3F1) (n=60/sex/group)	Four groups were tested at the doses of 0, 0.4, 2 and 10 ppm MeHgCl. Overall mean daily intake of MeHgCl: 0.0382, 0.174, 0.859 mg/kg/day for males and 0.0332, 0.166, 0.752 mg/kg/day for females (diet) of	104 wk	This study was conducted in B6C3F1 mice with the same dosages used in previous study (Hirano *et al.*, 1986) in ICR mice, to compare the results of both studies (equivalent to the TG 451 on the carcinogenicity (OECD)). At 10 ppm of MeHgCl: increased mortality in males but not in females. Renal epithelial tumors were observed in 16 of 60 males: 13 were diagnosed as carcinomas and 5 as adenomas (as quoted in the publication; the discrepancy was not	Mitsumori 1990

	Methyl Mercury Chloride		explained). One female exhibited an adenoma at 10 ppm but there was not any carcinoma in females at any doses level. At 2 ppm: one male exhibited an adenoma but there was not any carcinoma at this dose level. At 0.4 ppm of MeHgCl: no renal tumors were observed neither in males neither in females. Epithelial cell degeneration and interstitial **fibrosis** in **kidney**, with on-going regeneration of the tubules present were observed in 59 of 60 males and 56 of 60 females at 10 ppm but the renal damage was more prominent in males than in females. Similar nephropathy was also observed in males of the 2 ppm group. The morphological features of the renal epithelial tumors observed in male M6C3F1 mice of the 10-ppm group in the present study were similar to those induced in male ICR mice treated with 10 ppm MeHgCl (Hirano *et al.*, 1986). General toxicology: neurological signs from posterior paresis to paralysis were first observed at week 59 in males (33 of 60) and at week 80 in females (3 of 60) of the 10 ppm group. A marked increase in mortality was observed for males of the 10 ppm group after 60 weeks. The final survival rate of males in this group was 17%, in contrast to 48% in the male's controls.	

4.10.2 Carcinogenicity: inhalation

No data available.

4.10.3 Carcinogenicity: dermal

No data available.

4.10.4 Human information

A study (Yorifuji *et al.* 2007) was performed to explore the association between MeHg and malignant neoplasms on two groups of population because each group was contaminated differently. Exposure group 1 (Minamata and Ashikita regions, on the east side) was contaminated from the late 1930s to 1968 and exposure group 2 (Amakusa, region of the west side) was contaminated from 1959 to 1968. There were 92525 and 152541 residents in each group in 1960 respectively. The results show in both exposure groups a **positive** association with **leukemia** (ASMRs, 95% CI using data from reference population 1, on the whole period = 1.80 (1.57-2.06)), a **negative** association with **gastric cancer** and **no association with other cancers**.

4.10.5 Other relevant information

No data available.

4.10.6 Summary and discussion of carcinogenicity

Although a rat study is negative, three studies consistently report renal tumours in male mice at doses as low as 10 ppm (0.859 mg/kg bw/d for males and 0.752 mg/kg bw/d for females in Mitsumori, 1990 and approximately 1.4 mg/kg bw/d in Hirano, 1986).

For information, methylmercury was classified in Group 2B by IARC in 1993 because of sufficient evidence in experimental animals.

In humans, a study performed on the population of Minamata showed a positive association between MeHg exposure and leukemia.

4.10.7 Comparison with criteria

The CLP criteria for classification in Carc. 1A or 1B are as follow:

Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence;
Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2).
Such evidence may be derived from:
– human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
– animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).
In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

Classification in category 1A or 1B for the carcinogenicity is not appropriate because on the one hand, we do not have convincing data in human to classify in category 1A and on the other hand, the data are not sufficiently convincing to classify in category 1B, based on strength of evidence.

The CLP criteria for classification in Carc. 2 are as follow:

*"The placing of a substance in **Category 2** is done on the basis of **evidence** obtained from **human and/or animal** studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."*

Overall, based on animals studies performed in the same laboratory, methylmercuric chloride induced renal epithelial cell adenoma and carcinomas in one specie: mice and one sexe: male. In humans, MeHgCl could be responsible for the increased incidence of leukemia.

Based on the fact that tumors are seen in one specie and one sexe on different experiments performed in the same laboratory, classification in category 2 for the carcinogenicity seems to be the more appropriate classification.

4.10.8 Conclusions on classification and labelling

A classification Carc. 2 – H351 in CLP regulation is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

IARC classified the related substance, *methylmercury* in Group 2B by IARC in 1993 because of sufficient evidence in experimental animals.

In an oral carcinogenicity study in rats administered methylmercuric chloride in the diet, there was no increase in tumour incidence at any level. However, three other studies consistently reported renal tumours in male mice at dietary doses as low as 10 ppm.

In humans, a study performed on the population of Minamata showed a positive association between methylmercury exposure and leukaemia.

Based on the fact that renal epithelial cell adenoma and carcinoma were observed in one species (mice) and one sex (males) in different experiments performed in the same laboratory, the DS considered that Carc. 2; H351 would be the most appropriate classification.

Comments received during public consultation

One MS supported Carc. 2.

A second MS supported Carc. 2 provided that the renal tumours in male mice were statistically significant. The DS confirmed that the increased incidences in renal tumours were statistically significant in the three studies and provided further data (refer to Additional key elements, below).

A third MS asked why the positive association with leukaemia was not weighted more strongly for classification. In response, the DS explained that the following confounding

factors were discussed by the authors:

- Endemic HTLV-1 infection,
- Potential for confounding by other carcinogens (e.g. benzene, smoking, radiation).

In addition, the DS noted that the present study also has several limitations according to the authors:

- Ecological effect estimates might fail to reflect biologic effects at the individual level,
- Disproportionate increase in rate of leukaemia because of surveillance,
- Data only after 1961 (no assessment of early contamination),
- No determination of sex-specific ASMR (Age Standardised Mortality Ratio),
- No assessment of population mobility.

Additional key elements

The key results from 3 studies of carcinogenicity in mice are summarised below - they were provided by the DS in response to the Public Consultation.

(i) Mitsumori et al., 1981

Number of ICR strain mice with tumors						
Sex	**Males**			**Females**		
Dose level (ppm)	0	15	30	0	15	30
No. of mice examined	37	16	1	44	30	0
Kidney adenoma	1	5	0	0	0	0
kidney adenocarcinoma	0	11***	0	0	0	0

*** Fisher's exact test p < 001

(ii) Hirano et al., 1986

Number of ICR stain mice with tumors								
Sex	**Males**				**Females**			
Dose level (ppm)	0	0.4	2	10	0	0.4	2	10
No. of mice examined	58	59	58	59	59	60	60	60
Kidney adenoma	1	0	0	3	0	0	0	0
Kidney adenocarcinoma	0	0	0	10***	0	0	0	0

*** p < 0.001

(iii) Mitsumori et al., 1990

Male and female B6C3F1 mice were given 0, 0.4, 2 or 10 ppm methylmercuric chloride in the diet. There were significant increases in the incidence of renal adenoma and/or carcinoma (16/60) and tubular cell hyperplasia (14/60) in males of the 10 ppm group, as compared to the control group. The incidence of chronic nephropathies also increased in males of the 2 ppm group. There was a marked increase in mortality at 10 ppm: the final survival rate for males was only 17%, in contrast to 48% of concurrent controls.

Assessment and comparison with the classification criteria

In a 2-year non-guideline study to investigate renal carcinogenicity, rats (25/sex/group) were given dietary doses of 0, 0.1, 0.5 and 2.5 ppm methylmercuric chloride (0, 0.01, 0.05 and 0.25 mg/kg bw). No increase in tumour incidence was observed at any dose of methylmercuric chloride.

In a 78-week study of renal carcinogenicity, ICR strain mice (60/sex/group, equivalent to OECD TG 451) were fed diets of 0, 15 and 30 ppm (equivalent to 0, 2.14 and 4.3 mg/kg bw). The majority of mice at the top dose died by week 26 due to neurotoxicity. This dose was clearly above the Maximum Tolerated Dose (MTD). Tumours were observed in the kidney in males only. The incidence of adenoma was 1/37, 5/16, 0/1 at 0, 15 and 30 ppm, respectively, whilst adenocarcinoma was observed in mid dose males only (11/16 mice).

In a second study in ICR mice (60/sex/group), methylmercuric chloride was administered in the diet for 2 years (equivalent to OECD TG 451) at dose levels of 0, 0.4, 2 and 10 ppm (0, 0.06, 0.3 and 1.4 mg/kg bw). An increase in renal tumours was observed in males only at the top dose. The incidence of adenoma in males was 1/58, 0/59, 0/58 and 3/59 at 0, 0.4, 2 and 10 ppm, respectively. Adenocarcinoma was observed in 0/58, 0/59, 0/58 and 10/59 males at 0, 0.4, 2 and 10 ppm, respectively. There was no increased mortality in any of the treatment groups; the top dose did not appear to have been above the MTD.

In the third study of carcinogenicity in B6C3F1 mice (equivalent to OECD TG 451), methylmercuric chloride was administered in the diet at 0, 0.4, 2 or 10 ppm for 2 years. Renal epithelial tumours were observed in 16/60 males at the top dose (equivalent to approx. 0.86 mg/kg/d (13 carcinomas and 5 adenomas – the numerical discrepancy has been noted but not explained, it may be that some males at the top dose had multiple tumours). In addition, an adenoma was observed in 1 female at the top dose and 1 male at the mid dose. Morphologically, the renal epithelial tumours observed in this study were similar to those in the 2-year mouse carcinogenicity study presented above. Epithelial cell degeneration and interstitial fibrosis, with ongoing regeneration of the tubules, was present in 59/60 males and 56/60 females at 10 ppm. The damage was more prominent in males, but it is unclear why males developed tumours and females did not.

The discrepancy between the findings in rats and mice and the sex-specific effect in mice has not been possible to explain given the available data. However, there is no evidence to suggest that the findings in male mice are not relevant to humans.

Very limited details of a Japanese study of 2 human populations exposed to methylmercury through contaminated food prior to 1968 was summarised very briefly in the CLH report. A positive association with leukaemia was found in both populations, but

no details were provided about the possibility of confounding by other factors or about the control groups against which these populations were compared. RAC agrees with the DS that no firm conclusions about causality can be reached from these studies and that they do not provide sufficiently robust evidence to support classification of methylmercuric chloride as a carcinogen.

In conclusion, the available data provide strong evidence of carcinogenicity in male mice; therefore classification for this hazard class is appropriate. Since tumours were found in one sex and one species, RAC agrees with the DS that Carcinogenicity Category 2; H351 would be most appropriate classification. The limited available human information is not considered sufficient to justify a more severe classification, especially given the uncertain nature of the results and that the potential for confounding by other carcinogens does not appear to have been adequately controlled.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Studies presented in the TC C&L dossier:

Species	Route	Dose	Exposure time (h/day)	N° of generat° exposed	Observations and Remarks	Ref.
Reproductive toxicity studies on two generations in Mink :						
Mink (male and female) (for female, G1: n=46 G2: n=47) (for male : n=29)	Oral	0.1; 0.5 and 1 mg Organic Mercury /kg in food (fish) No free mercury diet was constituted due to nonavailability of noncontaminated fish G1 exposed by food G2 exposed *in utero*, by lactation and		2 For G1 exposition began at 8 months of age; mating at 10 and 20 months of age. For G2 exposition began *in utero;* mating at 10 months of age.	No significant statistical difference was found according to the various nutrient factors (p = 0.0001). At 0,5 and 1 mg/kg diet: statistically significant decrease (p = 0.005) of proportion of females G1 giving birth (dose-effect relationship), compared to 0.1 mg/kg diet. For the females G2, statistically significant decrease (p = 0.005) of proportion of females giving birth at 1 mg/kg only. At 0,1 and 0.5 mg/kg diet: nine G1 and G2 females died. They exhibited loss of appetite and apathy, but didn't demonstrate	Dansereau *et al*, 1999

		food when they began to eat solid food. No negative control group presented in this study because impossible to make a diet with freshwater fish noncontaminated by MeHg. 1 mg/kg food regarded as a kind of positive control group because this concentration is considered to be toxic for mink			any neurological clinical signs. For the author there is no increased mortality for G1 and G2 females, and no neurological signs at 0,1 (considered as control group) and 0.5 mg/kg. At 1.0 mg/kg: increased mortality in G1 (30/50) and G2 (6/7) females, with neurological signs.	
<u>Reproduction toxicity studies on mating trials in mouse and in rats:</u>						
Mouse (n = 42)	Oral	0; 1.1 and 2.2 mg methylmercuric chloride /kg bw/day	48 days (30 days before mating to GD 18)	1	General toxicity : At 0 and 1.1 mg/kg bw/day : no effect At 2.2 mg/kg bw/day: Significantly smaller decreased maternal weight gain at ($p < 0.01$). At 1.1 mg/kg bw/day : Prolonged length of the estrous cycle by 11% compared to control group. At 2.2 mg/kg bw/day : Prolonged length of the estrous cycle by 27% compared to control group.	Nobunaga et al, 1979
Mouse (Swiss Webster) Males exposed	Oral	0, 1, 2.5, and 5 mg methylmercuric chloride /kg bw per day (n = 10-13 per group)	Experiment 1: 7 days Experiment 2 : 5 days	1	General toxicity experiment 1: - At 1 and 2.5 mg/kg/day : no evidence of toxic effects - At 5 mg/kg bw per day : 2 of 13 died General toxicity experiment 2 - At 1 and 2.5 mg/kg/day : no evidence of toxic effects	Khera, 1973 (b)

Females unexposed (males caged with 3 females and separated per mating trial)		experiment 1 : 10-12 males per dose group and 7 mating trials experiment 2 : 12-13 males per dose group and 7 mating trials			- At 5 mg/kg bw per day : no apparent toxic effect No effect on fertility showed in the 7-days experiment and suggested (no significant effect in the 5-days experiment. However, for the author, the lack of reproducibility in these two mouse experiments and the two acute rat experiment makes the suggestion of a mercury-induced antifertility effect in mice unconvincing.	
Rat (Wistar) Males exposed Females unexposed (males caged with 2 females and separated per mating trial)	Oral	0, 1, 2.5, and 5 mg methylmercuric chloride /kg bw per day experiment 1 : 15-20 males per dose group and 14 mating trials experiment 2 : 15 males per dose group and 7 mating trials	A single daily dose for 7 consecutive days	1	General toxicity : For all doses, no adverse effect on the behaviour or the rate of body weight gain Fertility : The averaged incidence of pregnancy over all mating trials indicated a significant dose effect ($p<0.01$): 42% at 5 mg/kg, 50% at 2,5 mg/kg, 51% at 1 mg/kg and 57% in control group. The number of viable embryos per litter decreased significantly ($p=0.01$) when 2.5 or 5 mg/kg bw per day was given for 7 days (no effect on preimplantation rate).	Khera, 1973 (b)
Rat (Wistar) Males exposed Females unexposed (males caged with 2 females and separate	Oral	0, 0.1, 0.5 and 1 mg methylmercuric chloride /kg bw per day (14-19 males per dose group and 17-21 mating trials)	30 days and 90 days	1	General toxicity : - At 0.1 and 0.5 mg/kg/day : no evidence of toxic effects - At 1 mg/kg/day : depressed rate of body weight gain after 70 days of dosing and during the next 10 days, mild severe motor disturbances in 5 of 18 rats. 10 days after, one of the affected rats died. The number of viable implants decreased after dosing for 25-30 days at 1 mg/kg bw/day and after 85-90 days at 0.5 mg/kg bw/day. The preimplantation losses are	Khera, 1973 (b)

d per mating trial)					dramatic at 1 mg/kg bw/day (more than two fold increase after 85-90 days).	
Repeated toxicity studies: effects on the organs of the reproduction						
Male Wistar Rats (n=20)	Oral	2.2 mg/kg bw/day MeHg (n=10 control group; n=10 contaminated group)	8 weeks, in tap water	1	The body weight and the testis weight were altered in poisoned rats (decrease of 30% of the body weight between the control and the contaminated group; and decrease of 12.6% of the testis weight between the control and the contaminated group $p < 0.001$). But the ratio of testis weight to bw was not different to that of control rats. The plasma testosterone decreased dramatically in intoxicated rats (10.68 ng/ml and 0.26 ng/ml respectively in control group and intoxicated group, $p < 0.001$). With this, the concentration of testosterone in the interstitial and seminiferous tubules fluids dropped respectively about 73% (112.0 ± 18.0 versus 416.25 ± 20.50; $p<0.001$) and 55% (107.0 ± 20.05 versus 234.68 ± 29.73) in intoxicated rats in comparison with the controls. The decrease in plasmatic testosterone seems to be due to the fall in the secretion of testosterone (73%) by the Leydig cells, confirmed by the decrease of testosterone in the interstitial fluid. The disorder of the synthesis of testosterone could result in a reduction in the activity of enzymes implied in the biosynthesis of testosterone. No effect was observed on epididymal sperm count after 60 days of exposure to Methylmercury ($3.146 \pm 0.323 * 10^9$ g^{-1} epididymis versus $2.944 \pm 0.346 * 10^9$ g^{-1} epididymis). No histological changes were observed in the testes, neither in Leydig cells nor in seminiferous	Moussa H. *et al.*, 2010

					tubules.	
Adult male Wistar rats (n=60)	Oral	0;0.5;1.0 or 3.0 mg/kg/bw/day MeHg	Daily for 14 days	1	MeHg treatment at 3.0 mg/kg bw/day reduced body weight gain of 4.2% between initial and final body weight (7.2% of increase of body weight for the control), absolute and relative weights of the seminal vesicle of 31.6% and 27.8% respectively and increased relative kidney weight of 18%. The 0.5 mg/kg bw/day treatment produced a rise (of 30%) in vas deferens weight. MeHg did not markedly alter epididymal or testicular weight. The 0.5 mg/kg bw/day treatment produced a significant rise in the number/proportion of sperm with **head abnormalities** (control: 0.5(0-1) vs. 0.5 mg/kg bw/day MeHg: 2.25(1.12-5.37)**). **Sperm motility** significantly **decreased** in type A sperm (mobile with progression) while type B (mobile without progression) and type C (immobile) sperm motility **increased** in MeHg-treated groups. MeHg treated at 1.0 mg/kg displayed a significant elevation in daily sperm production. In contrast, there was a **reduction in sperm quantity** in the caput-corpus epididymis at all doses and an acceleration of sperm transit time at 1.0 and 3.0 mg/kg bw/day. The rate of spermatogenic process was not significantly affected by MeHg treatment (data not known). Treatment with MeHg produced a significant reduction in serum testosterone levels at the highest dose. No histopathological changes were observed on the morphological structure of the	Fossato *et al*., 2011

