NEUROTOXIC EFFECTS OF MERCURY: REGIONAL EPIDEMIOLOGICAL STUDIES

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Mercury (Hg) is an extremely hazardous pollutant to human health and its toxicity differs depending on the chemical form of intake, although the central nervous system and kidney remain the two main target organs. Hg can be present in the environment in the chemical forms of elemental, inorganic and organic mercury.

Elemental mercury (Hg^0), which is liquid and highly volatile at room temperature, is emitted into the atmosphere in the form of vapour from numerous anthropogenic and natural sources. Excluding occupational exposures, the main source of human exposure is the vapours released by amalgam from dental fillings.

Human studies have shown that 70-80% of inhaled Hg^0 is absorbed by the lungs and passes into the circulatory stream for distribution throughout the body. Very little Hg^0 is absorbed through the gastrointestinal tract (0.04%) probably due to its rapid oxidation in the gastric tract into the mercuric cation ($Hg^{(2+)}$) and subsequent binding to the sulphydryl groups (-SH) of proteins. Skin absorption is also negligible (2% of lung absorption). Once inside the red blood cells, Hg^0 is rapidly oxidised to $Hg^{(2+)}$ by the enzyme catalase and accumulates in this form in various tissues, first and foremost the kidneys. Due to its high fat-solubility, Hg^0 is able to cross the placental barrier, while it is only excreted in small amounts in breast milk. Following exposure, the half-life of Hg^0 concentration in the blood ranges from 3 to 18 days.

Inorganic forms of mercury (IHg) can have oxidation states +1 and +2 and result from the union of Hg with non-metallic elements such as chlorine (Cl)sulphur (S) and oxygen (O). Compounds corresponding to the +2 oxidation state (mercurous compounds) are predominantly covalent in nature, tend to hydrolyse in aqueous solution, volatilise with heat and are easily reduced to the +1 state (mercurous compounds) and to Hg^0. Compounds corresponding to the +1 oxidation state (mercurous compounds), in which two Hg atoms are bonded by a covalent metal-to-metal bond to form the $Hg^{(2+)}$ dimer ion, are ionic in nature, form less frequently and tend easily to disproportionate $Hg_2^{(2+)} \rightarrow Hg + Hg^{2+}$.

Toxic effects due IHg exposure have been associated mainly with occupational exposures; in the general population, the main source of IHg exposure Hg^0 vapours released from dental fillings. Less than 10% of dietary IHg intake is absorbed from the gastrointestinal tract.

At the blood level, $Hg^{(2+)}$ is more than 50% transported in the plasma and has a half-life of approximately 28 days. Hg^{2+} is unable to cross the blood-brain barrier and placenta while it is excreted in breast milk bound to serum albumin and casein.

There are several organic mercury compounds of which the most dangerous and widespread is monomethylmercury or methylmercury (MeHg). MeHg has no industrial applications but is

originates from the methylation of $Hg^{(2+)}$ cations by certain anaerobic bacteria living in soil and marine, lake and river sediments.

Most of the MeHg in the environment is a product of the respiration of sulphur-reducing anaerobic bacteria. In the methylation reaction, an atom or an ionic group of an Hg compound (oxidation state +2) is replaced by a methyl group ($-CH_3$), giving rise to the monomethylmercuric cation ($CH3Hg^+$). The monomethylmercuric cation rapidly combines with anions such as chloride ion (Cl^-), hydroxide (OH^-), the nitrate (NO^-) and other anionic groups typically found in humic and fulvic acids in soils and sediments.

The neurotoxicity of MeHg is best known as a result of a number of collective poisoning incidents in the last century, the first of which involved the city of Minamata in Japan in the 1950s and 1960s, where the consumption of fish contaminated with Hg released into the bay waters by an industrial complex for the production of acetaldehyde affected over 12,000 people with serious consequences (Tsubaki & Irukayama, 1977). Those intoxicated manifested neurological disorders characterised by ataxia, paresthesia, visual field weakening, hearing loss, difficulty in articulating words, mental disorder, and in severe cases paresis and death. Children born to women who had consumed contaminated fish showed marked neurological abnormalities, mental retardation and cerebral palsy at birth even in the absence of detectable toxic effects in their mothers. Hg levels measured in children's hair were between 10 and 100 ppm, while no measurements were available on maternal hair. Another episode of MeHg intoxication occurred in Iraq between 1971 and 1972. Exposure in this case occurred through the consumption of bread prepared using seeds treated with MeHg as a fungicide. Again, children exposed in utero to MeHg (particularly during the third trimester of gestation) showed the highest sensitivity, manifesting severe mental retardation, muscular hypotonia and ariflexia. In both episodes of intoxication there was latency period (16-38 days in Iraq and several years in Japan) between exposure to MeHg and the onset of neurotoxic symptoms (Harada, 1995).

