

**FINAL REPORT**

**TO: INTERNATIONAL ACADEMY OF ORAL  
MEDICINE AND TOXICOLOGY**

**ON BEHALF OF FUNDERS, INCLUDING THE PARKER  
HANNIFIN FOUNDATION**

**MERCURY EXPOSURE AND RISKS FROM DENTAL  
AMALGAM, PART 2: CUMULATIVE RISK  
ASSESSMENT AND JOINT TOXICITY: MERCURY  
VAPOUR, METHYL MERCURY AND LEAD**

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## **EXECUTIVE SUMMARY**

Regulatory guidance for evaluating potential cumulative risks from concomitant exposure to chemical mixtures was reviewed. Following this guidance, the toxicological and related data were evaluated for mercury vapour (Hg<sup>0</sup>), methyl Hg and lead (Pb). That evaluation focused on reported similarities in individual and combined target organ, mechanism of action, toxicokinetics and toxicodynamics for these substances. This information and the potential for combined exposures to these substances were considered in order to determine if risks associated with the concurrent exposures to these metals should be evaluated jointly, rather than independently as is currently practiced.

Concurrent exposure to Pb, MeHg and Hg<sup>0</sup> does occur in the US population. A large proportion - 1/3<sup>rd</sup> - of the US population is concurrently exposed to Hg<sup>0</sup>, methyl Hg and Pb on a daily basis. All of these substances share the following characteristics:

- All are absorbed in large proportion into systemic circulation;
- All cross the blood-brain and placental barriers;
- All cause neurological toxicity;
- Exposure to all of these substances currently exceed individual reference exposure levels for many in the US population.

85.7 million Americans currently exceed the California EPA's reference blood Pb level of 1 ug Pb per deciliter of blood (1 ug/dL or 10 ug/L). For Hg<sup>0</sup>, 101.5 million Americans currently exceed the urine Hg concentration associated with the reference exposure level of 0.06 ug/m<sup>3</sup> recently developed in Canada. For methyl Hg, 1.8 million Americans exceed the reference blood level of 8 ug/L established in Canada for protection of neurological outcomes, particularly relating to neurodevelopmental effects in the fetus.

The weight of available evidence suggests that risks posed by concurrent exposure to combinations of these 3 substances should be assessed as additive. In keeping with ATSDR guidance, the calculation of a Hazard Index (sum of Hazard Quotients) is recommended where individual HQ values  $\geq 0.1$  are determined for Pb, MeHg and/or Hg<sup>0</sup>. The Hazard Index should not exceed a value of 1.0 to be reasonably assured of public health. From the 2003-04 NHANES data, the number of Americans in whom exposure exceeds 1/10<sup>th</sup> of the appropriate reference exposure level (i.e., where HQ > 0.1) are as follows:

- Pb (reference level = 10 ug Pb/L blood; CalOEHHA, 2009): 121,744,106
- MeHg (reference level = 8 ug/L blood; Legrand et al. 2010): 62,150,604
- Hg<sup>0</sup> (reference level = 0.3 ug/L urine; derived from Richardson et al. 2009): 121,677,708

Following USATSDR guidance, the Hazard Index, that combines (sums) the Hazard Quotients of the 3 substances, was determined for each of the NHANES participants having concurrent levels of these substances in blood or urine above analytical detection limits. The Hazard Index should not exceed a value of 1.0 to be reasonably guaranteed of public health protection. The number of Americans with a Hazard Index > 1 for Pb, MeHg and Hg<sup>0</sup> combined, was 121,677,708, or 1/3<sup>rd</sup> of the US population.

Hg<sup>0</sup>, methyl Hg and Pb should never be assessed for their population health risks on a chemical-by-chemical (independent) basis as is now the routine practice. Efforts should be made to establish toxicity equivalency factors for these 3 substances to support the assessment of concurrent exposures and joint toxicity, similar to toxic equivalency schemes developed to assess risks from other groups of chemicals with similar mechanisms of action, such as polycyclic aromatic hydrocarbons and polychlorinated dibenzo-p-dioxins and furans.

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## **1. INTRODUCTION**

Risks posed by concurrent exposure to multiple chemicals (mixtures) has long been a concern to the regulatory and risk assessment communities. However, little practical progress has been made, with the exception of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-p-dioxins and furans. For these substances, toxic potency equivalence factors have been devised (USEPA, 1989; USEPA, 2009), to permit the amalgamation of the toxicities of several related chemicals into a single toxicity metric. However, for all other chemicals, risk assessments are conducted on a chemical-by-chemical basis unless it can be demonstrated that the target organ and mechanism of action of 2 or more chemicals is the same (Health Canada 2010). This determination is seldom if ever undertaken.

Lead, Hg and methyl Hg present a unique situation in that these 3 substances are all known to cross the blood-brain barrier and to cause neurological toxicity. For all 3, that toxicity has been observed in humans, not just in animal studies. Given that they affect the same target organ, it seemed appropriate to apply current regulatory guidance to evaluate their joint toxicities, towards determining if these substances should continue to be independently assessed for health risks, or if a new combined approach should be considered.

### **1.1 Simultaneous Exposure To Hg<sup>0</sup>, Methyl Hg And Pb**

There is indirect evidence that the US population is concurrently exposed to various forms of Hg and Pb. Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the USATSDR and USEPA are required to prepare a list of those substances commonly occurring at facilities on the National Priorities List (NPL; also known as Superfund Sites) and to prioritize these substances according to potential threat to human health. Pb and Hg were ranked #2 and #3, respectively, on the latest (2007) CERCLA Priority List of Hazardous Substances (ASTDR 2010a) (see <http://www.atsdr.cdc.gov/cercla/>).

In a separate ranking process, the USATSDR publishes a Completed Exposure Pathway (CEP) list, which links sources of contamination to receptor populations and presents information on the number of hazardous waste sites for which a CEP has been determined for a substance. In their 2007 CEP list, the USATSDR ranked lead at #1 and mercury at #8 (see <http://www.atsdr.cdc.gov/cep/07cep.html>).

Indirect evidence aside, there is direct quantification of this simultaneous co-exposure to Hg<sup>0</sup> (in amalgam fillings), to methyl Hg (in fish) and to Pb (in air, food, and consumer products) occurring in the US population. This concurrent exposure was evident in the New England Children's Amalgam Trial during which co-exposure to Hg<sup>0</sup> (Maserejian et al. 2008; determined by urine analysis) and Pb (Surkan et al. 2007; determined by blood analysis) was recorded. Further evidence of this concurrent exposure is provided by the National Centre for Health Statistics (NCHS), through that agency's National Health and Nutrition Examination Surveys (NHANES). Blood and urine chemistry data collected as part of the 2003-04 NHANES survey are summarized in Table 1. Table 1 presents data on survey participants who had at least one filled tooth surface and also had:

- concentrations of Hg in urine > detection limit (DL); **and**
- concentrations of inorganic Hg in blood > DL; **and**
- concentrations of organic (methyl) Hg in blood > DL; **and**
- concentrations of Pb in blood > DL.

A total of 1008 NHANES participants satisfied these criteria. Based on the statistical population weighting factors provided by the NCHS in association with the analysis of Hg in urine, these 1008 NHANES participants represent a total of 121,744,105 members of the US general population who have these concurrent exposures.

Summarized immediately below is some general information about Pb, to provide background and context. This is followed by similar information for methyl Hg. Similar information on Hg<sup>0</sup> is presented in Part 1 of this report.

**Table 1. Summary of data from NHANES (2003-04) on simultaneous presence of Hg in urine, inorganic and methyl Hg in blood, and Pb in blood.**

Age Group	NHANES Participants <sup>1</sup>	Statistic	Filled tooth surfaces	Urine Hg (µg/L)	Blood Pb (µg/dL)	Blood Inorganic Hg (µg/L)	Blood Methyl Hg <sup>1</sup> (µg/L)
	(N)		(N)				
Children (72-155 months)	75	mean	8.2	1.44	1.52	0.43	0.63
		st. dev.	8.0	1.67	0.96	0.30	0.67
		minimum	1	0.15	0.5	0.30	0.1
		maximum	49	9.51	6.1	2.0	3.9
Adolescents (156-251 months)	188	mean	8.2	1.42	1.13	0.37	0.71
		st. dev.	8.8	1.64	0.65	0.17	0.78
		minimum	1	0.16	0.4	0.3	0.1
		maximum	86	9.86	3.8	1.5	5.3
Adults (252-719 months)	470	mean	19.3	1.58	1.73	0.40	1.53
		st. dev.	15.6	3.31	1.33	0.20	1.89
		minimum	1	0.14	0.3	0.3	0.1
		maximum	117	50.2	16.1	1.9	15.3
Seniors (≥ 720 months)	275	mean	28.1	0.80	2.43	0.37	1.53
		st. dev.	21.3	0.75	1.51	0.14	1.58
		minimum	1	0.15	0.3	0.3	0.1
		maximum	107	4.42	11.3	1.2	10.5

1. No adjustment for population weighting.
2. Derived as (total Hg in blood) – (inorganic Hg in blood); due to different analytical methods for total versus inorganic Hg, some values of methyl Hg in blood ≤ 0; all NHANES participants with calculated blood methyl Hg values ≤ 0 omitted.

## **1.2 Lead (Pb)**

It is estimated that health effects from environmental lead exposure account for 1% of the global burden of disease, placing it in 16<sup>th</sup> position in terms of leading global health risk factors (Fewtrell *et al.*, 2004). Environmental lead exposures are thought to account for about 13% of all cases of Mild Mental Retardation (MMR) in children; MMR has an incidence rate in North America of about 1 in 1,000 (Fewtrell *et al.*, 2004).



At the present time, the production of batteries, used predominantly in the automotive industry, comprises the single largest global market for refined lead following the virtual elimination of lead in household paints, gasoline additives, and solder in food cans (OECD 1993; Keating and Wright 1994; Keating 1995).

### 1.2.1 Pb in the environment

Of the heavy metals with atomic number > 60, lead is the most abundant in the earth's crust (Adriano, 2001). Lead occurs naturally in bedrock, soils, tills, sediments, surface waters, ground waters, and sea water (Reimann and de Caritat, 1998). As a result of its natural presence in the environment, lead also occurs naturally at low levels in foods, due to its uptake from soil into plants, its uptake from water and sediments into fish, and its uptake into animals that consume plants and other animals (Adriano, 2001). Due to lead's limited leaching potential, soils and sediments are considered to be important sinks for lead compounds and contamination is generally regarded as irreversible and permanent unless removed through remediation (US ATSDR, 2007b).

Due primarily to industrial development and human activities, Pb is a common and ubiquitous contaminant, particularly of the urban environment. In Canada, ambient air levels of lead have declined by 97% between 1970 and 2008 (Environment Canada, 2010). Trends in the US are expected to be the same.

Pb levels in indoor air are greater than those observed out of doors. Indoor levels generally range from about 0.1 to 75 µg/m<sup>3</sup> (US EPA, 2010b). Lead-based paint is currently the most significant source of lead in indoor air in North America (US EPA, 2010c). Lead particles from this source are dispersed when the paint degrades and contaminates dust that is re-suspended into the indoor air.

Related to indoor air, studies of the indoor environment have consistently implicated household dust as a major source of lead exposure, particularly for children (Lanphear *et al.*, 1996; Lanphear *et al.*, 1998; Rabinowitz *et al.*, 1985a; Roy *et al.*, 2003; Rhoads *et al.*, 1999). Levels of lead in indoor dust, particularly in older houses, are strongly influenced by the presence of lead-containing paints (Jacobs *et al.*, 2002; US HUD, 2001; Farfel and Chisolm, 1990; US CDC, 2009; Jacobs *et al.*, 2000). However, high

concentrations of lead in house dust have also been reported near point sources such as smelters ( Meyer *et al.* 1999a,b; Roy *et al.*, 1993; Hertzman *et al.*, 1990).

The young are particularly at risk of elevated exposure to lead in settled dust due to behavioral patterns. Toddlers and children play and crawl on the ground and floor, engage in hand-to-mouth activity, mouthing of objects and indiscriminate eating of food items dropped or found on the ground or floor (US EPA, 2008).

The concentration of lead in surface waters and groundwater unaffected by pollution sources is generally low. More than 99% of all publicly supplied drinking water in the US contains less than 0.005 ppm of Pb (US ATSDR, 2007a). However, drinking water can become contaminated as it moves through municipal water distribution systems and residential or other plumbing systems, where lead is introduced as a result of dissolution from lead pipes and lead service connections, lead-based solders used to join copper pipes within homes and buildings, and from plumbing fixtures made of lead-containing brass components (Health Canada, 1992). These issues are most prevalent in older residential areas built prior to the introduction of regulations limiting and/or banning the use of lead in pipes, solders and fixtures.

In the U.S., the FDA reports on the Pb content of the US commercial food supply via regular total diet surveys (US FDA, 2010). Pb also occurs in breast milk (Almeida *et al.*, 2008; Sowers *et al.* 2002; Gundacker *et al.* 2002; Friel *et al.*, 1999; Gulson *et al.* 1998a; Rabinowitz *et al.* 1985b), and has also been reported in infant formulas (Dabeka 1989). Dietary supplements and vitamins can also be contaminated with Pb, due in part to the source from which these are derived (Bourgoin *et al.* 1993; Scelfo and Flagel, 2000; US FDA, 2008).

Numerous consumer products, from children's toys to paints and cigarettes have been reported from time to time to contain varying levels of lead. Governments act on this information to inspect, analyze, and when necessary, to recall and ban products that present unacceptable public health risks.

### 1.2.2 Pharmacokinetics of Pb

The uptake, distribution and excretion of lead are described in detail by the US Agency for Toxic Substances and Disease registry (US ATSDR, 2007a). The brief synopsis that follows is based largely on US ATSDR (2007a) unless otherwise cited.

#### Absorption

It is reported that from 3 to 10% and from 40 to 50% of lead ingested with food is absorbed into the the adult and juvenile body, respectively. The absorption of lead from ingested media other than food (such as water, soil, dust) is variable but is strongly influenced by food intake, with much higher rates of absorption occurring during periods of fasting. Age, sex, hormonal and nutrient status, as well as life-stage and behavioural patterns are all associated with changes in the rate of lead uptake (bioavailability) from the gastrointestinal tract.

Gastrointestinal absorption of soil-borne lead is much lower than that of soluble lead compounds. Adult volunteers fed lead contaminated soil absorbed 26% of the delivered dose while fasting, which was approximately half the absorption rate for soluble lead. In contrast, when accompanied by food intake, soil-borne lead absorption decreased to approximately 2.5%. Direct data on the gastrointestinal absorption of soil-borne lead in children is not available. However, work by von Lindern *et al.* (2003) predicted the bioavailability of lead in soil/dust to be 18% in children (range of 12 to 23%) based on regression analysis with varying soil concentrations. Based on a review of *in vivo* studies on the absorption of lead from soil and soil-like material in immature swine (US EPA, 2007), absorption averaged 60% relative to that of absorption of soluble lead from food or water. This translates into absolute absorption rates from ingested soil in adults on the order of 5%, and in children of approximately 25%.

When inhaled, lead is deposited in either the upper or lower respiratory tract, depending on particle size; larger lead-bearing particles deposit in the upper respiratory tract, whereas lead-bearing particles  $\leq 1\mu\text{m}$  in aerodynamic diameter and lead fumes reach the lower respiratory tract. Of the lead deposited in the alveoli, 95% is absorbed. No data for absorption following inhalation in children are available; however, their respiratory uptake of lead is likely to be comparatively greater on a body weight basis.

Lead associated with soil particulate is assumed to have a low rate of dermal absorption, generally 1% (OME, 2008). However, there is evidence for higher dermal permeability of soluble inorganic lead compounds and organic lead compounds from *in vitro* studies conducted with excised skin. As much as 30% of certain soluble inorganic lead salts applied in water may be retained in skin layers and/or be absorbed into the blood stream. The rank order of lead absorption rates through excised skin from humans and guinea pigs was as follows: tetrabutyl lead > lead nuolate (lead linoleic and oleic acid complex) > lead naphthanate > lead acetate > lead oxide (nondetectable) (Bress and Bidanset 1991).

#### Distribution in the body

Absorbed lead is divided into two general kinetic pools in the body: soft tissues and the skeleton. For soft tissues, including blood, the average half-life is 20–40 days (Gulson *et al.*, 1996). The skeleton represents the primary reservoir for lead in the body. For adults, about 94% of total body burden is present in bone, while in children the bone reservoir represents about 73% of total body burden. The half-life of lead in bone is measured in decades and is certainly more than 20 years (Klaasen, 1996).