					testis and the region containing the caput and the cauda epididymis.	
15 males Wistar rats	Orally in the drinking water	20 mg/L Group 1: mercuric chloride (HgCl2) (inorganic) Group 2: **methyl mercuric chloride (CH3HgCl)** Control group: demineralized water	For 12 weeks, Every second day	No study on several generations	No mercury deposits in tissue sections prepared from control group. Mercury deposits are present in the testes from animals in both experimental groups (no obvious difference in the density of mercury-stained cell bodies between these two groups). Mercury was found intracellularly in the seminiferous tubules, exclusively in the lysosomes of Sertoli cells, which closely associated with the developing spermatozoa. No mercury deposits were observed in the spermatogenic cell line. In the interstitial tissue, mercury staining was abundant in the **Leydig cells**, and more particularly in **lysosomes** but minor deposits were scattered in the cytoplasm. Lipid droplets were devoid of deposits. Mercury was present in the lysosomes of a few macrophages located in the interstitial tissue. Excessive accumulation of mercury in lysosomes might therefore damage essential cell function. The mercury deposits in **Leydig cells** might affect the **testosterone synthesis** in these cells.	(Ernst *et al.* 1991a)
Pekin ducks (*Anas platyrhynchos*)	Orally	One control group = 0 Group 1 = 0.5 mg/kg Group 2 = 5 mg/kg Group 3 = 15 mg/kg of MeHgCl	Daily, for 12 weeks	Only males ducks 1 generation	Testicular cells of Pekin ducks were examined by electron microscope. Results of Hg analysis in testicular tissue indicated that metal residue tended to increase (0.13 to 24.75 µg of Hg/g of tissue) with increased doses of CH3HgCl. There were no ultrastructural changes in the seminiferous epithelium of group 1 ducks (0.5 mg of CH3HgCl/kg of basal feed). Cellular damage	(McNeil and Bhatnagar 1985)

					was more extensive as the dosage of CH3HgCl increased from 5 mg/kg to 15 mg/kg of basal feed. There was a significant difference between the mean value of Hg in ducks from group 2 and 3, when compared with the control mean at 90% confidence level in a 2-sided Student's *t* test. Sertoli's cell, as a steroid producer, probably regulates local differentiating germ cells. **Ultrastructural** alterations were evident in **Sertoli**'s cells of groups 2 and 3, along with an increase in large lipid droplets, in which methylated mercury could be sequestering. Reduction in microtubules, microfilaments and smooth endoplasmic reticulum, along with distended Golgi complexes indicate the cellular detoxification machinery cannot cope with the highest concentration of CH3HgCl. Methyl mercury causes **disruption** of cellular **microtubules** in a concentration and time-dependent manner. **Primary spermatocytes** were the first germ cells to show extensive **degenerative** changes, and the severity increased with high dosages. Occurrence of degenerating cells in treated ducks was more marked than expected when compared with the control group. Groups of germ cells in synchronized meiosis showed no apparent cytokinesis. **Spindle formation** during metaphase was abnormal, probably because CH3Hg was bound to –SH groups or S-S bonds of microtubules of spindle.	
Mucuca fusciculuris	Orally by	0.025 mg/kg methyl Hg	Daily for 20 days	-	Mohamed et al. (1987) reported a decreased percentage of motile sperm and increased sperm tail	Mohamed et al.;

monkeys	gavage				defects in *Mucuca fusciculuris* **monkeys** dosed by gavage with 0.025 mg of methyl Hg per kilogram per day for 20 days.	1987
Rats	Intra peritoneal	5 pg/kg MeHg	Daily for 15, 30, 60, or 90 days	-	Alteration of spermatocytes and spermatides was observed in rats treated i.p. with 5 pg of methyl Hg per kilogram daily for 15, 30, 60, or 90 days.	Vaccharajani, K.D., et al., 1993
Male mice	Intraperitoneal	A single dose of 1 mg/kg MeHgOH	A single dose	-	A decreased fertility was observed in male mice dosed i.p. with a single dose of 1 mg/kg of methyl Hg hydroxide.	Lee, I. **P.,** 1975.

4.11.1.2 Human information

Studies presented in the TC C&L dossier:

Effects on fertility in men	
Relationships between male fertility and mercury levels in hair. The purpose of the study is to examine the relationship between human male infertility and mercury in **seafood**. Hair samples from 94 fertile and 117 subfertile Hong Kong residents were compared over four separate age groups. Two semen analyses were taken two weeks apart for each patient, including sperm count, morphology, motility, velocity and linearity. Azoospermic patients were excluded as they are infertile rather than subfertile and many of them have aetiologies distinct from subfertile men. A series of standard questions were asked concerning the consumption of fish (frequency and type of fish), cigarette smoking, the number of tooth amalgam fillings, the intake of Chinese herbal remedies (some containing heavy metals), the types of perms and hair colouring agents used and the duration of living in Hong Kong (only those living in Hong Kong for a minimum of 5 years were included in the study). **Results: The level of total mercury in hair of the 37 fertile non vegetarians tested from Hong Kong was 3.3 mg/kg. Age corrected estimates of risk indicated that compared with men with low levels of mercury in their hair, men with higher levels were twice as likely to be subfertile (relative risk = 1.95) and there was a dose-response trend that was highly significant (p < 0.0005).**	Dickman *et al*, 1999

New study added in the present dossier:

Objective: to compare blood mercury concentrations of infertile couples with those of fertile couples and to examine the relationship between blood mercury concentrations and seafood consumption among infertile couples (in Hong-Kong). The analysis was performed on 150 males and 155 females in the infertile group, compared with 26 males and 26 females in the control group. Among the infertile group, 95 couples	(Choy *et al.* 2002)

suffered from primary infertility; 40 males had abnormal semen and 30 couples had no known cause for their infertility. **Results:** Overall, the infertile group had significantly higher blood mercury concentrations than the control group. In the subgroup analysis of "infertile males with abnormal semen" and "infertile females with unexplained infertility" also had higher blood mercury concentrations than their fertile counterparts. Besides, blood mercury concentrations were positively correlated with quantity of seafood consumption. Infertile subjects with elevated blood mercury concentrations consumed a larger amount of seafood. **Higher blood mercury concentration** is associated with male and female **infertility**. Higher seafood consumption is associated with elevated blood mercury concentrations in the infertile population.	
In vitro incubation of fresh human semen with 20 µM methyl Hg resulted in an inhibition of sperm motility.	**Ernst, et al., 1991b**
Adverse effects on the male reproductive system, including oligospermia, teratospermia, asthenospermia, and reduced libido, have been observed in workers chronically exposed to organic mercury (mean duration of exposure, 10.5 years).	**Popescu, H. I., 1978.**

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Studies presented in the TC C&L dossier:

Species	Route	*dose mg/kg/day ppm **Conc. (mg/l)	Exposure time (h/day)	Exposure period : - number of generations or - number of days during pregnancy	Observations and remarks	Ref.
Mouse (n = 42)	Oral	0, 1.1 and 2.2 mg methylmercuric chloride /kg bw/day		48 days (30 days before mating to GD 18)	General toxicity : At 0 and 1.1 mg/kg bw/day : no effect At 2.2 mg/kg bw/day: Significantly smaller decreased maternal weight gain at ($p < 0.01$). At 0 and 1.1 mg/kg bw/day: no significant decreased in the number of total implants par dam. No significant retard in growth of surviving fetuses. At 1.1 and 2.2 mg/kg bw/day: significant incidences of fetuses with cleft palate ($p<0.05$). Malformations	Nobunaga *et al*, 1979

					to 17% of fetuses at the low dose and 55% at the high dose. At 2.2 mg/kg bw/day: significant decreased in the number of total implants par dam ($p<0.05$). Higher incidences of resorptions, dead embryos and dead foetuses. Significant retard in growth of surviving fetuses.	
Mouse BALB/C, C57BL/6J and C57BL/6Cr strains Variable groups: 8 to 22 individuals	Oral	3 mg MeHg/kg bw Controls: 3 equivalent volumes of Phosphate-buffer saline		GD 12-14	**Neuro-behavioural effects of MeHg on mouse offsprings subjected to a prenatal exposure** - Maternal toxicity: none of the mother died nor present overt neurological symptoms - Decrease in the bw in the male offspring of the 3 strains from week 3 to week 12 after birth. - The open field test revealed a decrease in the total locomotor activity in male from mother exposed to methyl mercury but no difference with control for C57BL/6J strain. Decrease in rearing only in C57BL/6Cr strain. Increase in grooming behaviour in C57BL/6J strain and a drastic decrease in BALB/C strain. - Significant change in locomotion in BALB/C only/: increase in central locomotion and decrease in peripheral locomotion - Home cage activity (spontaneous activity): decrease of spontaneous activity in the dark phase in BALB/C of the methylmercury group. Strong decrease in the light-phase spontaneous activity in C57BL/6Cr and C57BL/6J methymercury group compared with their respective control groups. - Morris water maze: prolonged latency in the C57BL/6Cr and C57BL/6J strains exposed to methylmercury. No significant effect on BALB/C methylmercury group. - Mercury concentration in tissue: higher Hg level in the brain and liver for all groups compared with control (less than twice levels of control	Kim *et al*, 2000

					groups)	
Mouse (pregnant Mice CD1 strain	Oral (gavage)	12,5 mg of MeHgCl/kg of bw ± 25 mg/kg Lead nitrate or 6 mg/kg sodium arsenite (combined exposure and no combined exposure)		Single prenatal exposure at GD10. Observations on fetuses at GD18	**Induction of physiological and anatomical effects in mice offsprings by a single exposure to MeHgCl at GD10**. All Hg-exposed groups (alone, binary or tertiary combinations) showed significant increase of mortality among females. Presence of some dams carrying completely resorbed litters. **Specific effects of MeHgCl on fetuses** - Decrease in average fetal bw/litter. - Skeletal defects : supernumerary and asymmetrical ribs, delayed ossification - Increase of incidence of cleft palates	Belles *et al*, 2002
Mouse C57BL/6 strain	Oral (drinking water)	Chronic treatment with 0, 4, 6 and 8 mg of MeHgCl/L (continuous exposure) (approximately 0, 0.8, 1.2 and 1.6 mg MeHgCl /kg bw)		GD2 to weaning	**Neuro-behavioural effects of MeHgCl on mice offsprings exposed prenatally and during weaning.** - Decrease in offspring survival at the age of 5 weeks in the 8 mg/L group compared to controls. Analysis of behavioral functions of offspring between the age of 6 and 12 weeks: - Decreased locomotion in the female offspring of all treated females groups. - Impairment of working memory in the females offspring of females treated with 6 and 8 mg/L **Maternal toxicity was not described**	Goulet *et al*, 2003

Rat (Fischer 344) (n =120)	Oral	0, 10, 20 and 30 mg methylmercuric chloride /kg bw (single dose) (n = 30 / dose group)		GD 7	Maternal body weight declined at all doses : At 10 mg/kg decreasing for 2 days and by 86 % of control on day 20 of gestation. At 20 mg/kg decreasing for 6 days and by 76% of control on day 20 of gestation. At 30 mg/kg decreasing during the experimental period without recovery and by 61.9%of control on day 20 of gestation. Maternal death on day 20 : At 0 mg/kg : 0 % At 10 mg/kg: 6.7 % At 20 mg/kg : 16.7 % At 30 mg/kg : 30 % The decrease in the number of live fetuses on day 20 of gestation. Survival rate of fetuses : At 0 mg/kg : 97.4 % At 10 mg/kg : 77.8 % At 20 mg/kg : 54.9 % At 30 mg/kg : 7.6 % A dose-dependent decrease in ossification centres was seen. The concentrations of mercury for the doses 0, 10, 20 and 30 mg/ kg were respectively 0.02, 2.6, 9, and 21 µg/g in maternal brain, 0.19, 3.5, 11, and 15 µg/g in fetal brain.	Lee *et al*, 1995
Rat (dams)	Oral (gavage)	0, 0.02, 0.04, 0.4, or 4 mg methylmercuric chloride /kg bw		GD 6-9	No sign of adverse effects in the pregnant rats. At 4 mg/kg bw, swimming behaviour of pups at 4-35 days of age was impaired and changes in the dendritic spines of the pyramidal neurones. At 0.04, 0.4 and 4 mg/kg bw, increased passiveness and decreased habituation to an auditory startle were observed 60-210 days postnatally, in pups.	Stoltenberg-Didinger *et al*, 1990
Rat Sprague Dawle	Oral (MeHg), Inhalation (Hg^0),	Oral : 2 mg MeHg/kg/day Inhalation 1.8	For 1,5 hour per day	4 groups of pregnant rats (12 animals per group):	No sign of adverse effects in the pregnant rats. **Offsprings were observed up to weaning:**	Fredriksson *et al*, 1996

y	And both oral and inhalation	mg Hg^0/m^3 air for 1.5 per day (equivalent to 0.1 mg Hg/kg.day) And combined exposures : MeHg+ Hg0 And control group		MeHg, Hg^0, MeHg +Hg^0 and control Pregnant rats: - orally during days 6-9 of gestation - inhalation during gestation days 14-19	- No difference between groups (including control) in clinical observations and developmental markers (body weight, pinna unfolding, surface righting reflex and tooth eruption). **Offsprings of 4-5 months were involved in behavioral tests:** - MeHg group: no significant functional alterations in the brain at the dose used. - those of dams exposed to Hg^0 showed hyperactivity in locomotion, rearing and total activity this effect is potentiated in the (MeHg + Hg^0) group - Exposure to MeHg and MeHg + Hg^0 vapours induces alterations of both spontaneous and learned behaviour reflected by delayed behavioural responses and deficits in spatial learning, and hyperactivity (ambulation and rearing) - MeHg seems to potentiate the effects of Hg^0.	
Rat	Oral (drinking water)	0, 0.5 and 6.4 ppm Hg as MeHgCl in drinking water resulting in daily intakes of 0, 40-50 or 500-700 µg/kg/day		Maternal exposure 28d and 49d before mating continued to postnatal day 16	Exposure to MeHgCl accelerates the decline in training performances. With aging, the behaviour may reveal consequences of MeHg exposure that had taken place early in development.	Newland *et al*, 2000

Rat Wistar	Oral	Adult female: diet containing 5 ppm Hg (as MeHg) during 8 weeks Then mating, same diet throughout gestation and after parturition Control group		4 females control and 10 exposed to MeHg Offspring were exposed via the mother prenatally and up to weaning (prolonged to d 30 postnatal) and then fed during 2 months with the same diet contaminated with MeHg.	On the day of parturition: concentration of Hg in the brain of newborns 1.4 times higher than in the mothers. During lactation, rapid **decrease** of Hg in the brain of the offspring (1/5 of concentration at birth). Elimination by milk limited. Gradual increase when the offspring is fed with diet containing MeHg. Behavioral tests on the MeHg exposed offspring and control at 5-6 weeks of age: deficit in motor coordination and learning disability in the MeHg group. Histopathological abnormalities observed in the cerebellum. Maybe induced by accumulation of MeHg especially during gestation period.	Sakamoto *et al*, 2002

Rat Long Evans	Oral (drinking water)	0, 0.5 and 6.4 mg/L Hg (as MeHgCl) resulting in daily intakes of 0, 40-50 or 500-700 µg/kg/day		Maternal Exposure during 28 or 49 days before mating and continued to postnatal day 16 **1.7 and 2.3-year-old offspring** **Experiment 1 =Group 1.7 year-old** Group 0: 10 (5 ♂) Group 0.5: 10 (5♂) Group 6.4: 11 (6♂) corresponding to 5, 5 and 6 litters **Experiment 2=Group 2.3 year-old** Group 0: 8 (4 ♂) Group 0.5: 7 (2♂) Group 6.4: 9 (5 ♂) corresponding to 4, 5 and 5 litters	1.7 and 2.3-year-old offspring behaviour observed. No overt maternal toxicity. Some evidence but not significant, of small litter sizes in the 500-700 µg/kg/d exposure group. Hg detected in neonatal brains for the 0.5 and 6.4 ppm groups, not in the 0 group. No difference between 28- and 49-day groups, so the groups were combined. No significant exposure via lactation. **For the group 1.7 year-old**: No effect of prenatal MeHgCl exposure. **For the group 2.3 year-old**: Retarded acquisition of choice in training tests The results were lower for the rats exposed prenatally to 40 µg/kg/d. Results described here replicate earlier similar observations on monkeys (squirrel) (see hereafter Newland 1994) but some important difference: in rodents, apparent interaction with age.	Newland *et al*, 2004
Mouse	Oral	2, 4 and 4,8 mg methylmercuric chloride /kg bw per day		GD 6-13	At 2 mg/kg bw : few malformations. At 4 mg/kg bw : decreased fetal weights and large increase in the frequency of malformations At 4,8 mg/kg bw : decreased fetal weights and large increase in the frequency of malformations Increased post-implantation loss	Fuyuta *et al*., 1978