Both adult and foetal brains are extremely sensitive to MeHg toxicity, although the developing nervous system is particularly reactive to the action of this toxicant. MeHg can easily cross the blood-brain barrier and the placental barrier via amino acid carriers. Infants and children can also be exposed to MeHg through milk.

In humans, 95 per cent MeHg ingested through food is absorbed from the gastrointestinal tract, from there it passes circulatory system where it is transported 90 per cent into the red blood cells through bonds with the -SH groups of haemoglobin and 10 per cent into the plasma. In the body, almost all MeHg is found bound to albumin, glutathione (GSH) or L-cysteine (L-Cys) and the formation of these complexes appears to be the basis for the greater or lesser absorption of MeHg within the various organs. The formation of complexes with D-cysteine (D-Cys), on the other hand, hinders the penetration of MeHg into the endothelial cells of the cerebral capillaries, whereas the formation of complexes with L-methionine (L-Met) abolishes it completely. In fish tissue, MeHg is predominantly found in the form of MeHg-L-Cys complexes, thus increasing the risk of contamination through fish consumption. Some of the MeHg that enters the brain is rapidly demethylated to the inorganic form Hg^{2+}; The mechanisms by which MeHg exerts its neurotoxicity have not yet been fully understood, but appear to be related to the ability of the Hg^{2+} cation to bind to the -SH groups of enzymes, ion channels and membrane receptors causing the inhibition of most important cellular antioxidant systems such as N-acetyl-L-cysteine (NAC)alpha-lipoic acid (ALA) and GSH as well as the production of free radicals. Within the central nervous system, MeHg accumulates within astrocytes with extremely long half-life and causes intense neurological dysfunction.

Excitotoxicity is a phenomenon of neuronal toxicity resulting from exposure to high concentrations of glutamate (Glu), the main excitatory neurotransmitter in the central nervous system.

Ionotropic postsynaptic Glu receptors are regulated by N-methyl-D-aspartate (NMDA) and once activated by their own ligand or other factors allow Na ions^{+} and Ca^{2+} to flow inside the neuron and K ions^{+} outside it. Excitotoxicity is one of the main causes of MeHg neurotoxicity and there is a very strong correlation between the areas of the brain characterised by high incidence of Hg-induced neuronal damage and the areas with the highest density of NMDA receptors. For example, cerebellar molecular and Purkinje cells, which are spared under conditions of chronic Hg poisoning, have a very low population of NMDA receptors. In contrast, cerebellar granule cells, which are a primary target in Hg-induced neurodegeneration, express a high density of NMDA receptors. A high density of NMDA receptors is also present in the primary visual cortex, a site particularly involved in MeHg-induced damage.

MeHg inhibits the penetration of Glu into astrocytes leading to an excessive concentration of excitatory amino acids in the extracellular fluid resulting in hyperstimulation of the post-synaptic neuron and destruction of the dendrite or the entire neuron by processes of necrosis and apoptosis.

In the presence of MeHg, the ability of astrocytes to absorb and remove excess Glu deteriorates, leading to an increase in NMDA receptor-mediated excitotoxicity. In particular, the astrocyte's system for neutralising Glu is highly sensitive to $Hg^{(2+)}$); the Hg^{2+} cation interacts with certain critical -SH groups located at specific sites in the astrocyte's cell membrane, inactivating the cellular systems underlying the uptake and disposal of excess Glu.

MeHg is also capable of altering the correct homeostasis of mitochondria through interaction with the -SH groups of specific proteins involved in the production/detoxification of free radicals.

The binding of MeHg to protein residues interferes with enzyme activity, slowing the entry of glucose into the cell and the activity of membrane ATP, reducing the sodium-potassium exchanges essential for proper functioning of the nervous system. Detectable damage is atrophy of the brain and cerebellum with a reduction in the number of neurons and an increase in interstitial tissue in the central nervous system and alterations in the myelin sheath of nerves in the peripheral nervous system.