#### Excretion

Lead is predominantly excreted via the urine (50 to 60%) and, to a lesser extent, by biliary excretion into the feces (Klaasen, 1996). Ingested lead which is not absorbed from the gastro-intestinal tract is also found in the feces.

#### Transfer of lead from mother to foetus and infant

Placental transfer of lead occurs in humans as early as the twelfth week of gestation, and uptake of lead by the foetus continues throughout development (Barltrop, 1969). The concentration of lead in umbilical cord blood is correlated with maternal blood lead levels in ratios that range from 0.8 to 1.0 (Angell and Lavery 1982; Moore *et al.* 1982; Gershanik *et al.* 1974; Lacey *et al.* 1985). The ratio of blood lead in the foetus to that of the mother is also about 0.8 to 1.0 (Angell and Lavery 1982; Gershanik *et al.* 1974).

Maternal lead is also transferred to infants via breastfeeding, although breast milk/maternal blood concentration ratios are generally less than 0.1. However, Ettinger *et al.* (2006) and Gulson *et al.* (1998a) reported a breast milk/maternal blood concentration ratio of 0.9. Lead in breast milk can contribute substantially to the lead content of infant blood, contributing approximately 40–80% (Gulson *et al.* 1998b).

### **1.2.3 Pb exposure**

Estimated daily intakes of Pb in the various segments of the North American population (represented by recent Canadian data) are summarized in Table 2. Exposure to lead through ingestion of indoor settled dust was the principal exposure pathway for infants, toddlers and children. For teens, adults and seniors, the principal pathway of lead exposure was through consumption of foods.

#### *Blood lead levels in the population*

Blood is the tissue most commonly used as a biomarker of human lead exposure (US ATSDR 2007a; Health Canada 1994). Blood lead levels are generally believed to reflect recent lead exposure, with 1 to 3 months of continuous exposure required to achieve a steady state (Ewers and Schipkötter 1991; Health Canada 1994).

Between 1976 and 1991, mean blood Pb levels in the US declined by more than 70% in all age groups and ethnic groups (Pirkle *et al.*, 1994). Further declines were reported from 1991 through 2002 (Schwemberger *et al.* 2005), with geometric mean blood Pb levels declining >30% overall (from 2.3 ug/dL to 1.6 ug/dL) for the US population over this later time span. From the 2003-04 NHANES data, an overall geometric mean blood Pb concentration of 1.5 ug/dL was determined for all survey participants (N=8373). For 2007-08, the overall geometric mean blood Pb concentration was further reduced to 1.35 ug/dL (N=8266).

Other body tissues such as bone, teeth, and hair have been used as indicators of longer term exposure to lead. However these tissues are not reviewed herein.

### **1.2.4 Overview of the toxicology of Pb**

A thorough review of the toxicology of Pb was recently completed on behalf of Health Canada by Azimuth (2010). A review of this subject is also provided by the US ATSDR

(2007a). Therefore, a detailed review of the toxicology of Pb will not be presented herein.

Most lead poisoning symptoms are thought to occur via common mechanisms of action such as lead's ability to mimic other biologically important metals (the most notable of which is calcium) (Kosnett, 2006). Lead also crosses the developing blood-brain barrier (i.e., in the foetus, infants and children) (US ATSDR, 2007a) and, once in the brain, lead binds to sulphhydryl (thiol) groups which ultimately leads to its effects on the central nervous system and detrimental outcomes on neurobehaviour and cognitive function. This ability of Pb to cross the blood brain barrier, its mechanism of action by which it binds to sulphhydryl groups, and its impacts on neurobehaviour and cognitive function, echo those for Hg<sup>0</sup> and MeHg.

The adverse health outcomes of Pb exposure have been well documented in the literature for a wide variety of tissues, organs and organ systems. These include the neurological system, cardiovascular system, reproductive system, haematopoietic system (blood), immune system and renal system (kidneys).

Of these various target organs and systems, developmental neurotoxicity is considered the key effect in children (manifested as decrements in IQ with increasing blood lead levels) and cardiovascular toxicity is the key effect in adults (manifested as increasing systolic blood pressure with increasing blood lead levels). No threshold for the toxic effects of lead has yet been determined, with toxic effects being observed to the lowest levels of exposure that have been (repeatedly) measured, particularly in relation to the neurodevelopmental effects and IQ in children (US EPA 2006; CalOEHHA, 2009; CalEPA, 1997; WHO 2009).

The US EPA has not established a toxicological reference value for Pb, relying instead on the blood lead "level of concern" in children of 10 µg/dL, established by the CDC in 1991 (US CDC, 1991). The toxicology of Pb has been recently re-evaluated by the California EPA, has established a reference blood Pb level of 1 µg/dL for children, which they predict is associated with a 1 IQ point decrement (CalOEHHA, 2009). Re-



evaluations are also ongoing by the CDC, and internationally in Canada, the European Union and elsewhere.

**Table 2: Estimated Daily Intakes (EDIs) of Lead ( $\mu\text{g}/\text{kg}\text{-day}$ ) by Environmental Medium and Age-Group (Based on SENES, 2010)**

Stats	Infant		Toddler	Child	Teen	Adult	Senior
<b>Indoor Air EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
Mean	$7.8 \times 10^{-4}$		$1.3 \times 10^{-3}$	$1.1 \times 10^{-3}$	$6.8 \times 10^{-4}$	$6.0 \times 10^{-4}$	$5.5 \times 10^{-4}$
SD	$1.9 \times 10^{-3}$		$3.3 \times 10^{-3}$	$2.8 \times 10^{-3}$	$1.9 \times 10^{-3}$	$1.4 \times 10^{-3}$	$1.3 \times 10^{-3}$
<b>Settled Dust EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
Mean	1.19		0.60	0.23	$7.9 \times 10^{-3}$	$8.6 \times 10^{-3}$	0.010
SD	13.2		3.46	1.33	0.034	0.047	0.11
<b>Ambient Air EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
Mean	N/A		$1.1 \times 10^{-4}$	$1.3 \times 10^{-4}$	$5.4 \times 10^{-5}$	$4.9 \times 10^{-4}$	$4.0 \times 10^{-5}$
SD	N/A		$6.6 \times 10^{-4}$	$4.4 \times 10^{-4}$	$2.3 \times 10^{-4}$	$1.6 \times 10^{-4}$	$1.6 \times 10^{-4}$
<b>Soil EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
Mean	N/A		$7.9 \times 10^{-3}$	$3.7 \times 10^{-3}$	$2.6 \times 10^{-4}$	$2.4 \times 10^{-4}$	$1.8 \times 10^{-4}$
SD	N/A		0.014	0.010	$5.3 \times 10^{-4}$	$5.4 \times 10^{-4}$	$5.6 \times 10^{-4}$
<b>Water or Breast Milk EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
	Breast fed	Non-breast fed					
Mean	0.43	0.038	0.032	0.022	0.016	0.020	0.023
SD	1.21	0.11	0.090	0.063	0.047	0.058	0.064
<b>Food EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
Mean	0.19		0.24	0.16	0.100	0.094	0.087
SD	0.26		0.13	0.097	0.068	0.066	0.049
<b>Total EDI (<math>\mu\text{g}/\text{kg}\text{-d}</math>)</b>							
	Breast fed	Non-breast fed					
Mean	1.81	1.42	0.88	0.42	0.12	0.12	0.12
SD	13.18	13.12	3.46	1.33	0.089	0.10	0.13



### **1.3 Methyl Mercury (MeHg)**

The toxicology, pharmacokinetics and general exposure to methyl Hg have been extensively reviewed by numerous national and international agencies. Reviews include US EPA (2001, 1997), US ATSDR (1999), and WHO (1990). The text that follows has been extracted and condensed from these sources unless otherwise noted.

#### **1.3.1 MeHg in the environment**

MeHg occurs naturally in the environment. It is readily produced from inorganic mercury in fresh and marine surface waters and sediments through the methylating action of certain microorganisms. MeHg generally constitutes no more than 25% of the total mercury in surface water; typically, less than 10% is observed. However, its propensity for bioaccumulation in the aquatic food chain results in the accumulation of relatively high levels in aquatic biota, particularly in higher trophic level fish. Piscivorous birds and mammals, including humans, can also accumulate high body burdens of MeHg, due to consumption of contaminated fish.

Virtually all of mercury in fish occurs in methyl form. Aquatic organisms rapidly absorb MeHg and store it for long periods of time in their muscle tissue, thus accumulating it to levels that are potentially toxic to humans and wildlife that eat fish and shellfish.

#### **1.3.2 Pharmacokinetics of MeHg**

MeHg in the human diet is almost completely absorbed into the bloodstream and distributed to all tissues within about 4 days. Maximum levels in the brain are reached after 5-6 days. In humans, the distribution of MeHg between red blood cells and plasma is about 20:1.

In humans, approximately 90% of the absorbed dose of MeHg is excreted in the feces. Excretion via the urine is relatively minor but slowly increases with time. Mercury is also excreted in breast milk of lactating women (discussed further below), as well as into the hair. Incorporation of MeHg into hair is irreversible, and blood to hair concentration ratios are about 1:250. As a result, hair analysis is a useful tool for monitoring exposure to MeHg. Segmental analysis of hair may be used to provide a historical record of exposure patterns (Richardson and Currie, 1993).



MeHg is converted to inorganic mercury in humans and experimental animals. When MeHg crosses the blood-brain barrier this conversion traps mercury in the brain leading to a much longer half-life for elimination from the brain than for other tissues of the body (Lorscheider et al. 1995). MeHg also crosses the placental barrier into the fetus, with MeHg concentrations in cord blood generally being the same or higher than in maternal blood.

The rate of excretion of mercury in both laboratory animals and humans is directly proportional to body burden. The biological half-life for MeHg in fish-eating humans is 39-70 days (average approximately 50 days). The relatively long half-life for MeHg in the body results partly from resorption of MeHg secreted into the bile (hepatobiliary cycling). MeHg-glutathione complex secreted into the bile is reabsorbed from the gallbladder and intestines into the blood. This cycle is terminated when intestinal microflora demethylate the MeHg to Hg<sup>2+</sup> which is poorly absorbed from the gastrointestinal tract.

Lactating females have significantly shorter half-times for MeHg excretion than non-lactating females, due to excretion via the breast milk. The ratio of mercury in breast milk to mercury in whole blood was approximately 1:20 in women exposed to MeHg via contaminated grain in Iraq between 1971 and 1972. The proportion of total mercury as MeHg in human breast milk is about 50% (Oskarrson et al., 1996). The mercury content of breast milk is proportional to the mercury content of plasma, but the ratio of concentration in breast milk to concentration in blood generally averages about 0.2, and seldom exceeds a ratio of 1. For inorganic Hg, the breast milk to blood concentration ratio is about 3 times greater on average than that for MeHg, and in some cases the concentration of inorganic Hg in breast milk can exceed that in the blood of the mother, by up to about 4 times.

In the case of continuous exposure, a single-compartment model with a 70-day half-time predicts that the whole-body steady state (where intake equals excretion) will be attained within approximately one year and that the maximum amount accumulated will be 100 times the average daily intake.

### 1.3.3 Exposure to MeHg in the US population

Estimates of MeHg exposure in the US population are presented in Table 3. The primary source of human exposure to MeHg is through consumption of contaminated fish and seafood. Consumption of non-fish food items, drinking water and other potential minor sources and routes of MeHg exposure account for less than 0.1% of total exposure. MeHg and/or total Hg are generally non-detectable in the majority of non-fish foods.

Infant postnatal exposure to MeHg through ingestion of breast milk is a pathway of potential concern. MeHg is excreted in breast milk, with the concentration increasing as the amount of fish consumption.

### 1.3.4 Toxicology of MeHg

MeHg is a highly toxic contaminant that can cause a variety of adverse health effects. Toxicity has been observed in adults exposed through consumption of contaminated food. Toxic effects and subtle neuropsychological effects have been seen in children exposed *in utero* when their mothers consumed contaminated food while pregnant. A reference dose (RfD) of 0.1 ug MeHg/kg-day was established by the US EPA. This RfD is based on changes in neuropsychological measures in children exposed to MeHg *in utero*.

MeHg-induced neurotoxicity is the effect of greatest concern when exposure occurs to the developing fetus. MeHg exposure adversely affects a number of cellular events in the developing brain both *in utero* and post-natally. Minamata disease in children and adults included impairment of the peripheral vision, disturbances in sensations ("pins and needles" feelings, numbness) usually in the hands and feet and sometimes around the mouth; incoordination of movements as in writing; impairment of speech; impairment of hearing; impairment of walking; and mental disturbances.

In 1965, an additional methylmercury poisoning outbreak occurred in the area of Niigata, Japan. The signs and symptoms of disease in Niigata were strongly similar to the disease in Minamata.

Two outbreaks of MeHg poisoning occurred in Iraq (late 1950s, early 1970s) following consumption of seed grain that had been treated with a fungicide containing methylmercury. Unlike the long-term exposures in Japan, the epidemic of methylmercury poisoning in Iraq was short in duration, but the magnitude of the exposure was high. As in the Japanese poisoning epidemics, the signs and symptoms of disease were predominantly those of the nervous system: difficulty with peripheral vision or blindness; sensory disturbances; incoordination; impairment of walking; slurred speech; and in some cases, death.

Infants born of mothers who had consumed the methylmercury-contaminated grain (particularly during the second trimester of pregnancy) showed nervous system damage even though the mother was only slightly affected herself.

The current U.S. EPA RfD for methylmercury is 0.1 ug MeHg/kg-day, and was based on data on neurologic changes in Iraqi children of mothers that had eaten methylmercury-contaminated bread during pregnancy. Key neurological impairments in these children (exposed *in utero*) included: inability to walk two steps without support by two years of age; inability to respond to simple verbal communication by age 2 years among children with good hearing; scores on physical examination by a neurologist that assessed cranial nerve signs, speech, involuntary movements, limb tone, strength, deep tendon reflexes, plantar responses, coordination, dexterity, primitive reflexes, sensation, posture, and ability to sit, stand, walk, and run; and assessment of mental development or the presence of seizures (based on interviews with the child's mother).

Table 3. Estimated daily intake of MeHg in the US population (from US EPA, 2001).

	Summary of Exposure (mg/kg-day)							
Population group	Ambient Water	Drinking Water	Non-fish Dietary Items	Freshwater/ Estuarine Fish	Marine fish	Air	Soil	Total Exposure
Children Age 0-14 Years	$5.0 \times 10^{-9}$	$6.5 \times 10^{-8}$	0	$1.3 \times 10^{-3}$	$4.2 \times 10^{-4}$	$5.5 \times 10^{-9}$	$5.9 \times 10^{-9}$	$1.7 \times 10^{-3}$
%Total Exposure	0.0003%	0.0038%	0.0000%	76.0198 %	23.9755%	0.0003%	0.0003%	
Women of Childbearing Age	$4.5 \times 10^{-9}$	$5.8 \times 10^{-8}$	0	$6.4 \times 10^{-4}$	$2.0 \times 10^{-4}$	$2.6 \times 10^{-9}$	$1.3 \times 10^{-9}$	$8.4 \times 10^{-4}$
%Total Exposure	0.0005%	0.0069%	0.0000%	76.1844%	23.8076%	0.0003%	0.0002%	
Adults in General Population	$4.3 \times 10^{-9}$	$5.6 \times 10^{-8}$	0	$6.5 \times 10^{-5}$	$2.7 \times 10^{-5}$	$4.6 \times 10^{-9}$	$1.3 \times 10^{-9}$	$9.2 \times 10^{-5}$
%Total Exposure	0.0047%	0.0608%	0.0000%	70.6014%	29.3267%	0.005%	0.0014%	

In the studies on subtle neuropsychological effects in children published to date, there has been no definitive separation of prenatal and postnatal exposure that would permit dose-response modeling. That is, there are currently no data that would support the derivation of a fetal RfD or child RfD versus a general population RfD. Therefore, the RfD is intended to protect the population in general, not only the developing fetus or child.

Also, although the US EPA RfD is considered protective for fetal and developmental neurological effects, there is still concern that other less-studied effects may occur at lower doses. There is also concern (based on recent reports on the Minamata, Japan, population) that exposure *in utero* or in childhood could result in subtle impairments that would not be detectable until middle age or older.