Rat	Oral	2, 4 and 6 mg methylmercuric chloride /kg bw per day		GD 7-14 or 18-20	No effect at 2 mg/kg bw. At 4 mg/kg bw : malformations consisting mostly of cleft palate and vertebral defects in the offspring of dams At 6 mg/kg bw : Resorptions, deaths. Malformations consisting mostly of cleft palate and vertebral defects in the offsprings of dams.	Fuyuta *et al*., 1978
Mouse (Swiss-Webster female) (n = 148)	Oral	0, 0.001, 0.01, 1, 5 and 10 mg methylmercuric chloride /kg bw per day (single daily dose in food)		GD 6-17	At 0.1, 0.01 and 0.001, no effect At 1 mg /kg bw/day, no evident toxic effects in the dams. Number of live pups and survival after 28 days of age not affected but there was transitory inhibition of cerebellar cellular migration from the external granular layer. At 5 mg /kg bw/day, no evident toxic effects in the dams, but reduction of the number of live pups, and live-born pups died within 2 days. At 10 mg /kg bw/day, all the dams died (Results statistically significant)	Khera *et al*., 1973 (c)
Rat (Wistar strain female) (n = 175)	Oral	0, 0.002, 0.01, 0.05, 0.25 mg methylmercuric chloride /kg bw per day (single daily dose in food)		Exposure between female weaning and whelping	At 0.002, 0.01 and 0.05 mg /kg bw/day, no effect At 0.25 mg /kg bw/day, no toxic effects evident in the dams. No apparent adverse effect on fetuses, and the only abnormality seen postnatally was eyelid lesions associated with hardening of the lacrymal glands. The dose-response relationship was significant (p = 0.01)	
Semi-domesticated mink (Mustela vison)	Oral	Feeding with fresh water fish Contaminated inMe Hg 3 diets: 0.1, 0.5, 1.0 mg/kg of food total mercury **no control group**: no diet without Hg		2 generations of female mink (G1 and G2) **G1 females:** 20+20+20 with respectively each of 3 diets: during 60 days before	**Reproductive effects** **G1 and G2 females**: - no effect on gestation length - decrease in whelping percentages with increasing dose of Hg but not statistically significative - no effect on litter size **G1 and G2 kits** no influence on the survival of G1 and G2 kits between birth and weaning. no influence on growth of G1 and G2 kits between birth and day 35. Kits had higher liver Hg	Danserau *et al*, 1999

				mating **G2 females**: Kits born from G1 groups Exposure of kits in utero and /or throughout lactation and then through diet contaminated in Hg. **G1 Kits** After 430d of exposure, G1 females (20 of 0.1 ppm, 20 of 0.5 ppm, 6 of 1.0 ppm) mated with 0.1 ppm Hg males **G2 Kits** After 300 days of exposure, G2 females (20 of 0.1ppm, 20 of 0.5ppm, 7 of 1.0ppm) mated with 0.1 ppm Hg males Exposure of G1 and G2 kits in utero and /or lactation	concentration than non exposed kits (in another study) Transfer of Hg through placenta and/or milk but did not affect growth. **Adult mortality among G1 and G2 females** **Increased mortality in G1 (30/50) and G2 (6/7) females exposed at 1.0 ppm Hg diet, with neurological signs.** 0.1 and 0.5 ppm Hg diets: no increase mortality for G1 and G2 females, and no neurological signs Accumulation of Hg in the liver is dose-dependant Reproductive function of mink exposed to Hg could interact with other environmental factors.	
Monkeys (Macaca fascic	Oral, mixed with apple juice.	0 (n=8), 50 (n=7), 90 (n=7) µg methylmercury	One year. 124 days before	1 generation Treatment began	Maternal Toxicity: Four females receiving 90 µg/kg bw/day and one receiving 50 µg/kg bw/day showed signs toxicity (change in the sucking response,	Burbacher *et al*, 1984

ularis, Females) (n = 22)		hydroxide/kg bw per day	breeding, and during pregnancy, on a daily basis	approximately 124 days (equivalent with 4 menstrual cycles) before mating. Female bred to nontreated males.	finger movement loss, gross motor in coordination and blindness) were terminated. No effect on the menstrual cycle, conception rate, or size of offspring at birth, but a maternal blood concentration > 1.5 µg/ml decreased the number of viable deliveries and a concentration > 2 µg/ml is toxic to the dams. In addition, reproductive failure was associated with a significantly higher mean blood Hg concentration than reproductive success. The results of this investigation indicate that reproductive dysfunction is one of the earliest effects of MeHg administration in adult female. Increased blood Hg concentrations were associated with decreased fertility and increased early spontaneous abortion.	
Monkeys (Macaque Female)	Oral	0.04 or 0.06 mg methylmercury /kg bw per day		For 198-747 days before mating Infants were separated from their mothers at birth and were tested 210 and 220 days after conception (50-60 days after birth).	Randomness in visual attention to novel stimuli The mean maternal blood concentrations at birth were 0.84 and 1.04 µg/ml, and the blood concentrations of the offspring were 0.88 and 1.7 µg/ml	Gunderson *et al*, 1988
Squirrel monkeys		maternal blood concentrations of 0.7 and 0.9 mg methylmercury /ml		Offspring exposed to methylmercury during the second half or the last third of pregnancy	Reduced sensitivity to changes in the source of reinforcement, indicating learning impairment at five to six years of age	Newland *et al*, 1994

Porcine	oral	0, 0.5, 5 mg methylmercuric chloride /kg of food		Over pregnancy and lactation Growing of gilts and barrow; one couple per treatment	No significant variation of the number of born piglets per gilt, of their birth weight; of the number of born dead piglets, or abnormal piglets. (No teratogenic effect likely related to the anatomy of the placenta in Pigs: six intervening placental tissues between fetal and maternal blood of the porcine).	Chang *et al*, 1977
Cat	Oral (gavage)	0.03, 0.083, 0.25, 0.75 mg methylmercuric chloride/kg /day : corn oil suspension in gelatine capsules		GD 10-58	Cats were killed on day 59 of gestation: - at lower levels (0.03 and 0.083 mg/kg): no sign of poisoning in maternal cats - at 0,25 mg/kg : decrease in body weight of 12 of 13 cats - at 0.25 and 0.75 mg/kg: increased incidence of abortion and fetal osseous anomalies : in surviving fetuses (at the dose 0.25 and 0.75) there was reduced neuronal population in the external granular layer of the cerebellum. - at 0,75 mg/kg: vomiting, ataxia, convulsions, etc. and death within 32 days (4 cats)	Khera, 1973 (a)
New studies added in the present dossier:						
Monkeys (Macaca fascicularis)	Oral Substance tested: methylmercuric chloride.	n = 4 for the control **group (0)** For the postnatal exposed **group (1):** n = 5 infants exposed to 50 µg/kg/day and examined at 18 years old. For the *in utero* + postnatal exposed **group (2)**: n = 20 females	**Group (1):** 5 days per week on day 1 of life until 7 years. **Group (2):** females exposed 3 times per week; infants	For the *in utero* exposure **group (2):** exposure from the beginning of the gestational period and continuing postnatally. 2 generations tested (mothers and infants).	Assessment of the vibration sensitivity: Of the monkey exposed **postnatally only**, one monkey exhibited **normal** somatosensory function while three monkeys exhibited substantially **elevated** vibration thresholds. One monkey exhibited **difficulties to learn the task,** and were tested at the both hands: he had extremely impaired vibration sensitivity in the fingers of both hands even at the lowest frequency tested. The difficulties to learn the task seem to be caused by the **severely reduced perception of the vibratory** stimulus since this monkey were able to learn previous task for auditory tests.	Rice *et al.*, 1995

		(5/dose) exposed to 0, 10, 25, 50 µg/kg/day. Infants dosed at the same doses of their mother and examined at 15 years old.	dosed 5 days per week until 4 or 4.5 years.		For the ***in utero*** **plus postnatal** exposed monkeys, one monkey was clearly unimpaired; another exhibited slightly elevated thresholds for two of the five frequencies tested; both monkeys from the lower dose group exhibited impairment at all but the lowest frequency. These results suggest **permanent** impairment in vibration sensitivity, after a long term exposure and **even after a long period without treatment,** beginning during developmental period.	
Monkeys *(Macaca fascicularis)*	Maternal oral ingestion of methylmercuric chloride added to a small amount of juice	0, 10, 25, 50 µg/kg/day 5 monkeys for the high dose, 2 for the intermediate, 1 at the low dose;	*In utero*: 3 times per week (by the exposed mother). Postnatally: 5 days a week until 3.5-4.5 years. After, dosing was discontinued.	Experiment on the second generation (infants). Beginning at 11 to 19 years of age.	Assessment of the auditory function: At the high dose, thresholds were elevated in both ears, at all frequencies and more particularly at the highest frequencies. These effects were more severe at 19 years of age than at 11 years of age. At the middle dose, thresholds were more elevated at 19 years than 11 years at all but the highest frequency. At the low dose, no impaired thresholds were observed at 11 years compare to the control while at 19 years, thresholds were elevated in the both ears at all frequencies but the highest frequencies.	(Rice 1998)
Offspring of the Adult female macaque Mon	Maternal Oral ingestion mixed with apple juice	0, 50, 70, or 90 µg/kg/day of MeHg hydroxide Control group: n=9 MeHg	Daily exposure of the adult females, 7 days per week.	Prior to and throughout pregnancy. Experiment on the second generation (infants), between 11 and 14.5	Assessment of the visual function: Results indicate a significant loss of contrast sensitivity functioning in exposed monkeys, particularly for higher frequency visual images, 11-14 years after cessation of exposure. The loss of contrast is permanent in adult primates exposed to chronic but moderate	(Burbacher *et al.* 2005)

keys (*Macaca fascicularis)*		exposed group: n=12		years old.	dose MeHg during gestation but clinically normal at birth. This finding indicates that *in utero* exposure to MeHg can have **irreversible** effects on sensory functioning long after cessation of exposure.	
Sprague Dawley female rats	Intragastric intubation at 1ml/kg bw	Control: n=8; Group 1: n=8, 4 mg/kg. Group 2: n=8, 8 mg/kg.		On gestational day (GD) 15	At 4 mg/kg MeHg, there is an increase in the neuronal vulnerability in the cerebral cortex, and a **reduction of cell viability** and **apoptotic cell death**. At 8 mg/kg MeHg, necrotic death of cortical neurons and degeneration of neuritic processes was observed. This dose level caused a significant deficit in the retention performance in a memory task. MeHg effects are dose dependent and hight MeHg levels impaired the developing brain with consequent permanent behavioral dysfunctions.	(Ferrar o *et al.* 2009)
OLA 129/ C57 BL/6 strain mice (wild type) and MT-Null (knockout) mice	Orally by diet: 5 µg/g during gestation, starting at GD0 through 10 days after delivery at PND10	N=6-7 per group Group of wild type mice at 12 weeks and at 52 weeks of age Group of MT null mice at 12 weeks and at 52 weeks of age			This study included metallothionein (MT) knockout mice because studies have suggested the potential susceptibility of this strain to the **neurodevelopmental toxicity of MeHg.** **Open Field locomotor activity (OPF)**: At **12 weeks** of age, a significant ($p<0.001$, ANOVA) longer distance was traveled by the MT-Null mice compared to the wild type mice. Strain was also a significant factor for the proportion of the central area locomotion: this was higher in MT-Null females exposed to MeHg than in the control (no observed in the other strain mice). At **52 weeks** of age, the strain * Hg interaction was highly significant ($p<0.001$) in an ANOVA of locomotion distance; MeHg exposure was associated	(Yoshida *et al.* 2008)

					with decreased locomotion distance in wild type mice and with increased distance in MT-null mice. A strain-wise two-way ANOVA (with sex and Hg as the factors) revealed that only Hg was significant in both strains ($p<0.01$). **PA**: At 12 weeks of age, no consistent effect of MeHg was observed regardless strain or sex. At 52 weeks of age, a significant effect of MeHg between both strains on learning in MT null mice was observed: this group showed significantly shorter latency times compared to control mice. **Morris Water Maze (MWM):** no effects of MeHg at 13 weeks. At 52 weeks of age, the both strains showed a longer latency, hampering learning performance. The most important observation of the study was that the effects of low-level MeHg exposure were detected only at later stages in the lives of the mice. Except for the central area occupancy in **OPF** in **MT-null females**, no statistically significant effects of MeHg were observed in any of the three behavioral tests (OPF, PA, MWM) at 12 weeks of age. In contrast, **significant effects** were observed in **all three tests** at **52 weeks** of age.	
Mouse : ARE-hPAP transgenic mice backcrosse	Orally, via drinking water	0.5 mg/kg/day (0.47 mg/kg/day) of MeHg n=6-8 per group Continuing exposure		From GD7 until day 7 after delivery Two sessions of experiments: 1)5-15 weeks old (=youngs) 2)26-36	No behavioral changes are observed in **female** offspring exposed to MeHg during development. Results on male offspring behavior: **Locomotor activity** (spontaneous locomotion): no differences in distance covered over 1 h at both ages (data not shown).	(Onishchenko *et al.* 2007)

d to C57 BL/6 /Bkl				weeks old (=adults)	**Motor coordination** (accelerating rotarod tests): not affected at both ages by MeHg exposure ($F_{1.12}$ = 0.287; p = 0.60 and $F_{1.12}$ = 0.066; p = 0.80, repeated measures ANOVA for young and adult mice, respectively). **Behavioral parameters studied in the IntelliCage:** in the young animals, the average latency to first visit into the corner chamber was **three fold longer time** (9.7 ± 1.9 s) for the MeHg exposed mice and 3.3 ± 0.9 s for the control animals. Less visits for the **MeHg exposed mice** over the first 30 min period after introduction in the intellicage (11.6 ± 0.6 and 15.0 ±1.7 for MeHg exposed and controls, respectively; $F_{1.12}$ = 4.75; p < 0.05, one-way ANOVA). Less activity in the young exposed animals during "sunset" and "night". Lower number of visits in the dark period in the young **MeHg exposed** group than in the control group both in the new environment ($F_{1.12}$ = 6.8; p < 0.05, repeated measures ANOVA) and the familiar home environment ($F_{1.12}$ = 5.0; p < 0.05, repeated measures ANOVA). Difference in the adults group in exploratory behavior when the environment was new but no difference when the environment was familiar. **Spatial learning** (Morris Water Maze): No significant difference between treated or control groups (in escape latency or swim length). **Depression-like behavior** (forced swimming test): significant longer immobility time of both ages than control animals ($F_{1.11}$ = 8.336; p < 0.05 and $F_{1.11}$ = 4.991; p <0.05, one-way ANOVA for young and adults animals, respectively).	

Adult male and female C57 BL/6 +/+ wild type mice	Orally daily dose of 0.01 mg/kg bw of methylmercury (95% of methylmercuric chloride into water) (total dose of 0.11 mg/kg)	For motor and coordination tests: n = 13 males and n = 8 females for the control group; n = 15 males and n = 4 females for the exposed group; For the spatial learning assessment: n = 9 males and n = 8 females control and idem for the control group. Pups exposed to the substance from GD8 to GD18 for 11 days For motor and coordination tests: Start at 1 month of age. For the spatial learning test: start at 6 months of age.			Offspring weaned at 30 days of age and coordination and activity testing commenced at 2 months of age. **Sex difference:** in the **open field**, females reared less than males. No other sex effects were observed in the other tests. In the **footprint** analysis, MeHg exposed mice demonstrated a significantly narrower foot angle in comparison to control mice ($F_{1.36}$=10.66, P<0.005). No main effect of exposure was observed on the distance between the two hind feet or stride length. In the **rotarod task**, all animals increased time spent on the rotarod across days (two factors ANOVA: exposure*day) ($F_{2.74}$=15.45, P<0.0001) but there was a main effect of exposure such that MeHg exposed mice spent significantly less time on the rotarod than the control mice ($F_{1.37}$=8.72, P<0.01). When analyzing the entire 30 min session, the effects of MeHg on behavior were no significant. In the **open field task**, analyses of the initial 10 minutes revealed a significant main effect of exposure on each performance measure, such that the exposed mice moved less overall (rearing: $F_{1.36}$=10.45, P<0.01; total distance traveled: $F_{1.36}$=8.07, p<0.01) and spent less time in the center of the quadrant than did controls ($F_{1.36}$=8.862, P<0.01). Only the **Morris water maze** task commenced at 6 months of age. Swim speed did not differ between groups across days (cue training: $F_{1.32}$=0.19, p>0.05; place training: $F_{1.30}$=1.16, p>0.05). Analyses of place training trials across days revealed that all mice improved	(Montgomery *et al.* 2008)

					with respect to spatial learning over the course of training ($F_{5.150}$=9.026, P<0.001) but there was a main effect of exposure such that MeHg exposed mice were impaired relative to control mice ($F_{1.32}$=4.52, P<0.05). Time spent in target quadrant were significantly increased for the both groups during the 30 seconds interpolated probe trials ($F_{2.64}$=3.753, P<0.05) but on the last 10s of the 30s probe trials, MeHg exposed mice demonstrated less accurate searches for platform over the duration of the probe trials than controls ($F_{1.30}$=6.522; P<0.05). Despite the low level exposure used here, gait, assessed by footprinting and the ability to maintain balance on a rotarod were both significantly impaired in MeHg exposed mice relative to controls. The current results of the morris water maze task show that low level prenatal MeHg exposure impairs the ability to learn and recall the spatial location of a hidden platform in adult mouse offspring.	
Mice of the C57 BL/6 strain	Orally, in palatable food, methyl mercury chloride (MeHgCl)	N=20 in the chronic group N=20 in the bolus group Chronic condition 1.4 µg/g (bw/day) from GD1 to 18. Bolus condition 0.85 µg/g bw/day plus bolus dose of 6 µg/g bw/day on all days except GD12 and GD16			**Locomotor activity in the open field**: overall, the results yielded a modest indication that the chronic group had slightly decreased vertical movement on the last 2 days of testing but no other measure in that test further supported an impact of methylmercury. **Motor coordination**: the most consistent effect is shown in the climbing test: the time taken to climb to the top of the apparatus was longer for the both experimental groups than the control ($F_{2.55}$=4.24; P<0.001). The other motor coordination tasks did not significantly show effects	(Liang *et al.* 2009)

					of MeHg. **Emotional reactivity:** Emergence: no significant effect. (measure of anxiety) **Elevated plus maze:** no significant effect but a tendency for the chronic group to show increased anxiety (increased time spent in the closed arm). In the **radial arm maze**, both groups had impaired spatial learning abilities: there was a group effect in the number of errors made by animals ($F_{2.55}$=4.27; P<0.02). Post-hoc tests revealed significant difference between the chronic and the control group. A similar trend was observed for the bolus group but that difference was not significant because these animals performed as well or better than the control group on 10 of 20 days of testing. The second measure was the time to complete the maze: there was a group effect ($F_{2.55}$=10.2; P<0.001) which indicated that overall both experimental groups took longer than the control group to complete the task. Finally both experimental groups had impaired spatial learning abilities in the radial maze while learning of an operant task was not impaired. Taken together, the results generally revealed that **chronic group** produced the **largest impairments** in **all tasks** while the performance of bolus group was closer but not equivalent to the control group.	

4.11.2.2 Human information

Data are available for the effects on development in children exposed in utero during massive exposures of the mother or during lower exposures linked to a monotone diet (consumption of fish). Investigations of the possible neurodevelopmental effects of prenatal exposure to methylmercury are case series of children who manifested clinical signs of poisoning (Japan and Iraq) and then cohort studies of asymptomatic children considered to have 'low' exposure or at least exposure lower than that at which clinical signs and symptoms appear. The populations chosen were mostly those known to consume large amounts of fish, which contain variable amounts of methylmercury (Faroe Island, Seychelles…). When it is not specified, the concentrations of mercury in hair are expressed in total mercury.

