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Distribution of Mercury in Human Body Compartments - Testing the Indicator Function of Mercury in Hair

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Preface

During the second half of my studies *Nutritional Sciences* at the University of Vienna, I focused on the semi-elective module *Nutrition and Environment*, which amongst other lectures included seminars on nutrition and ecology, food production and environmental protection as well as on environmental analysis. To further improve and strengthen my knowledge in the fields I was most interested in - the impact of food production on the environment and environmental pollutants in nutrition - I knew I had to write my diploma thesis in a related field. Fortunately, Dr. Hans-Peter Hutter offered me the opportunity to support his work at the Institute of Environmental Health of the Medical University Vienna. The topic of my thesis was soon settled, it would deal with the mercury burden of the human body including the examination of human body tissues.

Structuring of the Thesis

The thesis is divided into two main parts, a literature survey on mercury and a description of the practical laboratory part and its evaluation.
The basic information on mercury of the literature part is on one hand based on scientific results and findings of specialized books as well as on papers published in pubmed.org. On the other hand I also included expert opinions like for example the statements of diverse environmental agencies on relevant topics.

Before I could start the practical lab work, the study had to be granted by the ethics committee of the Medical University of Vienna. The positive reply was given in June, the first sampling eventually started in August 2011. The samples for the study were provided by the Institute of Pathology of the Medical University of Vienna.
The laboratory part mainly took place at the Institute for Medical Genetics Vienna, where I was permitted to use the lab equipment that was needed to analyse the mercury content of human body tissues.

Since my advisor at the Institute of Medical Genetics had only worked with blood, urine and hair samples before, we had to get proper information on the amount of tissue needed for further analysis with Atomic Fluorescence Spectroscopy in the first place. Having read several scientific papers on the topic, but none of them including explicit information on the exact amount of organ tissue used for measuring the samples, we finally managed to get proper information from two different working groups after having contacted them via email.

All in all, I was permitted to obtain a perfectly smooth work flow, which above all was brought to an end just in time.

Acknowledgments

First of all, I thank my advisor Dr. Hans-Peter Hutter for giving me the great opportunity to write my thesis at an external Institute and for his support throughout the whole period of working and writing. He arranged the collaborations with all the other institutions and he was always approachable whenever I needed information (even during his stay in Africa). All in all, he made it possible for me to obtain an unobstructed workflow.

Next, I thank Prof. Dr. Jürgen König for taking on the supervision of my diploma thesis. He was immediately willing to oversee my thesis and additionally, he offered me his advice in case I required further instructions or information. I am especially thankful for his support with regard to the time pressure I had towards the end of my thesis.

Furthermore, I want to thank Dr. Claudia Gundacker, who not only provided the facilities to perform the analysis of human tissue at her laboratory at the Institute of Medical Genetics but who also showed me how to handle the equipment. At this point I also thank Matthias Scheinast, who helped me performing the analysis with the AFS apparatus.

Moreover, I wish to thank Prof. Dr. Susanna Lang, Dr. Klaus Aumayr and Dr. Felicitas Oberndorfer, pathologists at the Clinical Institute of Pathology at the AKH/MUW Vienna, who spontaneously agreed to do the sampling and thereby provided me with human body tissue for further analysis.

I also thank Prof. Dr. Karl Wittmann from the Instiute of Environmental Health for dependably answering all my questions on the practical lab part.

Last but not least, I am most grateful to my parents and my family, including Andi, for their unending support in every respect. They never stopped encouraging me in whatever my intentions and future plans were. Without their help I could not have realized my visions of life the way I did.

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I. INTRODUCTION

Heavy metals have been used by humans since early ancient times. One of the first mentions of mercury was by the Greek philosopher, doctor and naturalist Theophrastus (371-287 BC), who described in detail the manufacture of mercury by powdering cinnabar with vinegar in pots made of copper. (Vienecke-Janz et al., 2008)

The Romans are said to have used the metal as an ingredient in salves to alleviate dental pain in children whereas the Greek applied mercury as a cosmetic to lighten up the skin. (Järup 2003)

Throughout the centuries mercury has thus widely been used in industry, agriculture and medicine. For example, it has been provided for the treatment of the venereal disease syphilis from the 1300s to the late 1800s, which explains the origin of the phrase: “Two minutes with Venus, two years with mercury.” (O`Shea 1990)

In the 1970s, the organic compound methylmercury was also still used as a common fungicide for seed grain. (Järup 2003)

It was only during the second half of the 20th century that the poisoning effects of mercury were specifically analyzed. Earlier discoveries of the toxicity of methylmercury, which mostly occurred on workers during production processes, have regrettably been overlooked, ignored and disregarded over centuries. (D`ltri and D`ltri 1978)

In 1953, several cases of mercury intoxication emerged in the shore of Minamata Bay, Japan. The clinical picture, called the Minamata Disease, was officially reported in 1956. (Ekino et al. 2007) Mercury containing waste of the local chemical company *The Chisso Corporation* polluted the bay in the years 1932 to 1968, contaminating fish and shellfish in the seawater and therefore causing the disease in about 3.000 people, who frequently consumed the seafood. Affected individuals showed severe neurological deficits, in some cases the poisoning even lead to death. (Dobbs 2009)

Until today a lot of research has been done with regard to mercury and its effects on human health.

It is now an established fact that the element is a highly toxic heavy metal, which accumulates in the environment and which is therefore classified as *priority hazardous* by the EU Water Framework Directive. But despite all research that has been done until now, there are several questions left unanswered. Since it is for example still not possible to analyze the individual mercury burden of inner human body tissues in vivo, the results of this study shall contribute new findings to the latest research.

II. LITERATURE SURVEY

1. Basic Information on Mercury

1.1. Isotopes of Mercury

There are seven stable main isotopes of mercury in the environment:
^{202}Hg (29,86%), ^{200}Hg (23,10%), ^{199}Hg (16,84%), ^{201}Hg (13,18%), ^{198}Hg (9,97%), ^{204}Hg (6,87%) and ^{196}Hg (0,15%) (Gagnon 2011)
The half-lives of the 27 unstable isotopes vary from seconds up to years. Whereas half-life is no longer than one day for the majority of isotopes, it is 444 years in the case of ^{194}Hg.

1.2. Physiochemical Properties of Mercury

Mercury is classified as heavy metal with the atomic number 80. The common abbreviation for the element is the symbol Hg, derived from the Greek word hydrargyrum (hydr-: *watery* or *runny* and –argyros: *silver*).
Mercury exists in three forms: elemental mercury, inorganic mercury salts and organic mercury compounds. Elemental mercury or metallic mercury is liquid under standard temperature and pressure conditions and has a silvery white appearance. Because of its strong cohesion, droplets of liquid mercury show high surface tension on flat surface. Compared to other metals, the vapor pressure of mercury is very high (1.2×10^{-3} torr). It slowly evaporates into the air, forming odourless mercury vapor. Melting point of mercury is at -38.84° C, boiling point at 356.58°C. (Adolph 2007, Ibrahim et al. 2006) As well as every other metal, mercury can conduct electricity.

Naturally, there are various chemical compounds in the environment. The oxidation states of mercury are Hg^0, Hg^{1+} and Hg^{2+}. Since the element is able to build stable compounds with carbons like alkyl, alkoxyalkyl or aryl groups, mercury occurs in organometallic forms but also in inorganic forms, as it combines readily with halogens and sulphur at room temperature. (Holleman et al. 1995) In combination with tin, copper, gold or silver, the element forms alloys, the so-called

amalgams. Depending on the composition, the alloys are either liquid, doughy or solid. (Kommission Human-Biomonitoring 1999)

Tab. 1: Forms of inorganic and organic mercury

Inorganic	Organic
Mercuric chloride	Ethylmercury
Mercuric iodide	Methylmercury
Mercuric oxide	Merbromin
Mercuric sulphide	Merthiolate
Mercurous chloride	Phenylmercuric salts

(Neustadt and Pieczenik 2007)

Tab. 2: Reaction of mercury with some common reactants

Reactant	Conditions	Reaction products
Noble gases	In discharge tubes	HgAr, HgKr
Halogens	At room temperature	Mercurous halide
	on excess of hal.	Mercuric halide
Oxygen, air	At about 350°C	HgO (Hg, O_2 at temp. > 350°C)
Ozone	Room temperature	HgO
S, Se, Te	On heating	
Dry Hydrides HX (X = F, Cl)	> 200°C	HgX_2
H_2S, NH_3, PH_3, AsH_3 etc.	> 200°C	
ICl	Room temperature	$HgCl_2$, HgI
NO_2	Room temperature	$Hg_2 (NO_2)_2$
		$Hg_2(NO_3)_2$
Conc. H_2SO_4	Room temperature	Hg I, Hg II, sulfates
HNO_3	Room temperature	Hg I, Hg II, nitrites, nitrates
Ammonia solution	In air	Millons base

(Hutzinger 1980)

1.3. Mercury in the Environment

Sources of mercury in the environment are manifold. On one hand mercury can be found naturally, mainly in mineral form, on the other hand the anthropogenic emissions have rapidly increased throughout the last century and are of undeniably growing importance.

1.3.1. Natural Sources

In nature, the element mostly occurs as metallic mercury in mercury sulphide (HgS; trivial name: cinnabar). The corrosion of minerals in rocks and soil liberates the metal. Due to wind and water erosion mercury is eventually spread into the environment. Because of the ability of elemental mercury to form vapor, it can enter the atmosphere and thus reach distant regions. Figure 1 illustrates the Hg-cycle in the environment.

Other key causes for mercury distribution in nature are volcanic activities, geysers, thermal fluids, undersea vents, degassing of the earth mantle, emanation from the ocean and forest fires. (Hutzinger 1980) Most of the mercury, which accumulates in forest foliage, derives from the atmosphere. Fire releases the metal out of the leaves, and then mercury again enters soil and water. (Ericken et al. 2003)

Tab. 3: Global mercury emissions by natural sources estimated for 2008

Source	Mercury ($mg\ yr^{-1}$)	Contribution (%)
Oceans	2682	52
Lakes	96	2
Forests	342	7
Tundra/Grassland/Savannah/Prairie/Chaparral	448	9
Desert/Metalliferous/Non-vegetated Zones	546	10
Agricultural areas	128	2
Evasion after mercury depletion events	200	4
Biomass burning	675	13
Volcanoes and geothermal areas	90	2
Total	5207	100

(Pirrone et al. 2010)

In 1967 a simple experiment showed, that inorganic mercury salts can be transformed into methylmercury by microorganisms. (Jensen and Jernelov 1967) This way "(…) *methylation of mercury from all sources causes worldwide contamination of freshwater fish and seafood by methylmercury*" (Grandjean et al. 2010)

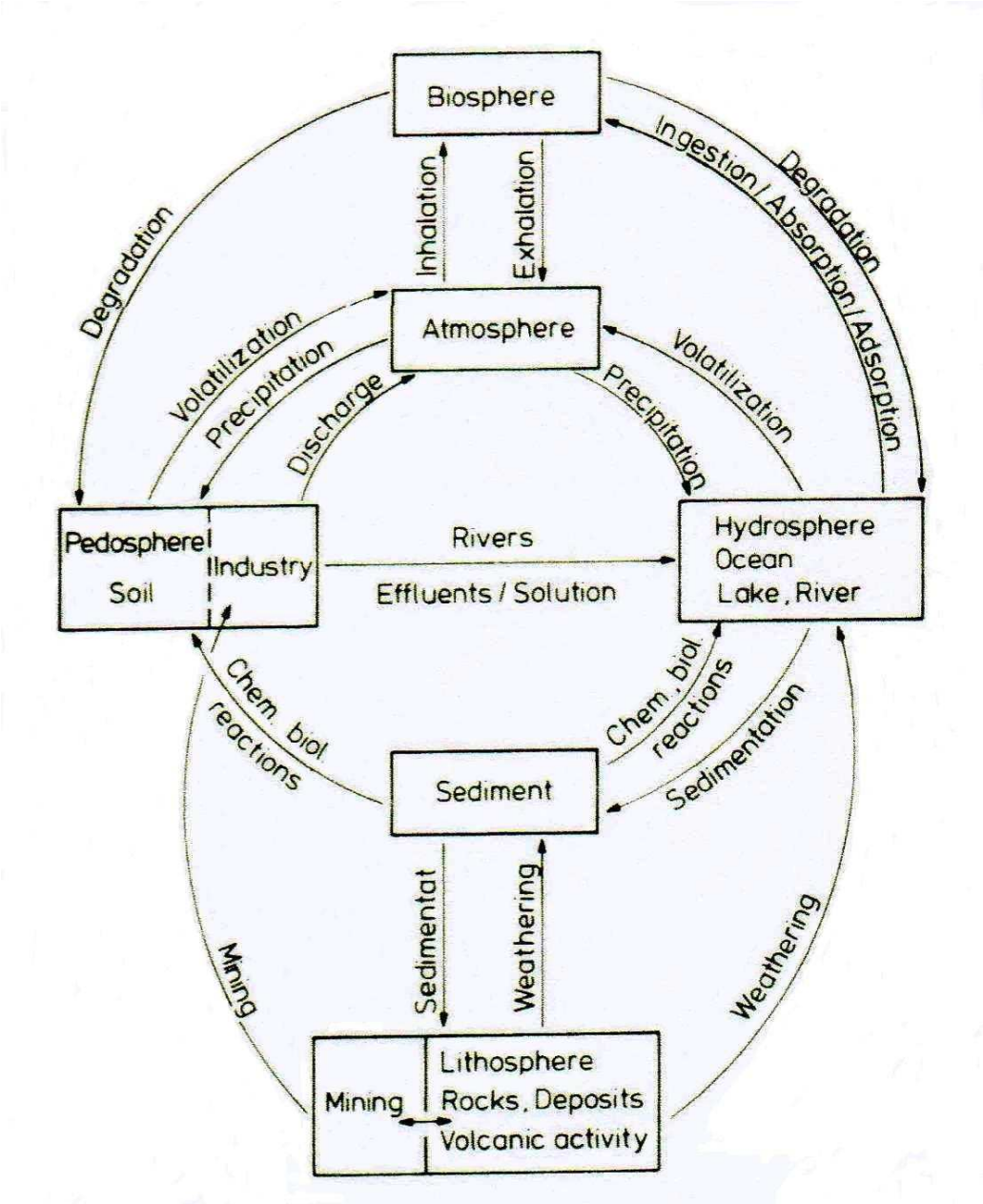


Fig. 1: Global mercury cycle (Hutzinger 1980)

1.3.2. Anthropogenic Sources

Today about two-thirds of the global mercury pool are considered to be anthropogenic. Only one-third derives from natural (geological) sources. (Hurley 2006)

Main anthropogenic causes for mercury release into the environment are coal-burning, caustic soda and cement production, the chlor-alkali industry and the disposal of mercury-containing products. It is also still used in gold mining in various countries of Latin America. (Järup 2003)

According to information from the Canadian Government in 2010, 95% of the estimated 200.000 tonnes of mercury emitted to the atmosphere since 1890 is currently embedded in terrestrial soil stocks or part of the oceanic basin. (Environment Canada, 2010). Moreover, remote areas like the Arctic and Antarctic, thousands of kilometres away from major anthropogenic sources also show significant mercury load, which can be explained by transboundary atmospheric long-range transport.

