Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Methylmercuric chloride

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

CLH-O-0000001412-86-146/F

Adopted
15 March 2017

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: methylmercuric chloride
EC number: 204-064-2
CAS number: 115-09-3
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Belgium		MemberState	1
Comment received				
BE CA welcomes and thanks the French MSCA for submitting this proposal for harmonized classification and labelling. As a general comment, BE CA would like to emphasize the fact that no studies quality assessment is given and the potency of the effects (significance, severity) are not enough detailed. Thus it is not always easy to conclude on their reliability (impurity? Test guideline? GLP compliance?...) and if the study can be taken into account for the classification. BE CA can support the proposal for harmonized classification submitted by ANSES, on behalf of France MSCA in case the studies are sufficiently reliable.				
Dossier Submitter's Response				
Thank you for your support. Studies were considered reliable with restriction or supportive and have been assessed in a weight-of-evidence basis. Further details on some key studies have been provided in response to comment 3, 5, 10 and 12.				
RAC's response				
RAC shared the concerns expressed by the BE CA. However, it was possible to consider all the key endpoints by taking a weight of evidence approach.				

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2016	Norway		MemberState	2
Comment received				
General comments And Toxicokinetics, section 4.1, page 14 in the CLH report: We support the inclusion of this new entry in CLP Annex VI. It is a leftover from TC C&L. The toxicologically most relevant mercury (Hg) species for human exposure is methylmercury (MeHg). Based on toxicokinetics (TK) we support the use of MeHg				

documentation for the assessment of MeHgCl. A better description of the TK for MeHg in the report should be considered.

Dossier Submitter's Response

Thank you for your comment.

Please find below a summary of the available TK data on MeHg as provided in the EPA report of 1997.

ABSORPTION

Inhalation

Inhaled methylmercury vapors are absorbed through the lungs. Fang (1980) did not measure percent absorbed but showed a correlation between tissue mercury levels and both exposure level and duration in rats exposed to radioactively labelled methylmercury vapor.

Oral

Methylmercury is efficiently absorbed from the gastrointestinal tract. Approximately 95% of
methylmercury in fish ingested by volunteers was absorbed from the gastrointestinal tract (Aberg et al. 1969; Miettinen 1973). Similarly, when radiolabeled methylmercuric nitrate was administered in water to volunteers, uptake was greater than 95% (Aberg et al. 1969). Reports of the percentage of absorbed methylmercury distributed to the blood range from 1% to 10%. Following the ingestion of a single meal of methylmercury-contaminated fish, Kershaw et al. (1980) found that blood accounted for 5.9% of absorbed methylmercury, while Miettinen et al. (1971) found an initial value of 10%, decreasing to about 5% over the first 100 days. In a population that chronically ingested fish with high methylmercury levels, approximately 1% of the absorbed dose was distributed to the blood (Sherlock et al. 1982).

Dermal

Dermal absorption of the methylmercuric cation (CH Hg) (as the dicyandiamide salt) has also been observed in treated guinea pigs (Skog and Wahlberg 1964). Approximately 3–5% of the applied dose was absorbed during a 5-hour period. Absorption was measured both by disappearance of the applied compound and by appearance in kidney, liver, urine and blood.

DISTRIBUTION

Methylmercury is distributed throughout the body, easily penetrating the blood-brain and placental barriers in humans and animals (Clarkson 1972; Hansen 1988; Hansen et al. 1989; Nielsen and Andersen 1992; Soria et al. 1992; Suzuki et al. 1984). By contrast with elemental mercury, studies in rats indicate that methylmercury transport into tissues is mediated by the formation of a methylmercurycysteine complex (Aschner and Aschner 1990; Tanaka et al. 1991, 1992; Kerper et al. 1992). The complex is structurally similar to methionine and is transported into cells via a widely distributed neutral amino acid carrier protein. Methylmercury associates with water-soluble molecules (e.g., proteins) or thiol-containing amino acids because of the high affinity of the methylmercuric cation (CH Hg) for the sulfhydryl groups (SH)-. Complexes of methylmercury with cysteine have been identified in blood, liver and bile of rats (Aschner and Aschner 1990).
Al-Shahristani and Shihab (1974) calculated a "biological half-life" of methylmercury in a study of 48 male and female subjects who had ingested seed grain contaminated by organic mercurials. The half-life ranged from 35 to 189 days with a mean of 72 days; it was determined from distribution of mercury along head hair.

The blood half-life is 49–164 days in humans (Aberg et al. 1969; Miettinen et al. 1971) and 10–15 days in monkeys (Rice et al. 1989). Smith et al. (1994) determined a blood half-life of 32–60 days in a study of seven adult males given i.v. methylmercury. In the blood, methylmercury is found predominantly in the red blood cells (Kershaw et al. 1980; Thomas et al. 1986). In humans, the ratio of red blood cell methylmercury to plasma methylmercury is approximately 20:1. This ratio varies in animal species; the ratio is approximately 20:1 in primates and guinea pigs, 7:1 in mice, greater than 100:1 in rats and 42:1 in cats (Hollins et al. 1975; Magos 1987). Toxicokinetics of methyl mercury were studied in pigs after intravenous (i.v.) administration of the compound. The distribution of methyl mercury was slow taking 3-4 days to be completed. Blood elimination half-life was found to be 25 days. The apparent volume of distribution was 9.8 l/kg indicating pronounced tissue accumulation of methyl mercury. Highest mercury levels were found in kidney and liver, with lower contents in muscle and brain and very little in adipose tissue. The results indicate that from organs like liver and kidney methyl mercury is eliminated much more slowly than from the blood. Over a period of 15 days 16% of the dose administered was excreted with faeces and 0.9% in the urine (Gyrd-hansen 1981; Iverson 1974).

The clinical significance of the differences in the distribution of various forms of mercury in the blood is that it permits diagnosis of the type of mercury to which an individual has been exposed. Short chain alkyl mercury compounds such as methylmercury or ethyl mercury are very stable in the body, whereas long-chain compounds may be metabolized over time to the mercuric ion. The mercury distribution in the blood, therefore, may shift from a distribution characteristic of methylmercury to one more suggestive of inorganic mercury (Berlin 1986; Gerstner and Huff 1977). Mercury has been found in the umbilical cord of human newborns at levels comparable to maternal blood levels (Grandjean et al. 1992a). For lactating mothers, the clearance of mercury from the blood appears to be faster than for non-lactating women. Lactating individuals have a blood half-life of 42 days compared to 75 days for non-lactating females among a group of people who had consumed contaminated seed grain (Greenwood et al. 1978). This finding may be due to excretion of mercury via the milk, increased food intake by mothers (which enhances biliary excretion) and/or altered hormonal patterns in lactating mothers (which affect the excretion pattern).

