

# Toxicity

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31. In October 2024 the US ATSDR published a toxicological profile for mercury which characterises the toxicologic and adverse health effects of organic and inorganic mercury. Mercury compounds exhibit a wide range of toxic effects, targeting common cellular functions. These include disrupting intracellular calcium balance, the cytoskeleton, mitochondrial function, oxidative stress, neurotransmitter release, and DNA methylation. The array of toxic effects is due to the strong affinity of  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^{2+}$  for the thiolate anion, which leads to the formation of  $\text{Hg}^{2+}$  and  $\text{CH}_3\text{Hg}^{2+}$  S-conjugates. This allows inorganic and MeHg to bind to and interfere with the structure and function of enzymes, transporters, and proteins that rely on functional thiol groups (ATSDR., 2024).

## Inorganic mercury

32. Information on the health effects of inorganic mercury comes primarily from oral studies in laboratory animals, with supporting data from acute poisoning case reports in humans. No epidemiological studies specific for exposure to inorganic mercury salts have been identified; however, animal studies consistently report dose-related impairments in fertility in male and female rodents following oral exposure (ATSDR., 2024). Animal studies have also shown that the critical target organ for inorganic mercury toxicity is the kidney (NTP., 1993); other targets include the liver, nervous system, immune system, reproductive system (EFSA., 2012).

33. Generational studies in rats and mice have shown that reproductive capacity decreases in a dose- and duration-dependent manner following oral exposure to mercuric chloride (Atkinson et al., 2001; Szász et al., 2002; Khan et al., 2004; Huang et al., 2011; Lukačínová et al., 2011; Heath et al., 2009; 2012; Laaroussi et al., 2025).

34. The effects of mercuric chloride were examined via 2-generation reproductive and fertility study in Sprague Dawley rats (Atkinson et al., 2001). Parental animals (F0, n=20 per group) were exposed daily via oral gavage to mercuric chloride in water from pre-mating through mating, gestation and lactation, before selected offspring (F1, n=25 for control + low dose and n=15 for mid dose) were then mated to produce a 2nd generation (F2). The study design is outlined below (Table 1).

Table 1. Study design for 2-generation reproductive and fertility study in Sprague Dawley rats (Atkinson et al., 2001).

Group	Dose levels (mg/kg bw/d)	Pre-mating ( Exposure duration in days)	Mating ( Exposure duration in days)	Gestation ( Exposure duration in days)	Lactation ( Exposure duration in days)
F0 Males	0, 0.5, 1 or 2.0/1.5	60	21	21	21
F1 Males	0, 0.5, 1.02	60	21	21	21

<b>F0 Females</b>	0, 0.75, 1.5 and 3.0/2.51	16	21	21	21
<b>F1 Females</b>	0, 1.5, 2.52	16	21	21	21

Control was milli-Q water. Pre-mating periods were selected to cover one spermatogenic or oogenic cycle. (1) High dose levels were adjusted during pre-mating in F0 animals due to excess toxicity, after 43 days for males, and 27 days for females. (2) Due to insufficient numbers of offspring in the high dose group, no 2nd generation was possible in this group.

35. Toxicity (including clinical signs and mortality) was present in all dosed parental F0 groups but not repeated in parental F1 groups. Body weight was significantly lower in high dose F0 females vs. control from study week 7, without corresponding effects on food consumption. Impacts on various organ weights were reported in F0 and F1 animals, although only kidney weight remained significantly reduced when adjusted for body weight.

36. Impacts on fertility and development included reductions in fertility indices and number of pregnant animals, live births and 4-day survival indices versus control in all treated F1 and F2 animals. Mean number of live pups per litter and pup body weight was also significantly reduced at days 4, 7, 14, 21 in all mercuric chloride treated groups, and at day 0 in pups of high dose parents. Implant efficiency (number of pups born/number implantation sites in dam) was significantly reduced in all treated F0 and mid-dose F1 females. At weaning, body weight in all F1 males of mercuric chloride dosed groups was significantly lower than control, and did not recover. Reduced body weight gain was also reported in all treated F1 females until week 11. It is not possible based on the study design to determine whether one or both sexes are responsible for the adverse impacts on reproduction. The remaining parameters, including fertility indices and number of pregnant animals in the F1 generation, sex ratio of pups (F1 and F2), pup survival (F1 and F2 at 7, 14 and 21-days), mean number of live F2 pups, and post-partum dam weight (F0 and F1) were unaffected by treatment.