Even though North America and Europe considerably reduced their mercury emissions during the last 30 years, no significant change in the atmospheric pool of mercury has been detected. An explanation for this stagnancy can be either the increased pollution by other countries (Asia) and/or an unknown compensating factor. (Hurley 2006)

A decrease in the mercury content of the soil in Austria could however be noted. This happened most likely due to the existing eco-political measures that were devised by the European Union and the Austrian government to reduce the national heavy metal emissions and stocks. (Reisinger et al. 2009)

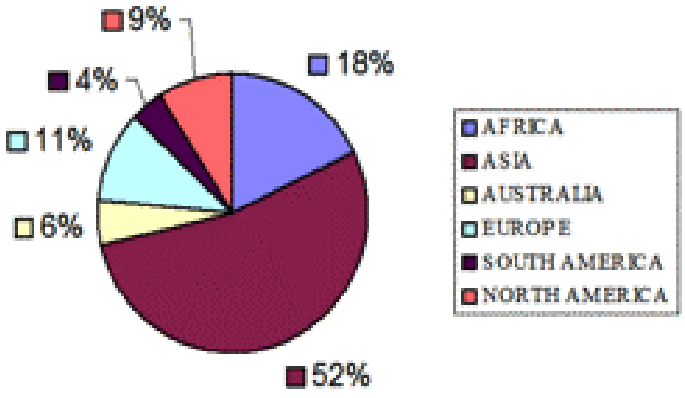


Fig. 2: Global emissions of total Hg from anthropogenic sources in the year 2000. Total emission: 2269 tonnes (Pacyna et al. 2006)

Tab. 4: Global mercury emissions from anthropogenic sources

Source category	Hg emission (mg yr^{-1})
Coal and oil combustion	810
Non-ferrous metal prod.	310
Pig iron and steel prod	43
Cement production	236
Caustic soda production	163
Mercury production	50
Artisanal gold mining prod.	400
Waste disposal	187
Coal bed fires	32
VCM production	24
Other	65
Total	2320

(Pirrone et al 2010)

1.4. Current Data on Mercury in Austria

National mercury stocks today contain about 47 tonnes of the metal. The main part is located in forests. The annual decrease is about 0.2 tonnes.

About 4 tonnes of mercury are imported annually.

2 tonnes reach private households every year in the form of amalgam fillings (about 1 tonne) and mercury-containing products (batteries, switches, lamps, electronic equipment etc.).

Every year about 10 tonnes of mercury waste accumulate in the form of junk goods, residual waste, waste water, and exhaust fumes. Mercury stocks on dumpsites add up to about 110 tonnes.

(Reisinger et al. 2009)

2. Use of Mercury in Industry and Medicine

Mercury features some very specific properties that make it a very attractive metal for industry and medicine. Its high density (13.5g per cc), its liquid state at room temperature and its strong volume expansion due to temperature variations offer some unique options in material processing. Moreover, the ability to conduct electricity and to form vapor, especially when current is applied, can be used for the fabrication of mercury-vapor lamps or fluorescent lights.

Sectors of industry that use mercury or mercury-containing products during manufacturing, are mainly the electrical Industry and the chlor-alkali industry where mercury is used as an electrode in the electrochemical process of manufacturing chlorine. (Umweltbundesamt 2009)

2.1. Mercury-containing industrial products

- Batteries (Mercuric Oxide Batteries, Alkaline)
- Vacuum lamps, energy saving lamps
- Catalysts
- Oscillators
- Detergents and Cleaners
- Thermometer, manometer, barometer
- Electrical equipment (conductor plates, cathode ray tubes, switches, relays)

(Umweltbundesamt 2009)

2.2. Mercury in medical products

- **Dental amalgam**: again the unique properties of mercury - especially the good binding capacity with the powdered alloy - make it a favoured ingredient for dental fillings. About half of a dental amalgam filling is made of liquid mercury; the other part is a powdered alloy of silver, tin, and copper. The role of mercury is to bind the composition and create a highly durable and solid filling. (WHO/IPCS 2003)

During the 1970s, mercury concentrations in some dental surgeries went up to 20 $\mu g/m^3$. Today levels have generally decreased to about one-tenth of those concentrations. (Järup 2003)

Even though dentists use more and more alternative tooth fillings, there are still about 18 tonnes of mercury contained in amalgam fillings in Austria today, making this the largest mercury stock in private households. (Reisinger et al. 2009)

- **Thimerosal** is an organomercury compound, containing 49% of ethylmercury (CH_3-CH_2-Hg^+). (Clarkson 2002) The beige-coloured powder is used as a preservative in cosmetic and pharmaceutical products in order to prevent them from microbial growth during storage and use. It also shows a very efficient antiseptic and antifungal effect.
Today Thimerosal is still contained in vaccination, ophthalmic (cleaning solution for contact lenses, eye drops) and nasal products, tattoo inks, antivenoms and cosmetic products (make up, make-up remover) (Schultz 2010)

- **Merbromin** is a mercury containing dye with antiseptic effect. It is sold as a two-percent solution (US & FR: Mercurochrome® / DE & CH: Mercuchrome®). In some countries, including Austria and Germany, the product is no longer available due to its content of mercury.

3. Absorption, Metabolism and Elimination in the Human Organism

The absorption ratio of mercury is determined by the form of the metal and its route of exposure. Elemental, inorganic and organic mercury each show distinctively different effects with regard to human health impairment.

3.1. Elemental Mercury

Poisoning by elemental mercury mainly takes place due to inhalation of the vapor, but may also occur due to ingestion of liquid mercury or dermal absorption. More than 75% of the mercury vapor stays in the lungs and is well absorbed within the organ. (Graebe and Pollack 1998)

Because of its mono-atomic structure, the mercury-specific high vapor pressure and its high lipophilicity, elemental mercury is characterized by a rapid diffusion through the alveolar membrane. It thus enters the pulmonary circulation very quickly. Animal experiments with mice and monkeys showed that significantly more mercury is transported to the brain after inhalation of elemental mercury than after intravenous injection of equivalent doses. (WHO/IPCS 2003) The toxic element eventually reaches different body tissues and organs via the blood circulation. Main targets of mercury accumulation are the central nervous system and the kidney.

Elemental mercury in the intestine is of rather harmless consequences, since it is hardly absorbed by the healthy gut (less than 0.1%) and excreted in the feces. (Goldmann 2001)

Cutaneous exposure rarely causes serious physical impairments, because of the rather low amount of absorption. Important to mention, however, is the local dermal reaction of the skin when mercury reaches the subcutaneous area. Elemental mercury as well as inorganic salts have been identified as sensitizing agents on the skin of sensitive persons. This is why direct skin contact of the metal as well as the contact with mercury containing vaccines or tattoo ink may cause extensive contact dermatitis and even distant skin lesions. The causative mechanisms of the immune system have not been identified yet.

In adults, the half-life of elemental mercury is 60 days (range: 35-90 days).

The biological half-life is yet different for the different organs. To some extent, mercury remains absorbed by the body. In brain and bones it may still be detected after years. (Dobbs 2009) The biggest part is excreted in feces (42%) and urine (52%), while only a minor part is exhaled. (Reichl 2009, Goldman 2001)

3.2. Inorganic Merucry Salts

Absorption routes of inorganic mercury salts are similar to those of elemental mercury, but the toxic effects of the pathways are remarkably different. In case of exposure, the intestine shows the highest absorption ratio, followed by the way of inhalation and dermal routes. (Ibrahim et al. 2006) Unlike metallic mercury, the inorganic salts are well absorbed by the gut and may cause serious corrosion to the gut mucosa. (Goldman 2001) As a result, ulceration and perforation in stomach and intestine may emerge and hence may lead to an increased absorption of the heavy metal. Main target organ of inorganic mercury salts is the kidney, where it accumulates in the proximal convoluted tubules. (Graeme and Pollack 1998) The biological half-life for inorganic mercury is about 40 days. (Dobbs 2009) Its elimination from the human body takes place via the kidneys (60%) as well as the feces (40%). (Reichl 2009)

3.3. Organic Mercury Compounds

In terms of organic mercury, humans are basically exposed to two different major compounds: methylmercury, which is primarily found in fish and seafood, has to be mentioned in the first place. Aside, ethylmercury has also become a widely discussed public health concern to humans since it is used as a preservative in a number of vaccines for preservation purposes.

Main difference between the two organic forms is their route of exposure. While methylmercury is ingested with food and readily absorbed via the intestine, ethylmercury may only be administered by medical injection. Again, primary target organ for both forms is the central nervous system.

Between the day of exposure and the onset of the first symptoms, a latent period of weeks or even months may pass by.

Both forms share equal properties such as their close chemical affinity and a similar initial distribution inside the human body. They also show similarities in the type of brain damage at high doses. (Clarkson et al. 2003)

But one should regard the distinctively different properties as well. As a matter of fact, methylmercury is the more potent compound with a longer biological half-life of about 50 days. Ethylmercury on the other hand is rapidly metabolized into inorganic mercury, which may be the reason why ethylmercury not only affects the central nervous system but also causes kidney damage. The biological half-life of ethylmercury in blood is 7 to 10 days and therefore much shorter than the half-life of methylmercury. (Clarkson et al. 2003) 90% of organic mercury is excreted via the feces, while only 10% are excreted via the urine. (Reichl 2009)

4. Toxicity

Mercury is biologically not essential and toxic to all organisms. The toxicity naturally depends on its chemical form, which determines the specifically different properties of the metal. (Dobbs 2009) The organic compound methylmercury is believed to be the most toxic form. (Järup 2003) Beyond that, the severity of poisoning by the heavy metal depends on additional factors like length and dose of exposure, route of absorption as well as gender, age and vulnerability of an affected person. (Dobbs 2009) One of the main target organs of mercury in the body is the central nervous system. Even though the central nervous system is protected by the blood-brain-barrier, the heavy metal gains access without any problems, which then leads to a variety of neurological symptoms after mercury exposure. (Dobbs 2009)

Since there is a wide range of possible effects on various body tissues, but no disease-specific symptoms, diagnosis and treatment of mercury intoxication turn out to be very difficult and treacherous.

Still, after cessation of exposure symptoms of mercury intoxication are reversible. (Järup 2003)

4.1. Elemental Mercury

In 1865, Lewis Carroll introduced the character of the *Mad Hatter* in his book *Alice`s Adventures in Wonderland*. The cause of *Mad Hatter's* insanity was the brain disease that oftentimes affected hat makers throughout the 19th century, who used liquid mercury to treat their hat felt. (Goldman 2001) This crazy character already indicates the hazardous effect mercury may have on human health.

As mentioned above, the absorption ratio of metallic mercury in the intestine is extremely low (less than 0.1%). Yet it may be increased by abnormal gut function such as decreased gut motility or fistulae, which restrain the ingested substances and thereby lead to prolonged exposure and increased absorption of the toxic metal. (Graebe and Pollack 1998)

Cutaneous reactions due to mercury exposure mainly occur with sensitive persons, or in case of high or chronic contact with the metal. Dermal manifestations of mercury poisoning may lead to well known clinical syndromes

like acrodynia (also known as pink disease), mucocutaneous lymph node syndrome, mercury exanthema, cutaneous hyperpigmentation or cutaneous granulomas. (Boyd et al. 2000)

Inhalation of mercury vapor has yet always been of major concern, especially for those, who frequently handle the metal during working procedures. The risk of serious lung damage increases with the amount of elemental mercury inhaled. High acute exposure affects the lung directly and consequently leads to inflammation of the bronchia, which may cause dispnoea and cyanosis. (Umweltbundesamt Fact Sheet) Extreme acute exposure may even result in acute necrotizing bronchitis and pneumonitis, which in some cases lead to death from respiratory failure. (Goldman 2001) On a cellular level, mercury interferes with a number of different metabolic processes, including protein phosphorylation, protein and nucleic acid synthesis, calcium homeostasis and oxidative stress. (Ibrahim et al. 2006).