Methylmercury transport across the blood-brain barrier in rats may involve an amino acid carrier (Kerper et al. 1992). Following acute exposure to methylmercury, most of the mercury in the brain is in the organic form; however, with chronic exposures, a greater amount of the mercury in the brain is in the inorganic form, suggesting that the rate of demethylation increases with long-term exposure (Aschner and Aschner 1990). Rice (1989a, 1989b) demonstrated that tissue half-life in the brain may be significantly longer than the blood half-life for methylmercury.
The bioaccumulation of methylmercury can be affected by age and sex (Thomas et al. 1982, 1986, 1988). After administration of methylmercury to rats, the females had higher peak levels of mercury in the kidneys, primarily as methylmercury, compared to the males; inorganic mercury levels did not differ significantly between the sexes (Thomas et al. 1986). Accumulation of mercury in the body is also found to be higher in neonatal rats (Thomas et al. 1988) than in adult rats (Thomas et al. 1982). Ten days after administration of methylmercury, 94% of the dose was still detected in neonates while _60% was retained in adults (Thomas et al. 1988). The longer retention of mercury in the neonates may be attributed to various factors including the high amount of mercury accumulated in the pelt of the neonates due to lack of clearance (Thomas et al. 1988) and the lack of a fully developed biliary transport system in the neonates (Ballatori and Clarkson 1982).

METABOLISM

Animal data

Methylmercury in the body is relatively stable and is only slowly demethylated to form mercuric mercury in rats (Norseth and Clarkson 1970). The demethylation appears to occur in tissue macrophages (Suda and Takahashi 1986), intestinal microflora (Nakamura et al. 1977; Rowland et al. 1980) and fetal liver (Suzuki et al. 1984). In vitro demethylation has been reported to involve hydroxyl radicals produced by cytochrome P-450 reductase (Suda and Hirayama 1992) or hypochlorous acid scavengers (Suda and Takahashi 1992). Organic mercury compounds with longer alkyl chains are more readily metabolized over time to the mercuric ion (Berlin, 1986).

Human cases

Methylmercury metabolism may be related to the latent or silent period observed in epidemiological studies from two methylmercury poisonings. During the latent period, both during and after the cessation of exposure, the patient feels no untoward effects. It is possible that a number of biochemical changes may take place in parallel during this period, and some may not be causatively related to the clinical outcome. Ganther (1978) has hypothesized that the carbon-mercury bond in methylmercury undergoes homolytic cleavage to release methyl free radicals. The free radicals are expected to initiate a chain of events involving peroxidation of lipid constituents of the neuronal cells. The onset of symptoms is delayed for the period of time that cellular systems are able to prevent or repair effects of lipid peroxidation. When the cellular defense mechanisms are overwhelmed, rapid and progressive degeneration of the tissue results. In the Iraqi poisoning incident, the latent period before toxic signs were noted varied from a matter of weeks to months. By contrast, in the Japanese poisoning incident, the latency was as long as a year or more. The difference in duration of the latent period may in part be due to the presence of selenium in the fish ingested by the Japanese population.

EXCRETION

Like inorganic mercury, methylmercury has a relatively long half-life of approximately 70–80 days in the human body (Aberg et al. 1969; Bernard and Purdue 1984; Miettinen 1973). Recently a shorter half-life of 44 days was estimated by Smith et al. (1994) in their study of seven adult males treated i.v. with methylmercury. In this study methylmercury and inorganic mercury concentrations in blood and excreta were determined separately based on differential extractability into benzene. The predominant species in the blood was methylmercury; there was no detectable methylmercury in the urine.
The long half-life of methylmercury in the body is due, in part, to reabsorption of methylmercury secreted into the bile (hepato-biliary cycling) (Norseth and Clarkson, 1971). In this cycle, methylmercury forms a complex with glutathione in the hepatocyte, and the complex is secreted into the bile via a glutathione carrier protein (Clarkson, 1993b). The methylmercury-glutathione complex in the bile may be reabsorbed from the gallbladder and intestines into the blood. When microorganisms found in the intestines demethylate methylmercury to form mercuric mercury, this cycle is broken, and fecal excretion of mercury from methylmercury occurs (Rowland et al. 1980). Mercuric mercury is poorly absorbed from the intestines, and that which is not reabsorbed is excreted in the feces. In humans, approximately 90% of the absorbed dose of methylmercury is excreted in the feces as mercuric mercury.
Excretion via the urine is minor but slowly increases with time; at 100 days after dosing, urinary excretion of mercury accounted for 20% of the daily amount excreted. The urinary

excretion of mercury may reflect the deposition of demethylated mercury in the kidneys and its subsequent excretion.

In animals, the predominant route of methylmercury elimination also is the feces (Farris et al.1993; Hollins et al. 1975; Thomas et al. 1987). As in humans, biliary excretion of methylmercury and its demethylation in gastrointestinal flora have been reported in rats (Farris et al., 1993) and in guinea-pigs (Komsta et al, 1983). After a single oral dose of methylmercury, the major elimination route was the feces (65% of the administered dose as inorganic mercury and 15% of the administered dose as methylmercury) and the minor route was urine (1% of the administered dose as inorganic mercury and 4% of the administered dose as methylmercury) (Farris et al. 1993). Female guinea pigs were dosed po with 1.0 mg CH3 203Hg/kg as methylmercuric chloride, 10 times over a 3-week period. Tissue distribution, excretion, and accumulation of inorganic and organic mercury were studied. The highest concentration of mercury was found in the kidney. The greatest decreases of mercury levels were observed in the small bowel, red blood cells, liver, and cerebrum. The half-life of whole body clearance, based on a single compartment model, was 31.6 days. Mercury in the kidney, liver, and cerebrum was bound mainly by nuclear and soluble fractions. The highest ratio of inorganic to total mercury was seen in the kidney, 60% of this being as inorganic mercury. Excretion of mercury in the feces was measured throughout the experiment. The relationship of organic to inorganic mercury was relatively constant at about 1:3. Data on the effects of methyl mercury on tissue concentrations of zinc and copper show that the only change in the copper content was a marked increase in the kidney (Komsta et al, 1983).

