Committee for Risk Assessment

RAC

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

Adopted

15 March 2017

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CLH-O-0000001412-86-146/F

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

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

Chemical name: **Methylmercuric chloride**

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

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

The proposal was submitted by **France** and received by RAC on **23 March 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **28 April 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 June 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-Rapporteur, appointed by RAC: **Ralf Stahlmann**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2017** by **a simple majority of all members present and having the right to vote**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	080-004-00-7	organic compounds of mercury with the exception of those specified elsewhere in this Annex			Acute Tox. 2 * Acute Tox. 1 Acute Tox. 2 * STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H330 H310 H300 H373 ** H400 H410	GHS06 GHS08 GHS09 Dgr	H330 H310 H300 H373 ** H410		* STOT RE 2; H373: ≥ 0,1%	A1
Dossier submitter's proposal	TBD	methylmercuric chloride	204-064-2	115-09-3	**Retain** Acute Tox. 1 **Add** Muta. 2 Carc. 2 Repr. 1A Lact. **Modify** Acute Tox. 2 Acute Tox. 2 STOT RE 1	**Retain** H310 **Add** H341 H351 H360Df H362 **Modify** H300 H330 H372 (nervous system, vision, kidneys)	**Retain** GHS06 GHS08 Dgr	**Retain** H310 **Add** H341 H351 H360Df H362 **Modify** H300 H330 H372 (nervous system, vision, kidneys)		**Remove** * STOT RE 2; H373: ≥ 0,1%	**Retain** Note 1 **Remove** Note A
RAC opinion	TBD	methylmercuric chloride	204-064-2	115-09-3	**Add** Carc. 2 Repr. 1A Lact. **Modify** Acute Tox. 2 Acute Tox. 2 Acute Tox. 2 STOT RE 1	**Add** H351 H360Df H362 **Modify** H330 H310 H300 H372 (nervous system, kidneys)	**Retain** GHS06 GHS08 Dgr	**Add** H351 H360Df H362 **Modify** H330 H310 H300 H372 (nervous system, kidneys)		**Remove** * STOT RE 2; H373: ≥ 0,1%	**Retain** Note 1 **Remove** Note A
Resulting Annex VI entry if agreed by COM	TBD	methylmercuric chloride	204-064-2	115-09-3	Carc. 2 Repr. 1A Lact. Acute Tox. 2 Acute Tox. 2 Acute Tox. 2 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H360Df H362 H330 H310 H300 H372 (nervous system, kidneys) H400 H410	GHS06 GHS08 GHS09 Dgr	H351 H360Df H362 H330 H310 H300 H372 (nervous system, kidneys) H410			1

Note: Hazard classes highlighted in grey in the row denoting the current Annex VI entry are not subject to assessment by RAC.

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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)**.

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**.

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 100 days	↑SBP after 4 weeks of exposure	N/A
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**.

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	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

		dose of 0.003 mg Hg/kg day.	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	Total hair mercury: 0.57 – 153.8 µg/g (mean: 13.5 µg/g)

			breaks in people with methylmercury levels < 10 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**.

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.

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.

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.

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.

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** for 30 days pre-mating until GD18 ***** calculated from published daily doses (in results section) in µmol MeHgCl (251.09 g/mol)	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 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)	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

		7 matings with 3 untreated virgins per male (Exp IV and V)	
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	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

		21 or 17 (high dose) matings with 2 untreated virgins per male	
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	no differences in body weight pre-mating decreased maternal bwg from GD3 at high dose

		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 MeHgCl (251.09 g/mol)	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
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	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.

		≈ 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	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**	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

		≈ 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 "chronic" and "bolus" regimens resulted in similar total amount of MeHgCl administered	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
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**	sig. decreased maternal bwg in high dose group and on some GD in other treated groups

		≈ 0, 2.0, 4.0, 6.0 mg/kg bw/d **Hg** GD7-GD14	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	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:

		Hg single dose on GD7	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	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)

		µmol/kg bw/d in low dose group and 500 to 700 µmol/kg bw/d in high dose group	
Newland, Reile, Langston 2004	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, 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 n = 2 per group (in utero group) n = 5 (postnatal group)	oral, sodium carbonate solution of methylmercuric chloride in syringe or corn oil solution of 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	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 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,	no differences in median reaction times evidence for an increase in impairment of auditory function in exposed monkeys relative to controls

		0.050 mg/kg bw/d **Hg** 3x per week + infants: 0, 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)*."

Fertility

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.

Development

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**.

ENVIRONMENTAL HAZARD EVALUATION

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.

Additional references

Bornhausen M, Müsch HR, Greim H. (1980) Operant behavior performance changes in rats after prenatal methylmercury exposure. Toxicol Appl Pharmacol;56:305-10.

Castoldi AF, Onishchenko N, Johansson C, Coccini T, Roda E, Vahter M, Ceccatelli S, Manzo L. (2008) Neurodevelopmental toxicity of methylmercury: Laboratory animal data and their contribution to human risk assessment. Regul Toxicol Pharmacol. 51:215-29

Iwai-Shimada M, Satoh H, Nakai K, Tatsuta N, Murata K, Akagi H (2015) *Methylmercury in the breast milk of Japanese mothers and lactational exposure of their infants.* Chemosphere 126:67-72

Sakamoto M, Kubota M, Matsumoto S, Nakano A, Akagi H (2002) *Declining risk of methylmercury exposure to infants during lactation.* Environ Res 90(3):185-189

Verschuuren HG, Kroes R, Den Tonkelaar EM, Berkvens JM, Helleman PW, Rauws AG,Schuller PL, Van Esch GJ. Toxicity of methylmercury chloride in rats. II. Reproduction study. Toxicology. 1976;6:97-106.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).