Early nonspecific signs include insomnia, forgetfulness, loss of appetite, and mild tremor, which is often misdiagnosed as psychiatric illness. Chronic intake may lead to the classic triad of progressive tremor, gingivitis and erethism, a syndrome characterized by red palms, emotional lability, and memory impairment. (Clarkson 1997) Salivation, excessive sweating, and hemoconcentration are accompanying signs of the autonomic nervous system. Mercury also accumulates in kidney tissues, directly causing renal toxicity, including proteinuria or nephrotic syndrome. Isolated renal effects may also be immunologic in origin. (Goldman 2001)

4.2. Inorganic Mercury Salts

Absorption of mercury salts in the gut may cause serious corrosion to the gut mucosa, which thereupon may result in gastrointestinal ulceration or perforation and hemorrhage. In many cases, this progression ends in circulatory collapse. (Goldman 2001)

Further effects of the breakdown of the intestinal mucosa barriers are extensive mercury absorption and transport to the kidneys, where the inorganic salts show their very toxic properties. Proteinuria, hematuria, glycosuria, oliguria, uremia, immunologic glomerulonephritis and acute renal failure are common consequences of acute inorganic mercury intoxication. Renal tubular acidosis and

nephrotic syndrome are symptoms of chronic absorption. (Graebe and Pollack 1998) Central neuropathy and acrodynia may also occur from mercury salt exposure. (Goldman 2001)

Another alarming presumption has emerged during the last decade, concerning the exposure of inorganic mercury compounds and the potentially correlated increased incidence of Alzheimer`s disease (AD). In 2007, a German research group stated:

> The available data does not answer the question, whether mercury is a relevant risk factor in AD distinctively. In sum, the findings from epidemiological and demographical studies, the frequency of amalgam application in industrialized countries, clinical studies, experimental studies and the dental state of Alzheimer patients in comparison to controls suggest a decisive role for inorganic mercury in the etiology of Alzheimer's disease. Other factors currently discussed as causes (e. g. other metals, inflammations, dietetic factors, vitamin deficiency, oxidative distress, and metabolic impairments) may act as co-factors.
>
> (Mutter et al. 2007)

4.3. Organic Mercury Compounds

Main source of methylmercury exposure for humans today is the consumption of fish and seafood. Organic forms of mercury are well absorbed within in the intestine and since it acts as a potent neurotoxine, it primarily affects the central nervous system, evoking severe health impairments. (Clarkson T.W. 1997)

In 1940, four cases of methylmercury intoxication were reported by Hunter, Bomford and Russel. The poisioning accrued due to fungicidal dusts at a working place, causing symptoms such as dysarthria, ataxia, constriction of the visual field and eventually causing death in the worst cases. The characteristic symptoms of mercury vapor poisoning, however, were not reported. Only the occurrence of tremors resembled the well-known symptoms of mercury vapor intoxication.

The autopsy of the deceased patients showed that the symptoms led back to atrophies of different brain tissues, amongst others the cerebellar cortical and bilateral cortical tissues. (Satoh 2000)

Later examinations of the brain revealed regional destructions of neurons in the visual cortex and cerebellar granule cells, involving paresthesias of the circumoral area and hand and feet as well as visual-field constriction and ataxia. (Clarkson et al. 2003)

Besides, the toxic properties of ethylmercury, the major component of thimerosal, have become a subject of concern for publicity during the last twenty years. Especially in terms of the significant increase in the diagnosis of autism and Alzheimer`s Disease throughout the last decades, ethylmercury was taken into consideration as a cause of the diseases.

It is indeed noticeable that the prevalence of autism increased from 5 in 10.000 to 60 in 10.000 in the United States in the early 1990s. Possible explanation for this scenario could have been the implementation of three additional thimerosal-containing vaccines for newborn babies during that time. (Mutter et al. 2005)

Studies focusing on the correlation of mercury exposure and the occurrence of autism are contradictory.

Holmes et al. concluded in 2003 that the metabolism of autistic people appeared to show a deficient efficiency in terms of mercury excretion. Results attested that the severity of the disease was inversely related to hair mercury levels. Even though the mothers of the autistic babies showed significantly higher levels of

mercury exposure, mainly due to Rho D immunoglobulin injections and amalgam fillings, the mean value of mercury concentrations of the hair in autistic babies was 0.47 ppm, while the mean value of the control group was 3.63 ppm.

There was as well no correlation between hair mercury levels and the mercury exposure of the mothers. In the control group, however, mercury levels of the hair were significantly correlated to their mother`s mercury exposure. (Holmes et al. 2003)

According to the results of this study, autistic babies were proven to have a malfunctioning of their bodies` mercury excretion. Whether this dysfunction also causes or increases the risk for the neurodevelopmental disorder is still not entirely analyzed.

5. Human Exposure

Because of the great variety of mercury sources in our environment, mercury intake is inevitable for almost everyone in modern society today. The metal is actually omnipresent and can be found in inorganic and organic form in food and as an additive in vaccines. Moreover, a large mercury stock exists in the mouths of most people due to the implementation of amalgam fillings for dental restoration. Main sources of mercury exposure are listed in the following.

5.1. Fish and Seafood

Because of its numerous nutritional benefits, fish is proclaimed to be part of a healthy and well-balanced diet. Especially the low amount of saturated fatty acids and high amount of vitamin D and essential omega-3 polyunsaturated fatty acids make fish an important component in human nutrition. In spite of these beneficial values, the highly toxic substance methylmercury has also become a hot topic in terms of fish and seafood

Because of its lipophilic properties, mercury is characterized by a high degree of bioaccumulation. Phyto- and Zooplankton concentrate both inorganic and alkylated mercury, which subsequently enters the food chain efficiently. (Hutzinger 1980) Therefore comparably high amounts of the metal can be found in animals, especially in the tissues of aquatic animals. Since the mercury burden of marine waters has remarkably increased throughout the last century, more and more mercury residues can be found in fish and seafood. The mercury levels naturally increase with size and age of the animal. Highest mercury contents can be found in predators such as pike and bass in freshwater and swordfish and shark in the oceans. (Clarkson et al. 2003) These species may even show a biological magnification of up to 100.000 times from the algae level. (Ahrenholt-Bindsley 1992) The chemical form of mercury in biological tissues like fish is the organic form methylmercury. The basic concentration of mercury in unpolluted sea water has been reported to be at least 0.1 ng per litre. (Fowler 1990) Geological and atmospheric mercury sources provide an additional natural input of the metal and therefore contribute to an annual increase of mercury concentrations in marine waters of about 100.000 tonnes. Although anthropogenic sources still represent a

minor part of the total mercury input, it may be the cause for serious environmental pollution in restricted domains like inland waters or coastal waters. (Kruse and Bartelt 2008) Near the industrial areas of New York Harbor, the water contained up to 90 ng per litre of dissolved mercury concentrations in 1990. (Fowler 1990)

In 2006, the *Eighth International Conference on Mercury as a Global Pollutant* took place in Madison, Wisconsin, USA. With regard to methylmercury in fish, the Conference Organizing Committee recommends:

> Fish can contain both methylmercury and beneficial omega-3 fatty acids. Methylmercury exerts toxicity and can also diminish the beneficial health effects of omega-3 fatty acids. As with mercury, there are large variations in the level of omega-3 fatty acids in fish. Selection of fish species for consumption should seek to maximize the intake of beneficial fatty acids while limiting exposure to methylmercury.
>
> (Hurley and Krabbenhoft 2006)

5.2. Tooth Fillings

In 1926, the German chemist Alfred Stock described a curious disease, which moderately started with slight symptoms but over the years became much worse. In the end, the suffering was almost unbearable. It was his own condition, Stock specified in his article *The Hazards of Mercury Vapor*. He suffered from chronic mercury intoxication for over 25 years. Eventually, he was one of the first to identify mercury as source of the disorders and also one of the first to describe the symptoms of mercury poisoning. With regard to dental tooth fillings, which were used since the early 1920s, Stock concluded: *It will then turn out to be likely that the reckless implementation of amalgams as tooth fillings was a fatal sin against humanity.* (Stock 1926)

Amalgam has been the material of choice for the restoration of broken teeth throughout the whole last century. The contained metals of an amalgam filling vary, but mercury has always been a steady component, because of its ability to bind the other powdered alloys. The content of the heavy metal quite often reaches 50%. (Clarkson 2002) Today, more and more dentists use alternative tooth fillings, like ceramic, gold or synthetic material. With the exception of the Scandinavian countries, the usage of mercury containing amalgams is yet not forbidden and still common in some dental procedures.

Even though a lot of research on this topic has been done over the past 30 years, results are still controversially discussed today. Several studies proved the release of mercury vapor out of amalgam fillings. (Ott et al. 1984, Vimy and Lorscheider 1985, Nylander et al. 1987, Aronsson et al. 1989, Marek 1992, Molin 1992, Björkman et al. 2007) A research group from New Zealand examined 172 people with regard to their amalgam fillings and found out that the mercury vapor concentration in the exhaled breath was high enough to be of chronic toxicologic hazard for some of the examined people. (Patterson et al. 1985)

The amount of mercury released depends on several additional factors: quality, composition and number of fillings, intensity and length of chewing, eating habits (hot, acidic) and ratio of mouth to nose breathing. (Kommission Human-Biomonitoring des Umweltbundesamtes 1999, Ferracane et al. 1995)

Breath takes the evaporated mercury into the lungs, where a big part enters the pulmonary circulation. A minor amount of vapor reaches the human intestine and is absorbed there. Via both routes of absorption, mercury eventually reaches

different body tissues and organs through blood circulation. In 2007, a research group from Norway discovered a significant correlation between the number of dental amalgam surfaces and the concentrations of inorganic mercury in blood and brain of 30 deceased people at the time of death. (Björkman et al. 2007)

Amalgam fillings thus permanently release small amounts of the toxic heavy metal. Average daily intake of elemental mercury from dental amalgams for an average person is about 3.8-21 µg. These amounts are yet not associated with an increased health risk. (IPCS 1990) A high number of amalgam fillings, optionally together with mercury intakes from additional sources, may nevertheless lead to chronic poisoning of the human body in the long term. (Clarkson 2002)

5.3. Vaccination

Thimerosal (TMS) is the most widely added preservative in more than 50 licensed vaccines. The most common thimerosal-containing vaccine is hepatitis B. (Egan 1999) As mentioned above, thimerosal contains up to 49.55% ethylmercury, which is bound to the thiol group of a salicylic acid. It was developed in 1927 and still today, there is not much information about the effects of ethylmercury (CH_3-CH_2-Hg^+) on human health, although it is assumed that its toxicity is similar to methylmercury (CH_3-Hg^+), which is a chemically very close compound. (Clarkson 2002) This is why TMS-containing vaccines are suggested to cause severe neurological impairments like autism or attention deficit hyperactivity disorder (ADHD), especially when administered to children during early stages of development. (Stratton et al. 2001)

Even though there are differences in route of administration, magnitude of doses and pharmacokinetics for ethylmercury, limits for intake are based on those for methylmercury. (Egan 1999)

Like on amalgam, different opinions exist on the harmlessness of thimerosal, although there have been several studies that tested and oftentimes confirmed thimerosal toxicity for many decades now. (Nelson 1966, Heyworth 1982, Royhans et al. 1984, Digar et al. 1987, Nascimento et al. 1990, Hilleman 1991, Lowell et al. 1996)

If the thimerosal compound (ethylmercury – salicylic acid) was stable, it could rapidly be transported to the kidney and lastly be excreted via the urine. Unfortunately, a breakdown to ethylmercury hydroxide and thiosalicylate may occur, after dissolution of the compound and reaction of the mercury radical with the hydroxyl ions of the thimerosal solution. The ethylmercury breakdown product is considered to be responsible for thimerosal toxicity, because of its high reactivity. (Geier et al. 2007)

In 1999, Egan outlined the evidence of thimerosal toxicity in a presentation to the FDA. According to the review, the contained ethylmercury may lead to local hypersensitivity reactions and show acute toxicity at high doses (including coma and death). For children, one of the most vulnerable groups, the paper recommended: "*Infant exposure to mercury from vaccines may be largely avoidable by using thimerosalfree products."* (Egan 1999) Today thimerosal has

been removed from all vaccines on the recommended childhood immunization schedule for children younger than seven years. (Stratton et al. 2001)

Even though the review left some very important unanswered questions ("*Is ethylmercury toxicity the same as methylmercury?"*), there has been no further research to provide new approaches.

