**Professor Jean-Bernard FOURTILLAN**
Chemical Engineer, Honorary Professor of Therapeutic Chemistry and Pharmacokinetics
Expert Pharmacologist-Toxicologist specialized in Pharmacokinetics with AFSSAPS (now ANSM)

# Mechanisms of neurotoxicity of heavy metals Hg and Al

## A-Mechanism of the neurotoxicity of Mercury and Thimerosal

Two neurotoxic forms of mercury can be administered to humans:
- **Silver-mercury dental amalgams**, an inorganic form of mercury
- **Thimerosal**, an organomercurial derivative

### I- Silver-mercury dental amalgams

Due to the antimicrobial properties of mercury, they **have been used to fill in decayed teeth**, for over 150 years. They release the mercuric ion **$Hg^{++}$**, which accumulate particularly in the kidneys and the **brain**, into the body. The silver-mercury dental amalgam arises from the reaction of liquid mercury with a metallic silver powder, which leads to the formation of a solid crystallized silver-mercury alloy. Renowned for its antibacterial properties, its durability, its ease of use and its low cost, it has long been the preferred solution in dentistry.
But, quickly, this use raised questions about the safety of chronic exposure to mercury. Proportional to the number of fillings in the mouth, the release of mercury can occur during condensation, polishing, installation and wear of amalgams.
An European regulation, dated May 17, 2017, banned the use of mercury dental amalgam for pregnant women, nursing mothers, and children under 15 years of age. This ban came into force on July 1, 2018.

#### What are the health hazards of mercury from dental amalgams?

Amalgam creates vapors of mercury, part of which is absorbed by the lungs. Mercury passes into the blood, crosses the blood-brain barrier **and accumulates in the brain**. It also crosses the placenta. Mercury $Hg^{++}$ ions that escape from amalgam accumulate in several organs, including the kidneys and **brain.** Some studies have long pointed out **a link between mercury dental amalgam and certain diseases** such as **multiple sclerosis, Parkinson's disease** and **Alzheimer's disease**. In a report published in 2015, in France, the National Agency for the Safety of Medicines and Health Products (ANSM) stipulated that *« **the lack of objective data and the absence of convincing arguments** do not allow a definitive decision on neurological risks or multiple sclerosis, neither on the impact on renal function, nor on a possible deleterious role on the health of children and adults, nor on the benefits of removing amalgams »*.

## II- Thimerosal

Due to its antimicrobial properties, it has been **used as a preservative in vaccines**. Thimerosal will release ethylmercury $^{+}Hg\text{-}C_2H_5$ ions into the body. Because of its bactericidal and antifungal properties, Thimerosal has been used as an **antiseptic**, for the disinfection of living tissues, in particular the skin covering, and for the same reasons as a **preservative in vaccines**.

This antimicrobial activity is attributed to the binding of mercury with the thiol groups (-SH) present, at all cellular levels, in enzymatic and structural proteins, as at the level of ribosomes. They are "thioloprive" agents. The ethylmercury $^{+}Hg\text{-}C_2H_5$ ion, released by Thimerosal, will combine with the thiol groups of certain cellular enzymes to form mercaptide-linked derivatives, as follows:

$$Enzyme\text{-}SH + {}^{+}Hg\text{-}C_2H_5 \rightarrow Enzyme\text{-}S\text{-}Hg\text{-}S\text{-}C_2H_5$$

**The thioloprive activity of Thimerosal is at the origin of its antimicrobial activity, for bacteria or fungi, but also of its high toxicity for all cells of the human body, as indicated by the results of acute toxicity ( class T+).**

The sodium salt of Thimerosal (sodium mercurothiolate) was marketed under the name of **Merseptyl®**, as an antiseptic solution for local application, by the GSK laboratory. **The marketing authorization was repealed in 1996, and the marketing cessation declaration was published on 03/19/1997.**