Studies presented in the TC C&L dossier:

Effects on development in children during massive exposure (poisoning cases)	

Poisoning by seafood in Minamata (JAPAN) because of pollution of sea by a chemical plant. Children born from women living in Minamata at the period of poisoning by seafood suffered mental retardation (100% of children), primitive reflex (100%), cerebellar ataxia (100%), disturbances in physical development and nutrition (100%), dysarthria (100%), deformity of the limbs (100%), hyperkinesia (95%), hypersalivation (95%), paroxysmal symptoms (82%), strabismus (77%) and pyramidal symptoms (pathological reflexes) (75%). Cerebral palsy was abnormally high. Mothers were first declared asymptomatic but subsequent examination showed mild symptoms of Minamata disease: sensory disturbances (100% of mothers), focal cramps (100%), mild ataxia (79%), auditory disturbances (75%), pain in the limbs (64%), constriction of visual field (57%), dysarthria (43%), tremor (39%). The degree of the symptoms was less severe than in their children. Mercury level in mothers' hair was analysed from 5 to 8 years after the birth : concentrations ranged from 1.82 ppm to 191 ppm while that of congenital patients ranged from 5.25 ppm to 110 ppm. Mercury level was measured in umbilical cord. In most cases, those whose MeHg content was 1.0 ppm or higher suffered from congenital Minamata disease but some with lower mercury levels were also congenital patients. The pathological findings of congenital Minamata disease are generally atrophy and hypoplasia of the brain cortex and abnormality of the cytoarchitecture, remaining matrix cells, hypoplasia of the corpus callosum, intramedullary preservation of the nerve cells, and dysmyelinisation of the pyramidal tract. In the cerebellum, hypoplasia of the granular cell layer and other layers as well as degeneration of granular cells (characteristic of Minamata disease) were also observed. The follow-up study revealed that children who first experienced mild symptoms had some symptoms alleviated : paroxysmal symptoms (attacks) decreased from 82% to 27%, salivation from 100% to 45%, primitive reflex and abnormal limb posture from 100% to 51%, and incoordination (ataxia) from 100% to 60%. However, dysarthria and intelligence disturbances were left unimproved and subjective signs that had not been previously observed such as headache, insomnia, arthrialgia, numbness, dizziness, and anorexia increased. 89 mothers living in the most contaminated area were interviewed later: in 272 pregnancies, there were 32 miscarriages, 9 stillbirths, 4 deaths within 1 week of birth, and 4 infants officially diagnosed with congenital Minamata disease. **Even among patients who had been already diagnosed with Minamata congenital disease, polydactalia, high palate, defective external acoustic meatus, microcomea and protrusion of the coccyx were observed.** **In a male child, polydactalia, syndactalia, an undescended right testis and an enlarged colon were found in addition of mental retardation. However, there were no remarkable neurological symptoms except clumsiness in movement. His parents had suffered from Minamata disease and 2.42 ppm MeHg in his umbilical cord were detected.** **In children born from parents with chronic Minamata disease, some orthostatic deregulation (24.6% of children) and disorders in bender's gestalt test (14.2%).**	Harada, 1995
Case report of a child exposed in utero following the consumption by his mother of meat contaminated with methylmercury. At the time, the mother began to ingest contaminated meat, she was 3 months pregnant. She ate no more contaminated meat after 6 months of pregnancy. Examination during the 7th month of pregnancy was within normal, neurologic findings and visual fields were normal. The urinary mercury levels were high during the 7th (0.06 ppm) and the 8th (0.18 ppm) months of pregnancy. The mother never suffered with symptoms. A 3 062 g male infant was delivered at term. **At 1 minute of life, he became dusky, gross tremulous movements of the extremities developed. These movements persisted for several days. The cry was weak and high pitched. In other respects, the child appeared normal on general and neurologic examinations. In the neonatal period, urinary mercury**	Snyder, 1971 Pierce *et al*, 1972

levels were high (2.7 ppm at 1 day of age, 2.0 at 4 days of age), decreasing to less than 0.01 ppm at 6 weeks of age and to less than 0.0005 ppm at 3 months of age. Blood electrolytes, glucose, calcium, magnesium and bilirubin were normal. **When examined at 6 weeks of age, the child was very irritable, with a high-pitched, weak cry, increased tone in the extremities and cortical thumb posturing.** An electroencephalogram was within the range of normal variation for age at the two examinations. Electromyography performed at 3 days and 6 weeks of age showed normal conduction velocities for age and normal muscle-action potentials. **At 6 months of age, the encephalogram was abnormal with the widespread occurrence of spike activity more abundant in the left central and parietal regions. Electromyography performed at 3 months of age remained normal for conduction velocities age and normal muscle-action potentials were observed. By 6 months of age, generalised myoclonic jerks developed and the electroencephalogram was markedly abnormal, with paroxysmal high-voltage spikes, polyspike and spike and slow-wave patterns. This activity was more marked over the right hemisphere but occurred bilaterally. At 8 months of age, the infant was hypotonic and irritable and had nystagmoid eye movements without evidence of visual fixation. The child remained blind and unable to sit up.** He was never breast fed. During his first 6 months, he was only fed with commercially prepared products.	
Relationships between maternal exposure to methylmercury and a possible impact on child development (IRAQ) Prenatal exposure to **methyl mercury** occurred during a large epidemic of methyl mercury poisoning in Iraq, resulting from ingestion of home-made bread prepared from wheat treated with a **methylmercury fungicide**. Body content would be expected to rise to a maximum at the end of approximately a two-months period of consumption. Thereafter, it should undergo an exponential decline to pre-exposure values at the end of one year, assuming a biological half-time in the adult human of 70 days. Thus, possibilities existed for prenatal foetal exposure, and for post-natal exposure of suckling infants due to transmission of methylmercury in their mother's milk. Infants born during the 12-month period immediately prior to the epidemic could have received methylmercury from maternal milk ingestion. Infants approximately 12 to 18 months of age at the time of epidemic might have also ingested contaminated bread. Infants born during and shortly after maternal consumption of contaminated bread received maximal exposure late in gestation and had a relatively large postnatal intake from milk. Infants born six to nine months after maternal consumption of methylmercury were maximally exposed early in pregnancy and received minimal intake from milk ingestion. In this study, all infants were exposed prenatally. C15 pairs mother-infant were studied from one month after cessation of ingestion of contaminated bread by mothers to 11 months later. Six of 15 mothers complained from symptoms linked to methylmercury toxicity. The most frequent symptoms were malaise, vague muscle and joint pains and loss of sensation in the perioral region and in the extremities. Motor weakness and exaggerated reflexes were observed in 5 cases. Visual changes (constriction fields, blurred vision, dimness) were reported in 4 cases. Ataxia, auditory changes and dysarthria also occurred. One woman whose child was severely affected refused examination but claimed to be quite well. Infants having blood levels in excess of 3,000 ppb were severely affected. One infant having a blood level of 1,053 ppb when examined was also severely affected. The lowest blood level associated with signs of poisoning was 564 ppb. Generally mercury blood levels are higher in children than in their mothers when they simultaneously have their blood collected. At least 6 of the 15 children had clinical evidence of poisoning. In the five infants severely affected, there was evidence of gross impairment of motor and mental development, with cerebral palsy, deafness and blindness in four. **Three of infants had microencephaly at an early age. In the 15 infant-mother pairs, only one pair was noted in which the infant had signs of poisoning and the mother claimed to be free of**	Amin-Zaki *et al*, 1974

any symptoms or signs of methylmercury poisoning.	
Longitudinal study (5 years) of children born just before the poisoning of farmers in Iraq following the contamination of bread by seeds which had been covered with a fungicide containing methylmercury (IRAQ). Those children were breast fed and were exposed via their mother's milk. 30 pairs mother-child were included in the study, 2 children died during it. Standard clinical tests were made to assess the general clinical state of the mothers and the children with special attention on the central nervous system functions. Vision and audition were assessed. The developmental milestones of the infants were evaluated according to Gesell's developmental screening tests. Motor development was considered to be delayed when the infant failed to sit without support by the age of 12 months, to pull himself to standing position by 18 months, or to walk 2 steps without support at the age of 2 years. Language development was considered to be delayed when, at the age of 2 years, a child with good hearing failed to respond to simple verbal communication. The 11 males and 19 females ranged from 1 month to 10 months at the time of exposure. **Abnormal neurological signs become more obvious with time. Hyperreflexia was found in only 8 of the 22 infants at the first examination, in 17 of the 22 infants at the second examination and in 16 of the 21 infants by the 5 year. The finding of an extensor Babinski's reflex was also higher in the second (8 patients) and third (7 patients) examinations than in the first examination (4 patients). In 5 children, walking was delayed beyond the age of 2 years. Hearing was normal but language development was delayed. The frequency of the children with impaired intelligence is 1/6 a frequency high enough to be noteworthy. The combination of delayed motor development, language development, language development, toilet training together with brisk deep tendon reflexes and sometimes a bilaterally positive Babinski's reflex was considered evidence of damage to the central nervous system.**	Amin-zaki *et al*, 1979; Amin-Zaki and Majeed, 1981
Mercury was measured in hair samples of mothers of children exposed to mercury during gestation to assess the level of exposure during the foetal period **(IRAQ).** Mothers from Iraq were exposed to methylmercury because of the consumption of bread made with seeds treated with a based methylmercury pesticide. The clinical evaluation of children was conducted via a questionnaire applied to the mother and the grandmother of the child to assess birth weights, the age at which the child was able to sit safe without help, to stand and walk unaided, and speak two or three meaningful words. Other questions concerned observations of involuntary movements, seizures, impaired vision or hearing, incoordination and the mother's overall impression of whether the child's physical and mental development had been normal. The physical examination of the child included observation, measurement of head circumference and body length, cranial nerve signs, speech, involuntary movements, limb tone, strength, deep tendon reflexes, plantar responses, co-ordination, dexterity, primitive reflexes, sensation, posture, and ability to sit, stand, walk and run. The clinical evaluation was made at home in the villages of rural areas of the country. The exact date of birth of children was not known but the month of birth could be assessed. 81 mother-infant pairs were studied. **Hair mercury concentrations of mothers ranged 1-674 ppm** (22 mothers having concentrations ranging from 154 to 674 ppm). Larger effects were seen in boys than in girls. **7 of the 28 most highly exposed children were reported to have seizures; none in the 53 less exposed children. All children with seizures had features of retarded early development. This was a dose-related effect. 4 children had very severe psychomotor retardation. All were in the highest exposure group.** A boy whose mother was contaminated during the third trimester of pregnancy experienced numbness and weakness of arms and legs for several weeks. These resolved and he had no persisting symptoms. The maximum hair mercury concentration of the mother during gestation was 404 ppm. Labour and home delivery were uneventful. The baby appeared to be normal at birth and was breast-fed by his mother. At 5 months, he began to have	Marsh *et al*, 1987

generalised convulsive seizures, which the occurred about once every three months but were not treated. When first seen, he was aged 3 years 1 month. The mother reported that he was severely retarded physically and mentally. He made sounds but spoke no words. He could not sit without support, stand or crawl. His head circumference was 45.8 cm, his length 75 cm. The pupils were equal and reacted well and the fundi appeared normal. He had alternative strabismus. There appeared to be deafness, even if he reacted to loud noises. There were constant athetoids movements of the arms. Limb tone was greatly increased, especially in the legs, where there were also adductor spasms. All deep tendon reflexes were increased. There was sustained ankle clonus, and the plantar responses were extensor. All limbs were weak. He could not hold his head erect. There were grasp reflexes and increased jaw reflexes. The main features were spastic quadriparesis, severe speech and mental retardation, some athetosis, and convulsive disorder. When re-evaluated at age of 5 years 9 months, there was no change.

The second case is a girl whose mother had transient numbness of limbs and headaches during pregnancy. There were no problems during labour or delivery. The maximum mercury hair concentration was 405 ppm. The baby was described as small and floppy. She was breast-fed by her mother who said that the child was mentally retarded, did not sit without support until 3 years of age, and began to talk at that time but her speech was extremely slow. There were no seizures. When first examined, she was 2 years 2 months old. Her head circumference was 45 cm, her body length, 76 cm. The pupils and fundi appeared normal and there were no cranial-nerve signs apart from deafness. She was unable to stand or walk, and had no speech. Tone was increased in the limbs and decreased in the trunk. The deep tendon reflexes were increased, with ankle clonus, and extensor plantar responses, dystonic posturing of the hands and ataxia in the arms. When reexaminated at age of 4 years 9 months, she walked with a wide-based ataxic gait. The deep tendon reflexes were normal. The right plantar response was extensor, the left flexor. Her speech was restricted to a few words. The other signs were unchanged. At age of 6 years 9 months, her head circumference was 49.5 cm, her height, 1.03 m. She exhibited no cranial-nerve signs. She appeared to be mentally retarded with little speech. Her gait was slow, spastic and ataxic and accompanied by prominent athetoid movements in the arms. There was no weakness, deep tendon reflexes were normal and plantars extensor. She had no sensory deficit. The main features were mental and speech retardation, ataxia and athetosis.

The third case is a boy whose mother was asymptomatic during pregnancy. The maximum mercury hair concentration was 418 ppm. The labour and delivery were normal. He appeared normal at birth but the first seizure occurred 7 days later and was followed by other seizures. When first seen at the age of 2 years 2 months, the mother stated that he was mentally retarded, could not sit or stand without support and had no speech. His head circumference was 43.5 cm, his body length, 78 cm. There were no cranial-nerve signs, apart strabismus. The posture was opisthotonic with adductor spasms. Deep tendon reflexes were increased and plantar responses extensor. At age 4 years 4 months, he remained unable to stand or walk and had no speech. The previous signs remained unchanged. His head circumference was 43 cm, his body length, 87 cm. The main features were mental and speech retardation, spasticity and convulsive disorder.

The last case is a boy whose mother was asymptomatic during pregnancy. The maximum mercury hair concentration was 443 ppm. The labour and delivery were normal. He appeared "very small" at birth. When seen at age of 3 years 1 month, he was described as normal by his mother. He spoke 2 or 3 words. There had been no seizures. He had just started walking and walked with a wide-based ataxic gait with hyperextension of the legs. There were no cranial-nerve signs apart from deafness. Limb tone and reflexes were decreased. Plantar reflexes were extensor. When seen 2 years later, his speech was dysarthric. There was ataxia of gait and limbs. His hearing appeared to be normal and there were no cranial-nerve signs. He exhibited

no weakness. Limb tone was mildly decreased. Plantar responses were extensor. The main features were mental and speech retardation, dysarthria and severe ataxia. Other children with less disabling signs had an upper motor neurone syndrome with increased tone, reflexes, extensor plantar responses, and developmental delay. The least affected children had a history of being slow in walking and/or talking with no definite neurological signs.	

Effects on development in children during lower exposures	
<u>Studies presented in the TC C&L dossier:</u>	
Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal ingestion: outcomes at 19 and 29 months (SEYCHELLES). The cohort consisted of 738 mother-child pairs at 19 months and 736 pairs at 29 months, representing 94% of the cohort of 779 pairs initially enrolled, and approximately 50% of all births in 1989. Infant intelligence was measured by the Bayley Scales of Infant Development (BSID) Mental and Psychomotor Scales. Both scales were given in Creole to each child at each session. To measure adaptive behaviours, a modified version of the BSID behaviour Record was completed at the 29 months session by the examiner administering the tests. All primary care-givers (defined as the family member in whose home the child spent more than five nights a week) were interviewed to obtain an interim health history and to ascertain family socioeconomical data. At the 19-month session, the Raven Standard Progressive Matrices (Raven, 1958) (a standard intelligence test) was administered to the primary caregiver. Prenatal exposure was assessed by measurement of the concentration of total mercury in a segment of maternal hair representing growth during pregnancy. Samples of 21 species of oceanic fish found in Seychelles were analysed for inorganic and methylmercury. The median prenatal mercury exposure of the cohort was 5.9 ppm (range = 0.5 – 26.7 ppm) in maternal hair. Cognitive developmental outcomes up to 2.5 years of age appear essentially normal following intrauterine exposure to a maternal hair mercury level of about 6 ppm through maternal fish consumption. **This cohort performed cognitive, perceptual, memory, motor, and language tasks as well as US toddlers. One functional behaviour (the examiner's subjective rating of the child's test session activity level) was related to maternal hair mercury levels in the mothers of male children: activity level decreased as maternal hair mercury level increased (p = 0.007). This outcome might represent a subtle influence of mercury on behaviour without detectable residual effects on cognition.**	Davidson *et al*, 1995
The cohort consisted of 711 mother-child pairs living in the Republic of Seychelles, representing 91 % of the 779 pairs originally included in the Seychellois **child development study (SEYCHELLES).** Each child was evaluated at 66 months (± 6 months). The test battery included : the General Cognitive Index of the McCarthy Scales of Childrens' Abilities to estimate cognitive ability; the Preschool Language Scale total score to measure both expressive and receptive language ability; the letter and Word Recognition and the Applied Problems subtests of the Woodcock-Johnson Tests of Achievement to measure reading and arithmetic achievement; the Bender Gestalt test to measure visual-spatial ability; and the total T-score from the child Behaviour checklist to measure the child's social and adaptive behaviour (this questionnaire was given to each child's primary caregiver). All tests were given in Creole. Pure tone hearing thresholds were tested using a portable audiometer. Caregiver's IQ was measured to minimise the	Davidson *et al*, 1998

effects of culture on the measurement of the child's IQ. When the children were between 42 and 56 months of age, the Home Observation for Measurement of the Environment Inventory for Families and Preschool Age children was administered during home visits. Prenatal exposure to mercury was assessed by measuring the concentration of total mercury in a segment of maternal hair representing growth during pregnancy. PCBs were also measured in serum of 49 children at the age of 66 months. Mercury in fish was measured in 350 samples. The median concentration for each of the 25 species ranged from 0.004 ppm to 0.75 ppm, with most medians in the range of 0.05 to 0.25 ppm. The mean maternal hair mercury level during pregnancy was 6.8 (SD = 4.5; range = 0.5 – 26.7 ppm) ppm (n = 711) **and the mean child hair** level at 66 months was 6.5 (SD = 3.3; range = 0.9 – 25.8 ppm) ppm (n = 708). No test indicated a deleterious effect of methylmercury exposure. **Four of the six measures showed better scores in the highest methyl mercury groups compared with lower groups for both prenatal and postnatal exposure. As mercury exposure is closely linked to consumption of fish, it seems that children with the highest exposure to mercury have also a best dietary pattern than other children which could explain why methylmercury exposure seems to result in better developmental outcomes in children.**	
Children of the Seychelles cohort (n = 738). At the 19 month examination, a questionnaire was applied to the child's parent or caregiver present during the tests to assess the age of walking without support and the age at which the child began to speak using words other than "mamma" or "dada" (SEYCHELLES). The measure of exposure during pregnancy was the mean of the total mercury concentration in segments of hair of the mother representing growth of the hair during the pregnancy. **Walking appeared at a later age as exposure increased in the range from 0 to 7 ppm (especially in male children) but appeared slightly earlier for exposure above 7 ppm which makes difficult to conclude to a cause and effect relationship. No influence of the level of exposure to mercury was seen concerning the age of talking.**	Axtell *et al*, 1998