In 2001, Magos wrote a review on ethylmercury toxicity, that summarized all known cases of human intoxication and evaluated the entire published data, but information remained the same and there is still insufficient scientific evidence that developmental neurotoxicity could result from thimerosal exposure. (Magos 2001)

The same year, the Immunization Safety Review Committee of the National Academy of Sciences Institute of Medicine wrote:

> The committee concludes that although the hypothesis that exposure to thimerosal-containing vaccines could be associated with neurodevelopmental disorders is not established and rests on indirect and incomplete information, primarily from analogies with methylmercury and levels of maximum mercury exposure from vaccines given in children, the hypothesis is biologically plausible.
>
> The committee also concludes that the evidence is inadequate to accept or reject a causal relationship between thimerosal exposures from childhood vaccines and the neurodevelopmental disorders of autism, ADHD, and speech or language delay.
>
> (Stratton et al. 2001)

5.4. Mercury Exposure of the Fetus and the Breast-Fed Infant

In the early 1970s, Iraqi farmers disseminated mercury-treated seed grain onto their fields. The consumption of home-made bread, baked of the contaminated grain, led to a series of methylmercury intoxications in the local population. It is suggested that almost 1000 people were affected, while only 370 were hospitalized. (Bakir et al. 1973) A number of clinical observations of that time allows an immerged insight into the effects and consequences of methylmercury poisoning, particularly on newborn babies and children. A survey of children, whose mothers were exposed to methylmercury during pregnancy, showed that methylmercury readily passes from mother to fetus. The study also indicates that the poisoning may result in physical impairment of the offspring, since six out of 15 babies showed clinical manifestations of mercury poisoning, five of them exceedingly severe with impaired motor and mental development. (Amin-Zaki et al. 1974)

Since the metal reaches the child via the umbilical cord, the fetus is exposed to methylmercury as well as inorganic mercury throughout the whole pregnancy. Based on the analysis of data from the Minamata disease, Sakamoto et al. discovered an important coherence of prenatal mercury exposure and an unequal sensitivity of the male and female sex in 2001. It seems that male fetuses were more susceptible to mercury intoxication as they showed a higher stillbirths ratio than their female counterparts. (Sakamoto et al. 2001) Animal experiments on mice also proved the higher sensitivity of male fetuses on mercury exposure. The administration of methylmercury led to significant neurobehavioral effects, particularly in the development of male mice. (Yoshida et al. 2011) Results of diverse animal experiments suggest that pre- and postnatal mercury exposure may hence affect the brain functions of the fetus. (Newland et al. 1996) It would, however, need further studies to prove the influence of prenatal mercury exposure and faulty fetal development.

The transmission of mercury from mother to child is yet undeniable and has been proven in many surveys over the years.

In 2001 a Swedish research group screened the mercury levels of the blood of 20 Swedish women before and after they gave birth. After childbirth, they also checked up on the blood mercury levels of the babies. Aside from giving blood samples, the women had to fill out a questionnaire about their fish consumption,

vaccination and dental care during the preceding 6 months and 13 weeks after childbirth. Results showed that methylmercury (MeHg) concentrations of the maternal blood strongly correlated with the concentrations of MeHg in cord blood and infant blood at 4 days, although the cord and infant blood levels were two times higher than those of maternal blood.

Maternal methylmercury increased from childbirth to 13 weeks postpartum, whereas methylmercury concentrations of the children declined from 4 days to 13 weeks after birth. (Björnberg et al. 2005)

The decrease of the infant blood levels during the breast-feeding period can be explained by the low mercury transfer through breast milk and the rapid growth of infants after birth. (Sakamoto et al. 2002)

Surprisingly, fish consumption had no influence on the mercury levels of maternal or infant blood, nor did dental care during pregnancy and breast-feeding period result in higher concentrations in the blood of the women.

Not surprising, however, was the significant correlation between the number of amalgam surfaces with the concentrations of inorganic mercury (I-Hg) in the maternal blood.

The values of I-Hg did not show any change before and after the women gave birth to their babies, the infant blood I-Hg levels correlated with those of their mother and decreased until 13 weeks of age. (Björnberg et al. 2005)

There is only vague information on the exposure to MeHg and I-Hg in breast-fed infants. Animal studies suggest that both MeHg and I-Hg are transported from blood plasma to breast milk primarily bound to serum albumin, with I-Hg also bound to casein. (Sundberg et al. 1999) No significant correlation was found between MeHg in the blood of the mother and mercury in breast milk. (Björnberg et al. 2005)

In summary, the resulting implication of the research findings can only be that, in order to protect the unborn child from possible negative health effects of mercury intoxication, exposure routes and mercury intake of the mother need to be minimized as much as possible.

To detect the mostly very subtle effects arising from prenatal exposure such as cognitive changes and delayed development in children is surely still a difficult task

and a huge challenge for researchers and medical scientists today. In their review of 2011, Greandjean and Herz summarized the complexity of the topic as follows:

> The outcome of developmental neurotoxicity may not be immediately apparent in the infant, but deficits will become evident later on as long-standing or irreversible dysfunctions. More generally, the delayed recognition of developmental neurotoxicity due to methylmercury heralds some limitations in scientific documentation that may lead to deficient prevention of neurotoxic exposures.
>
> (Greandjean and Herz 2011)

5.5. Energy Saving Lamps

In April 2009, the European Union published the regulation (EG) 244/2009, which determines the efficiency of regular lamps for household usage. Since commonly used bulbs did no longer achieve the newly regulated values, they have been completely replaced by so-called energy saving lamps (ESL). According to the information on the homepage of the European Commissioner for Energy Günther Oettinger, the durability of ESL is six to ten times higher whereas their current consumption is 75-80% lower than those of common bulbs. The change of lamps is supposed to save enough energy to supply eleven million households until 2020. (European Union 1995-2011)

Moreover, the usage of the ESL is said to reduce anthropogenic mercury emissions. Due to the application of regular household lamps 2.7 tonnes of mercury emissions accrued in 2007, because of the high power requirement (coal-burning) and the mercury-containing compact fluorescent lamps that were not recycled at the end of life. In 2020 emissions may increase up to 3.1 tonnes if no countermeasures are applied. Even though an ESL contains mercury as well, the allowed content and the current needed to run it are both lower, so that mercury emissions will significantly decrease. (Piebalgs 2009) Although this scenario is based on assumptions, it has become a very popular argument for proponents of the ESL and is by now commonly known as the *mercury-paradox*.

Current limit for the mercury content in ESL is 5 mg per lamp. For lamps with a power capacity of less than 50 watt, the limit will be reduced to 3.5 mg in January 2012. Maximum permissible values for lamps with a capacity lower than 30 watt will be 2.5 mg mercury per lamp starting January 2013. (Piebalgs 2009) Lowest mercury content in an ESL of today is about 1 mg. (Umweltbundesamt 2010)

The arguments for ESL seem convincing, nevertheless critical voices surfaced within the past three years. On one hand the health risks due to the mercury content of ESL have been reviewed, on the other hand the promoted advantages of the new bulbs have been inspected and the results of both objections are quite notable.

The first study on energy saving lamps and their potential health risk due to mercury emissions was initiated at the Maine Institute of Environmental Protection in 2008. Research interest was to measure the mercury concentrations after the

breakage of an ESL. Results showed significantly increased values of mercury in the surrounding air. Peak values were 50 – 100 µg/m^3. (Stahler et al. 2008) These results triggered great concern and demanded awareness, especially for vulnerable groups (children, pregnant women), which may get in contact with mercury emissions at these high values. Households including risk groups shall therefore always take precaution by using ESL with plastic coating.

Based on these results, the German Federal Environmental Agency also tested mercury emissions from broken ESL. Peak values exceeded the allowed value of 0.35µg/m^3 twenty times. (Umweltbundesamt 2010)

This is why in 2010 the German umbrella organisation *Verbraucherzentrale Bundesverband* demanded to defer the decided ban of regular lamps in households.

III. HUMAN BIOMONITORING

1. Background and Objective

Human Biomonitoring was initially designed for the monitoring of certain occupational groups, which were particularly exposed to pollutants at work. Today it is also used as a screening-method for the analysis of internal exposure to pollution in humans. Main objective of the examination is to determine substances (mainly pollutants) that are able to enter the human organism as well as the amount of those substances, which are consequently stored in different body tissues. Therefore it is necessary to examine different body samples, like fluids (blood, urine, breast milk) or tissues (hair, muscle/adipose tissue). Subsequently the substances and their metabolites are detected by various chemical and / or technical methods. Since Human Biomonitoring does not indicate the ratios of the substances in the target organs, the obtained material is called *indicator media* or *surrogate marker.* (Umweltbundesamt GmbH, 2011) (Reichl and Schwenk 2004)
The definition for Human Biomonitoring of the *The European Environment & Health Action Plan 2004-2010*, was adopted by the European Commission as “monitoring activities in human beings, using biomarkers, that focus on environmental exposures, diseases and/or disorders and genetic susceptibility, and their potential relationships". (Joas and Polcher 2009)

The method can be divided into two types of monitoring:

- *Human biological monitoring of exposure*: systematic examination (once or repeated) of certain substances and their metabolites from various body samples (blood, serum, breast milk, urine, teeth, hair)
- *Biological effect monitoring*: systematic examination (once or repeated) of biological parameters, which result from the exposure to chemical, physical or biological substances and which thus show a certain effect.

2. Scopes and Limits of Human Biomonitoring

- Scopes can be mainly differentiated into three domains:

1. Examination of a single person, who is exposed to a certain substance or who is assumed to be exposed to a certain substance
2. A quantitative examination of the pollutant exposure of a certain group of people or a certain population group during an epidemiological study
3. Examinations to detect specific trends in terms of pollutant exposure of humankind

- Limits of the human biomonitoring :

1. Human Biomonitoring can not differentiate between the different ways of entry or the sources of pollution. Therefore it is crucial to get additional information about the person's living habits.
2. This method can not be used for the analysis of pollutants, which already affect external and / or internal mucous membranes or pollutants, and are only absorbed in very small amounts.
3. Pollutants with short dwell time in the human organism can only be detected in a particular time period after absorption.
4. The exposure of substances, which occur or are excreted naturally in the human body, is usually not detectable.
5. There is not necessarily a correlation between the amount of pollutants in the indicator media and the amount of pollutants in the target organs.

(Kommission Human Biomonitoring 1996)

Overall human biomonitoring is an important method for evaluating internal pollutant exposure in human organisms as well as its effects on human health. Many environmental pollutants can thus be detected.

The method is already used in several countries to gather information on the human pollution level on an individual and national level. Moreover it can be used as an instrument for the promotion of health and environmental protection and to verify the effectiveness of political strategies and interactions.

IV. RESEARCH INTEREST

Recent research identified the main target organs of mercury and already showed correlations between mercury levels of different body tissues like blood and brain and between the number of amalgams in teeth and the mercury levels in blood. (Björkman et al. 2007) In 1995, Cernichiari et al. already demonstrated a strong correlation between the mercury content of maternal scalp hair, at the time of childbirth, to the mercury content in the brain tissues of the newborn babies. (Cernichiari et al. 1995) Moreover, Zareba et al. stated in 2008:

> […] hair levels are more likely to reflect brain levels as the mechanisms of uptake by the hair follicle very likely involve the same transport mechanisms that operate across the blood-brain barrier.
>
> (Zareba et al. 2008)

These approaches were mainly the origins of the considerations for this thesis.
Since the mercury burden of different human body tissues in living organisms is – except for hair – still not measurable today, we thought of analyzing human body tissues, whose mercury contents are well-established by now.
Aim of this study was the analysis of a correlation of the Hg-content of the hair and the Hg-content of inner organs (brain, kidney) to improve the understanding of the consequences of chronic mercury exposure in the human body.
Therefore, the Hg-contents of different human body tissues were analysed and subsequently compared to one another in order to detect possibly existing correlations of the values and in order to screen the potential indicator function of the hair with regard to the Hg-values of the inner organs.
Primary hypothesis of the study is hence to infer that the mercury content of the hair gives evidence for the mercury contents of inner organs.

V. MATERIALS AND METHODS

1. Specimen

The samples used for the examination of mercury in different human body tissues were descended from ten corpses that were provided by the Clinical Institute of Pathology at the AKH/MUW Vienna. Further handling of the samples was carried out at a laboratory of the Institute for Environmental Health, Centre for Public Health of the Medical University of Vienna. The Hg-content of the samples was eventually determined at the Institute of Medical Genetics of the Medical University of Vienna by Atomic Fluorescence Spectroscopy (AFS) technique.

1.1. Sampling

Samples were taken from five female and five male corpses at the age of 22 to 70 at the time of death. Bodies that showed any trait of iatrogenic mercury contamination or mercury contamination caused by force (e.g. an accident) were excluded from further examination.

In order to detect a correlation of different tissues, two samples per body were taken out of the brain (hypothalamus, corpus callosum) and the kidney (renal lobe, renal capsule) and were then put in a jar made of polycarbonate (Nalgene Labware).