In rat and monkey neonates, excretion of methylmercury is severely limited (Lok 1983; Thomas et al. 1982). In rats dosed prior to 17 days of age, essentially no mercury was excreted (Thomas et al. 1982). By the time of weaning, the rate of excretion had increased to adult levels. The failure of neonates to excrete methylmercury may be associated with the inability of suckling infants to secrete bile (Ballatori and Clarkson 1982) and the decreased ability of intestinal microflora to demethylate
methylmercury during suckling (Rowland et al. 1977).
Methylmercury is also excreted in breast milk (Bakir et al. 1973; Sundberg and Oskarsson 1992).
The ratio of mercury in breast milk to mercury in whole blood was approximately 1:20 in women exposed to methylmercury via contaminated grain in Iraq between 1971 and 1972 (Bakir et al. 1973).
Evidence from the Iraqi poisoning incident also showed that lactation decreased blood mercury clearance half-times from 75 days in males and nonlactating females to 42 days in lactating females; the faster clearance due to lactation was confirmed in mice (Greenwood et al. 1978). In mice, of the total mercury in the breast milk, approximately 60% was estimated to be methylmercury. Skerfving (1988) has found that 16% of mercury in human breast milk is methylmercury. Studies in animals indicate that the mercury content of breast milk is proportional to the mercury content of plasma (Sundberg and Oskarsson, 1992; Skerfving, 1988).

The role of the gallbladder in the disposition of methyl mercury was investigated in guinea pig, hamster, and macaque monkey. 203Hg-labeled methyl mercury or inorganic mercury (5 microM) and [14C]inulin were instilled into the in situ guinea pig or hamster gallbladder. After 2 h, only 27.6 +/- 7.0% of the methyl mercury remained in guinea pig gallbladder fluid as compared with 85.0 +/- 3.2% of the inorganic mercury and 90.7 +/- 4.5% of the [14C]-inulin. In the hamster, 42.5 +/- 4.5% of methyl mercury and 95% +/- 0.9% of inorganic mercury remained after 2 h. When the sulfhydryl-containing compounds L-cysteine, glutathione, and bovine serum albumin (20 microM) were added to the test solution, cysteine increased and albumin decreased absorption of methyl mercury. Ligation

of guinea pig cystic artery decreased gallbladder fluid absorption from 72.7 +/- 8.6 to 26.5 +/- 9.8% over 2 h but did not alter methyl mercury absorption. Bile was also sampled from gallbladders of four monkeys exposed chronically to CH_3HgCl and from three control monkeys. For one of the exposed and one of the control monkeys, bile was also collected from the common hepatic duct. In both methyl mercury-exposed and control monkeys, the concentration of methyl mercury in gallbladder bile was lower than in hepatic bile. In contrast, the concentration of inorganic mercury in gallbladder bile was four to seven times that of hepatic bile, suggesting that methyl mercury but not inorganic mercury was being reabsorbed. To assess the functional significance of methyl mercury reabsorption by the gallbladder, guinea pig cystic ducts were ligated, the animals were given $CH_3\ ^{203}HgCl$ (10 mumol/kg iv), and body burden of ^{203}Hg was measured over 16 days (Dutczak et al, 1991).

References

EPA (1997). Mercury Study Report to Congress, EPA-452/R-97-007, December 1997. Volume V: Health Effects of Mercury and Mercury Compounds.

RAC's response

Thank you for the additional information. This provides further useful background context for the proposal.

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Germany		MemberState	3

Comment received

The German CA agrees to the current proposal of the French CA for harmonised classification and labelling of methylmercuric chloride.

The French CA decided to focus the current proposal for harmonised classification and labelling of methylmercuric chloride on human health effects only.

The substance which currently has no specific classification/labelling-entry in Annex VI (Table 3.1) of the CLP Regulation is so far covered by the entry for organic compounds of mercury (Index No 080-004-007).

Methylmercuric chloride was already discussed within the previous legislative framework at the Technical Committee (TC) C&L between November 2005 and 2006. In October 2006 the TC C&L adopted the final recommendations for harmonised classification and labelling of methylmercuric chloride based on Directive 67/548/EEC criteria as:
Carc Cat 3; R40 - Muta Cat 3; R68 - Repr Cat 1; R61 – Repr Cat 3; R62 - T+; R26/27/28 - T; R48/25 - R64 N; R50-53, accompanied with the labelling:
Symbols: T+, N; R-phrases: 61-26/27/28-40-48/25-62-64-68-50/53; S-phrases: 53-45-60-61.
The French CA has implemented the final decision of the TC C&L regarding the toxicological classification/labelling of human health effects.

In addition, the French CA has considered new data as well as previously not considered data in the CLH report. These data did not lead to any changes of classification and labelling with regard to the evaluation of the human health hazards of methylmercuric chloride.

Further comments on the CLH report

Following information would be helpful:
- an assessment of the single studies regarding reliability (e.g. Kliemisch score),
- a statement about the guideline compliance/non-compliance (sometimes given),
- whenever adequate a statement as "positive, negative or equivocal",
- a separation of the studies in "Studies conducted with MeHgCl/MeHg/MeHgOH/etc. (since the CLH proposal is specifically about Methylmercuric chloride)

Section "4.1 Toxicokinetics of mercury compounds"

Please provide a comprehensive overview of the data regarding absorption, distribution, metabolism and excretion. While some information is available in this section other information is given in different sections.
Data regarding mercury concentration in tissue sections in the studies of e.g. Ernst et al. 1991 a, Kim et al. 2000, Lee et al. 1995, Gundersson et al. 1988 may add valuable details about distribution and elimination as might the results from the studies of Ernst et al. 1991 a, McNeil and Bhatnagar 1985 and Mohamed et al. 1987 that show effects in germ cells and/or associated tissues.

Dossier Submitter's Response

Thank you for your support on the classification proposal.

<u>Comments on CLH</u>
Most of the animal studies in the report have been performed with MeHgCl and were considered reliable with restriction. Animal studies performed with other mercuric compounds are considered as supportive data.