Relationships between a diet rich in fish and possible impact on child development were studied (SEYCHELLES). The cohort consisted of 779 children enrolled at the age of 6.5 months. Of these, 711 were available for the 66-months test battery. Prenatal exposure was measured in a segment of **maternal hair** corresponding to pregnancy. Postnatal exposure was measured in a scalp hair sample from each child at the time of the 66-month evaluation. The children's neurological and developmental status has been evaluated at 6.5, 19, 29 and 66 months of age using standard methods of assessment. The battery yielded to a total of six primary endpoints : the General Cognitive Index (GCI) of the McCarthy Scales of children's ability, the Preschool Language Scale Total Score (PLS) to measure receptive and expressive language, the Letter and word Recognition and the Applied Problems subtests of the Woodcock-Johnson Tests of Achievement to document reading and math readiness, the total error score from the Bender Gestalt test to estimate visual-spatial ability, and the total T score from the Child Behaviour checklist (CBCL) measuring social and adaptive behaviour. For all tests except the Bender and CBCL, an increase in the score is associated with an improvement in performance on the test. All tests were given in Creole, the language spoken in over 98% of Seychellois homes. For the PLS, the trend involved a decline of 0.8 points between 0 and 10 ppm followed by an increase (representing improvement) of 1.3 points above 10 ppm. For the CBCL, there was an increase of 1 point from 0 to 15 ppm and then a decline (improvement) of 4 points above 15 ppm. The GCI increased by 1.8 points through 10 ppm and then declined 3.2 points (representing worse performance) above 10 ppm. In every case, the trend changes direction so that an effect in one direction is followed by a trend in the opposite direction. **Overall, there was no clear evidence for consistent (across the entire range of exposure levels) adverse effects of exposure on the six developmental outcomes.** Authors think that apparent beneficial effects of exposure could be linked to the association of exposure to mercury and the nutritional benefits of fish consumption.	Axtell *et al*, 2000
Relationships between maternal exposure to methylmercury and a possible impact on child development (SEYCHELLES). The population under investigation consisted in 87 children from a pilot cohort living in the Republic of Seychelles. **These children were exposed to mercury during their foetal life because of the maternal diet based on seafood and during the rest of their life because of their own diet.** They reached the age of 108 months ± 6 months. Prenatal exposure was assessed by measurement of total mercury in a segment of maternal hair representing growth during pregnancy. 23 children had maternal hair levels ≤ 3 ppm, 23 between 4 and 8 ppm, and 41 children ≥ 9 ppm. 55 % of the children were male. Each child was administered a battery of tests assessing specific cognitive, visual motor and motor skills (13 subtests of the Wechsler Intelligence Test for Children- III, the California Verbal Learning test, The Boston naming test (BNT), the Beery-Buktenica Developmental Test of Visual Motor integration, the Design memory subtest of the Wide Range Assessment of memory and Learning, the Grooved Pegboard, the Trail making test, The Finger Trapping Test). Each test was standardised on western populations representing a wide range of socio-economic and cultural variation. All tests were translated in Creole, the language spoken at home by 98 % of the Seychellois children. The outcomes of this study show enhancement of performances on a number of neuropsychological tests associated with increasing prenatal exposure to methyl mercury in the range of exposures studied. There was a significant difference between gender with the Grooved pegboard and the test of Visual integration, with scores generally improved as exposure increases. **Only one test, the Grooved Pegboard, showed decreasing performance associated with increasing prenatal methyl mercury exposure in females. A secondary analysis including both prenatal and postnatal exposures showed evidence of only one adverse association between postnatal methylmercury exposure and the California Verbal Learning test short delay**	Davidson *et al*, 2000

subtest.	
The aim of this study is to identify adverse neurodevelopmental effects in a fish consuming population (SEYCHELLES) (an average of 12 meals per week). In 1989-90, 779 mother-child pairs were enrolled (about 50% of live births during this period. 643 children were investigated at the age of nine. **Prenatal methyl mercury exposure was determined from maternal hair** growing during pregnancy. Postnatal exposure was assessed by measurements of the mercury concentration of a 1cm hair segment closest to the scalp of the child at the age of 9. The mean prenatal total methylmercury exposure was 6.9 µg/g (SD 4.5). The mean postnatal hair concentration was 6.1 µg/g (SD 3.5). The correlation coefficient between prenatal and postnatal exposure was – 0.08 (p = 0.04). **Concentrations of total mercury in maternal** hair at delivery correlated highly with concentrations of mercury in brain samples taken at autopsy from Seychellois infants who died from natural causes. The mean age at testing was 107 months (SD 4). Individual tests measured intelligence (the Wechsler intelligence scale for children III full scale IQ); learning and achievement (the Woodcock-Johnson test of achievement, letter-word recognition, and applied problems subtests and the California verbal learning test); memory (the visual memory subtest of the wide-range assessment of memory and learning); motor functions (finger tapping, trailmaking, grooved pegboard, and most of the Bruininks-Oseretsky test of motor proficiency); language (Boston naming test); visual-motor integration (the Beery-Buktenica developmental test of visual motor integration and a test of haptic matching); and sustained attention (Connor's continuous performance test). Behaviour was assessed with the Connor's teacher rating scale and the parent-child behaviour checklist. Two of the 21 endpoints were associated with prenatal methylmercury exposure and developmental outcomes at 9 years of age. One association involved diminished performance (grooved pegboard non-dominant hand in males only) and the other an enhancement (hyperactivity index of the Connor's teacher rating scale). But according to the distribution of p values, authors conclude that both these outcomes are probably due to chance. **So authors conclude that the Seychelles Child development Study longitudinal assessments at 9 years of age indicate no detectable adverse effects in a population consuming large quantities of a wide variety of ocean fish.**	Myers *et al*, 2003
The aim of this study is to identify adverse neurodevelopmental effects in a fish (concentrated in methylmercury) consuming population (FAROE). A cohort of 1022 singleton births was assembled in the Faeroe islands during a 21-month period of 1986-1987. Mercury concentrations varied considerably. 15 percents of mothers had hair mercury concentrations above 10 µg/mercury whereas cord blood concentrations ranged up to 10 to 350 µg/l. However, obvious cases of congenital methylmercury poisoning were not found. Because the effects of foetal childhood exposure to methyl mercury are persistent, detailed examination of children with prenatal exposure to this neurotoxicant would be appropriate at school age. At this time, they have developed sufficiently to perform a wide variety of neurobehavioral tests, and they are able to cooperate for most functional tasks. 917 children were tested. Neuropsychological tests included Finger Tapping, Hand-eye co-ordination : reaction time on a Continuous Performance Test, Wechsler intelligence scale for children – revised digit Spans, Similarities and Block designs; Bender Visual Motor Gestalt Test; Boston Naming test and California Verbal learning test (children). Clinical examination and neurophysiological testing did not reveal any clear-cut mercury-related abnormalities. However, mercury-related neuropsychological dysfunctions were more pronounced in the domains of language, attention, and memory, and to a less extent, in visuospatial and motor functions. These associations remained after adjustment for covariates (especially PCB exposure) and after exclusion of children with mercury hair concentration above 10 µg/g. The effects on brain function associated with prenatal methylmercury exposure therefore appear widespread, and early dysfunction is detectable at exposure levels currently	Grandjean *et al*, 1997

considered as safe.	
Relationships between maternal exposure to methylmercury and a possible impact on child development (GUYANA). 156 children (and their 104 mothers) were examined in the Upper Maroni communities, 153 in Awala (115 mothers) and 69 in Camopi (51 mothers). Geometric means **for hair mercury** levels in children were 10.2 µg/g for children of Upper Maroni, 6.5 µg/g for children of Camopi and 1.4 µg/g for children of Awala. The geometric mean hair mercury levels for mothers were 12.7 µg/g for Upper Maroni, 6.7 µg/g for Camopi and 2.8 µg/g for Awala. No hair sample could be obtained from 39 mothers (14%) and 19 children (5%). There was no trend toward increasing mercury concentration with increasing age among children between 1 and 12 years old. 97 children between 9 months and 6 years old from the Upper Maroni Communities had neurological examination, 69 in Camopi and 82 in Awala. 103 children from 5 to 12 years old from the Upper Maroni Communities had neuropsychological examination and 103 in Awala. **There is an association between the level of exposure to mercury of the mother and the increased deep tendon reflexes, the poorer co-ordination of the legs, and a deficit in the Copying test score.** These associations held if the population is restricted to that in the exposed region but with a lower degree of significance, and they seemed to depend on the sex of the child. Although drawing errors in copying designs are part of normal development, frequent rotation errors after age 6 years are likely to result from insult in the parietal lobes of the brain.	Cordier *et al*, 2002
Relationships between mercury exposure during pregnancy and neurodevelopment among British child born in 1991-92 were studied. To evaluate their children, over 5000 mothers from the Avon Longitudinal Study of pregnancy and Childhood completed the Denver Developmental Screening test at 6 and 18 months after birth and the Mac Arthur Communicative Development Inventory at 15 months. Reported dental treatment and fish intake during pregnancy served as markers of exposure to mercury vapours and methylmercury respectively. Cord tissue mercury was measured for 1100 children. Total mercury levels were higher among those who had dental work and ate fish **but were not associated with developmental test scores**. Neither dental work nor fish intake was associated with decreased overall developmental test scores. Most developmental scores increased with fish intake (Mac Arthur : no fish = 123.5, fish < 1/week = 130.3, fish > 1/week = 129.8, trend $p < 0.02$).	Daniels *et al*, 2001
In Canada, a study of prenatal methylmercury exposure in 234 Cree Indian infants and children whose mean maternal hair level was 6 ppm showed that maternal exposure was related to abnormal muscle tone (deep tendon reflex) in male infants. In 73 children from New-Zealand whose maternal hair was above 6 ppm, early sensorimotor dysfunction was evidenced with the Denver Development Screening Test at the age of four. In 61 of these children involved in a subsequent study at the ages of 6-7 years, results showed that in children with poor score on the Denver development Screening Test at the age of 4, tended to have decreased scores on the WISC-R Intelligence test later in childhood. These neurobehavioral effects were associated with maternal blood methylmercury levels of only 20 to 80 ppb. **In Inuits whose source of contamination is the occasional consumption of highly contaminated whales, the foetal methyl mercury in cord blood averaged 80.2 ppb and the highest levels of exposures were related to decreased birth bodyweights of children.**	Gilbert and Grant-Webster, 1995
<u>New studies added in the present report:</u>	

Study performed on 780 children with mean blood MeHg concentrations of 0.5 µg/L that were enrolled in the Treatment of Lead (Pb)-exposed children clinical trial (TLC) with 396 children allocated to the succimer and 384 to the placebo groups. Almost all Hg in the blood (> 80%) was **methylmercury** (**MeHg**). The study examined the **postnatal** methylmercury exposure and cognition (IQ) and behaviour. The children's IQ and neurobehavioral were tested at the age 2, 5 and 7 years. There is a **weak**, **non-significant** but **consistently positive** association between **blood MeHg** and **IQ** tests scores in stratified spline regression and generalized linear model data analysis. The neuropsychological and behavioural test scores were **not significantly** associated with **MeHg** concentration. This study cannot determine a threshold above which postnatal MeHg exposure has detectable **neurological and developmental** consequences in children but we can conclude that at the present postnatal MeHg exposure level (**blood MeHg < 1µg/L**), adverse effects on children's cognition and behaviour were not detectable.	(Cao *et al.* 2010)
Study performed on 498 pregnant women which delivered on march 2004. Maternal hair levels less than 10 µg/g (median level of hair MeHg =1.96 µg/g). The results of the study indicated that prenatal exposure to methylmercury, even at low doses, at maternal hair levels of less than 10 µg/g (median level of hair THg = 1.96 µg/g, range of 0.29 to 9.35 µg/g) adversely affect neonatal neurobehavioral function (evaluated by the Neonatal Behavioral Assessment Scale, NBAS) in the 3 models (p=0.047 in model 1; p=0.01 in model 2; and p=0.01 in model 3) in a multiple regression analysis (negative relation to the motor cluster, Pearson product-moment correlation coefficients, hair THg (p=0.01)).	(Suzuki *et al.* 2010)

2000 US birth cohort. Analysis of the burden of mental retardation (MR) associated with **methylmercury** exposure emitted to the atmosphere by American electric generation facilities: it causes clinically significant mental retardation in hundreds of American babies born each year. The aggregate loss in cognition associated with MeHg exposure in the 2000 US birth cohort was estimated using two previously published dose-response models that relate increases in cord blood Hg concentrations (upper or equal in 3.5, 4.84, 5.8, 7.13, 15 µg/L) with decrements in IQ (losses of cognition of 0.22, 0.48, 1.39 IQ points respectively for the last three MeHg-concentrations). Downward shifts in IQ resulting from **prenatal exposure** to **MeHg** of anthropogenic origin are associated with 1566 excess cases of mental retardation annually, or 3.2% of MR cases in the US. After incorporating uncertainties in the relationship of IQ loss with increases in blood mercury levels and applying a conservative range of 1-1.7 for the true cord/maternal Hg ratio, between 376 and 14293 excess cases of MR, or 0.8% - 29.2% of MR cases in the US are associated with MeHg toxicity. After applying base-case assumptions and incorporating a 36% factor to specify the burden of anthropogenic MeHg exposure attributable to American sources, mercury emissions from American anthropogenic sources are associated with 564 cases of MR or 1.1% of MR cases in the US. After incorporating these assumptions, 68 – 5145 (0.1% - 10.5%) of MR cases in the US are associated with MeHg toxicity. After applying an additional fraction of 41% in this analysis to convert the burden of mental retardation attributable to all American emissions to the burden attributable to American electric power generation facilities, Hg from American power plants accounts for 231 cases of MR/year (range: 28-2109), 0.5% (range: 0.06%-4.3%) of all cases in the US.	(Trasande *et al.* 2006)
Study conducted on 300 mothers in the republic of Seychelles. Analysis of the **prenatal** effects of **methylmercury** exposure on child development from eating fish during pregnancy. The average prenatal MeHg exposure was **5.9 ppm** in maternal hair. 229 children were evaluated by the Bayley Scales of Infant Development-II (BSID-II) performed at 9 and 30 months of age, by the Psychomotor Developmental Index (PDI) score performed at 30 months. The primary analysis examined the associations between MeHg, maternal nutritional measures and children's scores on the BSID-II and showed an adverse association between MeHg and the mean PDI score at 30 months. Analyses of the association between the PDI score and only MeHg alone or nutritional factors alone showed only a borderline significant association between MeHg and the PDI at 30 months and no associations with nutritional factors.	(Davidson *et al.* 2008)

A cohort of 1022 births in the Faroe Islands. At age 14 years, 878 of 1010 living cohort members underwent detailed neurobehavioral examination. The same tests were performed at the age of 7. Exposure levels at age 14 years averaged about one-fifth of those experienced prenatally, although exposures at age 7 years were slightly higher. This study presents results on neuropsychological performance of adolescents with widely differing degrees of prenatal exposure to MeHg from maternal seafood diets during pregnancy and lower postnatal exposures to this neurotoxicant. Adverse effects were identified in regard to motor speed, attention and language. These findings are in accordance with the results obtained from examination of the same cohort members at age 7 years (Grandjean et al, 1997) and suggest that prenatal MeHg exposure is associated with enduring neurotoxic effects on CNS function.	Debes et al., 2006

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Effects on fertility

Animals
Numerous fertility animal studies show reproductive effects of methylmercury on several species, in both sexes (decreased mating success of male in rats and decreased sperm motility in monkeys and rats, sperm with abnormal head in rats, prolonged length of oestrous cycle of female mice and alteration of reproductive performance in mink, accumulation of mercury in seminiferous tubules in rats and in ovary in mice, decrease of plasma and serum testosterone in rats). Effects also occur at doses exerting no other toxic effects.

The old studies displayed contradictory results in terms of effects on fertility, wistar rats being the most responsive (Khera, 1973 (b)). The "new" studies performed on wistar rats (Ernst *et al.* 1991a; Moussa H. *et al.*, 2010; Fossato *et al.*, 2011, Vaccharajani, K.D., et al., 1993) but also in Pekin ducks (McNeil and Bhatnagar 1985), monkeys (Lee, I. **P.,** 1975) and mice (Mohamed et al. 1987) show that MeHgCl induce a decrease in testosterone biosynthesis, which affect spermatocytes formation.

Humans
A study describes effects of methylmercury on fertility, leading to subfertility in men (Dickman and al., 1999) but is not considered as sufficient to establish a clear causal link.

Another study (Choy et al., 2002) establishes a possible relationship between the infertility of couples in Hong-Kong and the high blood mercury level. The association between *in vivo* exposure to mercury and male infertility found in this study is biologically possible to be a causal relationship.

Effects on development

Animals

The studies on development show increase of embryonic lethality, decrease of fetus body weight and teratogenicity in rats (cleft palates, vertebral defects, histological abnormalities in the cerebellum, effects on lacrymal glands and ribs).
Neuro-behavioral effects are observed in mice (locomotion, memorisation), rats (locomotion, memorisation and hyperactivity) and monkeys (visual defects, impairment of the auditory function and of vibration sensitivity). In certain cases, adverse effects are unmasked by aging. The loss of contrast sensitivity functioning is persistent until 11-14 years after the cessation of the exposure (Burbacher, 2005) and also for the auditory function (Rice, 1998) and the vibration sensitivity (Rice, 1995) for which permanent impairments are observed after long term exposure, beginning during developmental period. These effects could be irreversible.
Besides, these effects appear even in absence of maternal toxicity in several studies.

The study of Onishchenko, designed for the chronic early life exposure of mice at low doses of MeHg (0.5 mg/kg/day), provide evidence for the long-lasting effects on learning ability and motivational behavior, with a persistent predisposition to depressive behavior.

These effects were observed only in male mice; the same gender-related difference is observed in the open field test of Montgomery. In contrast in the study of Yoshida, a sex-wise two-way ANOVA for the central area occupancy in OPF shows significant effects of MeHg only in MT-null females ($p<0.001$). Mechanisms underlying such gender-related differences are not clarified.

The long-lasting effects at low doses shown in the study of Onishchenko are consistent with the study of Yoshida which shows significant effects in the three neurobehavioral tests (OPF, PA, MWM) only at 52 weeks of age and not at 12 weeks. This is confirmed by the study of Liang: between two different lengths of treatments, the one chronic and the other acute treatment by bolus administration, the chronic group produced the largest impairments in all tasks while the performance of bolus group was closer but not equivalent to the control group.

The lack of effect on motor coordination of MeHg-exposed mice in the rotarod test (Onishchenko) is consistent with the study of Stringari *et al.*, 2006 (repeated subcutaneous exposure), showing that low doses of MeHg do not lead to gross motor deficit in mice. However, Montgomery showed that MeHg exposed mice spent significantly less time on the rotarod than the control mice, although in low doses administered during 11 days.

So, MeHg chronic exposure adversely affects mice at low doses (0.5 mg/kg/day) after exposure from gestational day 7 until day 7 after delivery, early in the life (at 5 to 36 weeks of age), with neurotoxic long-lasting effects on **motor coordination and neurobehavioral** (less intensive exploratory activity).

Humans
Effects of methylmercury are described on neurodevelopment: very severe effects appear in children exposed *in utero* during periods of poisoning via food (via bread in Iraq, via fish in Japan). Children are frequently deaf, blind, unable to sit or walk without help, unable to speak fluently. For children, the handicap seems to become more severe with aging. For other children, the handicap appears few months after birth. There is mental retardation, even when mothers experienced no or mild symptoms. So effects on neurodevelopment do not seem to be linked to maternal toxicity.