Exposure to high doses of MeHg results in sensory disturbances, visual field constriction, ataxia, cognitive impairment and death; the areas of the brain most affected are the primary visual cortex and *cerebellum*. Exposure to low doses is linked to hypoesthesia, ataxia, dysarthria, sensory disturbances and impairment of hearing and vision; Several studies have also shown that exposure to low concentrations of MeHg can increase abnormal folding of laproteina α -synuclein, the main component of the fibrillar plaques that characterise Parkinson's disease, and increase the secretion of the proteinβ -amyloid 1-40 and 1-42 involved in the aetiopathogenesis of Alzheimer's disease.

The inhibition of cellular antioxidant systems associated with the production of free radicals also seems to be linked to an increased risk of cardio- and cerebro-vascular diseases.

The half-life of MeHg in blood is 70 days in adults, 90 days in children and 46 days in breast-feeding women. MeHg is excreted 90% through the faeces and to a small extent through urine and sweat; MeHg excretion also follows the entero-hepatic cycle after demethylation in the intestine, slowing down the elimination process.

MeHg ingested by pregnant women through the diet can cause persistent damage to the central nervous system of the foetus even in the absence of toxic effects in the mothers, with detectable damage during both embryonic development and school age, leading in the developing foetal brain to faulty neuronal differentiation, division and migration through binding to the SH groups of tubulins, the main proteins that make up neuronal microtubules. The sensitivity of the developing fetal brain is 2 to 5 times greater than that of the adult.

Excluding occupational exposures, the main source of human exposure to MeHg is through the consumption of fish, seafood and marine mammals. MeHg is a highly toxic and bioavailable compound and once absorbed by primary producers it tends to bioaccumulate as it passes through the aquatic food chain. Large predatory fish at the top of the aquatic food chain appear to be the most important source of introduction of MeHg into the human body.

In fish, water-soluble MeHg enters both through the diet and through the gills, accumulating in the axial muscles. Hg levels in fish generally increase with age, size and weight; accumulation varies significantly between species and is assumed to be diet-related, being higher in freshwater and saltwater fish with higher trophic levels (Miklavcic *et al.*, 2011). Within the same trophic level, different fish may differ considerably in Hg content, and the Hg concentration in a fish cannot be reliably estimated based on concentrations determined in other environmental matrices such as water and sediment. MeHg half-life, if exposed aquatic organisms are moved to uncontaminated areas, ranges from 6 months for mussels (*Mytilus*) to 2 years for pike *(Esox lucius*) (Majori *et al.*, 1967).

Exposure to MeHg through fish consumption differs significantly from acute MeHg poisoning. Through fish consumption, the human body is exposed to extremely low concentrations of MeHg for extremely long periods of time. The benefits and risks of fish consumption during pregnancy have been debated for many years.

Fish provides some essential nutrients for the growth and development of the foetus' brain, but may contain varying amounts of MeHg. The true extent of the possible health risk from the presence of MeHg in fish, however, has not yet been clarified. Since toxicity of MeHg has been demonstrated even at low concentrations, exposure to this compound should be minimised, thus leading to a drastic decrease in fish consumption. In terms of prevention, however, this route is not completely feasible as fish is an essential component of balanced diet and provides important nutrients.

Although the various studies carried out internationally (Grandjean *et al*., 1998; Debes *et al.*, 2006; Hibbeln *et al.*, 2007; Davidson *et* al., 2008a; Davidson *et al.*; 2008b; Holmes *et al.*, 2009; Suzuky *et al* 2010; Bose-O'Reilly *et al.*, 2010; Llop *et al*., 2012; Strain *et al*, 2015; Van Wijngaarden *et al.*, 2017; Vejrup *et al.*, 2018) have not led to unambiguous results regarding the possible neurological effects caused by prolonged exposure to low doses of MeHg, the US *Environmental Protection Agency* (EPA) has determined
per il MeHg un valore di assunzione massimo quotidiano pari a 0,1 µg/kg di peso corporeo (EPA, 1997), the *Joint Expert Committee on Food Additives* (JEFCA) of the *FAO/WHO* (*Food and Agriculture Organisation/World Health Organisation*) established a provisional maximum weekly intake value (*Provisional Tolerable Weekly Intake*, PTWI) pari a 1,6 µg/kg di peso corporeo (corrispondente a 0,23 µg/kg per day) for the protection of the foetus from neurotoxic effects (WHO, 2004), while the experts of the *European Food Safety Authority* (EFSA) have
indicato un valore di assunzione massimo settimanale (TWI) più basso, pari a 1,3 µg/kg. La

differences tolerable intake values reflect different assumptions in risk assessment, as well as different methodologies in data analysis and uncertainty factors used (NRC, 2000).