No reference blood level has been published in the US, but Canada has proposed a reference blood level of 8 ug methyl Hg/L of blood (Legrand et al. 2010). This reference blood MeHg level has been formulated to protect children, pregnant women and women of childbearing age, particularly relating to neurodevelopmental effects in the fetus.

## **2. COMBINED TOXICITY ASSESSMENT: Hg<sup>0</sup>, MEHG AND PB**

### **2.1 Introduction**

This section includes a discussion of regulatory guidance (ATSDR and US EPA) for evaluating potential joint toxicity, resulting in cumulative risks, from concomitant exposure to chemical mixtures. Recommendations from these guidance documents were considered in an evaluation of toxicological data for mercury (mercury vapour [Hg<sup>0</sup>] and methylmercury [MeHg]) and lead (Pb), focusing on reported similarities in individual and combined toxicokinetics and toxicodynamics for each substance. This information and the potential for combined exposures to Hg<sup>0</sup>, MeHg and Pb, were considered in order to demonstrate how cumulative risks associated with combined exposures to these metals should be evaluated.

## **2.2** **What is Cumulative Risk Assessment?**

Cumulative risk assessment is defined as “...an analysis, characterization and possible quantification of the combined risks to health or the environment from multiple agents or stressors” (US EPA 2007b). Cumulative risk assessments emphasize evaluation of the potential for joint chemical toxicity and cumulative exposures, which includes consideration of:

- combined sources/releases, not all of which are located within a community
- joint behavior – grouped chemicals, environmental interactions, transformation
- sets of chemicals that coexist and/or move together
- sensitive subgroups and unique exposure activities
- combined chemicals and routes over time, considering sequencing
- combined amounts of various forms (potential impact on toxicokinetics)

## **2.3** **Why Consider Chemical Mixtures?**

Depending on their location and the time of day, individuals may be exposed to a range of chemicals present in outdoor and indoor air, water, soil, dust, food, and consumer products. Cumulative risk assessment attempts to account for possible risks from exposures to multiple chemicals in multiple media, rather than characterizing risks on the premise that exposure to one chemical via one pathway (e.g., ingestion of methyl mercury in fish) occurs in isolation of other chemicals and other pathways (e.g., inhalation of lead in indoor air).

As described in Section 1, co-exposure of the US population to lead and mercury occurs, and is well described and quantifiable. Lead and mercury (methylmercury and metallic mercury) are each capable of producing neurological effects (primary effect), with children exhibiting the most sensitive response to these substances. Within the body, Hg<sup>0</sup> and MeHg are converted to inorganic mercury (IHg), which, along with Pb, can also affect renal function (secondary effect). Toxicological interactions and responses following exposure to a mixture of lead and mercury is dependent on the dose received. Although the toxic effects of the individual substances are extensively researched and reported in the literature, considerably less information is available to describe responses following combined exposures to Hg<sup>0</sup>, MeHg and Pb.

### 2.3.1 What “guidance” is available for assessing mixtures?

Recent U.S. guidance and resource documents available to regulatory scientists and the risk assessment community for the purpose of assessing potential health hazards from exposures to chemical mixtures include:

- US Agency for Toxic Substances and Disease Registry (USATSDR). 2004. *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures*. ATSDR, Public Health Service, U.S. Department of Health and Human Services, Washington, DC.
- U.S. Environmental Protection Agency (U.S. EPA). 1999. *Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (External Scientific Peer Review Draft). Risk Assessment Forum, EPA, Washington, DC.
- USEPA. 2000. *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures*. Risk Assessment Forum, EPA, Washington, DC.
- USEPA. 2007b. *Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures.*, Office of Research and Development, USEPA, Washington, DC.

### 2.3.2 ATSDR guidance

The USATSDR (2004) is responsible for producing *toxicological profiles* for hazardous substances found at U.S. National Priorities List (NPL) sites and for substances related to federal sites under the Department of Defense (DOD) and the Department of Energy (DOE). The ATSDR has also been charged with developing methods to determine the health effects of combined exposures to more than one substance, when a substance occurs in combination with one or more other substances. To that end, a chemical mixtures program was developed.

Following the release of the USATSDR guidance, a series of *Interaction Profiles* were completed for priority mixtures of special concern to ATSDR (e.g., *Interaction Profile for Chlorpyrifos, Lead, Mercury, and Methylmercury*, ATSDR 2006). These profiles evaluate toxicity data available to describe a mixture or joint toxic action of chemicals in a mixture.

The ATSDR (2004) guidance recommends approaches for the assessment of potential hazards of combined exposures to chemical mixtures (noncarcinogenic and carcinogenic effects) on

public health. The approach for assessing noncarcinogenic effects of chemical mixtures is exposure-based and follows generally accepted risk assessment techniques, where Hazard Quotient (HQ) values (ratio of chemical exposure to exposure limit) are estimated for public exposure to each individual component of the mixture. An assessment of the potential for joint toxic action is recommended where individual HQ values for 2 or more components of the mixture equal or exceed 0.1. A significant additive and/or interactive health hazard is not predicted if HQ values for individual components are less than 0.1. Community-specific health outcome data and community health concerns should be taken into consideration when assessing the health implications and follow-up activities for public exposure to chemical mixtures (ATSDR 2004).

The ATSDR (2004) provide a flow chart outlining a *step by step procedure to exposure-based assessment of the joint action of chemical mixtures* which is reproduced here for non-carcinogenic effects (see Figure 1). Although it is preferable to have exposure and toxicity data for the mixture of concern (i.e., *Interaction Profile*), in the absence of suitable data a components-based approach is recommended, whereby the toxicity of the components are evaluated to determine if they have similar or different critical effects. Where components have similar endpoints in terms of critical effects or target organs, a hazard index method (i.e., adding HQ values for individual components) is recommended for characterizing potential risks associated with exposure to a chemical mixture.

An important component of the ATSDR guidance document for the assessment of chemical mixtures are qualitative weight-of-evidence (WOE) assessment methods designed to modify the hazard index to account for interactions among pairs of mixture components. Guidance is provided for classifying the WOE to determine if interactions among the components are additive, greater-than-additive, less-than-additive, or indeterminate. Additive effects indicate that, although both chemicals act on the same target, one chemical does not significantly affect the toxicity of the other. Greater than additive effects indicate that synergism or potentiation effects were observed at the target site in response to the presence of both chemicals.

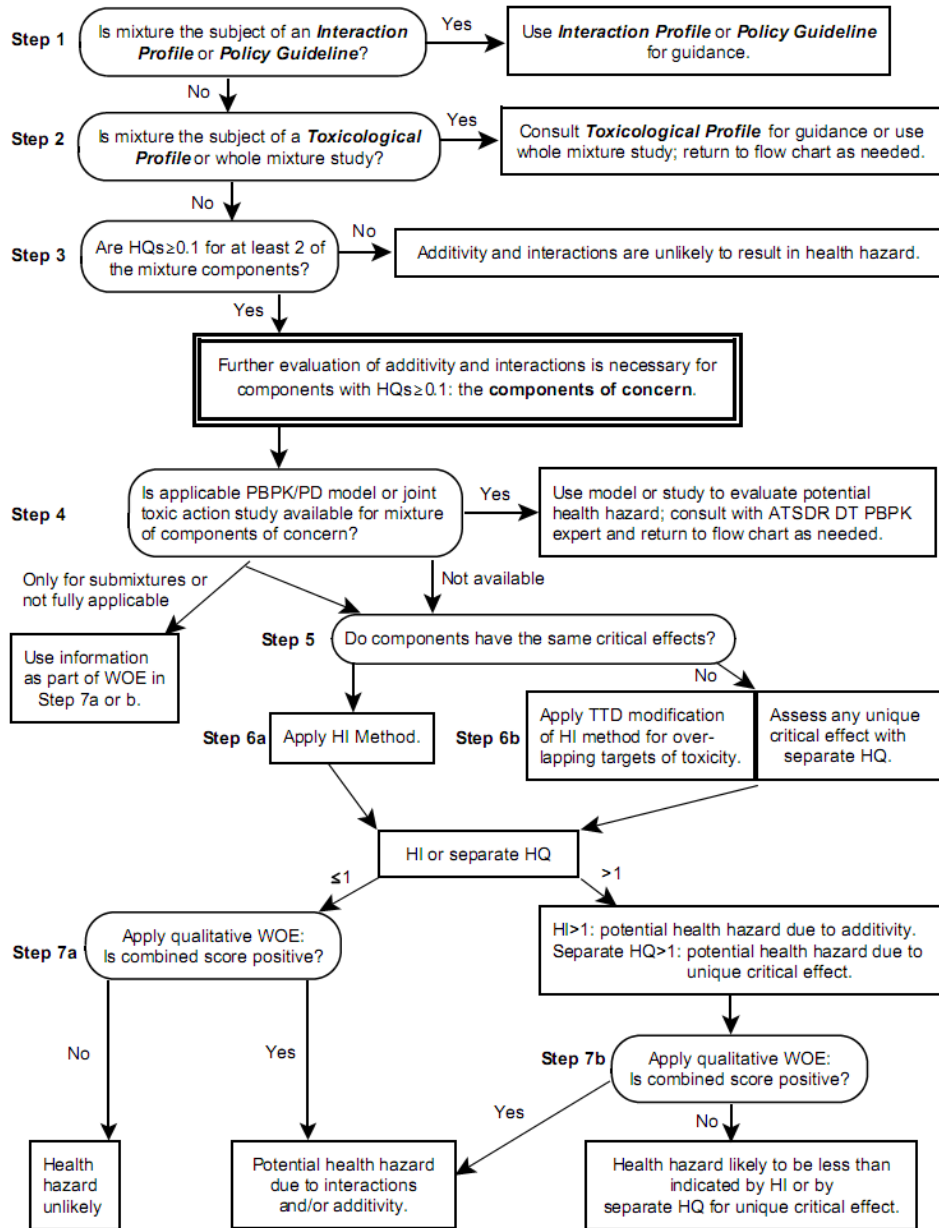
Determining how chemical components of a mixture will interact requires data to describe the mechanistic action of toxic effect for each chemical and illustrate action or interaction on the



same target to produce a similar (additive), greater than additive, or less than additive effect. This requires an understanding of chemical, biological, and physical processes at the molecular level and higher levels of biological or physiological organization. A weighting of the quality of the mechanistic data available to describe interactions is recommended which should reflect the extent to which the data indicates that chemicals will interact in a manner that significantly impacts the health of the exposed population.

The ATSDR binary WOE approach suggests that data relevant to joint action for each possible pair of chemicals in the mixture be evaluated for the determination of a qualitative binary weight-of-evidence (BINWOE) for chemical interactions. This should be a target-organ specific evaluation of toxicity, pharmacokinetic, and mechanism of action data for the individual chemicals and for interactions between each chemical pair. For a chemical pair A and B, the effect of chemical A on the toxicity of chemical B, and vice versa, should be considered. A basic assumption of this WOE method is that pairwise interactions will dominate the mixture, that is, the presence of a third chemical will not significantly change interactions between a pair of chemicals.

Figure 1 Strategy for Exposure-Based Assessment of Joint Toxic Action of Chemical Mixtures: Noncarcinogenic Effects (from ATSDR 2004)



### 2.3.3 US EPA guidance

The most recent publication by the USEPA (US EPA 2007b) was intended as a resource document (presentation of concepts, methods and data sources) for cumulative health risk assessments to serve US EPA Program Offices and Regions. Although not intended to be a guidance or a regulatory document, the document was also made available to the broader risk assessment community interested in locating data and approaches relevant to cumulative risk assessment. Since this most recent publication draws on earlier published information for chemical mixtures, a select summary of recommendations are provided here.

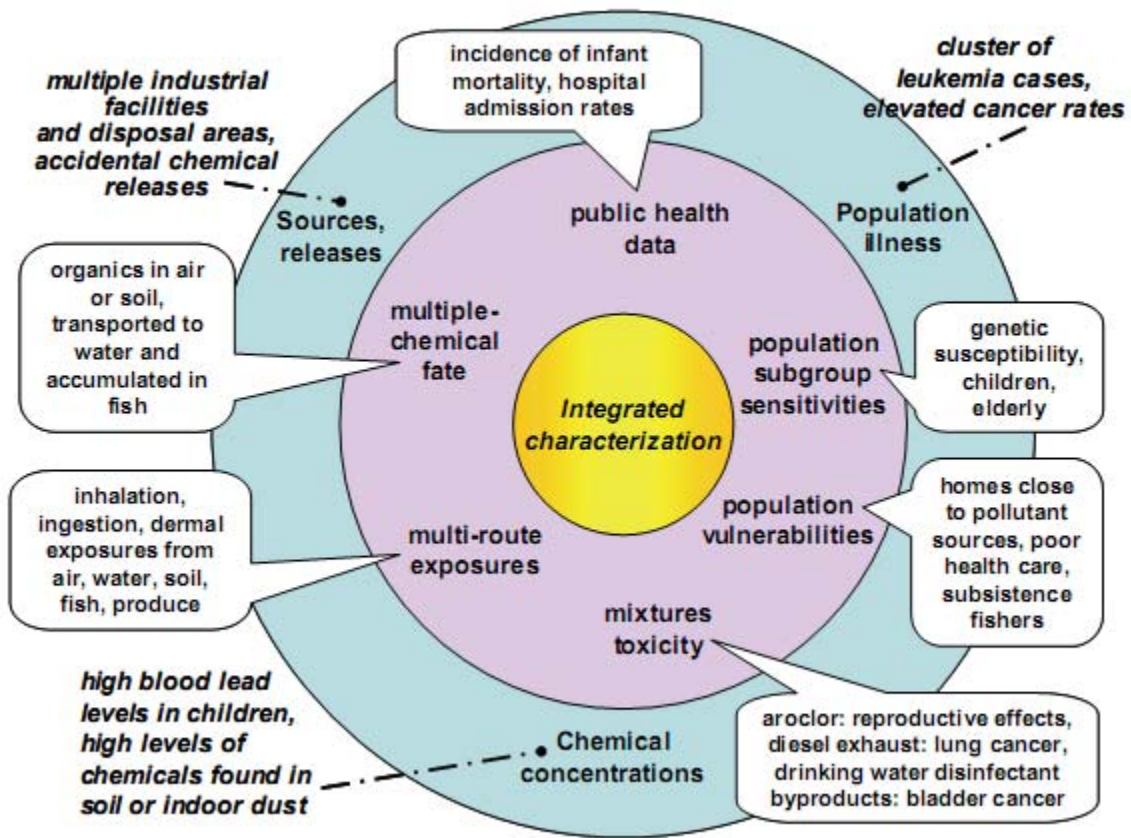
Cumulative risk analysis is appropriate for situations where populations are exposed to multiple chemicals. Three initiating factors for cumulative risk assessment identified by EPA (2007b) include:

- source releases: multiple pollutant sources or releases;
- chemical concentrations: elevated concentrations from environmental monitoring or biomonitoring of chemicals; and
- increased population illness in a community.

These initiating factors plus other data elements relevant to cumulative risk assessment are graphically illustrated in Figure 2, below (taken from EPA 2007b).

The future of cumulative risk assessment will likely incorporate biomonitoring data, (measured internal doses which reflect chemical exposure and absorption) which may provide key quantitative estimates of current chemical exposure levels within a population. This could include the NHANES data which currently publishes existing blood chemical or urine chemical concentration data. Challenges to interpreting and applying biomonitoring data to risk assessment include the task of integrating the biomonitoring data with exposure modelling information to identify the pathways, timing, and routes of exposure that will predict an individual's actual exposure (UE EPA 2007b).

Figure 2 Initiating Factors and Data Elements for Cumulative Risk Analysis (from US EPA 2007b)



For the assessment of exposure to multiple chemicals, the US EPA (2007b) recommends that chemicals be grouped together based on their potential for co-occurrence and joint toxic action, with an assumption that chemicals in these groups could produce toxicity by the same mode of action or affect the same target organ. Factors affecting the inclusion of chemicals within such toxicity groups include: pharmacokinetic parameters, persistence of the chemicals in the body and the formation of metabolites. Generally the identification of a common mode of action, and, to a lesser extent, common target organs, is the preferred method for grouping chemicals together for analysis of combined toxicity (US EPA 2007b).