To compare the Hg-content of the inner organs with the content of hair, a strand of hair was removed from each corpse. Hair strands were cut off in the occipital region using ceramic scissors in order to avoid metallic contamination. If long enough, the final hair sample included a three centimeter segment of hair closest to the scalp. (Gundacker 2007)

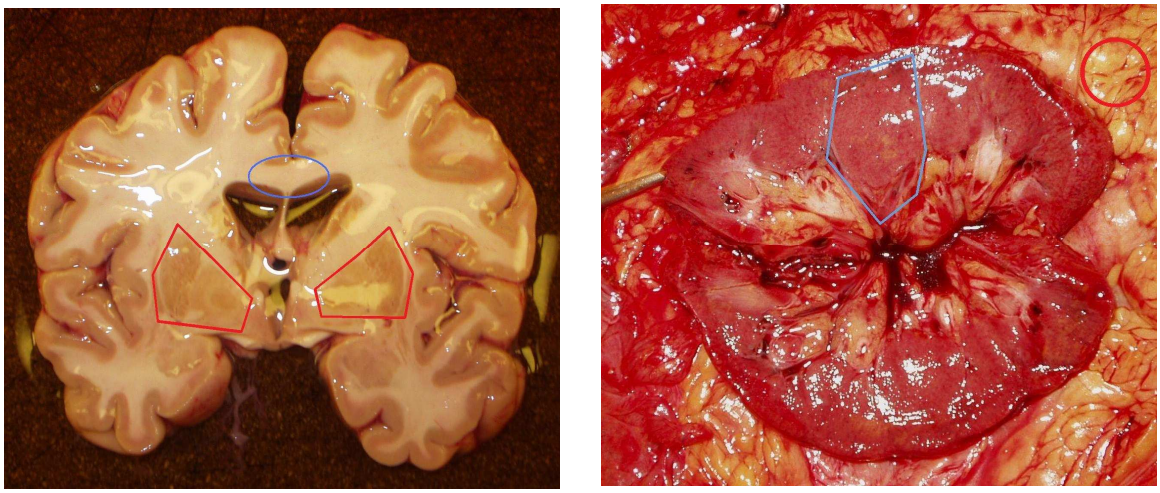
Tab. 5: Description of the specimen

Female	Proband Code	Year of Birth	Amalgam Fillings
F01	874	1956	7
F02	892	1958	---*
F03	968	1944	---*
F04	1040	1950	0
F05	1043	1988	---*

Male	Proband Code	Year of Birth	Amalgam Fillings
M01	920	1958	3
M02	1937	1953	---*
M03	941	1940	---*
M04	973	1947	---*
M05	1065	1952	---*

* amalgam fillings could not be noted due to rigor mortis

Fig. 3: Collected tissue samples of brain and kidney



Left: cross section of hypothalamus (red) and corpus callosum (blue)

Right: cross section of renal lobe (blue), renal capsule (red)

1.2. Sample Transport

Immediately after the sampling, the tissues were transported to a laboratory at the Institute of Medical Health, where they were put into a freezer at -21 °C. Hair samples were collected in regular envelopes, which were stored at room temperature.

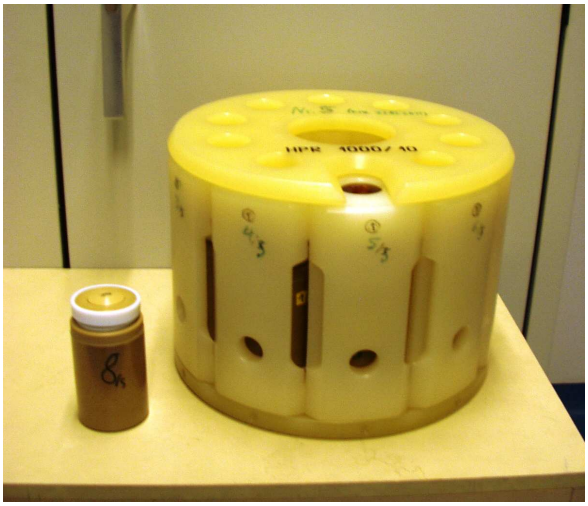
As the sample collection of all ten probands was completed, the frozen tissues were put into cooled bags and then were conveyed to the Institute of Medical Genetics for further handling.

1.3. Sample Preparation

In the beginning, the defrosted tissue samples were cut into tiny pieces with a ceramic knife while the hair samples were rinsed with deionized water. After that, precisely 0,5g of each tissue as well as the cleaned hair samples (3cm) were digested with a mixture of 2mL 65 vol.%HNO3 (Merck, Suprapur) and 0.75 mL 30% H2O2 (Merck, p.a.) in pressurized Teflon vessels in a microwave digestion unit for 40 minutes. After cooling down, the digested solutions were transferred to 20-mL volumetric flasks. The vessel was rinsed with 5 mL deionized water, which was then added to the solution. Finally the flask was filled up to 20 mL with deionized water. (Gundacker et al. 2007)

Fig. 4: Non-metallic lab tools

Fig. 5: Microwave digestion of the processed samples

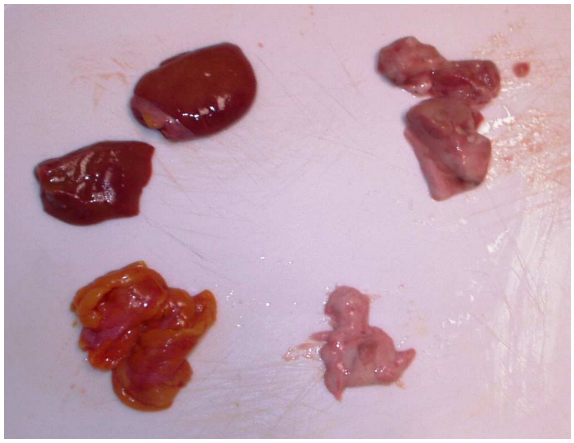


Carrier of the microwave vessels

Microwave MERCK mls 1200 mega

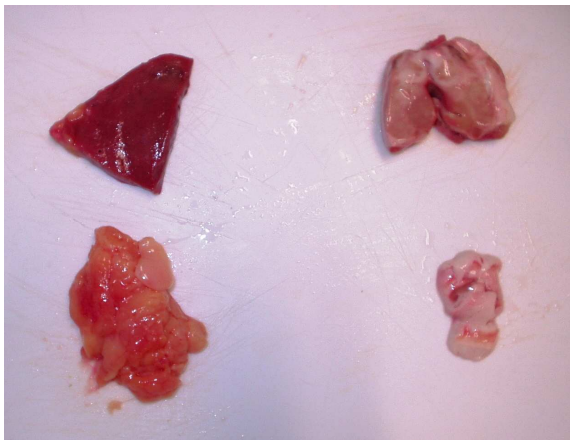
Fig. 6: Examples of tissue samples

left renal lobe
left: renal capsule
right: hypothalamus
right: corpus callosum



samples of proband F01

left renal lobe
left: renal capsule
right: hypothalamus
right: corpus callosum



samples of proband M04

2. Quantitative Determination of Mercury Levels in Brain, Kidney and Hair by Atomic Fluorescence Spectroscopy (AFS)

2.1. Principle of Mercury Determination with AFS

The process of the AFS technique can be divided into two main parts:

1. Atomization

A gas flow carries the vapor into heated regions where the sample molecules turn into ground state atoms. A hollow-cathode-lamp or a laser emits a beam of light into the atomic vapor, which excites the atoms.

2. Detection

The decay of the atoms of the excited electronic state results in the emission of photons and therefore in fluorescent light, whose single wavelengths can be selected by a monochromator. The detector then reads and amplifies the signal.

(Tissue B.M.1999)

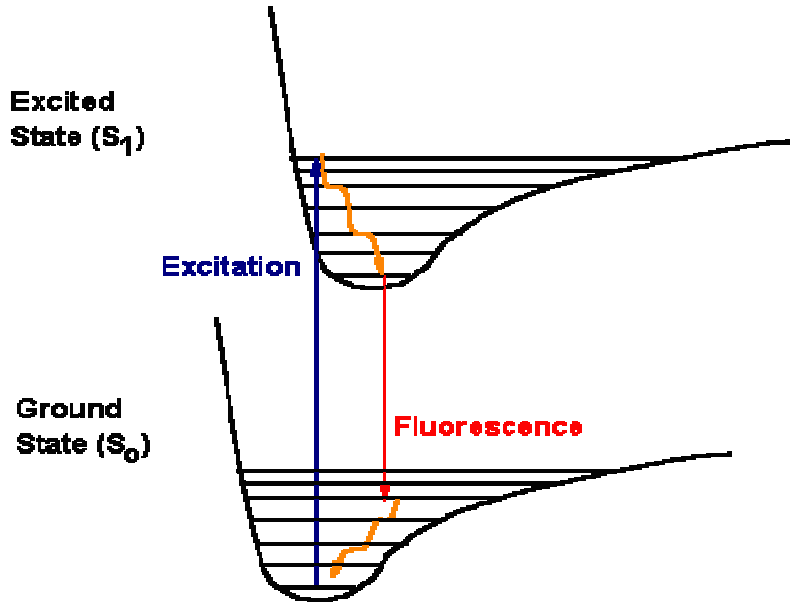
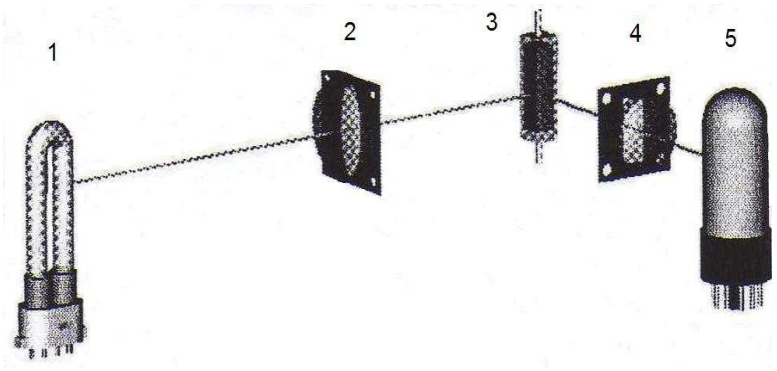


Fig. 7: Transitions between molecular electronic energy levels (The University of Adelaide 2011)

By adding a reducing agent to the sample solutions, the appearance of gases, which might have a quenching effect on mercury fluorescence, is minimized. The bottle with the reducing agent ($SnCl_2$) is connected to the apparatus before starting the analysis. Carrying gas for mercury in the AFS Mercur Plus is the inert gas Argon. (Analytik Jena AG 2007)

A low-pressure mercury lamp emits radiance at 253.7nm, which is exactly the range at which mercury atoms absorb light. Before the light strikes the sample solution in the cuvette, it has to pass a lens made of quartz crystal, which optimizes radiance efficiency. When coming to the cuvette, fluorescent radiation is deflected at a 90° angle and then again passes a biconvex quartz lens. The light is channelled and reproduced on a UV-sensitive photomultiplier. (Analytik Jena AG 2007)



1. low pressure mercury lamp
2. quartz lens
3. cuvette
4. quartz lens
5. receiver

Fig. 8: Optics scheme of AFS Mercur Plus (Analytik Jena AG 2007)

Main reason for mounting the source lamp for atomic fluorescence at an angle to the rest of the optical system is that the light detector only captures the fluorescence radiation this way and not the light from the lamp itself. (Analytik Jena AG 2007)

Because of the rather low background of the fluorescence signal, atomic fluorescence technique provides high measuring sensitivity. Besides, AFS allows an extremely precise analysis with a detection of values as low as ppm and even ppt.

2.2. Measuring Instruments and Settings

Mercury concentrations were determined by atomic fluorescence technique, using the AFS Mercur Plus (Analytik Jena AG) apparatus. Quality assurance was achieved by measuring blank test solutions and reference material (Seronorm Trace Elements Whole Blood, LOT 1003192). The reference material was prepared in the beginning and after each tenth sample preparation and was therefore all in all measured six times, in order to assure that no impreciseness occurred during the whole preparation process.

All metal contents were measured in triplicate by the working curve method. The limit of detection (LOD) was determined by the concentration equivalent to the threefold standard deviation of the signal of the blank solution (0.0024µg/kg). (Gundacker et al. 2007)

Fig. 9: AFS Mercur Plus

2.3. Experimental Procedure

After sample preparation, it was necessary to prepare a set of standard solutions, in order to get a straight line to which the sample results could be related.

Standard solutions emanated from a concentrated mercury stock solution (MERCK Certipur: $Hg(NO_3)_2$ in HNO_3, 2mol/L), which was used to make 100ml of a 100bbp solution. This Hg-solution was blended as follows:

Standard1: 2ml conc. HNO_3

Standard 2: 2ml conc. HNO_3 + 400µl Hg-solution

Standard 3: 2ml conc. HNO_3 + 800 µl Hg-solution

Standard 4: 2ml conc. HNO_3 + 1200 µl Hg-solution

Standard 5: 2ml conc. HNO_3 + 1600 µl Hg-solution

Standard 6: 2ml conc. HNO3 + 2000 µl Hg-solution

All standard solutions were filled up to 20ml with deionized water. Before starting the analysis, a bottle of HCl and a bottle filled with the reducing agent $SnCl_2$ were connected to the apparatus.

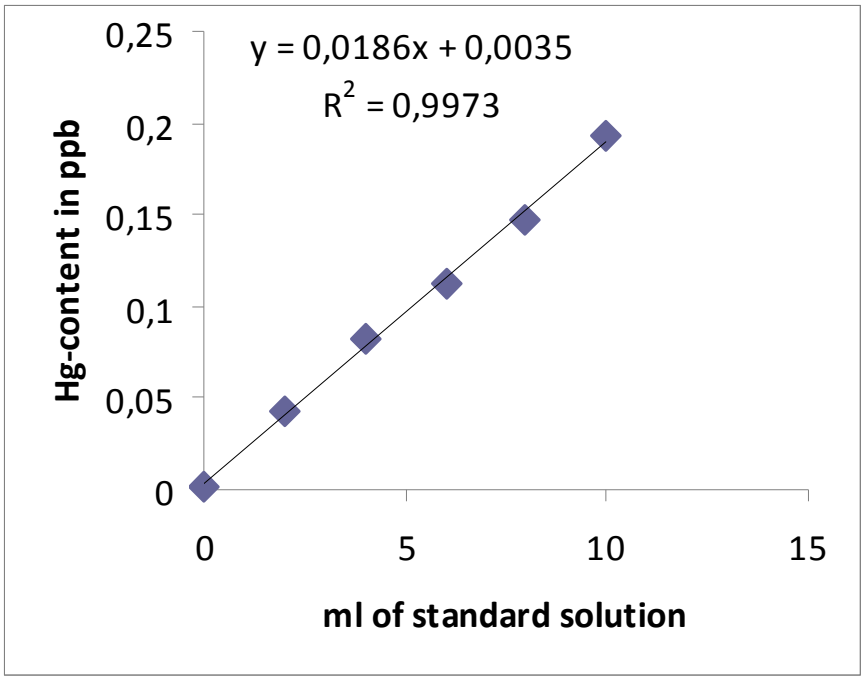
After that, all samples were filled into glass vessels, which belonged to the Mercur Plus and were then put into the measuring apparatus. Measuring was accomplished using a test record.