## III- Mechanism of neurotoxicity, in the short and medium terms, of mercury in dental amalgams and Thimerosal by potentiating and accentuating the Fenton / Haber-Weiss chemistry

It is unanimously accepted that the « oxidative stress », consecutive to the production of oxygenated free radicals, formed during aerobic respiration of the cells of our organism, constitutes the process of cellular aging, on which our life expectancy depends. These oxygenated free radicals, such as: $OH^{\bullet}$, $O_2^{\bullet -}$, $HO_2^{\bullet}$, $NO^{\bullet}$, $ONOO^{\bullet}$, $ROO^{\bullet}$, $RO^{\bullet}$, etc, have single electrons ($^{\bullet}$). These oxygenated free radicals are extremely reactive because, by seeking to pair, their single electrons cause a progressive destruction of the cells of the organism, in particular of the nerve cells (neurons, astrocytes). The production of these superoxides in the mitochondria of cells in the body causes damage to cellular mitochondrial DNA, especially in normal human neurons and astrocytes.

The mercuric ions **$Hg^{++}$,** released by **dental amalgams**, and the ethylmercury ions **$CH_3$-$CH_2$-$Hg^+$,** released by **Thimerosal**, **accelerate the formation of superoxides and oxygenated free radicals**. The mechanism is as follows: the **$Hg^{++}$** and **$CH_3$-$CH_2$-$Hg^+$** ions potentiate the chemistry of Fenton / Haber-Weiss in the mitochondrial matrix; which leads to the **production of superoxides and extremely neurotoxic oxygen free radicals**. **These two ions are therefore mitochondrial toxins in human neurons and astrocytes**. This finding is all the more important since the number of diseases, in which mitochondrial dysfunction is involved, is increasing rapidly (correlations with the increase in autism cases).

The **toxicology of Thimerosal** in normal human astrocytes (AHN) has been studied, paying particular attention to mitochondrial function and the formation of specific oxidants. It has been found that ethylmercury not only inhibits mitochondrial respiration, leading to a decrease in membrane potential when stationary, but also, at the same time as these phenomena, increases the formation of superoxides, hydrogen peroxide ($H_2O_2$) and oxygenated free radicals, such as the hydroxyl free radical $HO^{\bullet}$, generated by the **chemistry of Fenton / Haber-Weiss** in the mitochondrial matrix

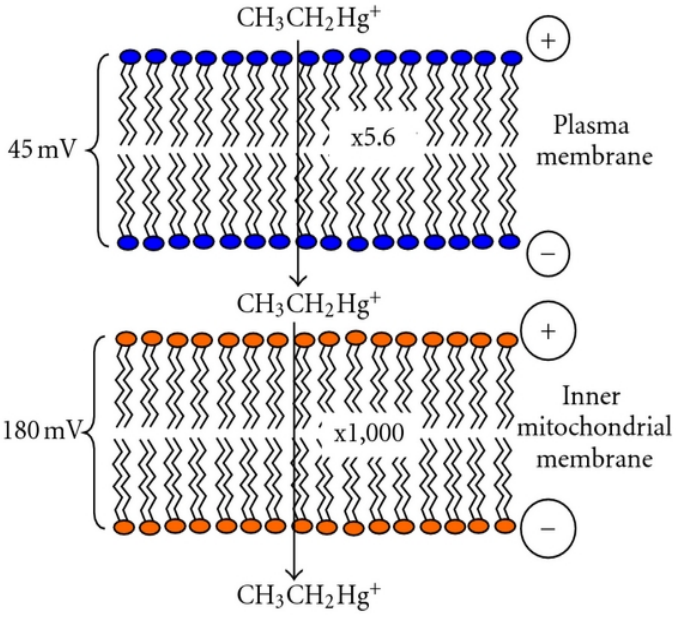
All this is corroborated by the video of **Pr F.L. Lorscheider** et al. (University of Calgary Faculty of Medicine), which clearly highlights **the role of mercury in the development of neurodegenerative diseases.**

Video: "**How Mercury Causes Brain Neuron Degeneration**"