Evaluating the potential for toxicokinetic or toxicodynamic interactions is an important step when considering exposures to multiple chemicals. The following definitions for toxicokinetic and toxicodynamic interactions were provided in US EPA (2007b):

- *Toxicokinetic interactions refer to alterations in the absorption, distribution, metabolism or elimination of a toxic chemical. For example, these interactions can be mediated by the induction or inhibition of enzymes involved in xenobiotic activation or detoxification.*
- *Toxicodynamic interactions encompass all interactions that do not directly affect absorption, distribution, metabolism or elimination of a toxic chemical. Toxicodynamic interactions affect a tissue's response or susceptibility to chemically mediated toxic injury. Modes of toxicodynamic interactions include, among others, depletion or induction of protective factors, alterations in tissue repair, changes in hemodynamics, and immunomodulation.*

As described in US EPA (2007b), it is important to consider whether observed toxicological interactions between chemicals could be classified as being direct or indirect. A direct interaction occurs when compounds are capable of altering the same biochemical pathway, cell type, or organ/tissue directly related to the toxic effect of the compound (i.e., competition for key metabolizing enzymes, receptor binding sites and lipid peroxidation leading to membrane damage and radical formation). Indirect interactions could result in alteration of the internal dosimetry/metabolism of other compounds through enzyme induction and/or glutathione depletion.

The importance of these interactions depends on the severity of the effects produced (US EPA 2007b). The effect of one compound within a mixture may only produce a slight decrement in function that can be recovered from or compensated by the tissue and that effect (direct or indirect) may not provide sufficient evidence for the assumption of interaction. Both direct and indirect effects may be reversible or compensated for by the cell (e.g., redundant cellular/biochemical systems) or may, over time and with repeated exposures, lead to toxic effects (e.g., glutathione depletion resulting in increased susceptibility). This underlies the importance of considering all available interaction information, coupled with the available dose-response data. Similar to ATSDR guidance, the US EPA (2007b) also describes WOE procedures for the influence of one chemical on the toxicity of another chemical.

## **2.4      *Mercury and Lead Additive/Interactive Toxic Effects***

### **2.4.1   ATSDR mixtures assessment**

The ATSDR (2006) developed an interaction profile for the mixture of chlorpyrifos (organophosphorus insecticide), lead, and mercury/methylmercury compounds, based on the potential for these compounds to produce neurological effects in exposed children. This profile evaluated data on the health effects and mechanisms of action for individual components of the mixture, as well as available data on joint toxic action for combinations of the components in this mixture. It is noted that Hg<sup>0</sup> was specifically and explicitly omitted from the group of Hg compounds evaluated in this interaction profile, due to the principle source of exposure, rather than based on levels of exposure, nor based on the toxicology of Hg<sup>0</sup> in general. As described in Section 1, co-exposure to Hg<sup>0</sup> with inorganic Hg (IHg) and MeHg occurs in the US population due to the prevalence of amalgam fillings. Regardless, the ATSDR concluded that there was a lack of clear evidence that *adverse effects* were occurring from amalgam fillings per se, so excluded this source and Hg<sup>0</sup> from consideration.

The results of the ATSDR (2006) binary evaluations of the interactive toxicity of Pb and MeHg were of interest to the current assessment. The critical effect of concern determined for combined exposure to Pb and MeHg was neurological, with children (i.e., developing nervous system) representing a sensitive subpopulation of concern. A BINWOE for chemical interaction was conducted for combinations of Pb and MeHg. The directions of interaction for the effect of Pb on MeHg neurotoxicity and the effect of MeHg on Pb toxicity were predicted to be additive.

In the studies reviewed Pb did not appear to influence the distribution of MeHg to the placenta and fetus in pregnant mice and there was an absence of evidence for greater than additive effects in studies on mice and ducks; however, one sequential study in rats did suggest potentiation of MeHg lethality by pretreatment with Pb.

A Hazard Index was recommended for the consideration of neurological effects from combined exposures to Pb and MeHg (as well as IHg and chlorpyrifos) from a contaminated or hazardous waste site, where individual HQ values are equal to or exceed 0.1. The assessment of potential joint toxic action was not considered necessary for individual HQ values below 0.1 or where HQ value exceeds 0.1 for only one component of the mixture. In the absence of an USATSDR MRL for Pb, the USATSDR (2006) recommended the use of a target-organ toxicity dose (TTD) of 10 µg Pb per dL blood for neurological effects (from USCDC 1991), to calculate a HQ value for exposures to Pb. The USATSDR also suggested that the HQ value for MeHg be determined by comparing an estimated daily chronic exposure dose (i.e., in mg/kg/day) to the chronic oral MRL developed by the ATSDR for MeHg (0.3 µg/kg/day). However, the CDC (1991) blood Pb level of concern is now out of date and should be replaced with the reference blood Pb level of 1 µg/dL from the California Office of Environmental Health Hazard Assessment (CalOEHHA 2009). Likewise, the USEPA reference dose (RfD) for MeHg of 0.1 µg/kg-day is preferable to the USATSDR MRL due to increased conservatism.

Where individual HQ values exceed 0.1, an HI for neurological toxicity of Pb and MeHg combined would be calculated as follows:

$$HI = \frac{EPb \text{ (predicted PbB in } \mu\text{g/dL)}}{\text{reference PbB}} + \frac{EMeHg \text{ (oral intake, mg/kg/day)}}{RfD}$$

It is recommended that the above HI process (exposure-based assessment of potential health hazard) be used as a screening approach in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns in the assessment of public health hazards associated with exposures to chemical mixtures (ATSDR 2006). Again, the subpopulation of greatest concern for neurological effects associated with this mixture includes infants, young children, and fetuses.

## **2.5** **Primary Literature Review**

### **2.5.1 Toxicokinetics**

A review of literature describing the absorption, distribution/storage, biotransformation and elimination of Hg<sup>0</sup>, MeHg and Pb is presented in Table 4 and summarized below.

The primary exposure route for Hg<sup>0</sup> is inhalation with over 80% of the inhaled dose rapidly absorbed across the lungs. The primary exposure route for MeHg is ingestion (generally in fish) which results in near complete (95%) absorption. Several factors may reduce the oral bioavailability of MeHg, including dietary fiber, phytate and selenium, as well as complexation of MeHg with selenium in the fish. Submicron sized particles of inorganic Pb are nearly completely absorbed following inhalation. The absorption of Pb following ingestion is higher in children (40–50%) compared to adults (3–10%). Factors affecting the oral bioavailability of lead include age, fasting, nutritional calcium and iron status (where fasting and deficiencies in calcium and iron will increase Pb availability) and the medium ingested (e.g., particle size, mineralogy, solubility, and lead species).

Hg<sup>0</sup> and MeHg are similarly distributed in the body, with both taken up by red blood cells and accumulated in the kidneys and hair. Inorganic Pb in blood also primarily occurs within red blood cells. Pb binds plasma proteins (e.g., albumin and  $\gamma$ -globulins), can complex with sulfhydryl compounds including cysteine and homocysteine and is often associated with  $\delta$ -aminolevulinic acid dehydratase (ALAD) within the red blood cell. Pb is transported to the liver, kidneys, lungs, brain, spleen, muscles, heart and ultimately distributed to bones, which act as a reservoir of Pb to the blood. Pb forms an extremely stable complex with potassium and is able to replace calcium in the primary crystalline matrix of bone - the calcium-phosphate salt hydroxyapatite. Conditions that increase bone resorption (e.g., pregnancy, lactation, menopause, and osteoporosis) will increase Pb levels in the blood.

Hg<sup>0</sup>, MeHg and Pb are all able to cross the blood-brain barrier, allowing for distribution to and accumulation in the brain. Both Pb and Hg may bypass the blood-brain barrier and enter neurons in the brain through retrograde transport.



Table 4. Summary of data and information on the toxicokinetics and of Pb, Hg<sup>0</sup> and MeHg.

Toxicokinetics	Absorption, Distribution, Biotransformation and Excretion Processes for Mercury Vapour, Methyl Mercury and Lead	Reference
Absorption – Hg	Primary routes of exposure to mercury are inhalation and ingestion. Metallic Hg (Hg <sup>0</sup> ) primarily absorbed via inhalation (over 80% uptake) with rapid diffusion through lungs. Methyl mercury (MeHg) nearly completely absorbed following ingestion of fish (95%). Oral bioavailability of methylmercury may be confounded by dietary factors (e.g., fibre, phytate) and complexation of methylmercury with selenium in the fish.	ATSDR 1999; Counter and Buchanan 2004; Peraza et al 1998
Absorption – Pb	Primary routes of exposure to lead are inhalation and ingestion. Inhaled submicron sized particles of inorganic lead are nearly completely absorbed. Lead absorption across GI tract is higher in children (40–50%) compared to adults (3–10%). Oral bioavailability of lead affected by individual physiological states (e.g., age, fasting, nutritional calcium and iron status) and medium ingested (e.g., particle size, mineralogy, solubility, and lead species).	ATSDR 2007; Peraza et al 1998
Distribution – Hg	Similar distribution of Hg <sup>0</sup> and MeHg in the body, both are taken up by red blood cells and the kidneys are a primary target for accumulation. Both forms of Hg can cross the blood-brain and placental barriers which allows for distribution and accumulation in the brain and fetus. Hg accumulates in hair following exposure to MeHg, Hg <sup>0</sup> , and inorganic Hg (IHg). Correlation between maternal hair mercury and mercury in fetal brain mercury levels. MeHg compounds undergo enterohepatic cycling whereby they are secreted in the bile, partly reabsorbed into the portal circulation and returned to the liver. Gender differences: higher tissue, blood and urine Hg levels reported in female versus male cadavers.  Long-term subclinical exposure of monkeys to methylmercury resulted in accumulation of inorganic Hg in the brain, with astrocytes and microglia containing higher deposits relative to other cell types.	ATSDR 1999; Clarkson 2002; Counter and Buchanan 2004
Distribution – Pb	Inorganic Pb in blood primarily occurs within red blood cells, travels in the blood to the liver, kidneys, lungs, brain, spleen, muscles, and heart. Ultimately distributed to bones (~90% of the total body burden in adults and ~73% in children). Bone lead exchanges with plasma lead and thereby acts as a reservoir to replace lead removed from the blood by excretion. Conditions that increase bone resorption will increase lead in blood (e.g., pregnancy, lactation, menopause, and osteoporosis).  During pregnancy lead can be mobilized from the bones of the mother and transferred to the fetus (i.e., across placenta).	ATSDR 2007

Toxicokinetics	Absorption, Distribution, Biotransformation and Excretion Processes for Mercury Vapour, Methyl Mercury and Lead	Reference
CNS Distribution Pb and Hg	<p>Retrograde transport may allow Pb and Hg to bypass the blood-brain barrier and accumulate in neurons. MeHg binds with cysteine to form a methylmercuric-cysteine complex that is recognized as methionine (an essential amino acid) by amino acid transporting proteins which allows free transport across blood brain barrier; a similar process may occur for neuron uptake.</p> <p>Hg localized to lysosomal dense bodies in neurons after retrograde transport; potentially inert if bound to selenium. IHg sequestered in nerve cells will remain for long periods. Pb uptake into neurons likely occurs via calcium channels.</p>	Aavidson 1994
Brain Storage and Protection – Hg and Pb	<p>Electron microscopic evaluation of human brains aged 70-80 years: Hg reported in cerebellum neurons and Pb in dopaminergic neurons. Protective roles reported for neuronal melanic pigments (“neuromelanins”) identified in neurons from the putamen, premotor cortex, and cerebellum regions of human brains (aged 70–80 years) which serve to remove reactive neurotoxic quinones and chelate and accumulate metals, including mercury and lead. Metals are accumulated with age and without turnover.</p> <p>High neuromelanin/tissue accumulation ratios reported for Pb and Hg – with neuromelanin in cerebellum reported to accumulate “enormous amounts” of Hg and neuromelanin in substantia nigra reported to accumulate high amounts of lead.</p> <p>Autopsy data from Minamata Bay victims exposed to high MeHg concentrations in fish revealed total Hg levels in the brain remained elevated 26 yrs after exposure.</p>	Zecca et al 2008  Zheng et al 2003
Accumulation of Hg in Brain following Prenatal Combined Exposure to MeHg and Hg <sup>0</sup>	<p>Study evaluated interactions between MeHg and Hg<sup>0</sup> exposures in neonatal rats exposed <i>in utero</i> to concentrations designed to model human exposures. At low MeHg doses, Hg<sup>0</sup> exposure increased pup brain Hg levels (opposite was true for high MeHg doses where Hg<sup>0</sup> exposure decreased pup brain Hg levels) compared to either type of Hg alone.</p> <p>Authors suggest the finding of higher Hg levels in fetal brains associated with combined Hg vapor and low dose MeHg exposures would be relevant to human exposures and could indicate elevated neurotoxic risks for fetuses from maternal coexposure to MeHg and Hg<sup>0</sup>.</p>	Ishitobi et al 2010
Bone Storage – Pb	<p>Pb stored in bones can remain for decades and is released to the blood stream as a result of broken bones and/or during pregnancy and breast feeding.</p>	ATSDR 2007
Biotransformation – Hg	<p>Hg<sup>0</sup> undergoes oxidation to Hg<sup>2+</sup> via the hydrogen peroxidase-catalase pathway, primarily in red blood cells but present in most tissues. Hg<sup>0</sup> in the brain is readily converted to Hg<sup>2+</sup> which cannot cross the blood brain barrier.</p> <p>MeHg will bind to the sulfur atom of thiol (-SH) ligands and generally occurs in the body as water-soluble complexes. When MeHg binds with cysteine (thiol containing amino acid) it becomes structurally similar to the essential amino acid methionine, and is able to cross through endothelial cells of the blood brain barrier, disguised as an amino acid via a carrier mediated system.</p> <p>To a lesser extent, MeHg is also converted to Hg<sup>2+</sup> in brain tissue, which may be a function of hydroxyl radical production by cytochrome P-450 reductase. There is evidence in mice that Hg<sup>0</sup> is exhaled following MeHg exposure - MeHg converted to Hg<sup>2+</sup> may be acted on by glutathione reductase to form Hg<sup>0</sup>.</p> <p>Tissue enzyme levels and gut microflora play a role in the demethylation of MeHg to Hg<sup>2+</sup>, which is then excreted in the feces.</p>	ATSDR 1999; Clarkson, 2000
	<p>Methylation of Hg by oral and intestinal microflora has been demonstrated <i>in vitro</i>.</p>	Heintze et al. 1983;

Toxicokinetics	Absorption, Distribution, Biotransformation and Excretion Processes for Mercury Vapour, Methyl Mercury and Lead	Reference
		Rowland et al. 1975; Yannai et al. 1991
Biotransformation – Pb	<p>Pb binds plasma proteins (e.g., albumin and <math>\gamma</math>-globulins) and can complex with sulfhydryl compounds including cysteine and homocysteine, often associated with <math>\delta</math>-aminolevulinic acid dehydratase (ALAD) within the red blood cell.</p> <p>Pb forms an extremely stable complex with potassium and is able to replace calcium in the primary crystalline matrix of bone, the calcium-phosphate salt hydroxyapatite.</p>	ATSDR 2007
Elimination/Excretion – Hg	<p>Hg<sup>0</sup> eliminated unchanged in expired air, sweat, saliva and as Hg<sup>2+</sup> in urine and feces.</p> <p>MeHg compounds undergo extensive enterohepatic cycling before being excreted (predominantly) as Hg<sup>2+</sup> in the feces, and to a lesser extent, urine. MeHg and IHg can also be eliminated through breast milk (approximately 16% of Hg in breast milk is MeHg).</p> <p>Younger rats exposed to IHg and MeHg demonstrated significantly higher Hg retention compared to older rats.</p>	ATSDR 1999; Counter 2004; Clarkson 2002
Elimination/Excretion – Pb	<p>Lead is excreted primarily in urine and feces regardless of the route of exposure. Minor routes of excretion include sweat, saliva, hair, and nails.</p> <p>Elimination half-lives of ~30 days and 27 years have been reported for inorganic lead in blood and bone, respectively.</p>	ATSDR 2007
Elimination – Pb and Hg	<p>Both Pb and Hg bind the cysteine sulfhydryl group (-SH) of glutathione – heavy metal-glutathione conjugate – subsequently metabolized and excreted in bile and urine – glutathione is the body's natural chelator and major mechanism for heavy metal detoxification and elimination</p> <p>Inorganic mercury, MeHg, and lead can all be excreted in breast milk.</p>	Rose et al 2008

Table 5. Summary of data and information on the toxicity and toxicodynamics and of Pb, Hg<sup>0</sup> and MeHg.