(see attachment: 2. Test Record)

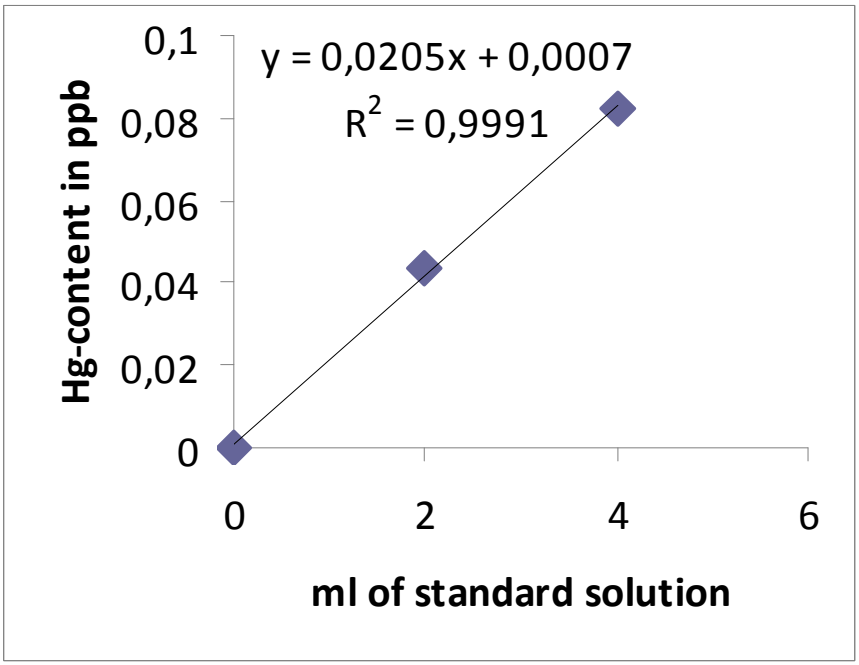
VI. DATA ANALYSIS

1. Description of the Obtained Material

The analysis of the different human body tissues showed following results:



Straight line for all six standard solutions



Straight line for standard solutions 0, 1 and 2 (more precise)

Tab. 6: Hg-content of the reference material in µg/kg (ppb)

1	2	3	4	5	6	Mean*
13.5	12.2	12.7	13.9	12.7	13.5	13.1

*Target range: 13.6-16.8, mean 15.2

Tab. 7: Hg-content of the female body tissues in µg/kg (ppb)

	F01	**F02**	**F03**	**F04**	**F05**
Hair	50.75	4.64	11.52	5.87	46.60
Corpus Callosum	0.07	0.07	0.62*	---*	0.46
Hypothalamus	4.88	---*	---*	0.09	2.33
Renal Lobe	97.94	1.28	0.10	5.81	9.26
Renal Capsule	---*	---*	---*	---*	---*

* test result below LOD

Tab. 8: Hg-content of the male body tissues in µg/kg (ppb)

	M01	**M02**	**M03**	**M04**	**M05**
Hair	7.34	17.48	7.31	46.26	108.11
Corpus Callosum	---*	0.17	---*	---*	---*
Hypothalamus	0.43	0.20	---*	0,40	1.44
Renal Lobe	1.03	---**	2.29	1.24	0.51
Renal Capsule	---*	---**	---*	---*	---*

* test result below LOD

** no kidney tissue available due to organ donation

Tab. 9: Mean Hg-content of the different tissues in µg/kg (ppb) (± sd)

	H	**CC**	**HT**	**RL**
Total	30.588 ± 33.03	0,077 ± 0.146	4.937 ± 11.78	11.946 ± 30.357
Women	23.876 ± 22.834	0.12 ± 0.193	1.46 ± 2.156	22.878 ± 42.12
Men	37.3 ± 42.673	0.034 ± 0.076	8.414 ± 17.666	1.014 ± 0.860