The EPA also calculated through a statistical model and using an uncertainty factor of 10, the *BenchMark Dose Level* (BMDL) that defines the *cut-off* between presumed harmlessness and toxicity; a total mercury concentration (THg) ofa 1,2 μg/g nei capelli materni and a THg concentration of 5 were chosen as cut-off values. ,8 μg/L nel sangue del cordone.

The BMDL value represents the lower 95% confidence limit of the dose that would increase the likelihood that 5% of the population would score abnormally on the Boston Naming Test, a neuropsychological test for school-age children that assesses the ability to choose the appropriate word for the expression of a particular concept. THg concentrations measured in hair are usually used as the *provisional tolerable weekly intake* s of main exposure in individuals for whom occupational or environmental exposures can be excluded and for whom the main source of exposure is dietary MeHg intake. In individuals consuming fish about 80 % of the Hg present in the hair is in the form of MeHg, therefore measuring the THg concentration gives a good estimate of the actual amount of MeHg absorbed by the hair follicle from the circulatory stream.

The concentration of THg present in hair samples reflects the concentration of MeHg present in the blood at the time the hair was formed. As the half-life of Hg in blood corresponds to about 50 days, the THg concentration in blood is a measure of recent exposure, while the THg concentration in hair allows us to recapitulate exposure from several months ago. The Hg concentration measured in cord blood, on the other hand, is an excellent *biomarker* for MeHg exposure in the foetus; MeHg is actively transported across the placenta and the Hg present in cord blood is almost entirely in the form of MeHg. Given that the umbilical cord develops predominantly during the second and third trimester of gestation, the concentration of MeHg in cord blood can be considered as an indicator of average fetal Hg exposure during the third trimester.

According to the tolerable daily intake value established by the EPA, a 70 kg woman potrebbe assumere al massimo circa 50 μg di MeHg a settimana; questo valore was calculated on the basis of the 95 per cent lower confidence limit of the dose that in the case of prenatal exposure could lead to abnormal neurodevelopmental test results and multiplied by an uncertainty factor of at least ten times.

Considering that the average concentration of MeHg in marine and freshwater fish is approximately 0.2 mg/kg, a 70 kg woman could consume approximately 35 g of fish per day or approximately one portion (150 g) of fish per week.

The *Food and Drug Administration* (FDA) and EPA have also issued a series of recommendations aimed both at women of childbearing age, with the aim of limiting fish consumption by avoiding in particular the consumption of predatory fish at the top of the aquatic food chain, and at pregnant women, with the aim of avoiding potentially harmful exposures to the foetus (US-FDA, 2001; HHS, 2004).

While uncertainty remains as to the minimum level of prenatal exposure to MeHg that causes detectable toxic effects on the foetal nervous system, the available literature provides consistent evidence of the neurotoxicity of prenatal exposures to MeHg corresponding to THg concentrations in maternal hair between 10 and 20 ppm.

The Gulf of Trieste is one of the areas most prone to Hg pollution in the entire Mediterranean (Covelli *et al.*, 2011; Emili *et al.*, 2011). The causes of this contamination can be attributed to the presence of cinnabar mining deposits in the nearby Slovenian town of Idrija,

the natural mercury mineral, which chemically is a mercury sulphide (oxidation state +2) with the crystallochemical formula HgS.

For years, the waters of the Idrijca stream, which flows through the town Idrija, drained the mercurial soils of the mining district and then flowed into the Soča river, which transported the contaminated sediments to its mouth in the Gulf of Trieste. The Isonzo River constitutes the main freshwater supply to the Gulf of Trieste, and the Isonzo-Idrijca system represents the link between the source of contamination and the sites of partial accumulation, i.e. central area of the Gulf of Trieste, and final accumulation, i.e. the Lagoon of Grado and Marano (Kotnik *et al.*, 2005). Mining at the world's second largest Hg mine lasted for approximately 500 years reducing gradually until to final closure in 1996. In total, more than 5 million tonnes Hg-mineralised rock were excavated, essentially HgS but also native Hg (Mlakar, 1974).