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
Lethality and Renal Toxicity – Pb and Hg individually and <b>combined</b>	Authors developed rapid testing procedure for acute lethality and renal toxicity of combinations of IHg and Pb. Tested effect of low dose Pb or Hg on the LD50 values for Hg or Pb, respectively. Adult rats were exposed by iv injection to Pb followed immediately by Hg. The LD50 for Pb decreased ~50-fold in the presence of a Hg dose of ~LD20 (i.e., increased lethality of Pb in presence of moderate Hg dose) while the LD50 value for Hg increased ~50-fold in presence of a Pb dose of <LD1 (i.e., low dose of Pb reduced lethality and was protective of Hg toxicity). Combined exposure to ~LD1 doses of Pb and Hg produce a synergistic toxic response in the kidneys (renal tubular necrosis) compared to lesions caused by single exposures to LD1 doses.	Schubert et al 1978; ATSDR 2006
Central and Peripheral Nervous Activity Pb and Hg (individually and <b>combined</b> )	<p>Acute, <i>in vivo</i> study in rats evaluating spontaneous and stimulus-evoked cortical electrical activity and stimulus-evoked peripheral electrical activity in response to high dose exposures to inorganic Pb and Hg (tested individually and in combination).</p> <p>Hg decreased frequency of spontaneous-evoked cortical electrical activity. Previous studies demonstrated Hg decreases choline acetyltransferase activity and binding of acetylcholine to the muscarinic receptor, which would reduce cortex activation. Hg increased stimulus-evoked cortical electrical activity. Previous studies reported Hg<sup>+</sup> inhibited glial uptake of glutamate and increased cortical excitation. To a lesser extent Pb also increased stimulus-evoked cortical electrical activity. Combined low dose Hg and Pb exposures resulted in a synergistic response.</p> <p>Hg decreased stimulus-evoked peripheral electrical activity (conduction velocity of tail nerve and tail nerve action potential). To a lesser extent Pb also decreased peripheral electrical. Combined Hg and Pb exposures resulted in a greater response compared to each individually. Previous reports suggest neuron and axon damage by Hg and Pb occurs via effects on cationic channels in membranes, which could account for the decrease nerve action potential.</p> <p>Hg exposure resulted in more significant effects on central and peripheral nervous activity following acute exposures. Synergistic effects on these endpoints observed following exposure to Hg and Pb may have been the result of blood brain barrier damage by Pb.</p>	Papp et al 2006
Auditory and Visual Evoked Potentials Pb and Hg	<p>Sensory evoked potentials used to detect/evaluate subclinical CNS effects. Study reported both Pb and Hg produced delays in brainstem auditory evoked potentials in workers with no neurological symptoms. Effects produced following long-term occupational exposures to either lead (mean 9.3 yrs, PbB 47.5 µg/dL) or mercury (mean 11.7 yrs, UHg 325 µg/g). Study also cites previous work reporting effects on somatosensory and visual evoked potentials following occupational Pb and Hg exposures.</p> <p>Review paper – reported delays in auditory and visual evoked potentials in children following neonatal exposure to MeHg (maternal hair-mercury concentrations &gt;10 µg/g)</p>	Discalzi et al 1993  Trask and Kosofsky 2000
Behavioural Changes Prenatal Coexposure to Hg <sup>0</sup> and MeHg <b>combined</b>	Behavioral function was tested in adult rats exposed <i>in utero</i> to MeHg (2 mg/kg/day during days 6-9 of gestation) and/or Hg <sup>0</sup> (1.8 mg/m <sup>3</sup> air for 1.5 h per day during gestation days 14-19). MeHg exposure alone did not alter behaviour, however Hg <sup>0</sup> plus MeHg significantly aggravated changes in behavioural function associated with Hg <sup>0</sup> , including effects on spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning. Authors suggest that Hg <sup>0</sup> potentiated MeHg neurotoxicity. Hg <sup>0</sup> levels in the study approximated values associated with 20 amalgam fillings.	Fredriksson et al 1996

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
Calcium Homeostasis Pb and Hg (individually and <b>combined</b> )	<p><i>In vitro</i> study on rat tissue brain microsomal membrane. Pb and Hg, tested individually, each inhibited inositol 1,4,5-triphosphate (IP3) mediated Ca<sup>2+</sup> release from isolated rat brain microsomes and inhibited re-uptake of IP3 released Ca<sup>2+</sup> into microsomes. IP3 mediates the transient release of Ca<sup>2+</sup> from intracellular stores. Ca<sup>2+</sup> is a neuronal cell messenger and plays a role in regulation of cellular metabolic processes. Alterations in Ca<sup>2+</sup> homeostasis can impair cell signaling leading to neuronal dysfunction and cell death.</p> <p>Several review studies have reported effects on neuronal signaling related to changes in Ca<sup>2+</sup> homeostasis as a result of Hg and Pb exposures. . Effects on Ca<sup>2+</sup> homeostasis are involved in neurotoxicity – cell signaling in neural cells affected by lower Hg and Pb concentrations than those required for overt cytotoxicity. These changes in cell signaling can compromise regulation of critical cell activities, including responses to hormones, neurotransmitters and growth factors.</p> <p>Peripheral synaptic systems:                      Pb and Hg both interfered with Ca-mediated synaptic transmitter release. Individually, Pb and to a lesser extent Hg, blocked depolarization-evoked transmitter release. Combined exposures to Pb and Hg increased the rate of spontaneous transmitter release.</p> <p>Rat brain synaptosomes:                      Pb blocked Ca<sup>2+</sup> channels on nerve terminals and inhibited the release of acetylcholine from stimulated nerve terminals. By comparison, Hg was relatively ineffective at blocking nerve terminal Ca<sup>2+</sup> channels.</p> <p>Pb and Hg affected spontaneous neurotransmitter release: Pb increased ionized Ca<sup>2+</sup> levels in nerve terminals with parallel increase in efflux of Ca<sup>2+</sup> from rat brain synaptosomes; different mechanism for Hg which may increase intracellular Na concentration by inhibiting Na/K ATPase or increasing permeability of plasma membrane to Na.</p> <p>Both Hg and Pb altered calmodulin activity which affects Ca-ATPase, a calmodulin dependent enzyme that pumps Ca from the cytosol into organelles such as mitochondria or out of the cell – altered Ca homeostasis by Pb and Hg attributed to significant inhibition of Ca-ATPase</p>	Pentyale et al. 2010  Wennberg 1994; Manzo et al 1995; Denny and Atchison 1996; Atchison 2003; Florea & Busseberg 2006; Johansson et al 2007 Gulati et al 2010  Foulke 1987
Oxidative Stress Associated Pb and Hg (individually)	Recent reviews on Hg and Pb toxicity have emphasized the effects of these metals on intracellular mechanisms designed to protect cells from oxidative stress. Pb and Hg are both capable of inducing oxidative stress following the generation of reactive oxygen species (ROS) and nitrogen species, thereby altering pro-oxidant and antioxidant homeostasis which can result in neurotoxicity. Hg has a high affinity for thiol (-SH) groups of the antioxidant glutathione, which provides intracellular defense against Hg-induced neurotoxicity. Neuron cell vulnerability to Hg may be due to lower levels of glutathione which predisposes the cell to ROS damage and activates apoptosis-signalling pathways. Pb has been shown to alter the activity of glutathione and antioxidant enzymes.	Flora et al 2008; Guzzi & LaPort 2008

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
	Mechanisms reported for neurobehavioural effects of MeHg in <i>in vivo</i> and <i>in vitro</i> studies include adverse effects on neurotransmitter systems, induction of oxidative stress and cell death and disruption of microtubules and intracellular Ca homeostasis. Study provides human and rat infant or neonatal Hg brain LOELs for neurodevelopmental effects.	Johansson et al 2007
Metal Toxicity and Autism Pb and Hg	Both Pb and Hg induce oxidative stress by depleting glutathione – major intracellular antioxidant. Each inhibits enzymes responsible for heme biosynthesis – Pb affecting δ-aminolevulinic acid dehydratase (ALAD) and Hg affecting coproporphyrin oxidase (CPOX) – which results in accumulation and excretion of abnormal porphyrin in urine. ALAD and CPOX polymorphisms are associated with increased blood levels of Pb and Hg, respectively. The frequency of polymorphisms of CPOX and ALAD as well as plasma glutathione levels were examined in 242 autistic children and 75 control children. An increased frequency of ALAD polymorphism and significantly lower plasma glutathione levels were reported for autistic children. The authors suggest the study results could indicate that children with the ALAD polymorphism may have increased susceptibility to lead toxicity during critical windows of prenatal and postnatal development which may contribute, in part, to an increased risk of developing autism.  Authors associated Hg-induced glutathione depletion, cellular mitochondrial damage, increased lipid peroxidation in protein, and DNA oxidation in the brain as major factors in conditions such as autism and Parkinson's disease.	Rose et al 2008          Gulati et al 2010
Metal Induced Apoptosis Pb, MeHg and Hg <sup>0</sup> (individually)	Review of metal-induced apoptosis - no one unifying mechanism. Pb-induced apoptosis may be related to calcium overload and enhanced by glutamate – greatest effects during later stages of brain development, during pruning of synapses. MeHg initiates lipid peroxidation and alterations in cell membranes. Hg <sup>0</sup> and MeHg damage microtubule assembly in the brain. Hg has a high affinity for thiol groups and Hg-induced apoptosis may result from binding to and altering a variety of enzyme systems.  Apoptosis is regulated during neural development by processes involving growth factors, cytokines, and neurotransmitters - interference with these processes could trigger degeneration of necessary, or promote survival of unnecessary, cells.	Rana 2008; Costa et al 2004
Neurotransmission Pb, MeHg and Hg (individually)	Study reviewed the effects of Hg and Pb on amino acid neurotransmitters in the CNS: glutamate, major excitatory neurotransmitter and γ-aminobutyric acid (GABA), major inhibitory neurotransmitter. Both Pb and Hg affected GABA by altering GABAergic system: MeHg decreased GABA-induced currents; IHg increased GABA-induced currents, and; Pb decreased GABA transport and altered GABA channel properties. Both Pb and Hg affected glutamate levels by altering properties of the N-methyl D-aspartate (NMDA) glutamate receptor and decreasing the ability of astrocytes to clear extracellular glutamate.  Reviews of neurochemical studies suggest similar effects of Pb, MeHg and IHg on parameters of neurotransmission, including: <ul style="list-style-type: none"> <li>- effects on catecholaminergic system – dopamine (Pb, MeHg, IHg)</li> <li>- decreased norepinephrine (Pb, IHg)</li> <li>- decreased GABA levels (Pb, MeHg)</li> <li>- decreased glutamate decarboxylase activity (Pb, MeHg, IHg);</li> <li>- increased monoamine oxidase activity (Pb, MeHg)</li> </ul> Within the nervous system Pb can affect cell metabolism, membranes, nerve cell metabolism and nerve transmission, myelin formation, and breakdown the blood-brain barrier. Hg also affects nerve transmission and nerve cell metabolism, targeting the cerebellum.	Fitsanakis and Aschner 2005          Wennberg 1994; Costa 1988

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
	<p>Glutamate itself is considered a neurotoxin/excitotoxin – involved in the pathophysiology of neurodegenerative disorders. Cytotoxicity from excessive glutamate is largely due to an increase in intracellular ROS – initial ROS production resulting from glutathione depletion with a much greater subsequent burst of ROS production attributed to mitochondrial electron transport chain. NMDA receptor antagonists can prevent glutamate neurotoxicity.</p> <p>Pb affects Ca<sup>2+</sup>-dependent proteins and neurotransmitters receptors – in particular protein kinase C and the NMDA subtype of glutamate receptor, both of which are involved in learning and cognitive functions and may interact significantly to account for altered functions.</p> <p>MeHg is form of Hg most studied wrt nervous system. Basis for harmful effects of Hg compounds attributed to high thiol reactivity. Mechanisms of Hg toxicity include inhibition of protein synthesis, microtubule disruption, increase of intracellular Ca<sup>2+</sup> with disturbance of neurotransmitter function, oxidative stress and triggering of excitotoxicity mechanisms. Hg damages CNS development, altering the structure and functionality of the nervous system.</p>	Segura-Aguilar & ., Kostrzewa 2004
Neuroimmunotoxicity Pb and Hg (individually)	<p>Study reported the detection and quantification of autoantibodies against the nervous system following exposure to Pb or Hg. Total of 51 workers evaluated - 27 exposed to lead in battery manufacturing (median PbB of 35 ug/dL) and 24 exposed to mercury in neon light manufacturing (median Hg in urine of 100 ug/g creatinine) were compared against unexposed controls.</p> <p>Significant increases reported in IgG and IgM autoantibodies against neurotypic and gliotypic proteins. As a result the authors deduced the following neuroimmunotoxic effects of occupational exposure to Pb or Hg (significant responses observed for both groups):</p> <ul style="list-style-type: none"> <li>- persistent axonal insult -&gt; increased anti-NF (neuroaxonal filament) autoantibodies</li> <li>- involvement of the CNS and astrocytes -&gt; increased anti-GFAP (astrocyte glial fibrillary acid protein) autoantibodies, and</li> <li>- secondary demyelination -&gt; increased anti-MBP (myelin sheath basic protein) autoantibodies.</li> </ul> <p>These autoantibodies were not expected to interact with surface antigens but may penetrate normal cells (e.g., neurons) resulting in degeneration and apoptosis.</p>	Shamy et al 2007
	<p>Review of humoral assessment of neurotoxicity and autoimmune mechanisms. Human and animal studies have reported antibodies to the nervous system as a result of exposure to Pb (blood) or Hg (urine). These exposures correlated with subclinical deficits in exposed workers and evidence of histopathological damage to CNS and PNS in rats.</p> <p>Autoantibodies (IgG primarily) to neuronal cytoskeletal proteins, NF and MBP were detected in male workers and in rats and mice exposed to Pb or Hg. Sera IgG against NF and MBP were significantly correlated with blood Pb or urinary Hg concentrations.</p> <p>Measurements of these autoantibodies could be used as markers for the neurotoxic effects of Pb and Hg.</p>	El-Fawal et al 1999

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
Astrocytes Pb and Hg (individually)	<p>Astroglia - first cells of brain parenchyma to encounter metals crossing the BBB, designed to concentrate metals for metabolic use. Metal transport/carrier proteins and metal dependent brain enzymes localized in astroglia possess physiologic properties that protect cytoplasm from metal-induced oxidative damage, including: high cytosolic levels of metallothionein which binds heavy metals through thiol groups (metallothionein levels in adjacent neurons are low); high cytosolic levels of glutathione - antioxidant which neutralizes free radicals and undergoes nonenzymatic conjugation with lead and mercury.</p> <p>Pb effects on cultured astrocytes: dose-dependent inhibition of glutamate synthetase (enzyme is key to glutamate and ammonia metabolism in the brain); interferes with Ca homeostatic mechanisms - binds and stimulates Ca-regulated enzymes including protein kinase C (PKC) and calmodulin.</p> <p>Relative level of accumulation of Hg species in CNS: MeHg &gt; Hg vapour &gt; IHg. Humans: MeHg accumulated in astroglia and microglia; Hg vapour accumulated in motor neurons. Monkeys: IHg accumulated in astroglia and microglia - support buffering role for astrocytes. Rats: MeHg and IHg accumulated in neurons with less in glial cells Squirrels: Hg vapour accumulated in neurons and astroglia</p>	Tiffany-Castiglioni and Qian 2001
	<p>Astrocytes play important role in CNS development, maintain neuronal homeostasis, regulate neuronal survival and function in neurotransmitter metabolism (e.g., removal of glutamate and GABA from the synaptic cleft). Hg is preferentially accumulated by astrocytes - astrocytic swelling – effects on neuronal homeostasis.</p> <p>Astrocytes provide glutathione precursors to neurons – which are vital in the defense of the neuron against free radical damage. MeHg inhibits glutamate uptake by astrocytes by inhibition of specific glutamate transporter → diminished glutathione levels in astrocytes → increased extracellular glutamate to levels that are toxic to neurons. MeHg induced ROS production exacerbates inhibition of glutamate transport - effects on astrocytes include reduced availability of precursors for glutathione synthesis in neuron cells – neurons more susceptible to MeHg induced oxidative stress.</p> <p>In the adult brain, astrocytes accumulate MeHg which interferes with glutamate uptake resulting in high levels of extracellular glutamate which can damage adjacent neurons. MeHg-induced damage to astrocytes during brain development is associated with effects on astrocytic guidance of neuronal migration. Disruption of the blood brain barrier by Pb likely mediated through injury to astrocytes.</p> <p>Review of neurotoxicology studies involving Pb and Hg. Astrocytes (glial cells) primarily involved in neuroprotection and/or repair – supportive satellite cells for neurons. Hg and Pb exposure can result in oxidative stress and neuroinflammation. Neuronal damage induced by ROS leads to glial reactivity - reactive glial cells increase ROS. ROS-induced protein oxidation promotes aggregation of synuclein in Parkinsons disease and insoluble beta-amyloid in Alzheimers disease -&gt; neuronal damage. Authors suggest Hg and Pb are associated with the major pathways and signs of neurodegeneration associated with Alzheimers and Parkinsons disease.</p>	Vitarella et al. 1996  Allen et al 2002  Costa et al 2004  Monnet-Tschudi et al 2006
Brain Barriers:	Both Pb and Hg accumulate in the blood-cerebrospinal fluid barrier (BCB) and BBB.	Zheng et al 2003;



Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
Blood-CSF Barrier (BCB) Blood Brain Barrier (BBB)	<p>Hg reported to be a general toxicant (direct damage) to the choroid plexus (BCB). The BCB uptake mechanism for Hg is not determined but may be similar to BBB (i.e., protein transport).</p> <p>Pb considered a selective toxicant to choroid plexus - impairs plexus regulatory pathways critical to brain development and function including: reduction in transthyretin (TTR) production and secretion (exclusively produced and secreted by choroid plexus) and activation of choroid plexus protein kinase C, which may contribute to Pb effects on TTR. The reduction in TTR production and secretion by the choroid plexus may impair transport of thyroid hormones from the blood to cerebral compartment which might account for loss of cognitive abilities associated with Pb exposure.</p>	Zheng 2001
Effects of Pb on Nervous System	<p>Pb can affect the nervous system by mimicking calcium action and/or disrupting calcium homeostasis which affects many cell-signaling pathways. In particular, Pb activates protein kinase C (PKC) which is involved in many processes important for synaptic transmission. The <math>\gamma</math>-isoform of PKC may be a target for lead neurotoxicity, this calcium-dependent form of PKC is neuron-specific and involved in long-term potentiation, spatial learning, and memory processes.</p> <p>The formation of the AP-1 transcriptional regulatory complex, which regulates the expression of the glial fibrillary acidic protein (GFAP) gene, is induced by PKC. Studies in rats have demonstrated that Pb can alter the expression of the GFAP gene, which is induced during periods of reactive astrocytic gliosis. The effects of Pb exposure are long-lasting if exposure occurred during brain developmental when PKC is present in the cytosol, rather than membrane bound as it occurs in mature brain microvessels.</p> <p>Pb-induced activation of PKC may impair brain microvascular formation and function, and at high Pb levels, account for defects in the blood-brain barrier. The particular sensitivity of the developing brain to Pb is likely associated with the immaturity of the blood-brain barrier and the accumulation of Pb in astroglia which sequester Pb.</p>	ATSDR 2007
Developmental Neurotoxicity MeHg and Pb	<p>Regulation of gene expression at the mRNA level was evaluated in cultured rat cerebellar cells to identify which cell types and which stages of neuronal development were affected by exposure to low concentrations of MeHg or Pb.</p> <p>Gene expression of markers of different developmental stages were studied, including the proliferation of progenitor cells during early developmental (neural precursor cells nestin and SRY-box containing gene 10 [Sox10]), neuronal differentiation (NF-68 and NF-200), functional maturation (N-methyl D-aspartate glutamate [NMDA] and gamma-amino butyric acid [GABAA] receptors), and astrocytic proliferation and differentiation (glial fibrillary acidic protein [GFAP] and S100<math>\beta</math>).</p> <p>Markers of neural precursor cell or glial proliferation (Sox10 and nestin) were both downregulated (Sox10) and upregulated (nestin) by low</p>	Hogberg et al 2010

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
	<p>concentrations of MeHg, whereas Pb did not significantly affect expression of either. An increase in nestin mRNA expression could indicate either neural precursor cell or glial proliferation as a result of MeHg exposure. Markers of the neuronal cytoskeleton proteins (NF-68 and NF-200) were downregulated by MeHg but not affected by Pb. Markers important to neuronal functional maturation - subunits of the main neuronal excitatory receptor (NMDA-R) and inhibitory (GABA<sub>A</sub>) receptor - were significantly decreased at the highest MeHg concentration tested, an effect that was attributed to neuronal cell death. Pb significantly downregulated GFAP, a marker for mature astrocytes.</p> <p>mRNA levels for astrocytic and neuronal markers of developmental neurotoxicity are induced by different mechanism of toxicity on neuronal and glial cells. Low concentrations of MeHg downregulated mRNA level for neuronal markers but did not change mRNA expression of astrocytic markers. Low concentrations of Pb decreased mRNA expression of astrocytic marker with neuronal markers less affected.</p> <p>Different genes involved in key developmental processes of neuronal and glial cells were affected by MeHg and Pb, however, combined exposure could exacerbate overall neurodevelopmental toxicity.</p>	
	<p>Review of studies on behavioral alterations associated with Pb and MeHg.</p> <p>Pb accumulates in hippocampus region of brain which is sensitive to hypoxia. Damage in this region may be secondary to metabolic changes. Evidence that Pb may alter catecholaminergic, particular dopaminergic, systems. Studies in monkeys suggest behavioral effects at blood Pb concentrations observed in children.</p> <p>MeHg considered a potent cytotoxin - prenatal exposure reduced myelination, delayed neuronal migration and resulted in loss of neurons in the cortex and cerebellum -&gt; permanent effects on learning and memory. Similar to Pb, MeHg affects catecholaminergic system - altered pharmacological sensitivity.</p>	Annau and Cuomo 1988
	<p>Developing CNS more vulnerable to injury from MeHg and Pb. Lack of fully developed blood-brain until ~ 6 months allows greater access to the developing brain. MeHg reported to interfere with neuron proliferation and migration during CNS development. Pb reported to interfere with synaptogenesis – the number of synaptic connections between neurons peaks around age two and is then trimmed back by ~ half.</p>	Rodier 1995
	<p>Review of developing neuropathology of MeHg and Pb – widely distributed environmental pollutants considered to be major neurotoxicants – low level persistent exposures linked to subtle behavioral effects. Estimates that environmental exposures result in learning, neurodevelopmental, or behavioral disabilities in up to 12 million children in the US.</p> <p>Hg and Pb both:</p> <ul style="list-style-type: none"> <li>- produce more severe effects in fetal and young brain compared to adult brain;</li> <li>- accumulate in and damage astrocytes;</li> <li>- damage cerebellum; and</li> <li>- affect glutamatergic function.</li> </ul> <p>Motor movements and, to an extent, speech, learning, emotions, and attention are associated with the cerebellum. Hg exposure affects neuronal migration during early brain development, possibly through interaction with the microtubular element of the cytoskeleton. Pb exposure during later stages of brain development affects neural differentiation, synaptogenesis and myelin formation.</p>	Costa et al 2004
	<p>Critical Processes in Nervous System Development Affected by MeHg and/or Pb:</p>	Rodier 1995; Rice

Toxicodynamics	Similar Effects of Pb and Hg (combined and/or separate exposures) - Emphasis on Neurotoxicity and Mode of Action	Reference
	<ul style="list-style-type: none"> <li>- Neural Proliferation (MeHg) - disruption of proliferation can also affect migration</li> <li>- Neuronal Migration (MeHg) – possible result of hindering repolymerization of microtubules</li> <li>- Neuronal Differentiation (MeHg and Pb)</li> <li>- Synaptogenesis (MeHg and Pb)</li> <li>- Myelination and Gliogenesis (Pb) - astrocytes and microglia become reactive after insults resulting in reactive gliosis – dependant on timing of insult, precedes major phase of proliferation and differentiation of astrocytes</li> <li>- Apoptosis (MeHg and Pb) - tightly regulated to target removal cells without inflammatory response, both Pb and MeHg shift the balance of neurotrophic signals regulating apoptosis – increase or decrease cell number in affected regions of the NS. Unregulated apoptosis -&gt; neurodegeneration -&gt; neurodegenerative diseases.</li> </ul>	and Barone 2000; Mendola et al 2002; Costa et al 2004
Long-Latency Delayed Neurotoxicity MeHg	<p>Definitive evidence of delayed neurotoxicity in monkeys for deficits in fine motor control during middle age (13 years) following low level exposure to MeHg (50 µg/kg/d) from birth through puberty (7 years) resulting in steady-state blood Hg concentrations of 0.7 ppm, with no evidence of neurological effects during the period of exposure or for 6 years after exposure (i.e., until “middle age” of 13 years).</p> <p>Possible that Hg stored in CNS continued to exert toxicity – brain retained significant amounts of Hg eight months after cessation of MeHg exposure. IHg previously reported to concentrate in reactive glia following chronic low level exposures to MeHg or Hg<sup>2+</sup>. Mechanisms of effects may include – continued toxic influence as a result of Hg storage; premature death of damaged cells, accelerated aging of cells required to compensate for damaged or missing cells.</p>	Rice 1996

Hg sequestered in nerve cells will remain for long periods, although Hg localized to lysosomal dense bodies in neurons after retrograde transport could potentially be inert if bound to selenium. Hg<sup>0</sup> undergoes oxidation to Hg<sup>2+</sup> via the hydrogen peroxidase-catalase pathway, which is primarily within red blood cells, but is present in most tissues, including the brain, where Hg<sup>0</sup> is readily converted to Hg<sup>2+</sup> (IHg) which cannot cross the blood brain barrier. To a lesser extent, MeHg is also converted to Hg<sup>2+</sup> in brain tissue, which may be a function of hydroxyl radical production by cytochrome P-450 reductase. There is evidence in mice that Hg<sup>0</sup> is exhaled following MeHg exposure which would suggest MeHg converted to Hg<sup>2+</sup> may be acted on by glutathione reductase to form Hg<sup>0</sup>.

MeHg will bind thiol ligands and generally occurs in the body as water-soluble complexes. When MeHg binds with cysteine (thiol containing amino acid) it forms a methylmercuric-cysteinyl, structurally similar to the essential amino acid methionine, and is able to cross through endothelial cells of the blood brain barrier disguised as an amino acid via a carrier mediated system. A similar process may occur for neuron uptake of MeHg. Pb is accumulated by neurons, likely via Ca channels. An accumulation of IHg was reported in the brain (predominately astrocytes and microglial cells) of monkeys following chronic subclinical oral exposure to MeHg.

Hg was reported in cerebellum neurons and Pb in dopaminergic neurons in human brains (70-80 year old) examined on autopsy by scanning electron microscope. Neuronal melanic pigments (“neuromelanins”) were identified in neurons from the putamen, premotor cortex, and cerebellum regions of the brain and likely protect the brain from heavy metals by chelating and accumulating these metals over time and without turnover. Autopsy data suggests that Hg and Pb were accumulated by neuromelanin in the cerebellum and substantia nigra, respectively.

Hg<sup>0</sup>, MeHg and Pb are all able to cross placental barriers, allowing for distribution to the developing fetus. During pregnancy Pb can be mobilized from the bones of the mother and transferred to the fetus. Measurements of maternal hair Hg levels have been demonstrated to correspond to fetal brain Hg levels. Interactions between MeHg and Hg<sup>0</sup> exposures were recently reported in neonatal rats exposed *in utero* to low MeHg combined with Hg<sup>0</sup> at concentrations designed to model human exposures; combined

MeHg and Hg<sup>0</sup> exposures significantly increased pup brain Hg levels (in contrast to either type of Hg alone) suggesting elevated exposure and potential neurotoxic risks to fetuses from maternal coexposure to MeHg and Hg<sup>0</sup>. Maternal IHg, MeHg and Pb are all eliminated via breast milk.

Both Pb and Hg bind the cysteine sulfhydryl group (-SH) of glutathione to form a heavy metal-glutathione conjugate which is subsequently metabolized and excreted in bile and urine. Glutathione is the body's natural chelator, responsible for heavy metal detoxification and elimination. Tissue enzyme levels and gut microflora play a role in the demethylation of MeHg to Hg<sup>2+</sup>, which is then excreted in the feces. However, MeHg compounds can undergo extensive enterohepatic cycling before being excreted (predominantly) as IHg in the feces and, to a lesser extent, urine. Hg<sup>0</sup> can be eliminated unchanged in expired air, sweat, saliva and as IHg in urine (predominately) and feces. Pb is excreted primarily in urine and feces regardless of the route of exposure. Minor routes of Pb excretion include sweat, saliva, hair, and nails.

Elimination half-lives of ~30 days and 27 years have been reported for inorganic lead in blood and bone, respectively. Age was demonstrated to play a role in the elimination of Hg in rats, with younger rats exposed to IHg and MeHg demonstrating significantly higher retention compared to older rats. Autopsy data from Minamata Bay victims exposed to high MeHg concentrations in fish revealed total Hg levels in the brain remained elevated 26 yrs after exposure.

Overall, the uptake, distribution and persistence of Hg<sup>0</sup>, MeHg and Pb allows for potential joint action in the body as a result of increasing target dose, particularly in the brain and kidneys of the exposed individual (and developing fetus), which can accumulate these heavy metals over time. Metabolic pathways present in the blood and brain can result in the oxidation of Hg<sup>0</sup> to IHg and the conversion of MeHg to IHg. Therefore it was considered appropriate to evaluate internal responses to IHg in addition to Hg<sup>0</sup> and MeHg. Mechanisms to protect the body from heavy metals are present, including glutathione conjugation and removal or inert storage within neuromelanins of the brain. Dietary factors, including adequate levels of selenium, fibre, phytate, calcium and iron, can also play a role in the protection against Hg and Pb toxicity.

## 2.5.2 Toxicodynamics

A review of literature describing the cellular and molecular events leading to a toxic response following exposure to Hg<sup>0</sup>, MeHg and Pb is presented in Table 5. This information is further summarized and discussed below.

Although every attempt was made to identify recent literature describing the potential for joint toxicity for Hg<sup>0</sup>, MeHg and Pb, the summary below does not provide an exhaustive review of the available literature. A number of the papers identified were reviews of other work, no attempt was made to identify and review the primary literature summarized in these reviews. Terms considered in various combinations for the literature search that resulted in the papers reviewed below include: combined, exposure, interaction, lead, methylmercury, mercury, mercury vapour, mixture, mode of action, neurotoxicity, and toxicity.

It is noted that time did not permit an in-depth review and discussion of papers involving non-neurological endpoints. The kidney, in particular, represents another target for the accumulation of Pb and Hg, however, the potential for joint effects within this organ system (i.e., papers obtained from a search for *combined AND mercury AND lead AND kidney AND toxicity*) were not included in the current discussion.

### 2.5.2.1 Lethality and renal toxicity

A rapid acute toxicity testing procedure in rats was developed by Schubert et al (1978) to examine the acute lethality and renal toxicity of combinations of IHg and Pb. The effects of low dose exposure to Pb or Hg on the LD50 values for Hg or Pb, respectively, were determined. Adult rats were exposed by iv injection to Pb followed immediately by Hg. The LD50 for Pb was decreased ~50-fold in the presence of a Hg dose of ~LD20 (i.e., increased lethality of Pb in the presence of moderate Hg dose) while the LD50 value for Hg increased ~50-fold in presence of a Pb dose of <LD1 (i.e., low dose of Pb reduced lethality and was protective of Hg toxicity). Combined exposure to ~LD1 doses of Pb and Hg produced what was reported as a synergistic toxic response in the kidneys (renal tubular necrosis) compared to the lesions caused by single exposures to LD1 doses.

### **2.5.2.2 Central and peripheral nervous activity**

An acute, *in vivo* study in rats evaluated spontaneous and stimulus-evoked *cortical* electrical activity and stimulus-evoked *peripheral* electrical activity in response to high dose exposures to inorganic Pb and Hg (tested individually and in combination) (Papp et al 2006). In this study Hg decreased the frequency of spontaneous-evoked *cortical* electrical activity. Previous studies had demonstrated that Hg decreased choline acetyltransferase activity and binding of acetylcholine to the muscarinic receptor, which would reduce cortex activation. In this study Hg increased stimulus-evoked *cortical* electrical activity. Previous studies reported Hg<sup>+</sup> inhibited glial uptake of glutamate and increased cortical excitation. To a lesser extent Pb also increased stimulus-evoked *cortical* electrical activity in this study. Combined low dose Hg and Pb exposures produced a synergistic increase in stimulus-evoked *cortical* electrical activity.