In cases of mothers who are exposed to lower doses of mercury via food but during a long period, several differences between the cohort studies may have contributed to the apparent discrepancy in the findings (absence of effect or no observed effect). The children were evaluated for neurobehavioural endpoints at different ages and with tests of different reliability. Moreover, the studies may also differ with regard to exposure to other factors that can affect the neurobehavioural development of children, and the intake patterns may have differed (more mercury eaten less frequently or less mercury consumed almost daily, nutritional status). Perhaps there is a dose dependent effect linked not to the total dose of mercury to which the foetus is exposed during the pregnancy but to peaks of concentrations of methylmercury that can occur during the pregnancy. That would explain why effects appear for massive exposures and for milder exposures but with peaks of concentrations of methylmercury in food.

All the studies performed on human tend to show the link between the MeHg exposure and the neurotoxic effects.

In the study of Cao et al., 2010, the association between blood MeHg and IQ tests scores is not significant but consistently positive although at low doses, effects on cognition and behavior were not significantly detectable.

The studies of Suzuki and Davidson show that even at low doses, prenatal exposure of MeHg adversely affects neurobehavioral functions. In the study of Trasande, the toxicity of MeHg is attributed to the atmosphere by American electric generation facilities, responsible for the mental retardation in prenatal-exposed babies.

Finally, Debes performed clinical studies on children of 7 and 14 years, showing adverse effects in regard to motor speed, attention and language.

Low doses of MeHg-exposure (prenatally or postnatally exposure) are responsible for neurotoxic effects on human, such as losses of points in IQ, mental retardation, attention deficit hyperactivity disorder and adverse effects on cognition, neurobehavioral and neuropsychological comportment. Besides, long-term exposure increases the risk of neurotoxic effects incidence, even at low doses.

Effects via lactation

In addition, taking into account the elements presented at chapter 4.7.2 (metabolism), methylmercury is eliminated in breast milk. The studies of Amin-Zaki et al. (1979, 1981) show neurological effects in breast-fed children (see chapter 4.1.3.2. and chapter 4.11.2.6). The concentration in breast-milk was up to 200 ng/g. This value is about 100 fold greater than the concentrations in milk of women from several non poisoned populations (see chapter 4.7.2).

4.11.5 Comparison with criteria

The CLP criteria for classification in **Repr.1A** are as follow:

"Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the

evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)."

Effect on fertility

Studies performed to show MeHg-effects on fertility in animals and humans are not sufficient to demonstrate a clear effect-causal link. Indeed, the studies performed on animals are not acceptable because of the choice of the species, the mink, in the study of Dansereau; because of the lack of reproducibility in the study of Khera and the study of Mohamed was performed with methyl mercury hydroxide. Finally, the study of Nobunaga is insufficient to prove the effect of MeHg on fertility. Moreover, the new studies added in the present dossier did not show this relationship more clearly and did not allow classifying Repr 1B in the CLP.

Effect on development

Based on animal studies, development is severely impacted in several species (rats, mice...).

The studies on development show teratogenicity in rats and neuro-behavioral effects in mice (locomotion, memorisation), rats (locomotion, memorisation and hyperactivity) and monkeys (visual defects).

Besides, epidemiological studies after exposure of children *in utero* show deleterious effects of methyl mercury on children neurodevelopment.

Classification in Repr.1A is appropriate as human data shows a causal relationship between *in utero* exposure to methylmercury and adverse effects on development.

A classification **Lact. Effects** – H362 is also required taking into account the possible poisoning of human populations (intake of methyl mercury by mothers could be toxic for the infants if they are strongly exposed via maternal milk).

4.11.6 Conclusions on classification and labelling

A classification **Repr.1A – H360Df** is proposed in the CLP regulation.

Effects via lactation
A classification Lact. Effects – H362 is proposed in the CLP regulation.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS presented a large number of studies dealing with the impairment of fertility or mammalian development after exposure to methylmercuric compounds. However, test substance designation in the CLH report is not consistent and the terms methylmercuric chloride (MMC or MeHgCl) and methylmercury ($MeHg^+$, which is an ion and can originate from other compounds such as methylmercuric hydroxide) or even mercury are often used interchangeably. Therefore, the rapporteurs checked the original publications for test compound details. Because a sufficient number of studies with methylmercuric chloride are available, animal studies with other mercury compounds are not considered for the

evaluation of the reproductive toxicity in this ODD.

Fertility and reproductive function

Studies in animals

Fertility studies using methylmercuric chloride reported by the DS date back to the 1970's and 1990's. They show reproductive effects of methylmercuric chloride on several species in both sexes (decreased mating success of male rats and decreased sperm motility in monkeys and rats, sperm with abnormal head in rats, and alteration of reproductive performance in mink, accumulation of mercury in seminiferous tubules in rats and in ovary in mice, decrease of plasma and serum testosterone in rats). Effects notably occurred at doses exerting no other toxic effects.

One study from the 1970's performed with mice and rats displayed contradictory results in terms of effects on fertility, Wistar rats being more responsive than mice. A study performed with mice for 48 days showed a dose-dependently prolonged oestrous cycle (11 und 27% in low and high dose groups, respectively), but also a significant effect on maternal body weight gain in the high dose group concurrent with lower numbers of implants, higher incidences of resorptions and higher numbers of dead embryos and foetuses.

Findings in humans

The DS presented one study which describes effects of methylmercury compounds on fertility, leading to subfertility in men, but considered it not sufficient to establish a clear causal link.

Another study establishes a possible relationship between the infertility of couples in Hong-Kong and a high blood mercury level, presumably through seafood [RAC notes that no concentrations were reported by the DS].

Overall, the DS concluded that data on the effects methylmercury compounds on fertility in animals and humans are not sufficient to demonstrate a clear causal link because of the choice of the species (mink), because of the lack of reproducibility and because of results insufficient to prove the effect of methylmercury compounds on fertility. The DS stated that the new studies added in the present dossier did not show this relationship more clearly and therefore do not allow for classification as Repr. 1B. The DS concluded that Repr. 2 is more appropriate.

Developmental toxicity

Studies in animals

The DS summarised that the studies on development show an increase of embryonic lethality, decrease of foetal body weight, and teratogenicity in rats (cleft palates, vertebral defects [RAC notes that cleft palates and vertebral variations were also reported in mice], histological abnormalities in the cerebellum, effects on lacrymal glands and ribs).

The DS reported observations on neurobehavioral effects in mice (locomotion, memorisation), rats (locomotion, memorisation and hyperactivity) and monkeys (visual defects, impairment of the auditory function and of vibration sensitivity).

In monkeys, the loss of auditory functioning was persistent until 11-14 years after the cessation of exposure. This was true also for the vibration sensitivity for which permanent impairments were observed after long term exposure, beginning during the developmental period.

In mice, a study involved two different treatment regimens, one called "chronic" and another with a lower chronic dose and additional two bolus doses, named "bolus" group. Both treatments resulted in a similar total amount of methylmercuric chloride administered *in utero*. The chronic group produced the largest behavioural impairments in all tasks.

Findings in humans

In summary, the DS described the effects of exposure to alkylmercury compounds on neurodevelopment in humans: severe effects appeared in children exposed *in utero* during periods of poisoning via food (via bread in Iraq, via fish in Japan). Children born to exposed mothers were frequently deaf, blind, unable to sit or walk without help, unable to speak fluently. There was mental retardation in infants, even when mothers experienced no or mild symptoms. Therefore, effects on neurodevelopment do not seem to be linked to maternal toxicity.

In a study conducted in the Seychelles, prenatal exposure to low doses of methylmercury compounds appeared to have adverse effects on neurobehavioral functions. Children in these studies were evaluated for neurobehavioural endpoints at different ages and with tests of different reliability. Moreover, the studies may also differ with regard to exposure levels and possible additional exposures to other neurotoxicants that can affect the neurobehavioural development of children, and the intake patterns may have differed.

These and other human studies show that low methylmercury exposures (prenatally or postnatally) produce neurotoxic effects in humans, such as losses of points in IQ, mental retardation, attention deficit hyperactivity disorder and adverse effects on cognition, and neuropsychological behaviour.

Overall, development was severely impacted in several species. Exposure of children during prenatal development showed deleterious effects of methylmercury on their neurodevelopment.

The DS concluded that human data show a causal relationship between *in utero* exposure to methylmercury and adverse effects on development and therefore proposed a classification Repr. Category 1A; H360Df.

Moreover, the DS proposed a classification for Lact. Effects; H362, taking into account the possible poisoning of human populations (intake of methylmercury by mothers could be toxic for the infants if they are exposed via maternal milk).

Comments received during public consultation

Three MS supported the classification as proposed by the DS. However, one of these questioned the reliability of the described studies and one mentioned the lack of clear evidence of reproductive toxicity of methylmercuric chloride in humans. Nevertheless, they supported the proposed classification since there is evidence of teratogenic potential of methylmercuric chloride in animals and neurodevelopmental effects in humans induced by organic mercury compounds. One MS noted that it is difficult to be sure if the substance should be assigned to Category Repr. 1B or 2 for fertility based on the information in the CLH report. This MS also supported classification for Lact. H362.

Additional key elements

Toxicokinetic considerations

Doses used in developmental toxicity studies differ considerably across species. To facilitate a comparison between rodents, non-human primates, and man a brief description of the major species differences in toxicokinetics of methylmercury compounds is provided here. The issue of extrapolation from animals to man including species differences in kinetics has been reviewed by Castoldi et al. in 2008.

Interspecies comparisons should consider the concentration at the target organ to bypass species-related differences in methylmercury kinetics. The latter include the red blood cell to plasma ratio (about 20 in humans, 10 in monkeys and mice, and as high as 300 in rats); the brain to blood distribution ratio (6.0 in humans, 2.6 in monkeys, 1.2 in mice and guinea pigs, 0.1 in rats). In developmental toxicity studies the phase of development during which the embryo or foetus is exposed is of major importance. Due to the long half-life of organic mercury compounds a single day treatment results in a prolonged exposure which must be taken into account when the results are interpreted. Half-life of methylmercury elimination in days is 45 to 70 in humans, 16 in rats, and 8 in mice. For example, rats must ingest 10-fold higher quantities of methylmercury compounds as compared to humans and non-human primates, to achieve similar brain mercury levels (Castoldi et al., 2008).

Concentration in human breast milk

Studies from Japan show that MeHg is transferred to breast milk (Iwai-Shimada et al. 2015; Sakamoto et al. 2002). About one fifth of the methylmercury amount measured in mothers' plasma was found in their milk. Mean concentrations measured in milk ranged from 0.21 to 0.45 ng/g. Therefore, breast fed children are exposed to methylmercury compounds.

Assessment and comparison with the classification criteria

Unless stated otherwise, all studies described below were sufficiently well conducted to merit inclusion in a weight of evidence analysis (e.g. Klimisch score 1 or 2).

Fertility and reproductive function

Animal studies

Reference	Species	Design	Results
Nobunaga et al. 1979	mouse strain: IVCS sex: female age: 60 days n = 14 per group	oral, via food 4 or 8 ppm in commercial chow 0, 4.0, 8.3 µmol/kg bw/d **MeHgCl *** (pre-mating) ≈ 0, 1.0, 2.1 mg/kg bw/d **Hg** 0, 3.5, 7.4 µmol/kg bw/d **MeHgCl *** (gestation) ≈ 0**,** 0.9, 1.9 mg/kg bw/d **Hg**	no differences in body weight pre-mating decreased maternal bwg from GD3 at high dose number of oestrous cycles > 4 days increased by: 0-11-27% lower no. of implants per dam, higher incidences of resorptions, dead embryos/ foetuses in high

		for 30 days pre-mating until GD18 ***** calculated from published daily doses (in results section) in µmol MeHgCl (251.09 g/mol)	dose group
Khera 1973b Experiment IV and V	mouse strain: Swiss Webster sex: male n = 10-13 per group	oral, gavage 0, 1.0, 2.5, 5.0 mg/kg bw/d **Hg** (0.0025 to 0.125% methylmercuric chloride in 0.5% Na_2CO_3) for 7 d pre-mating (Exp. IV) or 5 days during mating trial 3 (Exp V) 7 matings with 3 untreated virgins per male (Exp IV and V)	2/13 mice dead after 7 days of dosing in high dose group no toxic effects in other groups and in high dose group after 5 days of dosing no effects on fertility
Verschuuren et al., 1976b [added by the rapporteurs]	rat strain Wistar 4 groups of 20 female and 10 male rats mated to produce F1, subsequently F2 and F3 generation	oral, food 0, 0.1, 0.5, 2.5 ppm methylmercuric chloride in diet	no effect on fertility index, lactation index or on body weights of pups at day 21 pn; viability index (day 5 pn) was impaired at 2.5 ppm in F1 and F2
Khera 1973b Experiment I and II	rat strain: Wistar sex: male n = 15-20 per group	oral, gavage 0, 1.0, 2.5, 5.0 mg/kg bw/d **Hg** (0.0025 to 0.125% methylmercuric chloride in 0.5% Na_2CO_3) for 7 days pre-mating 14 (Exp I) or 7 (Exp II) matings with 2 untreated virgins per male	no adverse effects on behaviour and bwg of males Exp I: In the initial four mating trials a reduced portion of pregnant femaleswas observed in the high dose group (37% vs. 56% in controls) Exp II: results of Exp. I are confirmed for a low pregnancy rate in the high dose group in the initial four mating trials (34% vs. 55% in controls). [Note: variability of the pregnancy rates range from 43%

			to 87% in the 14 control groups in Exp I and from 17% to 80% in the 7 control groups in Exp II] distribution of resorption sites and *corpora lutea* similar to controls
Khera 1973b Experiment III	rat strain: Wistar sex: male n = 14-29 per group	oral, gavage 0, 0.1, 0.5, 1.0 mg/kg bw/d **Hg** (0.0025 to 0.125% methylmercuric chloride in 0.5% Na_2CO_3) for 125 days (80 in high dose) concurrent to mating 21 or 17 (high dose) matings with 2 untreated virgins per male	sig. depressed rate of bwg in high dose group after 70 days of dosing (dosing stopped at day 80 → bwg normalised after 25 days) on average decrease in no. of viable implants after 25-30 days at high dose and after 85-90 days mid dose distribution of resorption sites and *corpora lutea* similar to controls
Vachhrajani, Chowdhury and Dutta 1992	rat strain unknown sex: male age: 30 ± 2 days n = 6 per group	i.p. in saline 0, 0.005, 0.01 mg/kg bw/d **MeHgCl** ≈ 0, 0.004, 0.008 mg/kg bw/d **Hg** for 15, 30, 60 or 90 days	D15: spermatogenesis arrested in high dose group D30: highly distorted germinal epithelium in high dose group D60: clogging of spermatogenic cells in low-dose group highly distorted peritubular membrane in high dose group D90: cellular content in tubules decreased in both dose groups, most of spermatocytic nuclei karyorrhetic or karyolytic

The following text comprises a short description of studies compiled in the table above. To facilitate a comparison of studies all doses are given as Hg fraction from administered methylmercuric chloride.

In a mating trial study, 60 days old female IVCS mice were exposed orally via food to two different dose levels of methylmercuric chloride for 48 days before mating with untreated males until gestation day 18. There were no signs of general toxicity in controls and the low dose group. In the high dose group a significant decrease of maternal weight gain was

observed. The proportion of oestrous cycles longer than 4 days increased by 11% and 27% in the low and high dose group, respectively. A decreased number of implants per dam, higher incidences of resorptions as well as dead embryos/foetuses were observed in the high dose group.

Male Swiss Webster mice were exposed orally by gavage to 0, 1, 2.5 and 5 mg/kg bw/d Hg in two experiments for 7 days and 5 days, respectively. No signs of toxicity were observed in the first experiment, but 2/13 animals were dead at 5 mg/kg bw/d after 7 days. The second experiment showed no toxic effects at all. Both experiments did not show any impacts on fertility.

Four groups of 20 female and 10 male Wistar rats received a diet containing 0, 0.1, 0.5, and 2.5 ppm methylmercuric chloride. Animals were mated and F1 and F2 generations produced. No effect was exerted on fertility index, lactation index or on the 21-day body weights of pups, but the viability index (day 5) was impaired at 2.5 ppm in the F1 and F2 generations.

Male Wistar rats were exposed orally by gavage to 0, 1, 2.5 and 5 mg/kg bw/d Hg for 7 consecutive days pre-mating. Subsequently, 14 (Experiment I) and 7 (Experiment II) mating periods of 5 days followed. In the initial four mating trials a reduced portion of pregnant females was observed in the high dose group in both experiments (Exp. I: 37% vs. 56% in controls; Exp. II 34% vs. 55% in controls). There were no signs of general toxicity in neither dose group. Also, the number of viable embryos was reduced in both experiments during the first four mating periods (Exp. I: 6.1 vs. 10.2 in controls; Exp. II: 8.1 vs. 9.9 in controls). These non-conventional studies indicate a possible effect of methylmercuric chloride on fertility in rats.

In an additional experiment, male Wistar rats were exposed orally by gavage to Hg at 0, 0.1, 0.5 and 1 mg/kg bw/d for 125 days and for 80 days in the high dose group. General toxicity occurred in top dose only, manifesting in decreased body weight gain after 70 days and mild to severe motor disturbances at the following 10 days in 5/18 rats. After 90 days one of the affected rats died. There was a decrease in the number of viable implants in the top dose group after 30 days of dosing and in the mid dose after 90 days. Preimplantation losses were dramatic at 1 mg/kg bw/d (more than two fold increase after 90 days).

In a further study, male rats were exposed intraperitoneally to 4 or 8 µg/kg bw/d Hg for 15, 30, 60 or 90 consecutive days. Alterations of spermatocytes and spermatides were observed in treated rats over all test periods. This included an arrested spermatogenesis in the high dose group after 15 days, a highly distorted germinal epithelium in the high dose group after 30 days as well as clogging of spermatogenic cells after 60 days in the low dose group, and a highly distorted peritubular membrane in the high dose group. After 90 days the cellular content in tubules decreased in both doses and most of spermatocytic nuclei were karyorrhetic or karyolytic. Given the non-physiological nature of this route of administration, this study serves only to support the findings of the other studies in rats.

In summary, several animal studies show some effects of methylmercuric chloride on fertility. However, all studies have flaws and none was designed according to today's guidelines. Treatment of male rats resulted in a reduction of pregnancies during four mating periods of 5 days. This was shown in two independent experiments underlining the reliability of the result. However, variability of the pregnancy rates in controls was large. In mice, oral dosing for 30 days premating led to lower numbers of implants per dam. Further evidence for a possible effect on fertility comes from the observations that in rats sperm motility was decreased and in female mice oestrous cycle was prolonged. In

contrast, in a study over 3 generations of rats no effect was exerted on fertility index, but viability index was impaired. These findings provide a suspicion that methylmercuric chloride represents a hazard to reproductive functions, but clear cut evidence is lacking.