Please find below details on some key animal studies reliable with restriction use for classification of MeHgCl:

Endpoint	Study reference	Guideline compliance	Test material	Other details
4.2.1.1 Acute toxicity: oral	Yasutake, 1991	no	MeHgCl	-
4.7 repeated dose toxicity	Hirano, 1986	no	MeHgCl	-
	Mitsumori, 1990	similar to OECD 451	MeHgCl	-
	Vershuuren, 1976	no	MeHgCl	-
	Wild, 1997	no	MeHgCl	-
	Grotto, 2009	no	MeHgCl	-
	Chang, 1972	Secondary litterature	MeHgCl	-
4.9 Germ cell mutagenicity	Betti 1992	similar to OECD 473	MeHgCl	-
	Betti 1993	no	MeHgCl	-
	Bala 1993	no	MeHgCl	-
	Morimoto 1982	no	MeHgCl	-
	Costa, 1991	secondary litterature	MeHgCl	-
	Miller, 1979	no	MeHgCl	3/sex/group
	Mailhes, 1983	no	MeHgCl	21 females in control, 13 in

					positive control and 15 in treated group. 150 oocytes/group analysed	
		Verschaeve, 1984a	similar to OECD 478	MeHgCl	20 females/group	
	4.10 Carcinogenicity	Verschuuren, 1976	no	MeHgCl	25/sex/group	
		Mitsumori, 1981	similar to OECD 451	MeHgCl	60/sex/group	
		Hirano, 1986b	similar to OECD 451	MeHgCl	60/sex/group	
		Mitsumori, 1990	similar to OECD 451	MeHgCl	-	
	4.11.1 effects on fertility	Nobugata, 1979	no	MeHgCl	14 IVCS mice/group in control, low and high dose of MeHgCl	
		Khera, 1973	no	MeHgCl	Mouse : Exp I : 10-12 males/group exp II: 12-13 males/groups Rats: exp I: 12-20 males/group exp II: 15 males/group Exp III : 14-19 males/group	
	4.11.2 developmental toxicity	Nobugata, 1979	no	MeHgCl	14 IVCS mice/group in control, low and high dose of MeHgCl	
		Belles, 2002	no	MeHgCl	-	
		Goulet, 2003	similar to OECD 423	MeHgCl	mouse per groups : 0 (n=14), 4(n= 14), 6 (n= 34), or 8 (n= 14) ppm	
		Lee, 1995	Similar to OECD 414	MeHgCl	30 rats/group	
		Newland, 2000	no	MeHgCl	295 foetuses from 25 litters but not all were used in the study.	

	Newland, 2004	no	MeHg Cl	5-11 rats/group
	Fuyuta, 1978	Similar to OECD 414	MeHg Cl	10 mice/group 20 rats/group
	Khera, 1973	Similar to OECD 414	MeHg Cl	mice: 11-17/group rats: 9-13/group
	Rice, 1995	no	MeHg Cl	-
	Rice, 1998	no	MeHg Cl	-
	liang, 2009	no	MeHg Cl	20 mice/group

Toxicokinetics

See response to comments number 2 for summary of ADME of methylmercury. Please find below a summary of the mentioned studies link to distribution and elimination.

Maternal and fetal toxicity of methylmercuric chloride administered orally to pregnant Fischer 344 rats following 10, 20 and 30 mg/kg of methylmercuric chloride (MMC) on day 7 of gestation was reported by **Lee and Han in 1995**. Mercury content in maternal organs was highest in kidney, followed by blood, spleen, liver, and brain, while in fetal organs it was highest in liver. Fetal liver and brain contained more mercury than maternal liver and brain. However, fetal kidney retained less mercury than maternal kidney.

Kim et al in 2000 reported some neurobehavioral changes in rodents prenatally exposed to methylmercury. Pregnant mice of three inbred strains (BALB/c, C57BL/6J, C57BL/6Cr) were orally given methylmercury (MMC; 3 x 3 mg/kg body weight) during days 12-14 of gestation and allowed to deliver. Total mercury was determined in mice at 12 weeks of age. The mercury levels in the liver and brain were significantly higher in all three treated groups than those in their respective control groups. However, the mercury levels in the liver and brain of the MMC groups were not beyond twice levels in those of the control groups. In addition, the mercury levels in the various tissues of the control groups are thought to be derived from the food diet.

Gunderson reported in 1988 some deficits in juvenile and adult macaques after methyl mercury exposure in utero (50 or 70 µg/kg/day) included visual recognition memory. In this study, infant Macaca fascicularis were exposed prenatally to maternal subclinical levels of methylmercury (MeHg). Maternal blood level during each trimester of the pregnancy and at delivery were determined (n=9). Infant (n=9) blood MeHg blood level was determined at birth and after 5-month. The level of mercury in maternal blood decrease significantly over the course of pregnancy. According to the authors it is unclear whether this decrease was the result of maternal weight gain during pregnancy, increased uptake of MeHg by the fetus or a combinaison of these and other factors. It tooks approximately five months for the MeHg to clear from the blood of the infants.

In the study of **Ernst** published in 1991, the autometallographic silver enhancement technique has been used to demonstrate the ultrastructural localization of mercury in the testes of adult rats. Administration of mercuric chloride or methyl mercuric chloride in the drinking water (20 mg/L for 12 weeks) resulted in intracellular accumulations of mercury in the interstitial Leydig cells as well as in the Sertoli cells of the seminiferous tubules.

Mohamed et al. in 1987 published studies of the effects of MeHg on testicular function in Macaca fascicularis monkeys. In an *in vivo* study involving oral treatment of adult males Macaca fascicularis monkeys with MeHg for 20 weeks, changes in spermatozoal production, motility and morphology and in serum testosterone were followed before, during and after treatment. Blood samples were collected weekly for mercury analysis during a period of 20

weeks. Blood mercury levels in each of the treated animals increased with duration of intake. Animals blood mercury levels in the lower dose group showed more variability than in the higher dose group. During the recovery period, the clearance of blood mercury level followed a logarithmic curve for all the animals. The average half-life was 20.4 ±1.02 and 23.6 ±1.27 days for the animals in the high and low dose groups respectively. The difference in half-life between the two groups was significant.

References

Ernst E, Møller-Madsen B, Danscher G.Ultrastructural demonstration of mercury in Sertoli and Leydig cells of the rat following methyl mercuric chloride or mercuric chloride treatment. Reprod Toxicol. 1991;5(3):205-9.