Hg, and to a lesser extent Pb, decreased stimulus-evoked *peripheral* electrical activity (conduction velocity of tail nerve and tail nerve action potential). Combined low dose Hg and Pb exposures resulted in a greater response compared to each individually. Previous reports suggested neuron and axon damage by Hg and Pb occurs via effects on cationic channels in membranes, which could account for the decrease nerve action potential. Overall Hg exposure resulted in more significant effects on *central* and *peripheral* nervous activity following acute exposures. The authors speculated that synergistic effects on these endpoints observed following exposure to Hg and Pb may have been the result of blood brain barrier damage by Pb (Papp et al 2006).

### **2.5.2.3 Auditory and visual evoked potentials**

Sensory evoked potentials were used to detect/evaluate subclinical CNS effects following chronic occupational exposure to Pb or Hg (Discalzi et al 1993). In this study delays in brainstem auditory evoked potentials were reported in workers with no neurological symptoms that had been occupationally exposed to either lead (mean 9.3 yrs, PbB 47.5 µg/dL) or mercury (mean 11.7 yrs, UHg 325 µg/g). The study cited previous work reporting effects on somatosensory and visual evoked potentials following occupational Pb and Hg exposures.

In a review of developmental considerations of neurotoxic exposures, Trask and Kosofsky (2000) reported delays in auditory and visual evoked potentials in children following neonatal exposure to MeHg correlating to maternal hair-mercury concentrations below 10 µg/g.

#### **2.5.2.4 Behavioural changes: prenatal coexposure to Hg<sup>0</sup> and MeHg**

Behavioral function was tested in adult rats exposed *in utero* to MeHg (2 mg/kg/day during days 6-9 of gestation) and/or Hg<sup>0</sup> (1.8 mg/m<sup>3</sup> air for 1.5 h per day during gestation days 14-19) (Fredriksson et al 1996). MeHg exposure alone did not alter behaviour, however Hg<sup>0</sup> plus MeHg significantly aggravated changes in behavioural function associated with Hg<sup>0</sup>, including effects on spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning. The study authors suggested that Hg<sup>0</sup> potentiated MeHg neurotoxicity. Hg<sup>0</sup> levels in the study approximated values associated with 20 amalgam fillings.

#### **2.5.2.5 Calcium homeostasis**

Numerous review studies have reported effects on neuronal signaling related to changes in Ca<sup>2+</sup> homeostasis as a result of Hg and Pb exposures (Wennberg 1994; Manzo et al 1995; Denny and Atchison 1996; Atchison 2003; Florea and Busselberg 2006; Johansson et al 2007; and Gulati et al 2010). Alterations in Ca<sup>2+</sup> homeostasis are associated with changes in cell signaling in neural cells. These changes in cell signaling are observed at lower Hg and Pb concentrations than required for overt cytotoxicity and can compromise regulation of critical cell activities, including responses to hormones, neurotransmitters and growth factors.

In a recent *in vitro* study on rat tissue brain microsomal membrane, Pb and Hg, tested individually, each inhibited inositol 1,4,5-triphosphate (IP3) mediated Ca<sup>2+</sup> release from isolated rat brain microsomes and inhibited re-uptake of IP3 released Ca<sup>2+</sup> into microsomes (Pentyale et al. 2010). IP3 mediates the transient release of Ca<sup>2+</sup> from intracellular stores. As a neuronal cell messenger, Ca<sup>2+</sup> plays a role in regulation of cellular metabolic processes. Alterations in Ca<sup>2+</sup> homeostasis can impair cell signaling leading to neuronal dysfunction and cell death.



In a review of *in vitro* studies in rat brain synaptosomes, Foulke (1987) reported that Pb and Hg both interfered with Ca-mediated synaptic transmitter release. Pb blocked Ca<sup>2+</sup> channels on nerve terminals and inhibited the release of acetylcholine from stimulated nerve terminals. By comparison, Hg was relatively ineffective at blocking nerve terminal Ca<sup>2+</sup> channels. Pb increased ionized Ca<sup>2+</sup> levels in nerve terminals with a parallel increase in efflux of Ca<sup>2+</sup> from rat brain synaptosomes. A different mechanism was proposed for Hg, which may increase intracellular Na concentration by inhibiting Na/K ATPase or increasing permeability of plasma membrane to Na. Both Hg and Pb altered calmodulin activity which affects Ca-ATPase, a calmodulin dependent enzyme that pumps Ca<sup>2+</sup> from the cytosol into organelles such as mitochondria or out of the cell. Alterations in Ca<sup>2+</sup> homeostasis by Pb and Hg were attributed to significant inhibition of Ca-ATPase.

#### **2.5.2.6 Oxidative stress**

Recent reviews on Hg and Pb toxicity have emphasized the effects of these metals on intracellular mechanisms designed to protect cells from oxidative stress (Flora et al 2008; Guzzi and La Port 2008). Pb and Hg are both capable of inducing oxidative stress following the generation of reactive oxygen species (ROS) and nitrogen species, thereby altering pro-oxidant and antioxidant homeostasis which can result in neurotoxicity. Hg has a high affinity for thiol (-SH) groups of the antioxidant glutathione, which provides intracellular defense against Hg-induced neurotoxicity. Neuron cell vulnerability to Hg may be due to lower levels of glutathione which predisposes the cell to ROS damage and activates apoptosis-signalling pathways. Pb has been shown to alter (impair) the activity of glutathione and antioxidant enzymes.

Johansson et al (2007) reviewed the mechanisms reported for neurobehavioural effects of MeHg in *in vivo* and *in vitro* studies, which included adverse effects on neurotransmitter systems, induction of oxidative stress and cell death and disruption of microtubules and intracellular Ca homeostasis. The study provided human and rat infant or neonatal Hg brain LOAELs associated with neurodevelopmental effects.

### **2.5.2.7 Metal toxicity and autism**

Rose et al (2008) examined the association between autism and the effects of Hg and Pb on glutathione and the heme synthesis pathway. Both Pb and Hg induce oxidative stress by depleting glutathione, a major intracellular antioxidant, and each inhibit different enzymes in the heme biosynthesis pathway. Pb affects δ-aminolevulinic acid dehydratase (ALAD) and Hg affects coproporphyrin oxidase (CPOX), which results in the accumulation and excretion of abnormal porphyrin in urine. ALAD and CPOX polymorphisms are associated with increased blood levels of Pb and Hg, respectively. The frequency of polymorphisms of CPOX and ALAD as well as plasma glutathione levels were examined in 242 autistic children and 75 control children. An increased frequency of ALAD polymorphism and significantly lower plasma glutathione levels were reported for autistic children. The authors suggest the study results could indicate that children with the ALAD polymorphism may have increased susceptibility to lead toxicity during critical windows of prenatal and postnatal development which may contribute, in part, to an increased risk of developing autism.

In their review of mechanisms of heavy metal toxicity Gulati et al (2010) associated Hg-induced glutathione depletion, cellular mitochondrial damage, increased lipid peroxidation in protein, and DNA oxidation in the brain as major factors contributing to neurodegenerative conditions such as autism and Parkinson's disease.

### **2.5.2.8 Metal induced apoptosis**

Several studies have reviewed metal-induced apoptosis (Rana 2008; Costa et al 2004). Pb-induced apoptosis may be related to calcium overload and enhanced by increased extracellular glutamate, with greater effects from Pb exposures occurring during later stages of brain development (i.e., pruning of synapses). MeHg initiates lipid peroxidation and alterations in cell membranes. Hg<sup>0</sup> and MeHg both damage microtubules in the brain. Hg has a high affinity for thiol groups and Hg-induced apoptosis may result from binding to and altering a variety of enzyme systems. Apoptosis is regulated during neural development by processes involving growth factors, cytokines, and neurotransmitters and interference with these processes could trigger degeneration of necessary, or promote survival of unnecessary, cells (Rana 2008; Costa et al 2004).

### **2.5.2.9 Nerve cell transmission**

The effects of Hg and Pb on two amino acid neurotransmitters in the CNS: glutamate, major excitatory neurotransmitter, and;  $\gamma$ -aminobutyric acid (GABA), major inhibitory neurotransmitter, were reviewed by Fitsanakis and Aschner (2005). Both Pb and Hg affected GABA by altering the GABAergic system: MeHg decreased GABA-induced currents; IHg increased GABA-induced currents, and; Pb decreased GABA transport and altered GABA channel properties. Both Pb and Hg affected glutamate levels by altering properties of the *N*-methyl *D*-aspartate (NMDA) glutamate receptor and decreasing the ability of astrocytes to clear extracellular glutamate.

Glutamate itself is considered a neurotoxin/excitatoxin and is involved in the pathophysiology of neurodegenerative disorders (Segura-Aguilar & Kostrzewa 2004). Cytotoxicity from excessive glutamate is largely due to an increase in intracellular ROS, with initial ROS production resulting from glutathione depletion and a much greater subsequent burst of ROS production attributed to the mitochondrial electron transport chain. NMDA receptor antagonists can prevent glutamate neurotoxicity. Pb affects Ca<sup>2+</sup>-dependent proteins and neurotransmitters receptors, in particular protein kinase C and the NMDA subtype of glutamate receptor, both of which are involved in learning and cognitive functions and may interact significantly to account for altered functions. MeHg is the most common form of Hg studied in terms of effects on the nervous system. The basis for harmful effects of Hg compounds is attributed to high thiol reactivity. Mechanisms of Hg toxicity include inhibition of protein synthesis, microtubule disruption, increase of intracellular Ca<sup>2+</sup> with disturbance of neurotransmitter function, oxidative stress and triggering of excitotoxicity mechanisms. Hg damages CNS development, altering the structure and functionality of the nervous system (Segura-Aguilar & Kostrzewa 2004).

Within the nervous system, both Pb and Hg can affect nerve cell metabolism and nerve transmission, with Hg targeting the cerebellum. Reviews of neurochemical studies have suggested similar effects of Pb, MeHg and IHg on parameters of neurotransmission, including: catecholaminergic system – dopamine (Pb, MeHg, IHg); decreased norepinephrine (Pb, IHg); decreased GABA levels (Pb, MeHg); decreased glutamate decarboxylase activity (Pb, MeHg, IHg); and increased monoamine oxidase activity (Pb,

MeHg). The effects of Hg and Pb on neurotransmitter systems are likely due to interaction at multiple sites.

### **2.5.2.10 Neuro-immunotoxicity**

Autoantibodies against the nervous system were detected and quantified in workers following occupational exposure to Pb or Hg (Shamy et al 2007). A total of 51 workers were evaluated with 27 exposed to Pb in battery manufacturing (median PbB of 35 ug/dL) and 24 exposed to Hg in neon light manufacturing (median Hg in urine of 100 ug/g creatinine). Significant increases were reported in IgG and IgM autoantibodies against neurotypic and gliotypic proteins in exposed workers compared to unexposed controls. The authors deduced the following neuroimmunotoxic effects of occupational exposure to Pb or Hg (significant responses observed for both groups): persistent axonal insult -> increased anti-NF (neuroaxonal filament) autoantibodies; involvement of the CNS and astrocytes -> increased anti-GFAP (astrocyte glial fibrillary acid protein) autoantibodies; and, secondary demyelination -> increased anti-MBP (myelin sheath basic protein) autoantibodies. Although these autoantibodies were not expected to interact with surface antigens, they may penetrate normal cells (e.g., neurons) resulting in degeneration and apoptosis.

In a review of the humoral assessment of neurotoxicity and autoimmune mechanisms, El-Fawal et al (1999) reported evidence from human and animal studies of antibodies to the nervous system as a result of exposure to Pb (blood) or Hg (urine). These exposures correlated with subclinical deficits in exposed workers and evidence of histopathological damage to CNS and PNS in rats. Autoantibodies (IgG primarily) to neuronal cytoskeletal proteins, NF and MBP were detected in male workers and in rats and mice exposed to Pb or Hg. Sera IgG against NF and MBP were significantly correlated with blood Pb or urinary Hg concentrations. Measurements of these autoantibodies could be used as markers for the neurotoxic effects of Pb and Hg.

### **2.5.2.11 Astrocytes**

Tiffany-Castiglioni and Qian (2001) reviewed the functions of astrocytes and mechanisms of Hg and Pb toxicity on these cells. Astroglia are the first cells of the brain parenchyma to encounter metals crossing the BBB and are designed to concentrate metals for metabolic use. Metal transport/carrier proteins and metal dependent brain

enzymes localized in astroglia possess physiologic properties that protect cytoplasm from metal-induced oxidative damage, including: high cytosolic levels of metallothionein (levels in adjacent neurons are low) which bind heavy metals through thiol groups, and high cytosolic levels of glutathione, an antioxidant that neutralizes free radicals and undergoes nonenzymatic conjugation with Pb and Hg.

In cultured astrocytes, Pb produced a dose-dependent inhibition of glutamate synthetase, the enzyme key to glutamate and ammonia metabolism in the brain, and interfered with Ca homeostatic mechanisms by binding to and stimulating Ca-regulated enzymes including protein kinase C (PKC) and calmodulin. The relative level of accumulation of Hg species reported in the CNS was reported to be: MeHg > Hg<sup>0</sup> > IHg. In humans, MeHg was accumulated in astroglia and microglia while Hg<sup>0</sup> accumulated in motor neurons. In monkeys IHg was also reported to accumulate in astroglia and microglia. These data support a buffering role for astrocytes. Contrary to these results, rats accumulated MeHg and IHg in neurons with less occurring in glial cells and squirrels exposed to Hg<sup>0</sup> accumulated Hg in both neurons and astroglia (Tiffany-Castiglioni and Qian 2001).

Astrocytes play important role in CNS development, maintain neuronal homeostasis, regulate neuronal survival and function in neurotransmitter metabolism (e.g., removal of glutamate and GABA from the synaptic cleft). Hg is preferentially accumulated by astrocytes which results in astrocytic swelling and affects neuronal homeostasis (Vitarella et al. 1996). Astrocytes provide glutathione precursors to neurons, which are vital in the defense of the neuron against free radical damage. MeHg inhibits the uptake of glutamate by astrocytes by inhibiting a specific glutamate transporter. Diminished glutathione levels in the astrocytes results in increased extracellular glutamate to levels that are toxic to neurons. MeHg also induces ROS production which exacerbates the inhibition of glutamate transport and reduces the ability of astrocytes to provide precursors for glutathione synthesis in neuron cells, leaving neurons more susceptible to MeHg induced oxidative stress (Allen et al 2002).

In the adult brain, astrocytes accumulate MeHg which interferes with glutamate uptake, resulting in high levels of extracellular glutamate and damage to adjacent neurons.

MeHg-induced damage to astrocytes during brain development is associated with effects on astrocytic guidance of neuronal migration. The disruption of the blood brain barrier by Pb is likely mediated through injury to astrocytes (Costa et al 2004).

Monnet-Tschudi et al (2006) reviewed neurotoxicology studies involving Pb and Hg. Astrocytes are primarily responsible for the protection and/or repair of neurons. Damage to astrocytes as a result of exposure to Hg and Pb can result in oxidative stress and neuroinflammation. Neuronal damage induced by ROS leads to glial reactivity (reactive glial cells) which can increase ROS production. ROS-induced protein oxidation can promote the aggregation of synuclein (associated with Parkinsons disease) and insoluble  $\beta$ -amyloid (associated with Alzheimers disease), resulting in neuronal damage. The authors suggest that the effects of Hg and Pb on astrocytes and subsequently neurons are associated with the major pathways and signs of neurodegeneration related to Alzheimers and Parkinsons disease.

#### **2.5.2.12 Brain barriers**

Both Pb and Hg are accumulated in the blood-cerebrospinal fluid barrier (BCB) and BBB. Although the mechanism for Hg uptake by the BCB is not determined, it may be similar to that for the BBB (i.e., protein transport). Hg is reported to be a general toxicant (direct damage) to the choroid plexus of the BCB. Pb is considered to be a selective toxicant to the choroid plexus as it impairs plexus regulatory pathways critical to brain development and function including reduction in the production and secretion of the thyroid hormone transthyretin (TTR) (exclusively produced and secreted by the choroid plexus) and activation of choroid plexus protein kinase C, which may contribute to Pb effects on TTR. The reduction in TTR production and secretion by the choroid plexus may impair transport of thyroid hormones from the blood to cerebral compartment which might account for loss of cognitive abilities associated with Pb exposure (Zheng 2001; Zheng et al. 2003).