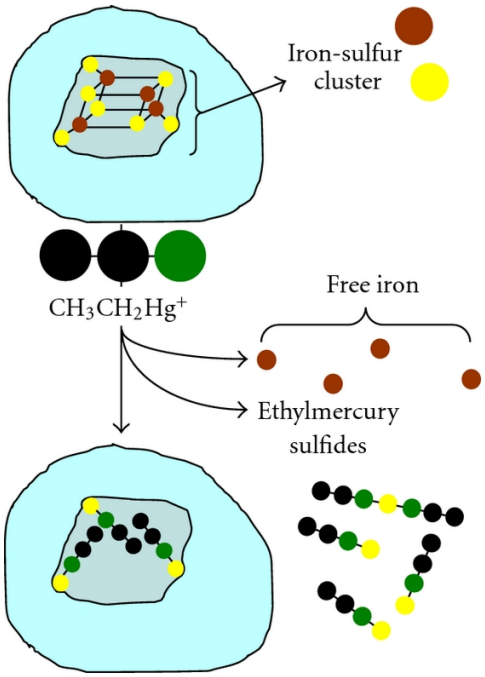
**<u>Application</u>: proposed mechanism for the neurotoxicity of the ethylmercury ion $CH_3$-$CH_2$-$Hg^+$ released by Thimerosal**

**1- As a lipophilic cation, the ethylmercury ion $CH_3$-$CH_2$-$Hg^+$ will be concentrated inside the neurons and astrocytes,** according to the plasma membrane potential of 45 mV, by a factor of 5.6 times, then Cytosolic ethylmercury will concentrate in the mitochondria by a factor of 1000 times, its diffusion being facilitated by the potential of mitochondrial membrane of approximately 180 mV (Figure 7-1).