Although the origin of Hg in the Idrija area is linked to the deposit, most of the metal found in the soils, surface sediments, and along the banks of the Soča river and its tributary Idrijca is Hg remobilised by mining activity, released into the atmosphere from the mine ventilation ducts, from the chimneys of the mineralised rock 'roasting' plant, and subsequently returned to the ground with precipitation. The ore processing residues themselves were abandoned near the plants along the banks of the watercourse. It is estimated that only 73 per cent the total 144,000 tonnes of extracted metal was recovered (Gosar *et al.*, 1997), while the remainder was dissipated into the surrounding environment. The contributions from the Isonzo area, dispersed mainly towards the west due to the anti-clockwise circulation system of currents characterising the northern Adriatic, contributed to an increase in Hg concentrations not only in sediments but also in the biotic component of the adjacent lagoons of Marano and Grado (Brambati, 1997).

In addition, the spillage of Hg into the Aussa river tributary of the Marano Lagoon, caused by the chlorine-soda plant of the Torviscosa (UD) industrial complex, began in 1949 and lasted until 1984, when suitable recovery and purification methods were adopted (Daris *et al.*, 1993). For these reasons, the lagoon environment appears to have undergone dual contamination, with different methods and times in relation to the two sources mentioned, compared to the Gulf of Trieste.

Initially, due to the widespread presence of mercury in environmental matrices, the Grado and Marano Lagoon was declared, by Ministerial Decree 468 of 18 September 2001, a "Remediation Site of National Interest". Subsequently, following the results of the sediment characterisation, by Decree of the Minister of the Environment and Protection of Land and Sea 222 of 12 December 2012, the perimeter of the "Marano and Grado Lagoon" site was redefined, excluding the entire lagoon perimeter from the previous perimeter.

In 1999, a small cohort study of newborns (North Adriatic Cohort) conducted by the University of Udine explored the relationship between prenatal exposure to MeHg through fish consumption during pregnancy and the neurological development of children born between 1999 and 2001 in 17 central and southern Friuli Venezia Giulia (FVG).

243 mother-child pairs were enrolled, 203 of which came from three countries located in the lagoon (Grado, Marano Lagunare and Carlino) characterised by a fishing-based economy, while the remaining pairs came from 14 countries located inland.

THg and MeHg concentrations were measured in hair and breast milk samples taken two months after delivery and in children's hair taken at 18 months, while information on the enrolled women's medical and occupational history, socio-demographic characteristics and family lifestyle, pregnancy, childbirth and the health status of the newborn was collected through a structured questionnaire administered to the women enrolled in the study.

The consumption of local and non-local fresh, canned and frozen fish during pregnancy and lactation and the consumption of fish by children at 18 months of age were investigated through the administration of a semi-quantitative questionnaire, which revealed the type, frequency and origin of fresh fish consumed by mothers and children during the summer and winter period. The neuropsychological development of 52 children born within the cohort was assessed at 18 months of age by administering the *Denver Developmental Screening Test* II (DDST II).

The THg concentrations measured in the mothers' hair samples were directly associated with the consumption of local fish and reached levels close to or above 10 ppm; furthermore, after adjustment for several potential confounding factors, the score on the DDST II fine-adaptive motor development assessment scale was inversely associated with the THg concentrations measured in the maternal hair samples (Barbone *et al.*, 2004).

Ascertaining the presence of neurobehavioural deficits associated pre- and postnatal exposure to Hg, as is also the case with other environmental chemical contaminants, is very complex and requires extremely long observation periods since neurotoxic effects can also occur at school and/or adolescent age, as emerged from two major cohort studies initiated in the 1980s in the Faroe Islands and the Seychelles Islands (Grandjean *et al.*, 1998; Myers *et al.*, 2003).