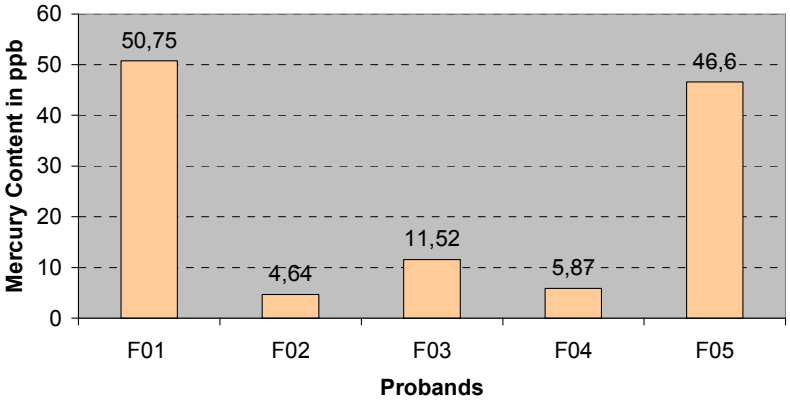
H = hair

CC = corpus callosum

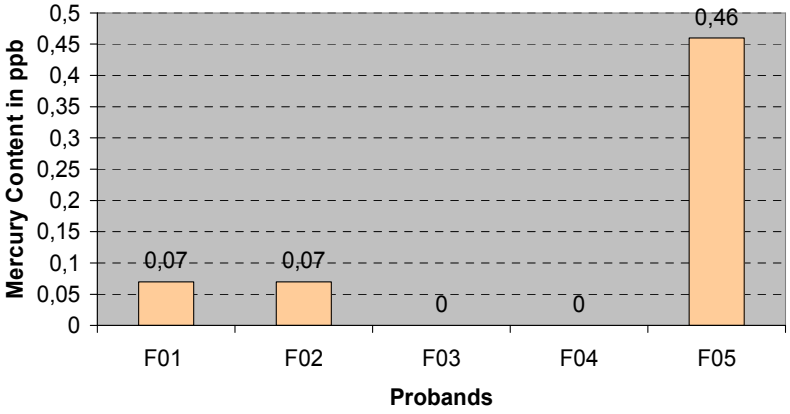
HT = hypothalamus

RL = Renal Lobe

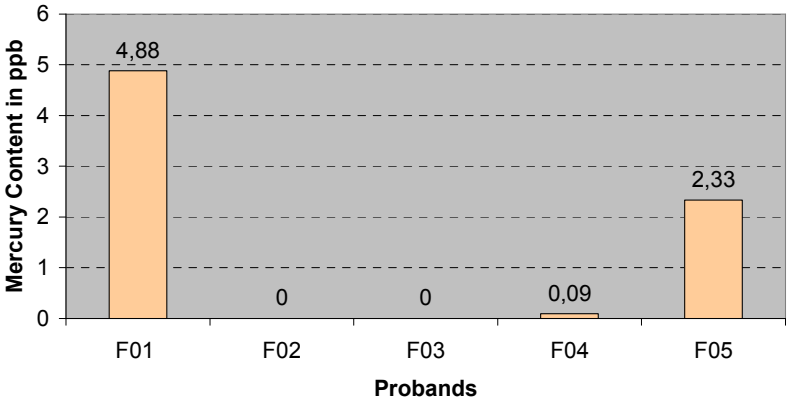
Mercury Content in Female Hair



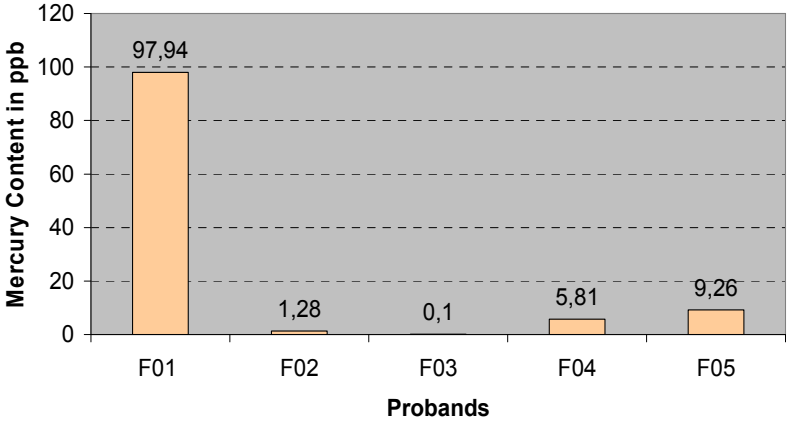
Mercury Content in Female C. Callosum



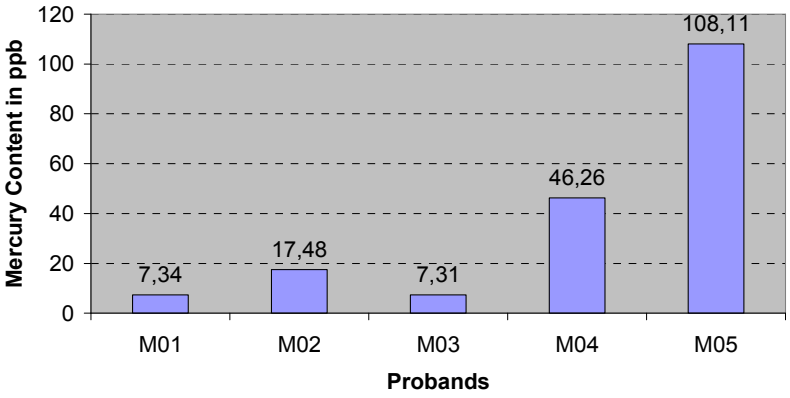
Mercury Content in Female Hypothalamus



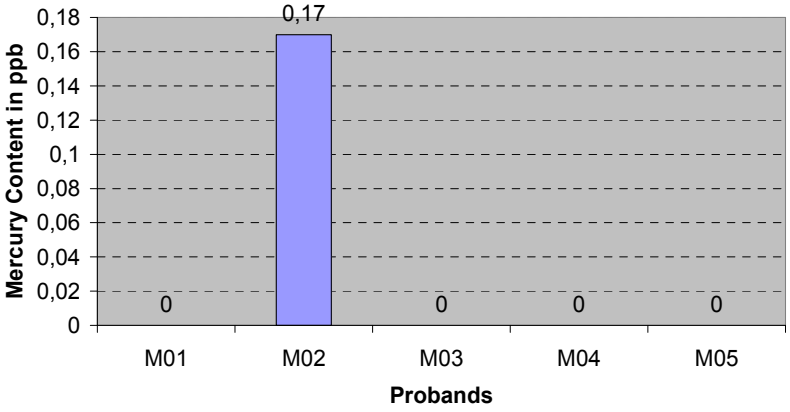
Mercury Content in Female Kidney



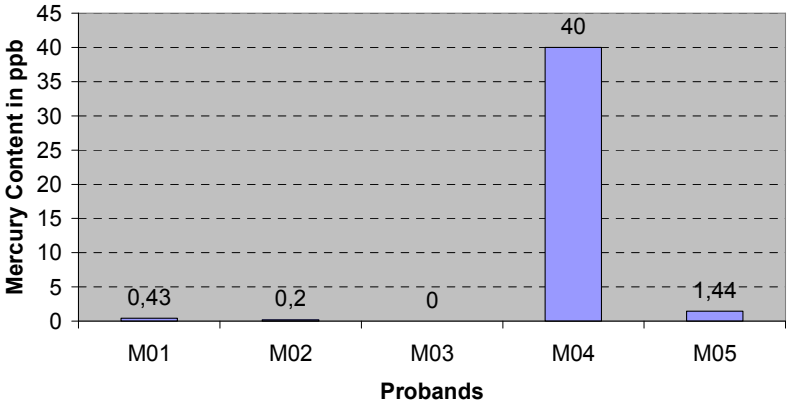
Mercury Content in Male Hair



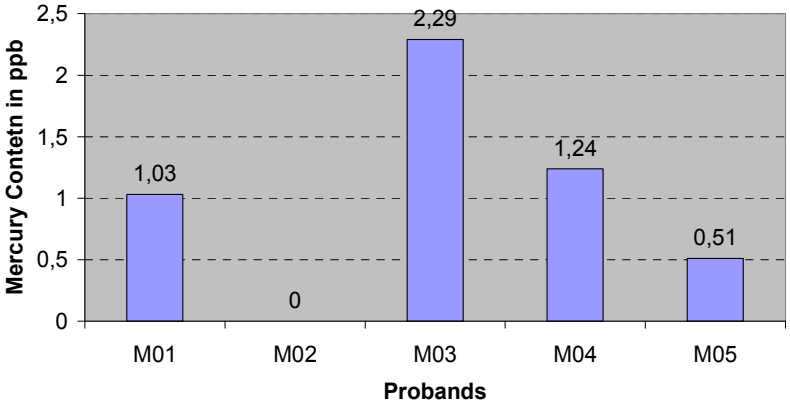
Mercury Content in Male C. Callosum

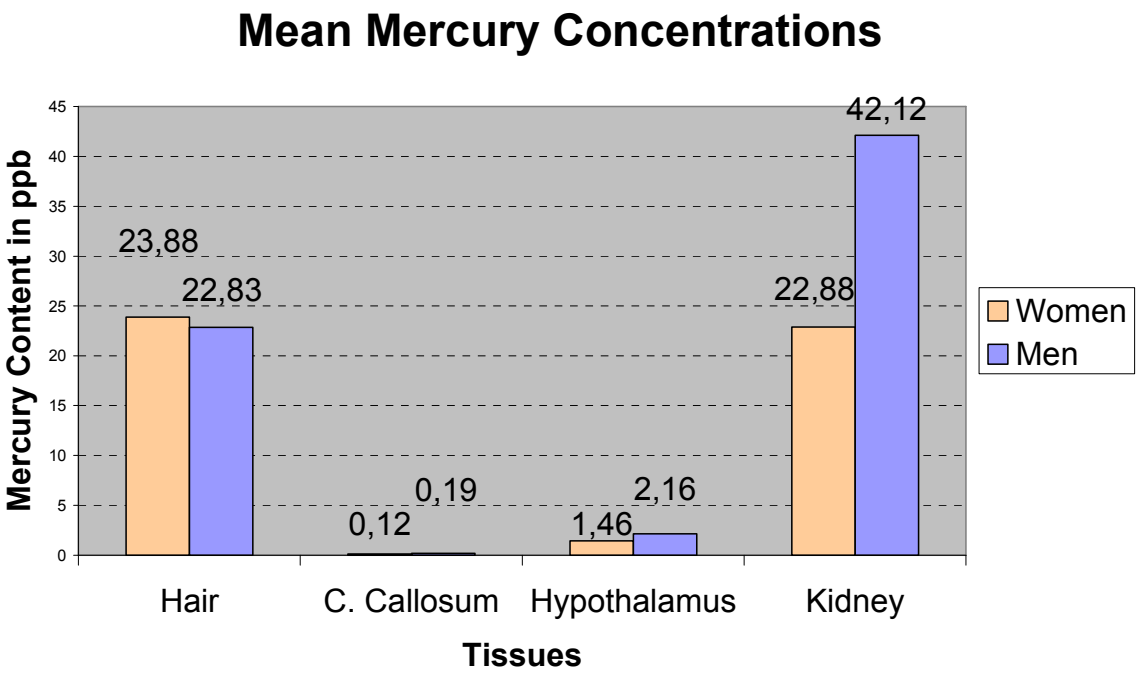


Mercury Content in Male Hypothalamus



Mercury Content in Male Kidney




Mean Mercury Concentrations
Mercury Content in ppb
45
40
35
30
25
20
15
10
5
0
23,88
22,83
0,12
0,19
1,46
2,16
22,88
42,12
Hair
C. Callosum
Hypothalamus
Kidney
Tissues
Women
Men

2. Statistical Analysis of the Mercury Content of Different Body Tissues

2.1. Kolmogorov-Smirnov Test

Kolmogorov-Smirnov-Test

		hair	cc	hypothalamus	kidney
N		10	10	10	9
Parameter of normality[a,b]	Mean	30.5880	.0770	4.9370	13.2733
	Standard Deviation	33.03228	.14568	12.41777	31.89017
Most extreme differences	Absolute	**.254**	**.319**	**.402**	**.439**
	Positive	.254	.319	.402	.439
	Negative	-.216	-.299	-.345	-.340
Kolmogorov-Smirnov-Z		.804	1.009	1.271	1.317
Asymptotic Significance (2-tailed)		.538	.260	.079	.062

a. normally distrubted.

b. calculated from the data.

Extreme differences that are bigger than 0.409 (n=10) or 0.43 (n=9) are not normally distributed (P = 0.05). In this case, kidney values are not normally distributed.

2.2. Spearman`s Rank Correlation Coefficient

Since kidney data are not normally distributed and since the sample size is rather low, correlation dimensions were calculated with the non-parametric Spearman`s rank correlation coefficient.

Formula for Spearman`s rank correlation coefficient

$$\rho = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)}.$$

Coefficient values are determined at a range between -1 for an absolute negative correlation and +1 for an absolute positive correlation. A Spearman`s correlation coefficient of 0 means, that there is no linear relationship between the two compared parameters.

Tab. 6: Classification of Spearman`s correlation coefficients

0	no correlation
0 – 0.2	very weak correlation
0.2-0.5	weak correlation
0.5 – 0.7	middle-sized correlation
0.7 – 0.9	strong correlation
> 0.9	very strong correlation

2.2.1 Correlation of the Hg-Levels of Hair and Brain

Correlations

			hair	hypothalamus
Spearman-Rho	hair	Correlation Coefficient	1.000	**.767****
		Sig. (2-tailed)	.	.010
		N	10	10
	hypothalamus	Correlation Coefficient	**.767****	1.000
		Sig. (2-tailed)	.010	.
		N	10	10

. Correlation is significant at **the 0.01 level (2-tailed).

Correlations

			hair	cc
Spearman-Rho	hair	Correlation Coefficient	1.000	**.206**
		Sig. (2-tailed)	.	.569
		N	10	10
	cc	Correlation Coefficient	**.206**	1.000
		Sig. (2-tailed)	.569	.
		N	10	10

Evaluation of the data resulted in a positive Spearman`s correlation coefficient of 0.767 for hypothalamus and hair and a coefficient of 0.206 for corpus callosum and hair.

This means a strong positive correlation between hypothalamus and hair, which was statistically significant (r_s = .767, p = 0.01).

Corpus callosum and hair, however, shows no measurable correlation.

2.2.2. Correlation of the Hg-Levels of Hair and Kidney

Correlations

			hair	kidney
Spearman-Rho	hair	Correlation Coefficient	1.000	**-.017**
		Sig. (2-tailed)	.	.966
		N	10	9
	kidney	Correlation Coeficient	**-.017**	1.000
		Sig. (2-tailed)	.966	.
		N	9	9

Spearman correlation coefficient for renal lobe and hair is -0.17 (p=0.966), marking a non-existing correlation of the two parameters.

2.2.3. Correlation of the Hg-Levels of Corpus Callosum and Hypothalamus

Correlations

			cc	hypothalamus
Spearman-Rho	cc	Correlation Coefficient	1.000	**.187**
		Sig. (2-tailed)	.	.604
		N	10	10
	hypothalamus	Correlation Coefficient	**.187**	1.000
		Sig. (2-tailed)	.604	.
		N	10	10

With a Spearman`s rank correlation coefficient of 0.187 and p=0.604, no correlation exists between corpus callosum and hypothalamus.

2.2.4. Correlation of the Hg-Levels of Corpus Callosum and Kidney

Correlations

			cc	kidney
Spearman-Rho	cc	Correlation Coefficient	1.000	**.647**
		Sig. (2-tailed)	.	.059
		N	10	9
	kidney	Correlation Coefficient	**.647**	1.000
		Sig. (2-tailed)	.059	.
		N	9	9

The p-value of the two parameters corpus callosum and kidney is bigger than 0.05, indicating a non-existing correlation.

2.2.5. Correlations of the Hg-Levels of Hypothalamus and Kidney

Correlations

			hypothalamus	kidney
Spearman-Rho	hypothalamus	Correlation Coefficient	1.000	**.305**
		Sig. (2-tailed)	.	.425
		N	10	9
	kidney	Correlation Coefficient	**.305**	1.000
		Sig. (2-tailed)	.425	.
		N	9	9

Correlation coefficient between hypothalamus and kidney is 0.305 (p=0.425), which also results in a non-correlation of the two parameters.

VII. DISCUSSION

Aim of this study was to analyse different human body tissues, in order to detect a correlation between the mercury concentrations of hair and those of the examined inner organs.

The evaluated Spearman`s correlation coefficients were strongly significant in only one of the six calculated cases. The correlation between hair and hypothalamus (r_s=0.767, P=0.01) showed a significant result.

As mentioned above, it has already been stated by Zareba et al., that hair mercury levels may reflect the mercury levels of the brain since transport mechanisms of mercury into the hair follicle resemble the transport mechanism of mercury, passing the blood-brain barrier. Therefore our findings would affirm the results of this study with regard to the correlation between hair and hypothalamus. (Zareba et al. 2008)

Furthermore, it would be advisable, to not only analyse the whole mercury concentrations, but to distinguish between anorganic mercury salts and organic methylmercury. This would increase the information value in terms of these different mercury forms and their behaviour in human body tissues, since this study only proves correlations between the whole Hg-contents of the human body but can not provide more specific information on correlations of methylmercury or anorganic mercury in different organs.

Such an analysis requests another specific analytical apparatus, the mass spectrometry. This analysis can be performed at The National Environmental Agency in Vienna. A continuative step after having finished this study will therefore probably be the more differentiated analysis of the different chemical mercury forms, in order to get more information on the different ways of mercury distribution in the human body.

All in all, it has to be said, that the sample size of this study was rather low, so that our findings may only indicate a tendency towards reality. Further research has to be done to clarify the remaining questions.

VIII. CONCLUSION

During the last sixty years it has clearly been proven that the exposure to mercury, regardless to its chemical form, affects the human body directly, which make it a serious threat for human health.

Even though it is positive to mention that most dentists abandoned mercury-containing amalgam fillings out of their surgeries and that even the application of thimerosal in vaccines is regarded critically by now, it is still not possible to avoid exposure completely, since there are various sources of the metal in the environment.

The mercury burden in the population is distinctively individual and therefore significantly varying. It is thus very unfortunate that there is still no possibility to diagnose the individual mercury burden. But it is also clear that no *in vivo*-analysis may be performed on the target organs of mercury - brain and kidney.

This is why mercury intoxication mostly remains undetected, especially low chronic intake with no clinically apparent symptoms, or, even worse, is misdiagnosed, for example as the symptoms of mental stress.

Aim of this project was to provide a new approach with regard to mercury distribution in human body compartments and with regard to possible existing correlations between the mercury content of the hair and the mercury content of the inner organs. Evaluation of the analyzed tissue samples, however, showed that such a correlation does not exist in most cases. Only the correlation between hair and hypothalamus was strong enough to be considered significant.

Aside, we confirmed that despite the lipophilic properties of methylmercury, no evidence of mercury accumulation was found in renal capsule tissue.

An individual rate of Hg-excretion or variable performances of the different chemical mercury forms in the body may be an explanation for the results of this study, but only a more differentiated analysis might ensure these assumptions.

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X. Attachment

1. Preparation Protocol

Aufschlussnummer: 1 -

Datum: 26.09.2011

Karussellnummer: ☐ 1 (+2) 5 4

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Autopsie ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	Verdünnung
T02 GB	1		2 ml 0,47 g	2 ml	0,75 ml	20,87 g
2GH	2		2 ml 0,57 g	2 ml	0,75 ml	20,82 g
2NL	3		2 ml 0,48 g	2 ml	0,75 ml	20,80 g
2NF	4		2 ml 0,51 g	2 ml	0,75 ml	20,83 g
Blind 1	5		2 ml /	2 ml	0,75 ml	20,88 g
Ref 1	6		2 ml 1ml	2 ml	0,75 ml	20,80 g
T01 GB	7		2 ml 0,52 g	2 ml	0,75 ml	20,82 g
1GH	8		2 ml 0,54 g	2 ml	0,75 ml	20,79 g
1NL	9		2 ml 0,51 g	2 ml	0,75 ml	20,80 g
1NF	10		2 ml 0,54 g	2 ml	0,75 ml	20,80 g

Anmerkungen:

Ref 1 (1ml)

Aufschlussnummer: 2 - Datum: 26.9.2011

Karussellnummer: 4 1 5 4

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Autopsie ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	
Blank	1		2 ml	2 ml	0,75 ml	20,82
Ref 2	2		2 ml 1mL	2 ml	0,75 ml	20,83
F06 GB	3	0,	2 ml 0,52g	2 ml	0,75 ml	20,80
F06 GH	4		2 ml 0,52g	2 ml	0,75 ml	20,80
F06 NL	5		2 ml 0,52g	2 ml	0,75 ml	20,81
F06 NF	6		2 ml 0,51g	2 ml	0,75 ml	20,89
F07 GB	7		2 ml 0,50g	2 ml	0,75 ml	20,87
F07 GH	8		2 ml 0,57g	2 ml	0,75 ml	20,85
F07 NL	9		2 ml 0,52g	2 ml	0,75 ml	20,84
F07 NF	10		2 ml 0,53g	2 ml	0,75 ml	20,81

Anmerkungen:

Aufschlussnummer: 3 - Datum: 26.9.2011

Karussellnummer: [ ] [1] [5] [4]

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Autopsie ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	
F08 GB	1		2 ml 0,51g	2 ml	0,75 ml	20,8g
GH	2		2 ml 0,50g	2 ml	0,75 ml	20,89
NL	3		2 ml 0,50g	2 ml	0,75 ml	20,89
NF	4		2 ml 0,53g	2 ml	0,75 ml	20,87
M01 GB	5		2 ml 0,53g	2 ml	0,75 ml	20,87
GH	6		2 ml 0,47g	2 ml	0,75 ml	20,84
NL	7		2 ml 0,55g	2 ml	0,75 ml	20,86
NF	8		2 ml 0,48g	2 ml	0,75 ml	20,82
Leer	9		2 ml	2 ml	0,75 ml	20,87
	10		2 ml	2 ml	0,75 ml	