Gunderson VM, Grant-Webster KS, Burbacher TM, Mottet NK. Visual recognition memory deficits in methylmercury-exposed Macaca fascicularis infants. Neurotoxicol Teratol. 1988 Jul-Aug;10(4):373-9.

Kim CY, Nakai K, Kasanuma Y, Satoh H. Comparison of neurobehavioral changes in three inbred strains of mice prenatally exposed to methylmercury. Neurotoxicol Teratol. 2000 May-Jun;22(3):397-403.

Lee JH, Han DH. Maternal and fetal toxicity of methylmercuric chloride administered to pregnant Fischer 344 rats. J Toxicol Environ Health. 1995 Aug;45(4):415-25.

Mohamed MK, Burbacher TM, Mottet NK. Effects of methyl mercury on testicular functions in Macaca fascicularis monkeys. Pharmacol Toxicol. 1987 Jan;60(1):29-36.

RAC's response

Thnak you; the additional information about the reliability and/or regulatory compliance of the toxicological studies is helpful.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Belgium		MemberState	4

Comment received

BE CA can agree that, according to the available data on male mice (Hirano, 1986; Mitsumori, 1990), MeHgCl could be a human carcinogen and a classification as Carc. 2 should be applied. Indeed, Hirano detected an increased incidence of renal epithelial cell adenocarcinomas in males and Mitsumori's study confirmed the results. Furthermore, Yorifuji (2007) detected a positive correlation between human exposure to methylmercury (MeHg) through a contaminated environment and leukemia, which confirms the concern on human carcinogenicity.

Dossier Submitter's Response

Thank you for your support.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2016	Norway		MemberState	5

Comment received

Section 4.10, p. 39-43:
We support the inclusion of this new entry in CLP Annex VI. It is a leftover from TC C&L.

The toxicologically most relevant mercury (Hg) species for human exposure is

methylmercury (MeHg). Based on toxicokinetics (TK) we support the use of MeHg documentation for the assessment of MeHgCl. A better description of the TK for MeHg in the report should be considered.
We assume that the findings of renal tumours in male mice receiving MeHgCl via the diet in three different studies are statistical significant compared to the controls. As this is not stated in the CLH report, it is difficult to assess the strength of evidence. If the findings are statistical significant, we agree with the proposed classification of Carc.2 (due to findings in one species, one sex, mostly benign tumours, and from same laboratory). Nephropathy was also observed in female mice but not development of renal tumours. The human data does not clarify the carcinogenic potential of MeHgCl much, even if an association with leukemia was found in one study.

Dossier Submitter's Response

Thank you for your support.

Toxicokinetics
See response to comments number 2 and 3.

Carcinogenicity
The increase incidence in renal tumors was statistically significant in the three studies. Futher details are provided below.

Results from Mitsumori et al., 1981

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

*** Fisher's exact test p < 001

Results from Hirano et al., 1986

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

*** p< 0.001

Results from Mitsumori et al., 1990

In proliferative lesions, there were significant increases in the incidence of renal adenoma and/or carcinoma (16/60) and tubular cell hyperplasia (14/ 60) in males of the 10ppm group, as compared to the control group. The incidence of chronic nephropathies also increased in males of the 2ppm group.

RAC's response

Thank you for provided a more detailed summary of the results of these key studies; this supports the proposal.

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Germany		MemberState	6
Comment received				
Section "4.10 Carcinogenicity" In section "4.10.4 Human information" the study of Yorifuji et al. 2007 is presented. Please provide the complete wording of the abbreviation ASMR at least once. In the publication the authors themselves state a number of confounding factors that narrow the value of the study and should be mentioned here. Otherwise it is likely not clear why the positive association with leukaemia that was found is not weighed more strongly for classification.				
Dossier Submitter's Response				
The abbreviation ASMR means for Age Standardized Mortality Ratio. The following confounding factors were disscused by the authors : - endemic HTLV-1 infection, - other leukemogens (e.g., benzene, smoking, radiation). However, according to the authors these confounding could not fully explain the increased leukemia ASMR. The present study has also several limitations according to the authors: - ecological effect estimates might fail to reflect biologic effects at the individual level, - increase leukemia because of surveillance, - data only after 1961 (no assessment of early contamination), - no determination of sex specific ASMR, - no assessment of population mobility.				
RAC's response				
Thank you for clarifying the relevance of the human data. It is evident that the key findings on carcinogenicity are to be found in the animal studies.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Belgium		MemberState	7
Comment received				
According to some in vitro studies, MeHgCl has the ability to induce a significant increase in chromosomal aberrations (Betti, 1992) and in SCEs (Bala, 1993), … Mutagenicity potential of MeHgCl could not be confirmed in vivo according to the TC C&L dossier data. However, human information allows to establish a correlation between the consumption of (Me)Hg-contaminated fish and significantly increased chromosomal aberrations, breaks and micronuclei in lymphocytes in vitro (Skerfving, 1974, 1970; Franchi, 1994; …). A tendency to increase the number of micronucleated cells, a significant increased number of cells in metaphase and a higher proportion of cells containing nucleoplasmic bridges were also detected after exposing human neuroblastoma cells to MeHgCl in vitro (Crespo-Lopez, 2007). Beside the lack of evidence in vivo, these positive in vitro results, the proposal to classify MeHgCl as Muta. Category 2 can be suitable.				

Dossier Submitter's Response
Noted.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Sweden		MemberState	8
Comment received				

p. 37-38 for all text below.

We agree that in vitro data show that methylmercuric chloride has a genotoxic potential.

In section 4.9.4 of the CLH report it is stated that two in vivo studies using the intraperitoneal route of exposure show effects on germinal cells and that, according to the CLP guidance 3.5.2.3.9, this route of exposure is not considered as relevant to the expected route of human exposure (mainly oral) to evaluate potential effects on germinal cells. We do not agree that this is actually expressed in 3.5.2.3.9, since the statement in 3.5.2.3.9 is "The relevance of the route of exposure used in the study of the substance compared to the most likely route of human exposure shall also be taken into account".