37. A further 2-generation reproductive toxicity study in Swiss albino mice examined the impact of chronic exposure to mercuric chloride via the maternal lineage (Laaroussi et al., 2025). Dams (F0, n=12) were given 40 ppm mercuric chloride in their drinking water (equivalent to approximately 6 mg/kg bw/day) or untreated water (control) from gestation day 0. The F1 offspring remained with their mothers until weaning; only female pups were retained for further analysis. F1 treated mice continued to receive treatment until they reached adulthood. At 10 weeks old, adult F1 females in both control and treatment groups were mated with untreated males to produce a second (F2) generation. The F1 dams were split into two groups: one which continued to receive treatment and another that was no longer exposed to mercuric chloride (n=12 per group). This created a second generation that was directly exposed to mercuric chloride (F2) through gestation, lactation and adulthood and another (F2') that was only indirectly exposed through the maternal gametes of their F1 mothers that were exposed to mercuric chloride during their development and adulthood. Maternal care, sexual maturation, fertility and hypothalamic function were evaluated (an area of the brain for which the BBB remains 'leaky' until later in development, making it more susceptible to toxicity where substances are small enough to pass through the incomplete barrier).

38. Although live birth index was unaffected across groups, reproductive dysfunction was observed in exposed females from development through adulthood (F1 and F2 generations). Litter size and lactation index (indicative of how successful dams are at rearing pups through lactation) were decreased versus control. A decline in maternal care behaviours was observed in all exposed groups, including reduced nursing frequency and grooming/licking behaviours. In all treatment groups, delayed vaginal opening (achieved in 80 % control versus 20 % F1, 10 % F2 and 50 % F2' animals at 33 days), and first oestrus (achieved by 44 days of age in 70 % control versus 30 % F1, 0 % F2 and 60 % F2') were reported. Furthermore, altered oestrous cyclicity and reduced mating interest was recorded in all directly and indirectly exposed animals (F1, F2 and F2'). Such effects in F2' females, exposed indirectly via the germline, are suggestive of transgenerational effects following mercuric chloride exposure. Finally, changes in key hypothalamic genes associated with control of the hypothalamus-pituitary-gonadal axis, neuroplasticity and inflammation were identified, indicating potential neuroendocrine disruption following mercuric chloride exposure in both directly and indirectly exposed animals. The COT note that the number of animals per group (n=12) is low in the Laaroussi et al. (2025) study and may be considered underpowered considering there were abortions and mortalities in the dams, and decreased numbers of pups per litter.

39. The impact of lifetime low-dose exposure to mercuric chloride via drinking water was explored in Wistar rats (Lukačínová et al., 2011). Male animals (n=10 per generation) originating from the parental, F1 and F2 generations of a reproductive trial were exposed to 1µM mercuric chloride via drinking water (treatment) or pure water (control) from 52 days of age for 156 weeks (3 years), with toxicity parameters measured every 26th week. Similar longtime average daily doses were calculated as 0.036, 0.036 and 0.035 mg/kg bw for animals from the parental, F1 and F2 generations, respectively. Mercuric chloride exposure led to a significant reduction in survival in all generations (controls 90-100 % versus treated 30-35 %). Leading causes of mortality included tumours and gastrointestinal haemorrhage. Increasingly shortened lifespan was reported in all generations (control >1100 days, F0 823 days, F1 736 days and F2 674 days). A similar pattern of increasing effect by generation from F0 to F2 was observed in all remaining parameters examined. Body weight was statistically significantly reduced, and food and water intake were increased (not statistically significant for food) in all generations. Total serum protein, serum albumin, transferrin (F1 and F2 groups only) and ferritin were significantly increased in mercuric chloride treated animals versus control, again with increasing impact by generation.

40. Data on reproductive hormones are limited to two sub-acute (60 day) oral gavage studies in Sprague-Dawley rats, whereby animals were exposed daily to low or high mercuric chloride (0.5, or 1.0 mg/kg bw/d), or deionised water control (Heath et al., 2009, 2012). Heath et al. (2009) took groups of 20 young female rats (30 days of age) and exposed them for 60 days. 10 females per group were then mated with untreated adult males; pregnant females were euthanised at approximately gestation day 13. In Heath et al. (2012) young male rats were exposed from 30 days of age for 60 days, before being bred with unexposed adult females (n=10 per group). Male animals were euthanised after 21 days of cohabitation; the females were observed for a further 21 days, with all pregnant animals being allowed to reach term. Selected general, reproductive and hormonal parameters were examined. The COT noted that the number of animals per group (n=10) is low in the Heath et al. (2009) and (2012) studies and may be considered underpowered.

41. Heath et al. (2009) reported a significant impact of mercuric chloride exposure on body weight gain, and on implantation number, but no impact on number of corpora lutea (a measure of ovulation rate). No impact on follicle stimulating hormone was identified following mercuric chloride treatment. However, significantly lower serum progesterone and significantly higher pituitary luteinizing hormone levels were identified in the high dose group, compared to

control. The authors suggested these results indicated mercuric chloride may have a disruptive effect on progesterone production by the corpora lutea (leading to impacts on viable implantations given progesterone's role in maintaining pregnancy), but the mechanisms are not understood.