Precisely for this reason, the neuropsychological development of 154 (63.3%) children born within the North Adriatic cohort, enrolled by the University of Udine between 1999 and 2001, was re-evaluated upon reaching the of 7/9 years, through the administration of the 'Wechsler Intelligence Scale for Children' (WISC III); during the neuropsychological evaluation, a hair sample was taken from each child, while information on the child's socio-economic status, medical history and family eating habits, health status and diet was updated by means of a self-completed questionnaire filled in by the mothers. THg concentrations measured in children's hair samples at 7 years of age were fairly correlated with THg concentrations measured in maternal hair taken at delivery (Spearman's correlation coefficient: $rs= 0.35$; $p < 0.0001$) and strongly correlated with fish consumption by the children ($rs = 0.50$, $p < 0.0001$). After adjustment for several potential confounding factors, children born to mothers with hair THg concentrations greater than or equal to 2000 ng/g showed total, verbal and performance Intelligence Quotient (IQ) scores 4-5 points lower than children born to women with lower THg concentrations, but these differences were not statistically significant. Consumption of fresh fish during pregnancy was positively associated with children's total and performance IQ scores but not with verbal IQ scores. The relatively low levels of Hg found in the biological samples of the mothers and children enrolled in the study did not provide evidence of high and extensive exposure to Hg in the test population and did not allow for the development of recommendations on fish consumption in pregnancy but indicated that it may be desirable to maintain THg concentration levels in hair <2000 ng/g (Deroma *et al.*, 2013).

In 2006, the University of Udine in collaboration with researchers from Slovenia, Croatia and Greece launched, as part of the PHIME (*Public health impact of long term low level mixed element exposure in susceptible population strata*) international research project, a prospective cohort study to enrol and monitor 2189 mother-child pairs residing in Mediterranean coastal areas potentially exposed through fish consumption to MeHg. Four different cohorts of newborns were recruited: in the city of Trieste, the city of Lijubljana in Slovenia, the city of Rijeka in Croatia and four Greek islands in the Aegean Sea.

The main objective of the study was to assess relationship between prenatal exposure to low doses of MeHg through a fish-rich maternal diet and the neuropsychological development of children (Valent *et al.*, 2013a). The enrolment of the Italian cohort started in April 2007 at the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Materno Infantile Burlo Garofolo in Trieste and ended in March 2009.

The subjects eligible for the study were recruited from a population of pregnant women who had been resident in FVG for at least five years, were of age, had no pathologies that could in any way affect the normal neurological development of their children, had single pregnancies and had a physiological course. 900 pregnant women between 20(a) and 22ª weeks' gestation were enrolled and of these 767 were still in the study at the time of delivery.

The deliveries took place between July 2007 and August 2009. The neurological and psychological development of the children born within the four cohorts was assessed at 18±2 months of age through the administration of the *Bayley test Scales of Infant and toddler Development-third edition* (BSID III) the international reference assessment tool regarding the cognitive, language, motor, socio-emotional and behavioural development of children from 1 to 42 months. The neuropsychological assessments, which covered the children's level of cognitive, language, motor and socio-behavioural development, were conducted between January 2009 and February 2011.

After excluding children born preterm (< 37ª weeks' gestation), those with congenital malformations or severe perinatal problems, and those with serious health problems that could impair their neurological development, BSID III was administered to 632 children.

In this study, the concentration of THg and MeHg was measured in the hair, venous blood and urine samples of the enrolled women with regard to prenatal exposure, and the concentration of THg and MeHg in cord blood and milk samples with regard to postnatal exposure. In addition to Hg, other heavy metals that can be ingested in the diet (lead, cadmium, arsenic, manganese, copper, zinc and selenium) were also taken into account, as well as nutritional factors considered beneficial for growth and development such as *polyunsaturated* fatty acids (PUFA), in which fish is rich. Other variables that may influence children's neurocognitive functions such as family environment, family socioeconomic status and maternal Intelligence Quotient (IQ) were not neglected during the investigation (Valent *et al.*, 2013a).