In the CLP guidance it is further stated "A positive result for somatic or germinal mutagenicity in a test using intraperitoneal administration only shows that the tested substance has an intrinsic mutagenic property, and the fact that negative results are exhibited by other routes of dosage may be related to factors influencing the distribution/metabolism of the substance which may be characteristic to the tested animal species. It cannot be ruled out that a positive test result in intraperitoneal studies in rodents only may be relevant to humans." …. "For instance, it may be difficult to reach a decision on whether or not to classify in the case where there are positive in vivo data from at least one in vivo test using intraperitoneal application but (only) negative test data from (an) in vivo test(s) using oral, dermal, or inhalative application."

We think this guidance should be taken into account when evaluating the in vivo data for methylmercuric chloride as follows:

There was a statistically significant ($P<0.01$) increase in hyperploidy in germ cells in Syrian hamster, indicating induction of numerical chromosome aberrations. A positive dominant lethal study in female mice indicating mutagenic effects (structural and numerical chromosome aberrations) in germ cells is also available, but since no data on maternal toxicity was reported we agree that this limits the value of the study for the evaluation. A study in cats orally exposed through the diet demonstrated a statistically significant ($P<0.05$) increase in nuclear abnormalities in the bone marrow. In conclusion, since one study using intraperitoneal exposure was positive for numerical chromosome aberrations in germ cells, and one study using oral exposure was positive for nuclear abnormalities in the bone marrow, the present case differs from the circumstances described in the CLP guidance referred to above, i.e. "For instance, it may be difficult to reach a decision on whether or not to classify in the case where there are positive in vivo data from at least one in vivo test using intraperitoneal application but (only) negative test data from (an) in vivo test(s) using oral, dermal, or inhalative application." In the present case there are 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, 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, i.e. interacts with a cellular structure in germ cells of crucial importance for normal segregation of chromosomes. Therefore it is not "difficult to reach a decision on whether or not to classify". Seeing that the data demonstrate 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, we think that classification in Muta. 1B rather than Muta. 2 could be considered.

Dossier Submitter's Response

The *in vivo* oral genotoxicity study in cats (Miller, 1979) is only considered supportive due to the limitations of the study:

- unusual test species,
- low number of animals,
- unusual measurement of positive animals (cats characterized as with or without the presence of 2 or more micronuclei),
- no dose-relation observed,
- no positive and negative controls.

Therefore, we agree with the necessity to classify the genotoxicity potential of methylmercuric chloride but we are in the opinion that Muta. 2 is more appropriate based on the *in vitro* findings and positive *in vivo* results in an oral study with limitations or studies with intraperitoneal route of exposure.

RAC's response

As discussed in the ODD, there are significant weaknesses in all of the in vivo studies that make it very difficult to conclude that methylmercuric chloride possesses this hazard. It is a possibility, but in RAC's view the necessary weight and strength of evidence to meet the criteria is lacking.

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2016	Norway		MemberState	9

Comment received

Section 4.9, p. 31-39:
Two in vivo tests on germ cells were available: Effects from IP dosing with MeHgCl were observed in a study where dominant lethal (DL) mutations caused increased pre- and postimplantation loss in mouse, and in another study an increase in hyperploid cells in germ cells in hamsters was observed. These in vivo germ cell studies are accompanied with genotoxic effects in vitro in somatic cells and in vivo findings in cat bone marrow as well as various observations in humans. In a weight of evidence analysis approach a genotoxic potential of MeHgCl in vivo is plausible. However we agree with the proposed classification Muta.2 as the administration route in the DL tests were IP, and maternal toxicity may be involved in the implantation losses but maternal toxicity was not reported.

Dossier Submitter's Response

Thank you for your comment.

RAC's response

One of these studies was NOT a heritable mutation test. As females were treated with the test substance, the possibility of a non-genotoxic cannot be excluded. The relevance of other test is also unclear given that damage was assessed in metaphase II chromosomes from hamster oocytes. Such a target is not proven as a reliable model for chromosomes is somatic cells and therefore the lack of relevance of the I.P. route of exposure is especially relevant in this instance.

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Germany		MemberState	10

Comment received

Section "4.9.1.1 Germ cell mutagenicity"

There appears to be information missing. Please supplement:

- Substance name/specification in the studies of Costa 1991 and Fiskejo 1979 (defined as Methylmercury chloride in the respective publications) and exposure/dose-information/statistical evaluation in the study of Costa 1991
- Possible confounding in the studies of Franchi 1994 and Amorim et al. 2000
- Information whether a negative control has been assessed in the in vitro studies (assessment of a negative control is mentioned only in the studies of Betti 1993 and Verschaeve 1984 a)

Dossier Submitter's Response

Please find below the missing information:

Substance name/specification

The study from Costa and al. is a review on $HgCl_2$ and Methyl-Hg Cl_2. We have no information on exposure/dose-information/statistical evaluation. This study is only considered as supportive data.

In the study of Fiskejo et al., 1979, two test materials were used, methyl mercury chloride and methoxymethyl mercury chloride. The test material comes from Casco and plantex, respectively, however, no purity data is given.

Confoundings in human studies

In the study of Franchi, 1994, the following confounding factors were evaluated: smoking and alcohol.

In the study of Amorim, 2000, the following potential confounding factors were taken into account: age, smoking habits, alcoholic beverages, malaria.

Negative controls

In the study of Betti et al., 1993, a negative control group was assessed in the study. Untreated controls received the vehicle only (DMSO)

In the study of Verschaeve et al., 1984a, a negative control was assessed.