Anmerkungen:

Aufschlussnummer: 4 - Datum: 27.9.11

Karussellnummer: [ ] [1] 2 [5] [4]

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Autopsie ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	
M03 GB*	1		2 ml 20,83	2 ml	0,75 ml	0,48
GH	2		2 ml 20,8	2 ml	0,75 ml	0,5
M04 GB	3		2 ml 20,8	2 ml	0,75 ml	0,53
~~NH~~	4		2 ml 20,8[79]	2 ml	0,75 ml	0,55
NF	5		2 ml 20,8	2 ml	0,75 ml	0,54
M06 GB	6		2 ml 29,8	2 ml	0,75 ml	0,52
GH	7		2 ml 20,83	2 ml	0,75 ml	0,51
NL	8		2 ml 20,79	2 ml	0,75 ml	0,534
NF	9		2 ml 20,83	2 ml	0,75 ml	0,53
M04 GH	10		2 ml 20,8	2 ml	0,75 ml	0,54

Anmerkungen:

Aufschlussnummer: 5 - Datum: 27.9.11

Karussellnummer: ☐ 1 (5) 4

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Autopsie ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	
M07 GB	1		2 ml 20,81	2 ml	0,75 ml	0,6
GH	2		2 ml 20,83	2 ml	0,75 ml	0,56
NL	3		2 ml 20,82	2 ml	0,75 ml	0,50
NF	4		2 ml 20,81	2 ml	0,75 ml	0,5
M08 GB	5		2 ml 20,84	2 ml	0,75 ml	0,65 ~~0,49~~
GH	6		2 ml 20,83	2 ml	0,75 ml	~~0,66~~ 0,51
NL	7		2 ml 20,80	2 ml	0,75 ml	0,53
Leer4 ~~Ref~~	8		2 ml 20,82	2 ml	0,75 ml	
Leer 5	9		2 ml 20,81	2 ml	0,75 ml	
Ref 5	10		2 ml 20,84	2 ml	0,75 ml	1mL

10,8*

Anmerkungen:

Aufschlussnummer: 6 - Datum: 27.9.11

Karussellnummer: [ ] [1] [5] [4]

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Haare ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	
F01	1		2 ml 0,53	2 ml	0,75 ml	20,82
F07	2		2 ml 0,61	2 ml	0,75 ml	20,88
F08	3		2 ml 0,53	2 ml	0,75 ml	20,81
M01	4		2 ml 0,37	2 ml	0,75 ml	10,84
M03	5		2 ml 0,51	2 ml	0,75 ml	10,83
M04	6	Rückstand	2 ml 0,79	2 ml	0,75 ml	10,8
M07	7		2 ml 0,20	2 ml	0,75 ml	10,81
M08	8		2 ml 0,58	2 ml	0,75 ml	10,85
Leer 6	9		2 ml	2 ml	0,75 ml	10,80
	10		2 ml	2 ml	0,75 ml	

10,8

Anmerkungen:

Aufschlussnummer: 7 - Datum: 28.9.2011

Karussellnummer: ☐ 1 (5) 4

Aufschluss – Protokoll für Flüssigkeiten

Probenmaterial: **Urin** ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Autopsie ☒

Probe – Nr.	Vessel	Anmerkung	Einwaage	HNO_3	H_2O_2	
M08 NF	1		2 ml 0,53	2 ml	0,75 ml	2981
Haar F06	2		2 ml 0,55	2 ml	0,75 ml	2983
Haar F07	3		2 ml 0,52	2 ml	0,75 ml	20,82
Leer 7	4		2 ml /	2 ml	0,75 ml	20,83
	5		2 ml	2 ml	0,75 ml	
	6		2 ml	2 ml	0,75 ml	
	7		2 ml	2 ml	0,75 ml	
	8		2 ml	2 ml	0,75 ml	
	9		2 ml	2 ml	0,75 ml	
	10		2 ml	2 ml	0,75 ml	

Anmerkungen:

2. Test Record

Datum: 28 . 9 . 2011 ANALYSENNAME: Gewebe – Autopsie

Messprotokoll

Element: [As] [Ca] [Cd] [Cr] [Cu] [Fe] [Hg] [Ni] [Pb] [Se] [Zn] [.....]

Methode: [FAAS] [GFAAS] [CVAAS] [CV Amalgam] [...AFS.....]

Methode (AAS Nr.): 1

Bearbeiter: Lukas Damjanovic __ Matthias Scheinast _____

Küvette getauscht am: _____ Firing times der Küvette: _____ h

Betriebsstunden der Küvette: ~ _____ h

Glühwendel getauscht am: _____ Betriebsstunden der Glühwendel: ~ _____ h

HKL getauscht am: _____ Betriebsstunden der HKL: _____ h

Matrix:

H_2O	:	6,25 ml
HNO_3	:	2 ml
H_2O_2	:	0,75 ml
.....	:	ml
.....	:	ml

Probenmaterial: **Haare** ☒ **Erde** ☐ **Fisch** ☐

Urin ☐ **Blut** ☐ **Medium** ☐ **Zellen** ☐

Sonstiges: Organgewebe ☒

Anmerkungen:

Referenz Level 1 Blood 2/10:	Hg: 0,4	Pb: 3,0
Referenz Level 2 Blood 1/15:	Hg: 1,0	Pb: 22,4
Referenz Urin 2/10:	Hg: 8,1	Pb: 8,1

Referenz Level 2 Blood 1/20 Hg : 0,765

Cup	Standard Nr.:	Konzentration:	RSD		Cup	Nummer	Probennummer	RSD
	Std 1	0 ppb				Sample 25	M03 GB	
	Std 2	2 ppb				Sample 26	M03 GH	
	Std 3	4 ppb				Sample 27	F07 GH	
	Std 4	6 ppb				Sample 28	F07 Haar	
	Std 5	8 ppb				Sample 29	F08 Haar	
	Std 6	10 ppb				Sample 30	M01 GB	
	Std 7					Sample 31	M01 GH	
	Std 8					Sample 32	M01 NL	
	Std 9					Sample 33	M01 NF	
	Std 10					Sample 34	M04 GB	
						Sample 35	Blind 2	
	Nummer	Probennummer				Sample 36	Ref. 2	
	Sample 1	F02 GB				Sample 37	Standard 1	
	Sample 2	F02 GH				Sample 38	Standard 2	
	Sample 3	F02 NL				Sample 39	Standard 3	
	Sample 4	F02 NF				Sample 40	M07 NF	
	Sample 5	F01 GB				Sample 41	M08 GB	
	Sample 6	F01 GH				Sample 42	M08 GH	
	Sample 7	F01 NL				Sample 43	M08 NL	
	Sample 8	F01 NF				Sample 44	M08 NF	
	Sample 9	F06 GH				Sample 45	F02 Haar	
	Sample 10	F06 GB				Sample 46	F06 Haar	
	Sample 11	Blind 1				Sample 47	Blind 1	
	Sample 12	Ref. 1				Sample 48	Ref. 1	
	Sample 13	F06 NL				Sample 49	M04 GH	
	Sample 14	F06 NF				Sample 50	M04 NL	
	Sample 15	F07 GB				Sample 51	M04 NF	
	Sample 16	F01 Haar				Sample 52	M06 GB	
	Sample 17	F07 NL				Sample 53	M06 GH	
	Sample 18	F07 NF				Sample 54	M06 NL	
	Sample 19	F08 GB				Sample 55	M06 NF	
	Sample 20	F08 GH				Sample 56	M07 GB	
	Sample 21	F08 NL				Sample 57	M07 GH	
	Sample 22	F08 NF				Sample 58	M07 NL	
	Sample 23	Blind 2				Sample 59	Blind 4	
	Sample 24	Ref. 2				Sample 60	Ref. 5	

%

Sample

61	Ref-Th (NAB) 06	1:15
62	M01 Haar	1:10
63	M03 Haar	
64	M04 Haar	
65	M07 Haar	
66	M08 Haar	
67	Blind 4	
68	Ref. 5	
69	Std 1 0ppb	
70	Std 2 2ppb	
71	Std 3 4ppb	

Abstract

Studien der letzten sechzig Jahre haben eindeutig erwiesen, dass eine Quecksilberexposition, unabhängig von der chemischen Form des Schwermetalls, negative Auswirkungen auf die menschliche Gesundheit hat.

Obwohl die großflächige Anwendung quecksilberhaltiger Zahnfüllungen reduziert wurde und auch Thimerosal in Impfungen bereits als kritisch betrachtet wird, ist es nahezu unmöglich, eine Aufnahme von Quecksilber zu vermeiden. Abhängig von der individuellen Ernährungs- und Lebensweise, ist jeder Mensch verschiedenen Quecksilberquellen unterschiedlich häufig und intensiv ausgesetzt. Die daraus resultierende individuelle Quecksilberbelastung kann bis heute nicht durch diagnostische Verfahren nachgewiesen werden. Eine *in-vivo*-Analyse der Zielorgane Gehirn und Niere ist jedenfalls nicht möglich. Eine Vergiftung durch Quecksilber bleibt daher meist unentdeckt, vor allem wenn es sich dabei um eine geringe chronische Aufnahme ohne klinisch manifeste Symptomatik handelt. Häufig kommt es auch zu Fehldiagnosen, wie zum Beispiel Belastungsstress.

Ziel dieser Biomonitoring-Untersuchung war der Nachweis einer Korrelation des Hg-Gehaltes von Haaren mit den Hg-Gehalten von Hirn- und Nierengewebe zur Verbesserung des Verständnisses von Nachweisen einer chronischen Hg-Belastung im menschlichen Körper. Die Auswertung der Ergebnisse zeigte allerdings, dass eine derartige Korrelation nur in einem der untersuchten Fälle stark genug war, um als signifikant angesehen zu werden. Eine Erklärung dafür kann die individuelle Ausscheidungsrate von Quecksilber im menschlichen Organismus oder aber die unterschiedlichen Verhaltensweisen der verschiedenen chemischen Formen von Quecksilber im Körper sein. Eine Überprüfung dieser Vermutungen kann jedoch nur durch weitere Untersuchungen erfolgen.

Abstract

During the last sixty years it has clearly been proven that the exposure to mercury, regardless to its chemical form, affects the human body directly, which make it a serious threat for human health.

Even though it is positive to mention that most dentists abandoned mercury-containing amalgam fillings out of their surgeries and that even the application of thimerosal in vaccines is regarded critically by now, it is still not possible to avoid exposure completely, since there are various sources of the metal in the environment.

The mercury burden in the population is distinctively individual and therefore significantly varying. It is thus very unfortunate that there is still no possibility to diagnose the individual mercury burden. But it is also clear that no *in vivo*-analysis may be performed on the target organs of mercury - brain and kidney.

This is why mercury intoxication mostly remains undetected, especially low chronic intake with no clinically apparent symptoms, or, even worse, is misdiagnosed, for example as the symptoms of mental stress.

Aim of this project was to provide a new approach with regard to mercury distribution in human body compartments and with regard to possible existing correlations between the mercury content of the hair and the mercury content of inner organs. Evaluation of the analyzed tissue samples, however, showed that such a correlation does not exist in most cases. Only the correlation between hair and hypothalamus was strong enough to be considered significant.

An individual rate of Hg-excretion or variable performances of the different chemical mercury forms in the body may be an explanation for the results of this study, but only a more differentiated analysis might ensure these assumptions.

Curriculum Vitae – Simone Spangler

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Personal Data

Date of Birth	5th of March 1985
Place of Birth	Regensburg
Nationality	German

Education and Training

Since Oct 2005	Studies of the Master`s Degree Programme *Science of Nutrition* at the University of Vienna, Austria
1995 – 2004	Secondary School Parsberg, Germany
Graduation	University-Entrance Diploma

Internships

08th Feb – 05th Mar 10	University Hospital of Pediatrics Vienna, Austria Head Laboratory and Laboratory for Metabolic Diseases Administration: Univ.-Prof. Dr. Kurt Herkner
15th Jul – 15nd Oct 09	Institute of Medical Microbiology and Hygiene University Hospital Regensburg, Germany Project: Cytomegaly-Virus, Project-Leader: Dr. Michael Nevels

07th Feb – 14th Mar 07	Seidl Confiserie GmbH Laaber, Germany Domain: Quality Assurance, Packaging, Fabrication

Further Occupations

since 15th Oct. 2010	Tutor at the Institute for Hygiene und Applied Immunology, Vienna Administration: Ao.Univ.-Prof. Dipl.-Ing. Dr. Mohammad Manafi
15th Jul 2010 – 15th Oct 2010	Student research assistant at the Institute of Medical Microbiology and Hygiene, University Hospital Regensburg, Germany; Project: Cytomegaly-Virus Project-Leader: Dr. Michael Nevels
01st Sept 2008 – 28th Sept 2008	Seidl Confiserie GmbH Laaber, Germany Domain: Fabrication

Additional Skills

Language skills	German	–	First language
	English	–	TOEFL IBT 108 credits
	French	–	DELF A2
	Swedish	–	Basic knowledge

Workshop	Basics about Communication and Coaching
Honorary post	Assistant at the *Friede Institute* Vienna Center for Dialogue and Integration