The composite scores obtained by the children in the neurodevelopmental test were correlated with maternal eating habits and lifestyle, possible environmental and/or occupational exposures and, above all, with the concentrations of contaminants detected in the biological samples analysed. The results of the Italian cohort showed that during pregnancy the enrolled women had consumed on average less than two portions of fish per week. The mean Hg concentrations measured in biological samples were quite low (THg in maternal hair: 1062 ng/g; THg in cord blood: 5.6 ng/g; THg in milk: 0.3 ng/g) and moderately correlated with women's reported fish consumption. The concentration of omega-3 PUFA measured in maternal venous blood correlated poorly with the fish consumption reported by the women. After adjustment for potential confounding factors, there was no evidence that Hg had an adverse effect on children's neurodevelopment, while the children's composite scores on the five BSID III rating scales were significantly correlated with socioeconomic factors and family lifestyle (Valent *et al.*, 2013b).

Through a meta-analysis performed on all four Mediterranean cohorts, the composite and scalar scores obtained by the children at BSID III and the THg concentrations measured in the biological samples were related. THg concentrations measured in maternal hair and cord blood samples were inversely associated with developmental level

fine and gross motor skills obtained by the children; however, no associations emerged between THg concentrations in the different biological samples considered and the children's scores on the Cognitive and Language Scale. The mean THg concentrations measured in hair, cord blood and milk samples from women enrolled in the other three Mediterranean cohorts were respectively: 381 ng/g, 2.1 ng/g and 0.3 ng/g in the Slovenian cohort; 968 ng/g, 5.1 ng/g and 0.3 ng/g in the Croatian cohort; and 1408.5 ng/g, 7.7 ng/g and 1.7 ng/g in the Greek cohort (Barbone *et al.*, 2019).

The neurodevelopment of 470 of the children born within the Italian cohort was re-evaluated at the age of 40 months; here too, the aim was to investigate the possible effects of exposure to low doses of Hg through the maternal diet, in particular through fish consumption, on the cognitive development of the children. In this case, potential beneficial effects of fish consumption were also taken into account by taking into account pre- and postnatal exposure to selenium (Se) and the role played by this essential element as an antagonist against heavy metals. The cognitive composite score obtained by the children at BSID III was related THg and Se concentrations measured in cord blood, venous blood taken during pregnancy from the enrolled women and breast milk.

After adjustment for potential confounding factors, no clear associations emerged between pre- and postnatal exposure to Hg and the neurodevelopment of the children examined, although some evidence emerged concerning the antagonistic role of Se in relation to the neurotoxic action exerted by Hg. The possible interactions between Se and Hg require further investigation (Castriotta *et al.*, 2020).

To date, scientific studies that have investigated how genetics may influence the neurotoxicity associated with Hg exposure have been few and far between (Llop *et al.*, 2015). Studies Drosophila have shown that ectopic CYP6G1 gene expression confers resistance to the neurotoxic action exerted by MeHg. In humans, the subfamily of the CYP3A gene, the human homologue of the CYP6G1 gene, consists of four distinct genes (CYP3A4, CYP3A5, CYP3A7 and CYP3A43) located on chromosome 7. These genes, mainly expressed in liver, kidney, intestine and placenta tissues, are responsible for the metabolism of various xenobiotics and are essential for the synthesis of fatty acids, molecules that are fundamental for the development of the nervous system. The expression of CYP3A genes in other tissues and in particular in the brain is poorly understood to date, although there is some evidence that genes of this subfamily, in particular the CYP3A7 gene, are expressed in the developing fetal brain (Pavek & Dvorak, 2008). Given that the demethylation process of MeHg constitutes the most limiting factor in its elimination from the human body, the potential role of the CYP3A4, CYP3A5, CYP3A7 and CYP3A43 genes in the metabolism of this toxicant was investigated.

In 2017, Llop *et al.*, analysed data from 6 different international court studies, including the previously described Italian and Greek PHIME Mediterranean cohorts, in order to investigate whether and how polymorphic variants in the CYP3A gene subfamily are able to influence the association between prenatal exposure to MeHg and children's scores on neuropsychological surveys. Data from 2639 children, 573 Italian children, 281 Greek children, 1160 children belonging to a Seychelles cohort (Strain *et al.*, 2015) and 625 Spanish children belonging to the INMA cohort (Guxens *et al.*, 2012) were analysed. The data collected, first analysed for each country and then combined through the meta-analysis approach, showed that the association between prenatal exposure to MeHg and neuropsychological development could be influenced, particularly in children belonging to the Mediterranean cohorts, by polymorphisms in the CYP3A5 and CYP3A7 genes (Llop *et al.*, 2017). Further studies will be needed to investigate and better understand this association.

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