42. Heath et al. (2012) reported mercuric chloride exposure had a significant impact on body weight of high dose but not low dose males vs. control at necropsy. High dose males had a significantly poorer impregnation rate than low-dose or control groups, and there was a loss of correlation between testosterone levels and time to impregnate. Epididymal sperm counts were statistically significantly reduced in all exposed groups vs. control, and all exposed groups had significantly lower testicular testosterone vs. control (a similar effect was identified in plasma testosterone, although this did not reach statistical significance). The authors concluded that at doses lower than those producing clinical toxicity, mercuric chloride was able to significantly impact male reproductive hormones and performance.

## **Methylmercury**

43. Studies in humans and animals provide some evidence that oral exposure to organic mercury has renal, cardiovascular, immune, reproductive, and developmental effects; however, neurological and neurodevelopmental effects are established as the most sensitive effects of oral organic mercury exposure (ATSDR., 2024).

44. JECFA and EFSA have repeatedly evaluated the safety of mercury (EFSA, 2004; 2012; FAO/WHO, 1966; 1970; 1972; 1978; 1988; 2004; 2007; 2011) and agreed that the most sensitive endpoint is neurotoxicity and that life *in utero* is the critical period for the occurrence of neurodevelopmental toxicity (FAO/WHO., 2004; EFSA., 2012). This makes pregnant women a susceptible population. The bioaccumulative properties and long half-life of MeHg mean that the blood concentration of MeHg at the time of becoming pregnant depends on the exposure to MeHg during the preceding year (COT., 2004).

45. Following the Japanese Minamata MeHg poisoning incident, adults exposed to high levels of MeHg exhibited symptoms including sensory disturbances in the distal parts of the extremities followed by ataxia, concentric contraction of the visual field, impairment of gait and/or speech, muscle weakness, tremors, abnormal eye movement, and hearing impairments (Sakamoto et al., 2018). Similar symptoms were reported for Iraqi adults affected

after a large-scale epidemic of MeHg poisoning from wheat seeds disinfected with MeHg in 1972-73. A total of 6000 people were affected resulting in 400 deaths (Bakir et al., 1973). A study on the Iraq population estimated mercury body burden thresholds (mg) at diagnosis for various symptoms: abnormal sensory perception, ~25 mg (equivalent to a mercury blood concentration of 250 µg/L); ataxia, ~50 mg; articulation disorders, ~90 mg; hearing loss, ~180 mg; death, >200 mg (Bakir et al., 1973).

46. The symptoms observed in fetal-type Minamata disease patients (22 typical severe cases) were mental retardation, inability to walk unaided, disturbances of coordination, speech, chewing and swallowing, and increased muscle tone (Sakamoto et al., 2018). Developmental effects such as polydactyly, syndactyly, craniofacial malformations, microcornea, undescended testicles, enlarged colon, and coccyx protrusion were also observed in fetal-type Minamata disease patients. Histopathological examination of samples from Japanese fetal-type Minamata disease patients revealed widespread and severe neuronal degeneration in the central nervous system (Akagi et al., 1998). In Iraq, the children most severely affected by MeHg poisoning manifested with severe sensory impairments, general paralysis, hyperactive reflexes and/or impaired mental development (Bakir et al., 1973).

47. EFSA and the COT have both highlighted dietary factors that can reduce or prevent MeHg toxicity, including n-3 long chain polyunsaturated fatty acids (LCPUFAs), selenium, iodine, choline and vitamin E (EFSA, 2012; COT, 2018). The dietary substance most extensively studied as a confounding factor in studies of mercury is selenium.

48. MeHg and inorganic mercury binding affinities for selenium (1045) are up to a million times higher than its affinity for sulphur (1039) in analogous forms (Dyrssen and Wedborg., 1991). The high affinity means that in the presence of mercury the availability of selenium is reduced and its biological functions compromised. The ability of MeHg to cross the placenta and BBB means that MeHg can specifically sequester selenium at the active sites of essential selenium-dependent enzymes (selenoenzymes) in fetal neuroendocrine tissues that lack adequate reserves of selenium because of their rapid growth. As intracellular concentrations of MeHg approach – and especially as they exceed – 1:1 molar stoichiometries with selenium in these vulnerable tissues, vital selenoenzyme activities. In addition, formation of insoluble mercury selenides depletes availability of selenium for subsequent cycles of selenoprotein synthesis. Selenoenzymes are vital for redox control as well as preventing and reversing

oxidative damage in the brain and neuroendocrine tissues (among other important biological functions) and their loss can have severe adverse effects (Ralston and Raymond., 2010).