RAC's response

Noted; the additional information is appreciated.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Belgium		MemberState	11
Comment received				
Repr. 1A – H360Df Fertility: Some marked effects were observed after exposing only male rats to MeHgCl before mating: a significant dose-dependent decrease on the average incidence of pregnancy and a significant decrease of the number of viable embryos per litter (Khera, 1973). Male rats exposed to MeHg had a significant decreased plasmatic testosterone level and an associated drop in testosterone concentration in the interstitial and seminiferous tubules fluids evoking a disorder in testosterone synthesis (Moussa, 2010; Fossato, 2011). Sperm parameters abnormalities were detected in the rat treated with MeHg (Fossato, 2011). Ultrastructural alterations of the Sertoli's cells and degeneration of primary spermatocytes were identified in Pekin ducks (Mc Neil and Bhatnagar, 1985). In human data, it was concluded that men with higher levels of mercury in their hair were nearly twice as likely to be subfertile (Dickman, 1999). Furthermore, higher blood mercury concentrations were associated with male and female infertility (Choy, 2002). Development: A significant incidence of fetuses with cleft palates and growth retardation were observed in mice exposed to MeHgCl (Nobunaga, 1979). MeHgCl also induced a decrease in the average fetal bw/litter, skeletal defects and cleft palates in another study on mice (Belles, 2002). Cleft palates were also seen in rats exposed to MeHgCl, with associated vertebral defects (Fuyuta, 1978). In mice exposed to MeHg, significant neurotoxicity such as changes in locomotion, decreased home cage activity, … was detected (Kim, 2000). In human data available, children exposed in utero, or through maternal milk, to an environment contaminated by organic mercury showed mental retardation, cerebellar ataxia, deformity of the limbs, and other abnormal neurological symptoms (Harada, 1995; Snyder, 1971; Marsh, 1987;…). According to the available data, BE CA agrees that a classification as Repr. 1A – H360Df is appropriate beside the lack of reproducibility of the studies and the clear evidence of the impact of MeHgCl on Humans since there is evidence of teratogenicity potential of MeHgCl on animals and neurodevelopmental effects in humans induced by organic mercury. Lact. Effects – H362 As mothers eliminate organic mercury through their milk, lactation effects on children neurological system were observed (Amin-Zaki, 1979, 1981). Therefore, BE CA supports the classification proposed as Lact. Effects – H362.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Sweden		MemberState	12
Comment received				
The Swedish CA agrees with the classification of methylmercuric chloride in Repr. 1A – H360Df, as specified in the proposal. Regarding developmental effects, the Swedish CA supports the rationale for classification of methylmercuric chloride as Repr. 1A – H360D "May damage the unborn child". Epidemiological studies after exposure of children in utero show a causal relationship with				

adverse effects of methyl mercury on child neurodevelopment. In addition, development is severely impaired in several species, with teratogenicity in rats and neuro-behavioral effects (locomotion, memorisation, hyperactivity, visual defects) in rats, mice and monkeys.

Regarding fertility, the Swedish CA agrees with the conclusion that the data as presented meet the criteria for classification of methylmercuric chloride as "Suspected of damaging fertility". Indeed, to consider classification on fertility as Category 1B, the adverse effects on sexual function would need to be even more clearly connected to impaired fertility, since some of the key studies show some deficiencies. Studies in rats, mice, monkeys and Pekin ducks show that methylmercuric chloride causes a decrease in testosterone biosynthesis, which affect spermatocyte formation. Furthermore, decreased mating success in rats, sperm with abnormal heads in rats, prolonged estrus cycle in mice, alteration of reproductive performance in mink, accumulation of mercury in seminiferous tubules in rats and in ovary in mice, and decreased sperm motility in monkeys and rats were reported. Since no histopathological changes were observed in testis and epididymis, it would seem that it is a late effect affecting the sperm motility. In addition, epidemiological studies indicate an association between mercury exposure and male infertility. To allow an even more robust rationale, the Swedish CA would welcome some clarifications and a more transparent reporting in a few cases:

• In general for the mating trials, it would be desirable to know the mating index, and paternal status such as parental toxicity, body weight gain, clinical observations, behavior and food consumption, to establish the relevance of specific fertility effects in the parent.
• Nobunaga et al., 1979: it would be interesting to know if the prolonged estrous cycle has a biological consequence in terms of pregnancy.
• Khera, 1973 (b): in the chronic study on rats, it could be interesting to know the number of preimplantation losses at 0.1 and 0.5 mg/kg bw/day, compared with control.
• Fossato et al., 2011: in observations and remarks on sperm motility, it is stated that "Sperm motility significantly decreased in type A sperm (mobile with progression) while type B (mobile without progression) and type C (immobile) sperm motility increased." Should this be expressed differently or does the motility actually increase in immobile sperm? "[…] Significant elevation in daily sperm production. In contrast, there was a reduction in sperm quantity". Does this suggest that the sperm production is not necessarily connected to sperm quantity?
• Mohamed et al., 1987: it would be valuable to know how many animals were included in the study and if there were any signs of general toxicity. In addition, in the dose column it is stated that the test substance is methyl Hg, in contrast to the text in the section "Comparison of criteria" where it is stated that the test substance is methyl mercury chloride.
• Popescu, 1978: it would be of interest to know how many workers were affected and at what doses.

The Swedish CA agrees with the rationale for classification of methylmercuric chloride in Lact. effects – H362, as specified in the proposal.

In addition to the studies included in the CLH report, the following references gives further support to the conclusion that methylmercuric chloride should be classified as Repr. 1A:
• Debes F, Weihe P, Grandjean P. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury. Cortex. 2016 Jan;74:358-69
• FAO/WHO. Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series

940, 2007, 1-104

Dossier Submitter's Response

Please find below some further details on these studies:

- Nobunaga et al., 1978

Only females were exposed in the study. Body weight, water and food consumption were recorded. Results are available in the CLH report. There is no details on behaviour and clinical signs in the publication. Indeed, males were only stated to be healthy.
In this study, when the mating trial were terminated day 56, the conception rate was 100% in dose group treated with MeHg and 90 % in the control group.

- Khera et al., 1973b

Some details were given in male status in the publication. In experiment I, at 5 mg/kg death were observed in 2 out of 13 mice. No adverse effects were observed on the behaviour or the body weight gain in male rats. In experiment II, at 1 mg/kg bw, body weight decrease was observed after 70 days onwards and motor disturbances were noted in 5/18 rats. At 0.1 and 0.5 mg/kg bw, no toxic effects were observed.
It is stated in the discussion of the publication that the numbers of resorption sites and corporea lutea in the tested groups in this study were no different from that of the controls.

- Fossato da Silva et al., 2011,

Four groups of 15 rats were used in this non-guideline study. The statement comes from the publication itself. According to the presented table on sperm counts in testis and epididymis, the following statistically significant changes were observed:

- Increase in relative sperm number in testis at 1 mg/kg (no dose relation);
- Decrease in absolute number of sperm in epididymis at 0.5 and 3 mg/kg (no dose relation);
- Decreased in relative number of sperm in epididymis at all dose tested (no dose relation);
- Dose-related decrease in sperm transit time in caput/corpus.

- Mohamed et al., 1987

In this study, 3 animals in each group were used. None of the animals showed any significant changes in the general conditions, activity or weight during the treatment period. No neurotoxic manifestation were reported. The test material use in this study was methylmercury. The reference to methyl mercury chloride in the "comparison of criteria" is a typo.