Findings in humans

Two studies from Hong Kong examined the relationship between mercury concentrations in hair or blood and infertility. No information was provided about the specific nature of the exposures that had occurred for the test subjects. Hair samples from 94 fertile and 117 subfertile men were collected in one study, in another case-control study, blood mercury levels of 26 fertile and 150 infertile couples were compared. Both studies showed that elevated mercury levels in hair (4.23 mg/kg vs. 3.33 mg/kg in controls) or blood (40.6 mmol/L in infertile men and 33.2 mmol/L in infertile women compared to 31.2 mmol/L and 17.5 mmol/L in controls, respectively) were positively related to infertility in men and women.

However, both of these studies are not suitable to claim an effect of methylmercuric chloride on human fertility. Analysis was restricted to mercury in hair or blood, and the association between mercury concentration and fertility was weak. Design of the studies is questionable. They do show, however, that mercury levels are increased in subjects with higher seafood consumption.

Developmental toxicity - laboratory studies with methylmercuric chloride

Reference	Species	Design	Results
Nobunaga et al. 1979	mouse strain: IVCS sex: female age: 60 days n = 14 per group	oral, via food 4 or 8 ppm in commercial chow 0, 4.0, 8.3 µmol/kg bw/d **MeHgCl** (pre-mating) ≈ 0, 1.0, 2.1 mg/kg bw/d **Hg*** 0, 3.5, 7.4 µmol/kg bw/d **MeHgCl** (gestation) ≈ 0, 0.9, 1.9 mg/kg bw/d **Hg*** for 30 days pre-mating until GD18 * calculated from published daily doses (in results section) in µmol	no differences in body weight pre-mating decreased maternal bwg from GD3 at high dose litter size decreased: 10.2-8.1-5.3 cleft palates: 0 / 92 19 / 114 (16.7%) 41 / 74 (55.4%) mean no. of ossified vertebrae: 13.6-10.9-11.0

		MeHgCl (251.09 g/mol)	
Khera and Tabacova 1973c similar to OECD TG414	mouse strain: Swiss-Webster sex: female n = 5-17 per group	oral, gavage 0, 0.001, 0.01, 0.1, 1, 5, 10 mg/kg bw/d **Hg** (methylmercuric chloride suspended in corn oil) GD6-GD17	all dams dead in highest dose group, no maternal toxicity evident in other dose groups 100% stillborn pups or dams that were unable to litter at 5 mg/kg bw/d low incidence of delayed cerebellar differentiation and focal transitory inhibition of energy metabolism at 1 mg/kg bw/d until PND14, afterwards normal cerebelli in all groups
Fuyuta, Fujimoto and Hirata 1978 similar to OECD TG414	mouse strain: C57BL sex: female age: (not less than 23 g) n = 10 per group	oral, gavage 0, 2.5, 5.0, 6.0, 7.5 mg/kg bw/d **MeHgCl** ≈ 0, 2.0, 4.0, 4.8, 6.0 mg/kg bw/d **Hg** GD6-GD13	sig. decreased maternal bwg in highest dose group live foetuses: 75-71-70-48-1 number of resorptions and deaths: 9.6-12.3-12.5-34.2-98.7% average pup bw: 0.95-0.91-0.78-0.75-[/] g (males) 0.91-0.88-0.73-0.80-0.70 g (females) cleft palates: 0-4.2-57.1-97.9-100% hydronephrosis: 0-5.4-5.7-20.0-0.0% fused thoracic vertebrae: 0-5.9-62.9-60.9-[/]% decreased ossification of supraoccipital bone: 10.8-47.1-82.9-91.3-[/]% absence sternum: 0-17.6-40.0-60.9-[/]%

Belles et al. 2002	mouse strain: CD1 sex: female n = 10 (control) n = 12 (exposed)	oral, gavage 0, 12.5 mg/kg bw/d **MeHgCl** ≈0, 10 mg/kg bw/d **Hg** single dose on GD10	(decreased bwg on GD0-8) no. of dead dams: 0/10-1/12 sig. decreased food consumption on GD10-18 and sig. decreased gravid uterine weight/sig. decreased av. foetal bw/litter delayed ossification (calcaneous): 4/49-37/49 cleft palate: 0/54-28/46
Goulet 2003 similar to OECD TG423	mouse strain: C57BL/6 sex: female age: 11-12 weeks n = 14 per group mid dose n = 34	oral, drinking water 0, 4, 6, 8 ppm = 0, 4.0, 6.0, 8.0 mg/L **MeHgCl** ≈ 0, 1.0, 1.4, 1.9 mg/kg bw/d **MeHgCl *** at start of dosing ≈ 0, 0.8, 1.1, 1.5 mg/kg bw/d **Hg** from GD2 to weaning ***** calculated from average body weight at 12 weeks (21 g) as published by Charles River Laboratories and estimated 5 mL/d drinking volume	offspring: percentage of 5-week survival decreased in the high dose group: 89.8-87.8-84.1-75.8% similar levels of Hg were measured in brain and liver tissue near birth. Brain concentrations rapidly decreased during nursing. no differences in fall latency on rotarod, spatial alteration in T maze, no impairment in the reference memory component in modified T maze horizontal exploration reduced, working memory in the modified T maze impaired in females of the mid and high dose group, but not in males

Montgomery et al. 2008	mouse strain: C57BL/6+/+ sex: male and female coordination tests: control n = 13 males n =8 females exposed n = 15 males **n =4 females** spatial learning tests: control n = 9 males n=8 females exposed n = 9 males n = 8 females	oral, food 0, 0.01 mg/kg bw/d **MeHg** ≈ 0, 0.008 mg/kg bw/d **Hg** (chow moistened with 95% methylmercuric chloride dissolved in H_2O) GD8-GD18	no differences in litter sizes, no. of resorbed/dead foetuses and foetal bw Hg content in brain of exposed females and foetuses at GD18 sig. higher than in controls, no difference in Hg content in 3 month old offspring exposed mice spent sig. less time on the rotarod, were sig. less active no differences between controls and exposed animals in Morris water maze, cue training and place training
Liang et al. 2009	mouse strain: C57BL/6 sex: female n = 20 per group	oral, food “chronic”: 0, 1.4 mg/kg bw/d **MeHgCl** ≈ 0, 1.1 mg/kg bw/d **Hg** on GD1-18 “bolus”: 0, 0.85 mg/kg bw/d **MeHgCl** ≈ 0, 0.68 mg/kg bw/d **Hg** on GD1-11, GD13-15, GD17-18 **and** 0, 6.0 mg/kg bw/d **MeHgCl** ≈ 0, 4.8 mg/kg bw/d **Hg** on GD12 and GD16	behavioural testing started at PN57 motor tasks: sig. increased times in climbing task in exposed groups, two other tasks not affected emotional reactivity: no difference in anxiety levels between groups learning and memory: sig. increased number of errors made by “chronic” group, tendency same for “bolus” group, but not sig. sig. increased times to complete a maze in both exposed groups learning ability and activity reduced in exposed groups

		"chronic" and "bolus" regimens resulted in similar total amount of MeHgCl administered	
Khera and Tabacova 1973c	rat strain: Wistar sex: female, male n = 35 per group	oral, food 0, 0.002, 0.01, 0.05, 0.25 mg/kg bw/d **Hg** (methylmercuric chloride suspended in corn oil and mixed with the diet) F0 immature until killed F1 from weaning to 20 days after breeding	F0 females: no differences in bwg, behaviour, pregnancy no differences in values for *corpora lutea*, ratios of total implantations to *corpora lutea*, ratios of live to dead foetuses (including resorption sites), foetal weight and skeletal anomalies F1: no difference in bwg and survival until weaning, pups in exposed groups showed higher incidences of ocular defects no changes in parameters of reproductive performance
Fuyuta, Fujimoto and Hirata 1978 similar to OECD TG414	rat strain: Wistar sex: female n = 20 per group	oral, gavage 0-2.5, 5.0, 7.5 mg/kg bw/d **MeHgCl** ≈ 0, 2.0, 4.0, 6.0 mg/kg bw/d **Hg** GD7-GD14	sig. decreased maternal bwg in high dose group and on some GD in other treated groups food and water consumption decreased dose-dependently; 9/20 in high dose showed neurotoxic signs (spasms, disturbance in gait, hindlimb crossing phenomenon) no maternal deaths reported live foetuses: 251-250-236-137 no. of resorptions and deaths: 4.9-3.5-5.2-42.4%

			average pup bw: 4.42-4.32-4.03-4.08 g (males) 4.14-4.13-3.82-3.87 g (females) cleft palates: 0-0-0-17.5% generalized edema: 0-0-0-78.8% brain lesions: 0-0-0-66.7% hydrocephaly: 0-0-5.9-14.5% absence of vertebral centra: 0-0-0-5.9% wavy ribs: 0-0-6.8-26.5% sternebral defects: 0-0-0-19.1% bilobed vertebral centra: 2.4-2.4-3.4-14.7%
Lee and Han 1995	rat strain: Fisher 344 sex: female n = 30 per group	oral, gavage 0, 10, 20, 30 mg/kg bw/d **MeHgCl** ≈0, 8, 16, 24 mg/kg bw/d **Hg** single dose on GD7	maternal bw decreased for 2 days in low dose group, for 6 days in mid dose group and throughout gestation in high dose group maternal death rate: 0-6.7-16.7-30% live foetuses: 298-224-145-18 average pup bw: 3.78-3.46-2.86-2.14 g (males) 3.72-3.21-2.80-1.75 g (females) dose dependently sig. delayed ossification in all

			treated groups
Bornhausen et al., 1980	rat Wistar performance test in lever boxes, offspring (4 months old), male and female, n = 10 per group	Oral, intubation MeHgCl 0 mg/kg 0.005 mg/kg 0.01 mg/kg 0.05 mg/kg	operant conditioning test: differential reinforcement of high rates (DRH) [DRH 2/1 = press lever two times within 1 second] performance deficits were found at 0.01 and 0.05 mg/kg deficits were most pronounced at increasing learning demand (DRH 4/2 and DRH 8/4)
Newland and Rasmussen 2000 (Newland and Reile 1999 for further details)	rat strain: Long-Evans sex: female n = 5 per group n = 10 in control group	oral, drinking water 0, 0.5, 6.4 mg/L **Hg** (as methylmercuric chloride dissolved in drinking water) ≈0, 0.045, 0.6 mg/kg bw/d **Hg** (mean)***** from 28 or 49 days pre-mating until PND16 ***** calculated as mean from published doses of 40 to 50 µmol/kg bw/d in low dose group and 500 to 700 µmol/kg bw/d in high dose group	no maternal toxicity observed 25 litters: 9-7-9 (not sig. tendency to small litter sizes in high dose group) offspring: no differences in bwg or survival exposure related decline in training performances at aging, median age at 50% decline: 980-780-500 days (estimated from Fig. 4 in publication)
Newland, Reile, Langston 2004	rat strain: Long-Evans sex: female	oral, drinking water 0, 0.5, 6.4 mg/L	no maternal toxicity observed 25 litters:

	n = 5 per group n = 10 in control group	**Hg** (as methylmercuric chloride dissolved in drinking water) ≈ 0, 0.045, 0.6 mg/kg bw/d **Hg** (mean)* from 28 or 49 days pre-mating until PND16 * calculated as mean from published doses of 40 to 50 µmol/kg bw/d in low dose group and 500 to 700 µmol/kg bw/d in high dose group	9-7-9 (not sig. tendency to small litter sizes in high dose group) offspring: no differences in bwg or survival, no effects on asymptotic or terminal performance exposed offspring showed retardation in the acquisition of choice at 2.3 years of age, no effect at 1.7 years
Stoltenberg-Didinger and Markwort 1990 (Klimisch score 3)	rat strain: Wistar sex: female n = ?	oral, gavage 0, 0.025, 0.05, 0.5, 5.0 mg/kg bw/d **MeHgCl *** ≈ 0, 0.02, 0.04, 0.4, 4.0 mg/kg bw/d **Hg** GD6-GD9 * DS reported 0, 0.02, 0.04, 0.4, 4.0 mg/kg bw/d **MeHgCl**	litter size "within normal range", no differences in physical landmarks, no differences in brain weights, no malformations sig. impaired swimming behaviour in first testing battery at highest dose males of highest dose group were less active distinct neuropathological changes of dendritic spines in highest dose group
Rice and Gilbert 1995	monkey strain: *Macaca fascicularis* sex: male and female age: 15 or 18 years n = 4 in control group	oral, sodium carbonate solution of methylmercuric chloride in syringe or corn oil solution of	impaired vibration thresholds in monkeys of all exposed groups, monkeys in low dose *in utero* group exhibited stronger impairment than monkeys in high dose group

	n = 2 per group (in utero group) n = 5 (postnatal group)	methylmercuric chloride in gelatin capsules *in utero* group: (mothers) 0.025, 0.050 mg/kg bw/d **Hg** 3x per week + (infants) 0.025, 0.050 mg/kg bw/d **Hg** 5 days per week until 4 to 4.5 years of age postnatal group: 0.050 mg/kg bw/d **Hg** 5 days per week until 7 years of age all monkeys tested 11 years after cessation of dosing	aberrant spatial and temporal vision, impairment of absolute threshold for pure high frequency tones in group exposed postnatally only BUT: impairment of different sensory systems not correlated within individuals
Rice 1998	monkey strain: *Macaca fascicularis* sex: male and female age: 11 and 19 years n = 1-2 per group	oral, sodium carbonate solution of methylmercuric chloride in syringe or corn oil solution of methylmercuric chloride in gelatin capsules mothers: 0, 0.010, 0.025, 0.050 mg/kg bw/d **Hg** 3x per week + infants: 0,	no differences in median reaction times evidence for an increase in impairment of auditory function in exposed monkeys relative to controls

		0.010, 0.025, 0.050 mg/kg bw/d, respectively **Hg** 5 days per week until 3.5 to 4.5 years of age	

The following text comprises a short description of studies compiled in the table above. To facilitate a comparison of studies all doses are given as Hg fraction from administered methylmercuric chloride.

Developmental toxicity

Animal studies

Sixty day old mice (female IVCS) were exposed orally via food to 1 and 2.1 mg/kg bw/d Hg for 48 days, starting 30 days before mating. In the top dose group there was a significant decrease in maternal weight gain from GD 3 and a significantly decreased number of total implants, higher incidences of resorption, dead embryos and dead foetuses, as well as a significant retardation in growth of surviving foetuses. In addition, in both dose groups there was a significant incidence of cleft palates (17% at low dose, 55% at high dose).

In another mouse study (female Swiss-Webster), doses of 0, 0.001, 0.01, 1, 5 and 10 mg/kg bw/d Hg were applied from GD 6-17 by food. There were no effects at 0.001 and 0.01 mg/kg bw/d. At 1 mg/kg bw/d a transitory inhibition of cerebellar cellular migration from the external granular layer was observed. At 5 mg/kg bw/d there was a reduction in the number of live pups, and live-born pups died within 2 days. At 10 mg/kg bw/d all dams died.

Female Mice (C57BL) were exposed to 0, 2, 4, 4.8 and 6 mg/kg bw/d Hg orally on GD6-13. At 6 and 4.8 mg/kg bw/d a significant increase of dead and resorbed embryos (98.7 and 34.2%, respectively) as compared to controls was reported. A decrease in foetal body weight was observed in all treated groups. Impact of 4.8, 4, 2 and 0 mg/kg bw/d Hg on the incidence of malformations were significantly higher than in the control group (97.9, 75.7, 11.3 and 0%, respectively). The most common malformation was cleft palate in foetuses from dams given 4 or 4.8 mg/kg bw/d (97.9 and 57.1%, respectively). A significant increase in the incidence of fused thoracic vertebrae was found at 4 and 4.8 mg/kg bw/d Hg. A significant increase in delayed ossification of the supraoccipital bone and in sternebral variation was observed at all doses.

Pregnant mice were exposed orally to 10 mg/kg bw/d Hg via gavage in a single dose on GD 10. In the exposed group 1 of 12 died compared to 0 of 10 in the control group. Some dams carried completely resorbed litters. Litters showed decreased average body weight as well as delayed calcaneous ossification and an increased incidence of cleft palates.

In an attempt to examine neuro-behavioural effects, female mice (C57BL/6) were exposed to approximately 0, 0.8, 1.1 and 1.5 mg Hg/kg bw/d orally via drinking water from GD2 to weaning. A decrease in offspring survival in the top dose group at the age of 5 weeks was

reported. At the age of 6 and 12 weeks decreased locomotor activity in female offspring of all treated females was reported. Female offspring of rats treated with 1.1 and 1.5 mg/kg also showed impairment of working memory.

Adult male and female mice (C57BL/6) were exposed orally to 0 and 0.008 mg/kg bw/d Hg daily for 11 days from GD8 to GD18. A motor coordination test and a test for the spatial learning were performed. It should be noted that in the motor coordination test series, a number of only 4 females and 15 males was used. Exposed mice demonstrated a significantly narrower foot angle in comparison to control ($F_{1.36}$ = 10.66, $p < 0.005$). Exposed mice spent significantly less time on the rotarod and showed impairments at the Morris water maze relative to control mice. Exposed mice also demonstrated less accurate searches for platform than controls.

Mice of the C57BL/6 strain were exposed orally to 1.1 mg/kg bw/d Hg via food from GD1 to GD18. A second group was exposed to 0.68 mg/kg bw/d Hg on all days except for GD12 and GD16, when a bolus dose of 4.8 mg/kg bw/d Hg was administered. Investigation was performed in a blinded manner. The first group showed slightly decreased vertical movement on the last two days of testing and both groups showed decreased motor coordination in a climbing test. Further motor coordination tasks revealed no significant effects. Both experimental groups showed impaired spatial learning abilities in the radial maze while learning of an operant task was not impaired.

Female Wistar rats were exposed to 0, 0.002, 0.01, 0.05 or 0.25 mg/kg bw/d Hg as a single daily dose via food on GD 6-17. In all treated groups a higher incidence of ocular defects was observed.

Female rats were exposed to 2, 4 and 6 mg/kg Hg orally on GD 7-14. Body weight gain in the dams of all treated groups was significantly lower than of controls, 9 out of 20 dams in the top dose showed signs of neurotoxicity such as spasms, disturbance in gait and hindlimb crossing phenomenon. Foetuses from each treated group weighed less than those from the controls. Incidence of cleft palate and generalised edema was significantly higher in the top dose group than in the control. 67% of the top dose foetuses had lesions in the white matter of the cerebrum. At 6 and 4 mg/kg some foetuses showed alterations like hydrocephaly (14.5 and 5.9%, respectively). The top dose showed incomplete ossification of vertrebral centra (5.9%) as well as of the sternum (19.1%).

Female Fisher 344 rats were exposed to 0, 8, 16 or 24 mg/kg bw/d Hg as a single dose on GD7 by gavage. A decrease in maternal body weight up to 61.9% of controls as well as a dose dependent increase of maternal death up to 30% were observed. The survival rate of foetuses decreased to 7.6% at 24 mg/kg bw/d. A decrease in ossification centres was seen in all treated groups. Mercury concentrations were up to 21 µg/g in the maternal brain and up to 15 µg/g in the foetal brain.