<u>Figure 7-1</u>




<u>Figure 7-2</u>

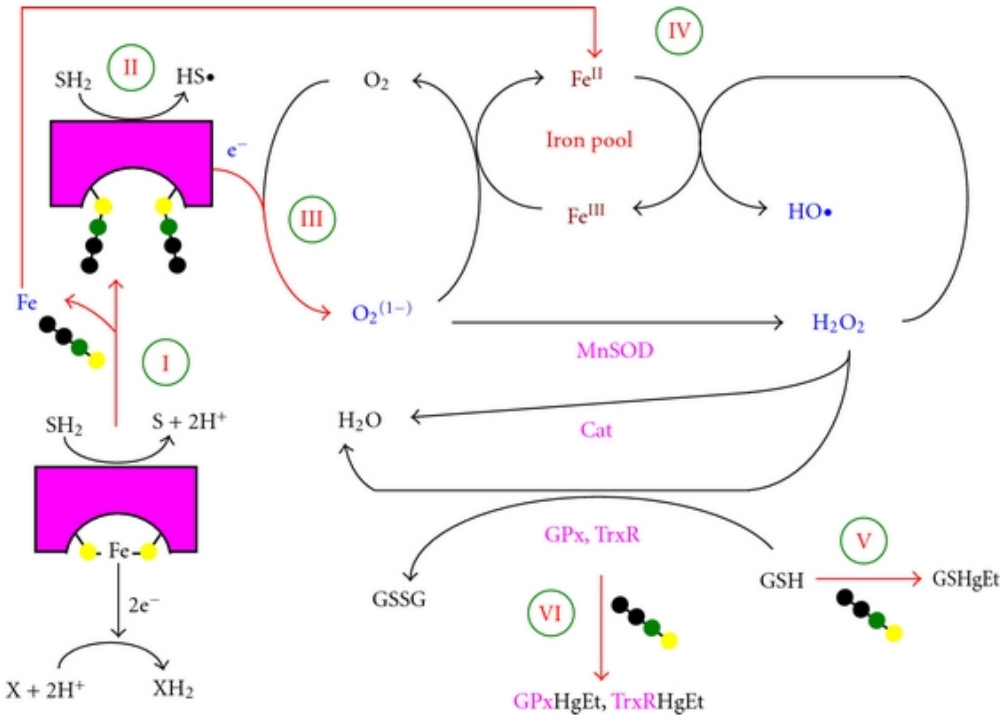



**2- Inside the mitochondria**

The ethylmercury ion $CH_3$-$CH_2$-$Hg^+$ will react and cap the R-S-H thiol / R-Se-H selenol functions, including the cysteine residues of the iron-sulfur clusters. The formation of ethylmercury sulfide adducts will not only cause enzyme inhibition, but also the release of free iron into the mitochondrial matrix (Fe) inside the mitochondria. (Figures 7-2 et 7-3 I).

The role of ethylmercury in the formation and detoxification of reactive oxygen species (ROS), is illustrated in Figure 7-3. The iron-sulfur clusters of oxidoreductases (for example, succinate dehydrogenase), when damaged by organic mercury, generate not only free iron (Figure 7-3 I), ferrous ions $Fe^{2+}$ (Figure 7-3-II), but also form species of intra-enzymatic carbon radicals (figure 7-3-II) which will react with molecular oxygen to give rise to superoxide, (Figure 7-3-III). Superoxide $\mathbf{O_2^{\bullet-}}$ can react with the generation of ion $Fe^{3+}$, or be destroyed by hydrogen peroxide, $\mathbf{H_2O_2}$, by mitochondrial Mn-SOD (**Manganese Superoxide Dismutase**). The ferrous ion $Fe^{2+}$, and the hydrogen peroxide $\mathbf{H_2O_2}$ react to generate the highly oxidizing radical, the hydroxyl radical $\mathbf{HO^{\bullet}}$ (according to the Fenton reaction, see Figure 7-3-IV), an agent involved in pathology and aging. Hydrogen peroxide levels would generally be lowered by mitochondrial antioxidants, including glutathione-dependent selenol / thio peroxidases, such as Glutathione Peroxidases GPx and Thioredoxines Reductases TrxR. However, these enzymes are inhibited by the organomercury indirectly by the depletion of glutathione, (Figure 7-3-V), and directly by the capping of the active site selenol / thiol by the organomercury, (Figure 7-3-VI)

Figure 7-3




**Legend** : $Fe^{II} = Fe^{2+}$ et $Fe^{III} = Fe^{3+}$

**Reminder on the Fenton reaction**: The most common oxidation reaction induced by iron complexes is the generation of oxygenated free radicals, such as **$HO^{\bullet}$** formed from **$H_2O_2$** by $Fe^{2+}$ salts according to the Fenton reaction (**Fenton / Haber-Weiss**)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-}$$

Thus, the release of iron catalyzes the chemistry of Fenton / Haber-Weiss, leading to the formation of the highly oxidizing oxygen free radical **$HO^{\bullet}$**. **$HO^{\bullet}$** has several targets, including permeability transition complex sensors and also mtDNA. Elevated **$HO^{\bullet}$** levels cause mitosis to stop, resulting in the release of cytochrome C from the mitochondria, and the initiation of apoptosis. We find that a consequence of exposure of ethylmercury to normal human neurons and astrocytes is damage to the mitochondrial genome, with an increase in DNA nicks and breaks and, above all, in the level of oxidized bases. Mitochondria typically have 150 copies of mtDNA and during aging or exposure to environmental stressors, the number of error-free copies of mtDNA is reduced. According to the theory of aging of free radicals of mitochondrial origin, the production of ROS by mitochondria leads to damage and mutations of mtDNA. These in turn lead to progressive respiratory deficits in the chain, which leads to an even greater production of reactive oxygen species (ROS), producing a positive feedback loop.