- Popescu, 1978

In this article Popescu HI made comments on articles on poisoning with alkylmercury compounds and gives some personal data. Popescu follow up three patients with mild occupational alkylmercury poisoning. The concentrations of methylmercury and ethylmercury in the air of working places exceeded 0.005 mg/m^3. The duration in occupation varied between six and eight years. When these patients were examined they had not been exposed for two to six months. Urinary excretion of mercury was between 340 and 480 µg/l.

Thank you for the new references supporting the proposal. In the study of Debes et al., 2016, prenatal exposure to mercury has been associated with adverse effects on child neurodevelopment. In a birth cohort of 1022 subjects from Faroe Islands, prenatal methylmercury exposure was assessed in terms of the mercury concentration in cord blood and maternal hair (1986-1987). Clinical examinations of 847 cohort members at age 22

years were carried out using a panel of neuropsychological tests that reflected major functional domains. Deficits in Boston Naming Test (BNT) and other tests of verbal performance were significantly associated with the cord-blood mercury concentration. Deficits were also present in all other tests applied, although most were not statistically significant.
RAC's response
Thank you to the SW CA and to the Dossier Submitter for the additional information; this is helpful.

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2016	Norway		MemberState	13
Comment received				
Section 4.11, p. 43-84: Mercury is bioavailable and reaches the Leydig cells and Sertolis cells and primary spermatocytes and effects on sperm is described. Further in mating trial studies with only males dosed, significant decreases in preimplantation losses and number of viable fetuses per litter occurred. Based on the information in the CLH report we find it somewhat difficult to be sure if the substance should be assigned to category Repr. 1B or 2 for fertility. For development it is clearly reprotoxic to animals and Repr.1B D is fulfilled. Neurodevelopmental toxicity is well documented in humans, so Repr.1A H360D is justified for development. As methylmercury is excreted via breast milk, we support classification with Lact. H362 as well.				
Dossier Submitter's Response				
Thank you for your comment. Some more details are provided in response to comment 12.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Germany		MemberState	14
Comment received				
Section "4.11 Toxicity for reproduction" Concerning many studies the number of animals/subjects is either not provided or just as a total number, not assigned to the single dose group. Please provide details.				
Dossier Submitter's Response				
Please see response to comments 3 for further details.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Belgium		MemberState	15
Comment received				
The LD50 of Methylmercuric chloride (MeHgCl) was < 20 mg/kg bw in C57Bl6 male mice, which were more sensitive than the females (LD50 > 50 mg/kg bw). LD50 in young and adult Sprague-Dawley rats were also < 50mg/kg bw. BE CA can agree to classify and maintain the existing classification as Acute Tox. 2 – H300. BE CA can agree to maintain the existing classification considering the toxicokinetics data on absorption via the inhalation and the dermal routes of exposure and some epidemiologic cases of occupational exposure.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
10.06.2016	Norway		MemberState	16
Comment received				
Section 4.2 Acute, p. 16-21, Section 4.3 STOT-SE, p. 21, Section 4.8 STOT-RE, p. 21-31: We support the classification for acute toxicity (oral), based on the available LD50 values. For acute toxicity via inhalation and dermal uptake we support the reasoning and the proposal for classification. STOT-SE: Based on the available data we support no classification for STOT-SE. STOT-RE1: Usually STOT-RE classification rests upon significant toxic effects in 90d or 28d guidelines studies in animals. In the CLH report non-guidelines animal studies of MeHgCl, human case studies, and animal studies on MeHg are discussed. Although most of the animal studies are non-guidelines studies, since the significant toxic effects are seen in animals at very low doses it is justifiable to classify MeHgCl as STOT-RE1 for the target organs nervous system and kidneys. For the immune system and cardio-vascular system the effects seems less pronounced in the animal studies and we agree that these should not be mentioned as target organs. The findings in the study of children with ADHD (Cheuk & Wong, 2006) shows an association between higher mercury blood level and ADHD, but this does not prove a causal relationship and cannot be used for STOT-RE classification. Also the study (Guallar et al., 2002) on the relationship between mercury in toe nails and the risk of myocardial infarction seems non-usable for classification. The case studies of humans experiencing blindness from MeHg exposure support the visual cortex in the central nervous system as a target organ for MeHgCl as well. We disagree that "vision" should be mentioned as a target organ, and think the vision effect is covered by the central nervous system since the effects arise from the visual cortex in the brain.				
Dossier Submitter's Response				
We agree to remove vision as it is covered by the central nervous system.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Belgium		MemberState	17
Comment received				
BE CA can support the proposal to classify methylmercuric chloride as STOT RE 1 since it is clear that the substance induces damages in target organs such as the kidneys, the nervous system… at doses below 10 mg/kg bw/d. BE CA can agree to change the hazard statement from H373 to H372.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2016	Sweden		MemberState	18
Comment received				
The Swedish CA fully agrees with the rationale for classification of methylmercuric chloride in STOT RE 1, H372 (nervous system, vision and kidneys, oral route), as specified in the proposal. Classification in Category 1 is applicable, since adverse neurotoxic effects such as visual constriction are observed in humans as reported in several studies (Harada, 1995; Amin-Zaki, 1974; Hunter, 1954; Stein, 1992; Ahlmar, 1948; Merigan, 1980; Jalili, 1961; NIOSH 94-116, 1994) (cf. paragraph 3.9.2.9.9 in CLP). In addition, significant nephrotoxic effects are observed in a 90-day repeated-dose toxicity study at ≤ 10 mg/kg bw/d by oral route in rat (Fowler, 1972) (cf. paragraph 3.9.2.9.6 in CLP). The following effects are considered to support classification for specific target organ toxicity following repeated exposure, in relation to CLP criteria, Annex I, paragraph 3.9.2.7.3: (a) Oral exposure in rats and mice resulted in substance-related deaths (2.4 mg Hg/kg for 29 days (Chang 1972);4.3 mg/kg bw for 26 weeks (Mitsumori 1981); (10 ppm (0.859 mg/kg in males) for 2 years (Mitsumori 1990)). (b) Significant functional 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 unknown). (e) Histological effects in the kidney including fibrosis by oral route in mice (2 ppm = approx. 0.1 mg/kg bw) (Mitsumori, 1990).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				