Female Long Evans rats were exposed to 0, 0.5 and 6.4 ppm Hg via drinking water resulting in daily intakes of 0, 45 and 600 µg/kg bw/d Hg. Exposure took place 28 and 49 days before mating and continued until postnatal day 16. Exposure accelerated the decline in training performance. The experiment was repeated under the same conditions, but with a longer observation period of 1.7 and 2.3 years. Exposed animals showed mercury deposits in the neonatal brains. A dose dependent retardation in the acquisition of choice was observed in the exposed offspring after 2.3 years.

Female Wistar rat dams were exposed to 0, 0.02, 0.04, 0.4 or 4 mg/kg bw/d Hg on gestation days 6 to 9. There were no adverse effects reported in the pregnant rats. In the

top dose group the swimming behavior of pups was impaired and changes in the dendritic spines of the pyramidal neurons were observed. At 0.04, 0.4 and 4 mg/kg bw/d an increased passiveness and decreased habituation to an auditory startle were observed.

Groups of monkeys (*Macaca fascicularis*) were exposed orally to 0 or 50 µg/kg bw/d Hg postnatally or to 0, 10, 25 or 50 µg/kg Hg *in utero* and postnatally. Of the 4 monkeys exposed postnatally only, 3 exhibited substantially elevated vibration thresholds at the assessment of the vibration sensitivity at 18 years of age. One monkey showed difficulties to learn the task and had extremely impaired vibration sensitivity in the fingers of both hands even at the lowest frequency tested. This monkey had no difficulties to learn a previous task for auditory tests. Therefore, the difficulties seem to be caused by the severely reduced perception of the vibratory stimulus. The group exposed *in utero* and postnatally was examined at the age of 15 years and showed different results. One monkey was clearly unimpaired, another one exhibited slightly elevated thresholds for 2 of the 5 frequencies tested. Both animals from the lower dose group showed impairment at all but the lowest frequency. These results suggest permanent impairment in vibration sensitivity after long term exposure.

For the assessment of the auditory function monkeys (*Macaca fascicularis*) were exposed to 0, 10, 25 and 50 µg/kg bw/d Hg^{2+} by ingestion. Exposure duration was 3 times per week *in utero* and 5 days a week postnatally until 3.5-4.5 years of age. The experiment was carried out on the infants, beginning at 11 and 19 years of age. At high dose, thresholds were elevated in both ears at all frequencies and particularly at the highest frequencies. These effects were more severe at 11 years of age. At 25 µg/kg bw/d thresholds were more elevated at 19 years. In animals of the lowest dose group, no impairment was observed at 11 years, while at 19 years, thresholds were elevated in both ears.

In conclusion, prenatal exposure to methylmercuric chloride causes external, visceral and skeletal malformations in mice as well as persistent neurological deficits at doses that are not associated with maternal toxicity.

In rats, a similar pattern of malformations was caused by methylmercuric chloride, consisting mainly of cleft palate, edema and brain malformations. In contrast to mice, gross-structural defects, such as cleft palate, were noted at doses that cause general toxicity. At lower doses methylmercuric chloride causes persistent neurobehavioural effects, such as operant learning changes.

Data from non-human primate studies are not convincing, due to the low number of animals examined and poor description of the results.

Findings in humans

The two major events of human poisoning with methylmercury (Minamata, Iraq) provide an insight into the clinical syndrome induced by high exposure to methylmercury in adults and in children exposed during pre- and/or postnatal development.

Individuals poisoned by methylmercury compounds through consumption of contaminated fish in Japan (Minamata) exhibited paresthesia, ataxia, sensory disturbances, tremors, impairment of hearing, and difficulty in walking. All children born from women living in Minamata at that period suffered from mental retardation, primitive reflex, cerebellar ataxia, disturbances in physical development, and dysarthria. Furthermore, most children

showed hyperkinesia, hypersalivation, paroxysmal symptoms, strabismus and pathological reflexes. The follow-up study revealed that some symptoms improved over time, some others did not. Mothers living in the most contaminated area were interviewed later: in 272 pregnancies, there were 32 miscarriages, 9 stillbirths, 4 deaths within the first week after birth and 4 infants with congenital Minamata disease.

In Iraq, exposure was due to the consumption of bread that was made with wheat treated with a mixture of organic mercury compounds as a fungicide. In that outbreak, the most common symptom in adults was paresthesia; the most severely affected individuals exhibited ataxia, blurred vision, slurred speech, hearing difficulties, blindness, deafness, and died subsequently. At least 6 of 15 children had clinical evidence of poisoning after prenatal exposure. In the 5 infants severely affected, there was evidence of gross impairment of motor and mental development, with cerebral palsy, deafness and blindness in 4. Three infants had microcephaly at an early age. A follow-up study reports on 32 infants, including the original 15, prenatally exposed to methylmercury compounds after 5 years. Nine deaths were recorded during the first 3 years.

In one case reported from New Mexico in 1971, a mother ingested methylmercury contaminated meat during the second trimester of pregnancy. The mother never suffered from symptoms and delivered a normal weight male infant at term. The child had gross tremulous movements of the extremities in the first days of life. The child was never breast fed, but urinary mercury levels were high (2.7 ppm) in the first days, decreasing to less than 0.01 ppm at 6 weeks. After 6 weeks, the child displayed an increased tone in the extremities and cortical thumb posturing. He subsequently developed generalised myoclonic jerks. At 8 months, the infant showed nystagmoid eye movements without evidence of visual fixation. At one year of age, the child was blind and could not sit up.

The Seychelles Child Development Study was a longitudinal study of the effects of pre- and postnatal mercury exposure through fish consumption. A total cohort of 779 mother-child pairs was enrolled in this study in 1989. Several publications report on the outcomes of developmental tests at different infant ages. The median prenatal mercury exposure of the cohort was 5.9 ppm (0.5 – 26.7 ppm) in maternal hair. Overall, none of the studies found a clear evidence for consistent adverse effects of exposure on the developmental outcomes. The authors think that apparent beneficial effects of exposure could be linked to the association of exposure to mercury and nutritional benefits of fish consumption. The outcomes are summarised below.

Walking appeared at a later age as exposure increased in the range from 0 to 7 ppm but surprisingly appeared slightly earlier for exposure above 7 ppm. No influence of the level of exposure to methylmercury was seen concerning the age of talking.

Cognitive developmental outcomes up to 2.5 years of age appeared essentially normal up to a maternal hair mercury level of 6 ppm. The childrens' activity levels decreased as maternal hair concentration increased. This outcome might represent a subtle influence of mercury on behavior without detectable residual effects on cognition.

At the age of 66 months, the results were related to the childrens' mercury levels in their hairs. For some of the tests impairments were observed at lower hair levels, but an improvement was observed when the mercury levels were higher.

At the age of 108 months, the study even showed enhancement of performances on a number of neurophysiological tests associated with increasing prenatal exposure to methylmercury. Only one test showed decreasing performance associated with increasing

prenatal methylmercury exposure in females. A secondary analysis including both prenatal and postnatal exposures showed evidence of only one adverse association between postnatal exposure and the test outcome.

At the age of nine, only two of 21 endpoints were associated with prenatal methylmercury exposure and developmental outcomes: decreased performance in the grooved pegboard using the non-dominant hand in males and higher scores in the hyperactivity index of the Conner's teacher rating scale.

Adverse neurodevelopmental outcomes were identified at the Faroe population consuming fish. A cohort of 1022 single births during 1986-1987 was assembled. Mercury concentrations in the cord blood ranged from 10 to 350 µg/L. Obvious cases of congenital methylmercury poisoning were not found. In a series of tests, mercury-related neurophysiological dysfunctions were pronounced in the domains of language, attention and memory and at a lesser extent in visuospatial and motor functions.

In Canada, a study of prenatal methylmercury exposure in 234 infants whose maternal hair level of MeHg was 6 ppm showed that exposure was related to abnormal muscle tone in male infants. In Inuits whose source of contamination is the occasional consumption of highly contaminated whales, the methylmercury concentration in cord blood averaged 80.2 ppb and the highest levels were related to decreased birth bodyweights.

The human poisoning events demonstrate that methylmercury is a developmental toxic compound in man. However, in no case humans had been exposed to methylmercury chloride. Methylmercury was detected – besides other organic derivatives as well as inorganic mercury – in the blood and in tissue samples from the victims of these mass intoxications.

Lactation Effects

Data describing effects of methylmercuric chloride on pups mediated exclusively by breast milk are not available. However, methylmercury is present in breast milk and it is reasonable to assume that toxic effects can be induced by this way.

Conclusion, comparison with criteria

The CLP criteria for classification in Repr. Category 1A read as follows: "*Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)*."

<u>Fertility</u>

No data are available showing an effect of methymercuric chloride on fertility in humans. Standard animal studies are not available. However, some findings relevant to this differentiation have been reported. Considering the inconsistency of effects on fertility occurring at high dose levels which produce general toxicity Repr. 2 is more appropriate than Repr. 1B.

<u>Development</u>

Human development can be affected by organic mercury compounds. Severe

developmental neurotoxic effects have been described in several poisoning events with organic mercury fungicides. RAC agrees with the DS that studies with other methylmercury compounds are regarded as supporting evidence for methylmercuric chloride toxicity and supports classification as **Repr. 1A**.

In the absence of specific studies addressing possible effects via lactation, but based on pharmacokinetic data RAC concurs with the proposal by the DS to classify methylmercuric chloride for **Lact. Effects; H362**.

4.12 Other effects

STOT RE 2, in CLP

The rationale that has justified STOT RE 2, in CLP in the existing generic entry is not specifically known. However, according to the criteria in Annex VI, STOT RE 2 applies when data are not sufficient to justify a classification STOT RE 1, in CLP. Here, STOT RE 1 is warranted and STOT R2 (R33 in DSD) may therefore be removed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier. Based on the priority defined by CLP, French CA decided to focus the CLH report of hand-over substances on CMR effects only and to propose their harmonisation consistently with what was discussed at TC C&L. Therefore, the environment effects are not considered for their harmonization in this CLH report. However, we consider that the classification for the environment endpoints coming from the generic entry of the mercury compounds should apply.

RAC evaluation of aquatic hazards (acute and chronic)

The substance is covered by the entry in the CLP Regulation with index no 080-004-00-7. This entry contains classification for Aquatic Acute 1 H400 and Aquatic Chronic 1 H410. It is proposed by the DS that these classifications are transferred to the entry for methylmercuric chloride. RAC has not assessed these hazard classes.

6 OTHER INFORMATION

No other information available.

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8 ANNEXES

ANNEX I

Extract from summary records of TC C&L meetings

TC C&L meeting of November 2005

Organic Mercury compounds:

Methyl mercury [3] (CAS number: 22967-92-6)

Methyl mercuric chloride [4] (CAS number: 115-09-3)

Current classification T+; R 26/27/28 – R33 - N; R50-53
Classification proposal T+; R 26/27/28 – R33 – Repr Cat 1; R61 – Repr Cat 3; R62 – R64 - N; R50-53

ECBI/09/05 [1] and Add. 1 [2], 2 [3, 4]

During the discussion a number of issues were raised by member states including the relevance of R64 and the appropriate classification for acute toxicity. **France agreed to revise their proposal in the light of these points.**

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Summary Record

Technical Committee on Classification and Labelling of Dangerous Substances

Meeting on Health Effects of Pesticides, Biocides, Existing Chemicals, New Chemicals and General issues

Hotel Concorde, Arona, 21 - 24 March 2006

Methyl mercury [3] (CAS number: 22967-92-6)

Methyl mercuric chloride [4] (CAS No: 115-09-3, EC No: 204-064-2)

Current classification T+; R 26/27/28 – R33 - N; R50-53
Classification proposal T+; R 26/27/28 – R33 – Repr Cat 1; R61 – Repr Cat 3; R62 – R64 - N; R50-53

ECBI/09/05 Rev 1, Add 1 Rev 1 and Add 2 Rev 1 – revised proposals for mercury compounds.

In **November 2005** member-states held a general discussion of the proposal and commented on a number of issues including the appropriate classification for acute toxicity and the need for R 64.

France summarised their revised proposal commencing with consideration of methyl mercury. There was good animal data to support T+; R 28 but no relevant information to support the current classification for skin or inhalation toxicity. In terms of repeat dose toxicity there was both human poisoning experience and animal data to justify T; R 48/25. Again there was no dermal or inhalation data and R 33 was not appropriate in view of R 48. For mutagenicity there was sufficient data to justify Muta Cat 3; R 68. Carcinogenicity data supported Carc Cat 3; R 40 with several studies showing effects in male mice. For reproductive toxicity there was human evidence to support Repr Cat 1; R 61 but classification for fertility effects was not justified because of maternal toxicity.

Acute and repeat dose toxicity

Member states broadly supported the French proposal and agreed to T+; R 28, T; R 48/25 It was agreed that R 26 and R 27 would be further investigated during the follow-up period to see if the evidence which led to the original classification with these phrases could be identified.

Carcinogenicity

In discussion of carcinogenicity Germany wondered if the mutagenicity evidence would lead to Category 2 classification but overall member-states preferred to classify with Carc Cat 3.

Mutagenicity

Member States agreed to Muta Cat 3; R 68.

Reproductive toxicity

Member States also supported the French proposal for Repr Cat 1; R 61 but disagreed that there was no case for classification for fertility. It was agreed to assign Repr Cat 3; R 62. It was also agreed that MS supporting R 64 could make a proposal during the follow up procedure.

Conclusion:

Discussion closed with agreement by the TC C&L to the following: **Carc Cat 3; R 40, Muta Cat 3; R 68, Repr Cat 1; R 61, Repr Cat 3; R 62, R64, T+; R 28, T; R 48/25.** T+; R 26/27 would be considered during the follow up period.

Member States agreed that the same classification as for methyl mercury would apply to methyl mercuric chloride.

Follow-up:

MS that would support no deletion of the current classification with T+; R26/27 should react in the Follow-up period. F sent in their rational for the R26/27 classification in ECBI/09/05 Add. 4. MS should react in case this information changed any classification agreed at the meeting. BE agreed with France (ECBI/09/05 Add.4) when they were proposing to keep skin and the inhalation route of exposure for acute toxicity.

NL sent in a proposal to classify with R64 (ECBI/09/05 Add. 3). In case other MS disagree to this proposal they should react during Follow-up II. DK and F agreed to the NL proposal.

The S proposal (ECBI/09/05 Add. 5) sent in during the Follow-up period to read across for other end-points between the mercury compounds should also be discussed at the next meeting.

Conclusion (Follow-up):

T+; R26/27 should be re-considered at the next meeting and classification with R64 should be confirmed. In addition the possibility to read across for other end-points based on the S proposal will be discussed in October 2006.

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TC C&L meetings of October 2006

Monomethyl mercury compounds (Index No: 080-004-00-7)

Methyl mercury (CAS number: 22967-92-6)

Methyl mercuric chloride (CAS No: 115-09-3, EC No: 204-064-2)

Current classification T+; R 26/27/28 – R33 - N; R50-53
Classification proposal T+; R 26/27/28 – R33 – Repr Cat 1; R61 – Repr Cat 3; R62 – R64 - N; R50-53

ECBI/09/05 Rev 1,	F, revised proposals for mercury compounds.
ECBI/09/05 Add 1 Rev 1	F, revised proposals for mercury compounds.
ECBI/09/05 Add 2 Rev 1	F, revised proposals for mercury compounds.
ECBI/09/05 Add. 3	NL, Proposal for the classification of methyl-mercury with R64
ECBI/09/05 Add. 4	F, Additional elements on mercury compounds
ECBI/09/05 Add. 5	S, proposal to read across to other mercury compounds
ECBI/09/05 Add. 6	S, Mercury study report of the US EPA.

In **November 2005** member-states held a general discussion of the proposal and commented on a number of issues including the appropriate classification for acute toxicity and the need for R 64.

In **March 2006** the discussion was closed with agreement by the TC C&L to the following: **Carc Cat 3; R 40, Muta Cat 3; R 68, Repr Cat 1; R 61, Repr Cat 3; R 62, R64, T+; R 28, T; R 48/25.** T+; R 26/27 would be considered during the follow up period. Member States agreed that the same classification as for methyl mercury would apply to methyl mercuric chloride.

During the **follow up procedure** several MS supported the T+; R26/27 classification, NL distributed a proposal for R64 classification and S proposed to read across for other endpoints as well. T+; R26/27 should be re-considered at the next meeting and classification with R64 should be confirmed. In addition the possibility to read across for other end-points based on the S proposal will be discussed in October 2006.

Acute toxicity:

F supported to keep T+; R26/27 because there is respiratory and dermal absorption. The other Member States agreed.

R64:

NL proposed to classify with R64. The TC C&L agreed.

Read across:

S appreciated the work by F. A proposal was sent in by **S**, proposing to read across to other mercury compounds. In the human body elemental mercury was oxidised and methyl mercury was reduced. These were reactions within the body. Different forms of mercury would be metabolised into the same compound within the body. Therefore **S** suggested that read across to other mercury compounds should be applicable.

The level of chronic toxicity was difficult to determine, but because there was evidence existing from the literature similar labelling for all compounds was warranted for chronic toxicity by oral route. The acute toxicity by all three routes should also be considered. For reprotoxicity endpoints it were relevant for both fertility and development. Methyl mercury had been classified with Repr. Cat. 1; R61 and Repr. Cat. 3; R62. This classification should also be applied to the other mercury compounds. Most difficult was to determine the level of toxicity for chronic toxicity.

F said that in general at reading across for the different end-points the TC C&L must be careful because the different substances do not behave the same in the body. They are not equally soluble and it is not easy to read across between one and the other. **F** explained for which substances they agreed or did not agree to read across between mercury compounds.

DE understood that S wanted to have more data included. That could be supported but at this point read across could not be discussed.

ECB proposed to come back at the next meeting with proposals for the other mercury compounds. **BE** also wanted to take better care on how to apply read across in this case.

S, F, DK and **BE** agreed to look into different endpoints for classification for additional mercury compounds and then come back with a proposal to the TC C&L.

Conclusion:

In October 2006 in was confirmed that T+; R26/27 should still be applied. R64 was added based on a proposal from NL (ECBI/09/05 Add. 3). The TC C&L then agreed to the final classification proposal: Carc. Cat. 3 ; R40 - Muta. Cat. 3 ; R68 - Repr Cat 1; R61 - Repr Cat 3; R62 - T+; R26/27/28 - T ; R48/25 - R64 - N; R50-53, accompanied with the labelling: Symbols: T+, N; R-phrases: 61-26/27/28-40-48/25-62-64-68-50/53; S-phrases: 53-45-60-61.

The classification proposals for other inorganic mercury compounds will be placed on a future meeting agenda when the classification proposals have been provided from the volunteering Member States.

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