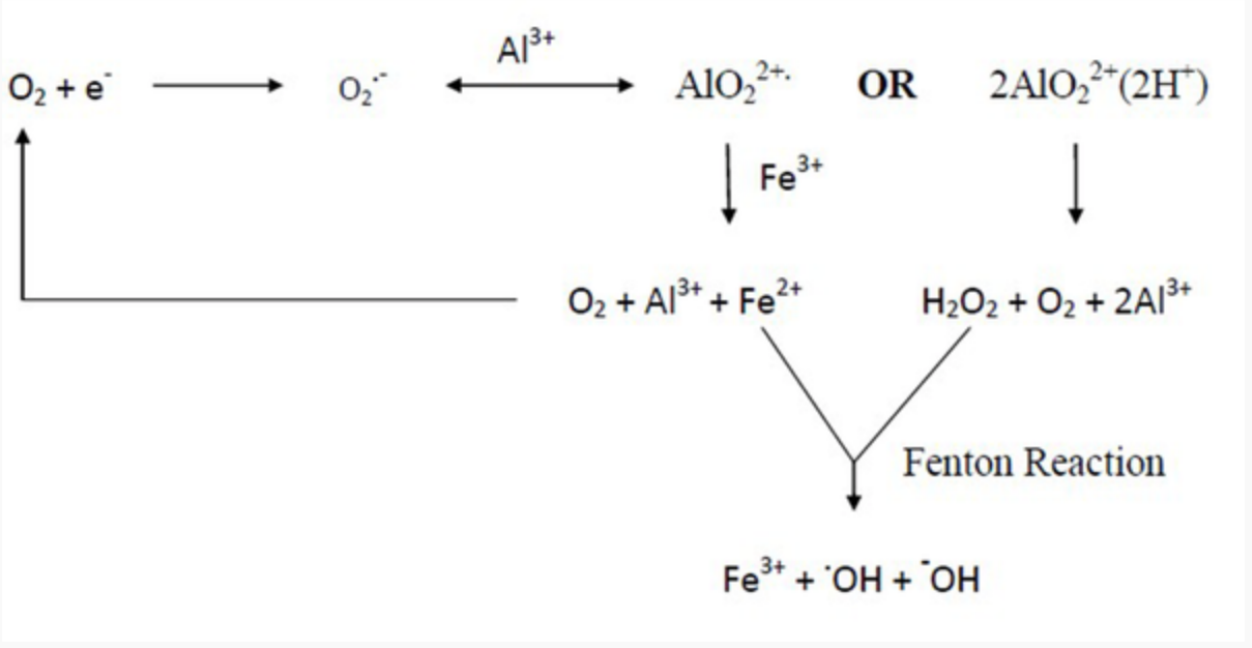
The results of this study show that **ethylmercury is a mitochondrial toxin in human neurons and astrocytes**. We think this discovery is important, especially since the number of diseases in which mitochondrial dysfunction has been implicated is increasing rapidly.

**Note:**

**The neurotoxicity mechanism of the mercury ion $Hg^{2+}$,** released by **mercury-silver dental amalgams**, is **similar to that** of the ethylmercury ion released by **Thimerosal**

## B- Mechanism of aluminum neurotoxicity

Aluminum accelerates oxidative stress in vivo and in vitro (Kanti Das T, Wati M R, Fatima-Shad K. Oxidative Stress Gated by Fenton and Haber Weiss Reactions and Its Association With Alzheimer's Disease, *Arch Neurosci.* 2015 ; 2(2):e60038). The semi-reduced radical ions of aluminum superoxide ($AlO_2^{2+}$) are produced by the reaction of $Al^{3+}$ with superoxide $O_2^{-}$. $AlO_2^{2+}$ then reduces $Fe^{3+}$ to $Fe^{2+}$ and promotes oxidative stress through the **Fenton and Haber-Weiss reaction**.




**Damage to DNA and neuronal structures**

The **•OH** hydroxyl radical, produced by the Fenton reaction, plays an essential role in damage to neuronal structures (**neurotoxicity which first affects the few dopaminergic neurons of the *Substantia nigra***) and to the nucleic acids of all cells of the organism.