49. Animal studies have demonstrated that high maternal MeHg exposures diminish fetal brain selenium and brain selenoenzyme activities to around 30% of normal (Watanabe et al., 1999a,b). The consequences of MeHg exposure in these studies and those of Ralston et al. (2007; Ralston, 2008) were directly proportional to dietary mercury:selenium molar ratios, which need to be significantly lower than 1:1 to prevent impaired maternal export of selenium to the fetus and adverse neurodevelopmental outcomes in the offspring (Newland et al., 2006; Reed et al., 2006, 2008).

50. Studies that have found associations between MeHg exposure from seafood consumption and neurodevelopmental impairments have uniformly involved consumption of foods containing mercury in molar excess of selenium e.g., pilot whale (5:1 reported by Julshamn et al., 1987) or varieties of shark (>2:1, reported by Kaneko and Ralston, 2007). In contrast, studies examining the effects of maternal exposure to MeHg from typical varieties of ocean fish (Davidson et al., 1998; Myers and Davidson, 1998; Hibbeln et al., 2007; Oken et al., 2008; Lederman et al., 2008) have not found adverse effects, but have instead found beneficial effects from increasing seafood consumption (Hibbeln et al., 2007; Oken et al., 2008; Lederman et al., 2008). In the UK, multiple examinations of the Avon Longitudinal Study of Parents and Children cohort – as of 2023 14,833 unique mothers and 15,447 associated pregnancies enrolled (Major-Smith et al., 2023) – have all reported either no significant associations or beneficial associations between prenatal mercury exposure and child outcomes, especially in mothers that had eaten fish during pregnancy (Golding et al., 2022; Dack et al., 2023).

51. The International Agency for Research on Cancer (IARC) concluded that elemental mercury and inorganic mercury compounds are not classifiable as to their carcinogenicity to humans (Group 3), but that MeHg compounds are possibly carcinogenic to humans (Group 2B). These conclusions were based on inadequate evidence in humans for mercury and mercury compounds, inadequate evidence in experimental animals for elemental mercury, limited evidence for carcinogenicity of mercuric chloride in experimental animals (forestomach tumours in rats), and sufficient evidence for carcinogenicity of methylmercuric chloride in experimental animals (kidney tumours in male mice) (IARC, 1993). The U.S. Department of Health and Human Services has not classified the potential for



elemental mercury, inorganic mercury compounds, or MeHg compounds to cause cancer in humans (NTP, 2016).

52. The neurological effects of MeHg in animals include sensorimotor dysfunction, vision and hearing deficits, impaired learning, and memory, along with clear signs of neurotoxicity such as clumsiness, motor incoordination, lethargy, hindlimb crossing, tremors, ataxia, and partial paralysis. Both developing humans and animals are more vulnerable to MeHg-induced neurotoxic effects compared to adults (ATSDR., 2024). Animal studies also consistently show that exposure to MeHg leads to dose- and duration- dependent decreases in offspring survival, increased fetal malformations and variations (including cleft palate, skeletal malformations, and hydronephrosis), and reduced fetal weight (ATSDR., 2024).

## Recently published literature

53. The previous discussion paper ([TOX/2025/03](#)) on mercury in the maternal diet included a comprehensive literature review on the toxicological effects of inorganic and organic mercury exposure including summaries of recent reviews and toxicologic/epidemiologic studies identified therein. The literature review predominantly covered reproductive toxicology – i.e., pregnancy outcomes and effects on maternal health – in addition to blood pressure, biomarkers and epigenetic effects of mercury exposure.

54. An additional literature search was performed to specifically identify recent publications on the Faroe Islands (Oulhote et al., 2019) and Republic of Seychelles birth cohorts (Wahlberg et al., 2018; Xu et al., 2019; Zareba et al., 2019; McSorley et al., 2020; Yeates et al., 2020; Cediell Ulloa et al., 2021; Strain et al., 2021; Love et al., 2022; De Paula et al., 2023; Wesolowska et al., 2024). The first of the numerous observation cohort studies established in the Faroe Islands and Republic of Seychelles began over three decades ago (Weihe and Grandjean., 2012; Shamlaye et al., 2020). The goal of these studies has been to understand potential adverse health effects in children raised by mothers with high seafood diets and thus high exposures to marine contaminants such as MeHg. The results of these studies have been crucial to deriving health-based guidance values (HBGVs) for MeHg and inorganic mercury by leading authorities JECFA and EFSA (search terms in Annex B).

55. The previous COT statement on MeHg in the infant and child diet (COT., 2018) included a similar literature search for the 2012-2018 period (year of

last EFSA evaluation to year of COT discussion); hence the most recent literature searches specified years 2018-2025.

56. Upon review of the recent literature, the COT concluded that the data confirmed the current knowledge on the toxicity of inorganic and MeHg and did not constitute a basis for revising the current HBGVs. The section below, therefore, describes the JECFA and EFSA evaluations and derivations of HBGVs for MeHg and inorganic mercury.