#### **2.5.2.13 Effects of Pb on nervous system**

As reviewed by the ATSDR (2007), Pb can affect the nervous system by mimicking calcium action and/or disrupting calcium homeostasis which affects many cell-signaling pathways. Specifically Pb activates PKC which is involved in many processes important for synaptic transmission. The calcium-dependent  $\gamma$ -isoform of PKC, which is neuron-

specific and involved in long-term potentiation, spatial learning, and memory processes, may be a specific target for Pb neurotoxicity. PKC induces the formation of the AP-1 transcriptional regulatory complex, which regulates the expression of the glial fibrillary acidic protein (GFAP) gene. Studies in rats have demonstrated that Pb can alter the expression of the GFAP gene, which is induced during periods of reactive astrocytic gliosis. The effects of Pb exposure have been reported to be long-lasting if exposure occurred during brain developmental when PKC is present in the cytosol, rather than membrane bound as it occurs in mature brain microvessels. Pb-induced activation of PKC may impair brain microvascular formation and function, and at high Pb levels, account for defects in the blood-brain barrier. The particular sensitivity of the developing brain to Pb is likely associated with the immaturity of the blood-brain barrier and the accumulation of Pb in astroglia which sequester Pb.

#### **2.5.2.14      *Developmental neurotoxicity***

Numerous studies have reported on the potential toxicity of Pb and Hg to the developing nervous system (Wennberg 1994; Rodier 1995; Rice 1996; Annau and Cuomo 1988; Costa 1988; Rice and Barone 2000; Mendola et al 2002; Costa et al 2004; Hogberg et al 2010). These studies are discussed below.

In a study on the regulation of gene expression at the mRNA level, Hogberg et al (2010) tested the effects of MeHgCl (MeHg) or PbCl<sub>2</sub> (Pb) on the expression of astrocytic and neuronal markers in cultured rat cerebellar cells in order to identify which cell types and which stages of neuronal development were affected. Markers of neural precursor cell or glial proliferation (Sox10 and nestin) were both downregulated (Sox10) and upregulated (nestin) by low concentrations of MeHg, whereas Pb did not significantly affect expression of either. An increase in nestin mRNA expression could indicate either neural precursor cell or glial proliferation as a result of MeHg exposure. Markers of the neuronal cytoskeleton proteins (NF-68 and NF-200) were downregulated by MeHg but not affected by Pb. Markers important to neuronal functional maturation - subunits of the main neuronal excitatory receptor (NMDA-R) and inhibitory (GABA<sub>A</sub>) receptor - were significantly decreased at the highest MeHg concentration tested, an effect that was attributed to neuronal cell death. Pb significantly downregulated GFAP, a marker for mature astrocytes.

In summary, the study evaluated changes in the mRNA level of genes for key neuronal and glial developmental processes, including proliferation, differentiation and maturation. Low concentrations of MeHg downregulated mRNA levels for neuronal markers (Sox10, NF-68, NF-200, NMDA-R, and GABA<sub>A</sub>) but did not change mRNA expression of astrocytic markers (GFAP and S100 $\beta$ ), whereas low concentrations of Pb decreased mRNA expression of astrocytic marker (GFAP), with neuronal markers less affected. Tested individually, MeHg and Pb affected different genes involved in the differentiation and maturation of neuronal and glial cells, however, the authors did postulate that combined exposure could exacerbate overall neurodevelopmental toxicity (Hogberg et al 2010)

Annau and Cuomo (1988) conducted a review of studies on behavioral alterations associated with exposure to Pb and MeHg. Pb was reported to accumulate in the hippocampus, a region of the brain sensitive to hypoxia and therefore damage in this region may be secondary to metabolic changes. The catecholaminergic systems, in particular the dopaminergic system, was affected by Pb exposures with studies in monkeys suggesting behavioral effects occur at blood Pb concentrations that have been observed in children. MeHg is considered a potent cytotoxin, with exposures during prenatal development resulting in reduced myelination, delayed neuronal migration and loss of neurons in the cortex and cerebellum. These actions can lead to permanent effects on learning and memory. Similar to Pb, MeHg has been reported to affect the catecholaminergic system.

Hg and Pb are widely distributed environmental neurotoxicants and low levels of persistent exposures to each have been linked to subtle behavioral effects. Costa et al (2004) report on the developing neuropathology of MeHg and Pb, both of which are considered to produce severe effects in fetal and young brains (compared to adults), accumulate in and damage astrocytes, damage cerebellum, and affect glutamatergic function. Motor movements and, to an extent, speech, learning, emotions, and attention are associated with the cerebellum. Hg exposure affects neuronal migration during early brain development, possibly through interaction with the microtubular element of the cytoskeleton. Pb exposure during later stages of brain development affects neural



differentiation, synaptogenesis and myelin formation (Costa et al 2004). The authors cite an estimate of 12 million children in the US with learning, neurodevelopmental, or behavioral disabilities that can be attributed to environmental exposures.

MeHg and Pb exposures can damage the CNS at various stages of development (Rodier 1995; Rice and Barone 2000; Mendola et al 2002; Costa et al 2004). Critical processes in nervous system development that are affected by MeHg and/or Pb include: 1) Neural Proliferation: MeHg involved in disruption of proliferation which can also affect neuronal migration, the next developmental stage; 2) Neuronal Migration: MeHg may affect neuronal migration through hindrance of the repolymerization of microtubules; 3) Neuronal Differentiation: this stage of development is affected by both MeHg and Pb; 4) Synaptogenesis: affected by both MeHg and Pb, normally the number of synaptic connections between neurons peaks around age two and is then trimmed back by approximately one-half; 5) Myelination and Gliogenesis: Pb exposure can produce reactive astrocytes and microglia (reactive gliosis) which, dependant on the timing of insult, precedes a major phase of proliferation and differentiation of astrocytes; 6) Apoptosis: a tightly regulated process to target the removal of cells without an inflammatory response, both Pb and MeHg shift the balance of neurotrophic signals regulating apoptosis which can increase or decrease cell numbers in affected regions of the nervous system. Unregulated apoptosis is associated with neurodegeneration and neurodegenerative diseases.

#### **2.5.2.15 Long-latency delayed neurotoxicity**

Definitive evidence for long-latency delayed neurotoxicity was demonstrated in a study evaluating the effects of chronic MeHg exposure in monkeys (Rice 1996). The monkeys were exposed from birth through puberty (7 years) to 50 µg/kg/d MeHg, which resulted in a steady-state blood Hg concentration of 0.7 ppm during the period of dosing. No signs of overt toxicity were reported in routine clinical assessments of sensory and motor function from infancy till about 4 years of age, however, deficits in fine motor control were observed during middle age (13 years). The authors noted that significant amounts of Hg remained in the brain eight months after cessation of MeHg exposure and months after HgB levels in the blood were below analytical detection. Previous studies reported IHg concentrated in reactive glia following chronic low level exposures to MeHg or IHg.

The authors suggested that the observed delayed neurotoxicity may be attributed to continued toxic influence as a result of Hg storage, premature death of damaged cells, and/or accelerated aging of cells required to compensate for damaged or missing cells.

### 2.5.3 Summary of joint toxic effects

The joint toxic effects for combined exposures to combinations of Hg<sup>0</sup>, MeHg, IHg and/or Pb, as reported in the literature and reviewed above are highlighted in point form below. Again, considering that internal metabolic processes result in the conversion of Hg<sup>0</sup> and MeHg to IHg, it was considered appropriate to evaluate responses to IHg in addition to Hg<sup>0</sup> and MeHg. Although these reported joint effects appear to act on similar targets, it is very likely that different mechanisms are involved, some of which have been described or postulated by the study/review authors as outlined above and in Table 5.

#### Similar target - synergistic effects – combined exposures to Pb and IHg

- Increased stimulus-evoked cortical (CNS) electrical activity and decreased stimulus-evoked peripheral (PNS) electrical activity (Papp et al 2006)
  - *in vivo* response, rats, *low dose* Pb and IHg
  - effect of Pb may be related to BBB damage

#### Similar target - potentiation effect – combined exposures to MeHg and Hg<sup>0</sup>

- Neurological/behavioural and spontaneous motor activity effects in adult rats exposed in utero to MeHg alone or in combination with Hg<sup>0</sup> (Fredriksson et al 1996)
  - Neurobehavioural and motor activity effects observed with combined MeHg and Hg<sup>0</sup> exposures but not observed with MeHg exposure alone.

#### Similar target, similar effect – combined exposures Pb and Hg

- Pb and Hg affected spontaneous neurotransmitter release from rat brain synaptosomes via *different mechanisms* (Foulke 1987)

#### Similar target, similar effect – individual exposures

- Delays in brainstem evoked potentials in humans:



- Auditory evoked potentials – adults with no neurological symptoms, chronic occupational exposure to either Pb or IHg (Discalzi et al 1993)
- Auditory and visual evoked potentials - children, neonatal exposure to MeHg (Trask and Kosofsky 2000).
- Astroglial accumulation of metals/oxidative damage/Ca homeostasis -> neuronal damage/apoptosis
  - Pentyle et al. (2010): *in vitro*, rat brain microsomes - Pb and IHg -> altered Ca<sup>2+</sup> homeostasis-> Ca = neuronal cell messenger -> regulation of cellular metabolic processes -> neuronal dysfunction -> cell death/apoptosis
    - Numerous studies support effects on neuronal cell signaling by Pb and IHg as a result of changes in Ca homeostasis (Wennberg 1994; Manzo et al 1995; Denny and Atchison 1996; Atchison 2003; Florea and Busselberg 2006; Johansson et al 2007; Gulati et al 2010)
  - Flora et al 2008; Guzzi and La Port 2008; Johansson et al 2007; Monnet-Tschudi et al 2006; Rose et al 2008; Gulati et al 2010: *in vivo* and *in vitro* studies - Hg, Pb, and MeHg - oxidative stress/generation of ROS and reactive nitrogen species -> neurotoxicity/ neurodevelopmental effects
    - Altered antioxidant enzymes
    - Glutathione depletion
    - ROS production -> glial reactivity
  - Rana 2008 and Costa 2004: *in vivo* and *in vitro* studies - Hg<sup>0</sup>, Pb, and MeHg - metal induced apoptosis -> no one mechanism
    - Altered Ca<sup>2+</sup> homeostasis
    - Excess extracellular glutamate (synaptic cleft)
    - Lipid peroxidation/alteration – cell membranes
  - Fitsanakis and Aschner (2005); Segura-Aguilar & Kostrzewa (2004) - glutamate, major excitatory neurotransmitter in the CNS - Pb and Hg altered NMDA (glutamate receptor) and decreased the ability of astrocytes to clear extracellular glutamate – NMDA involved in learning and cognitive functions.



- Tiffany-Castiglioni and Qian 2001 – *in vitro* culture astrocytes accumulate Pb, MeHg, IHg and motor neurons accumulate Hg<sup>0</sup> - Pb inhibits glutamate synthetase and affects Ca homeostasis by binding and stimulating PKC and calmodulin
- Vitarella et al. 1996; Allen et al 2002; Costa et al 2004 – astrocytes – Hg – astrocytic swelling and effects on neuronal homeostasis - MeHg inhibits glutamate uptake – induces ROS production – increased neuron susceptibility
- Alteration of enzymes involved in heme biosynthesis -> accumulation and excretion of abnormal porphyrin in urine (Rose et al 2008)
  - Pb acts on ALAD
  - Hg acts on CPOX
- Effects on parameters of neurotransmission (Fitsanakis and Aschner 2005; Wennberg 1994; Costa 1988)
  - effects on catecholaminergic system – dopamine (Pb, MeHg, IHg)
  - decreased norepinephrine (Pb, IHg)
  - decreased GABA levels (Pb, MeHg)
  - decreased glutamate decarboxylase activity (Pb, MeHg, IHg)
  - increased monoamine oxidase activity (Pb, MeHg)
  - increased GABA-induced currents (IHg), and
  - decreased GABA transport and altered GABA channel properties (Pb)
- Production of autoantibodies against neurotypic and gliotypic proteins observed in human and animal studies - indicative of neuroimmunotoxic effects in the absence of neurological symptoms (Shamy et al 2007; El-Fawal et al 1999)
- Toxic effects on choroid plexus of BCB (Zheng et al 2003; Zheng 2001)
  - Hg – general toxicant
  - Pb – specific toxicant – reduced TTR production
- Effects during significant stages of neurodevelopment were affected by Pb and/or MeHg (Hogberg et al 2010; Annau and Cuomo 1988; Rodier 1995; Costa et al 2004; Rice and Barone 2000; Mendola et al 2002)
  - Neural Proliferation (MeHg)
  - Neuronal Migration (MeHg)
  - Neuronal Differentiation (MeHg and Pb)

- Synaptogenesis (MeHg and Pb)
- Myelination and Gliogenesis (Pb)
- Apoptosis (MeHg and Pb)

Although not tested in the studies described above, it is noted that Hg<sup>0</sup> was reported to potentiate the neurodevelopmental effects of MeHg in adult rats exposed *in utero* to MeHg and Hg<sup>0</sup> (i.e., neurological effects not observed from MeHg exposure alone) (Fredriksson et al 1996).

#### **2.5.4 Weight of evidence for interactions**

A qualitative WOE analysis of interactions among mixture components was completed for the purpose of the current assessment. It is noted that an exhaustive review of the available literature (and details within primary studies reported with review papers) was beyond the scope of the current assessment. Therefore, the WOE analysis presented here should be considered preliminary and requiring validation.

The available data generally were insufficient to consider the effect of “chemical A” on “chemical B” versus the effect of “chemical B” on “chemical A”. Therefore, the WOE evaluation for chemical interactions was limited to observations for effects associated with exposure to a chemical pair (e.g., responses to Pb and MeHg combined) to determine if interactions for the two components were additive, greater-than-additive, less-than-additive, or indeterminate. It is noted that there were insufficient data on the effects of Hg<sup>0</sup> per se, however, there was sufficient data to describe the effects of IHg (i.e., Hg<sup>0</sup> metabolite) which, for the purpose of this assessment was considered to be potentially representative of Hg<sup>0</sup> exposures.

As per ATSDR (2006) guidance, the direction of interaction was classified as one of the following:

- = Additive
- > Greater than additive
- < Less than additive
- ? Indeterminate

The quality of the data in terms of mechanistic understanding was classified as follows:

- I. Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.
- II. Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.
- III. Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.

The quality of the data in terms of toxicological significance was classified as follows:

- A. The toxicological significance of the interaction has been directly demonstrated.
- B. The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.
- C. The toxicological significance of the interaction is unclear.

The toxicodynamic interaction data for Pb, MeHg, and Hg<sup>0</sup> and the direction of interaction for the combined effect was evaluated according to ATSDR (2004) and is summarized in Table 6.

Table 6. Summary of interaction assessment for Pb, MeHg and Hg<sup>0</sup>.

Binary mixture	Effect	Suggested interaction	Binary weight of evidence assessment (after USATSDR 2004) <sup>1</sup>
Pb and Hg <sup>0</sup> (as IHg)	Increased stimulus-evoked cortical (CNS) electrical activity and decreased stimulus-evoked peripheral (PNS) electrical activity in response to Pb and IHg	Synergism	> IA
Pb and Hg <sup>0</sup> (as IHg)	Spontaneous neurotransmitter release resulting from exposure to Pb and IHg.	Additive	> IA
Pb and Hg <sup>0</sup> (as IHg)	Delays in brainstem evoked potentials in humans (Pb, IHg, MeHg)	Additive	= IB
Pb and Hg <sup>0</sup> (as IHg)	Alteration of enzymes involved in heme biosynthesis leading to accumulation and excretion of abnormal porphyrin in urine (Pb and IHg)	Additive	= IA
Pb and Hg <sup>0</sup> (as IHg)	Production of autoantibodies against neurotypic and gliotypic proteins in the absence of neurological symptoms (Pb, Hg);	Additive	= IA
Pb and Hg <sup>0</sup> (as IHg)	Toxic effects on choroid plexus of BCB (Pb, IHg)	Additive	= IB
Pb and MeHg	MeHg (alone) produced similar effect to Pb and IHg, therefore inferred	Additive	= IIB
Pb and MeHg	Effects during significant stages of neurodevelopment (Pb, MeHg).	Additive	= IA
MeHg and Hg <sup>0</sup> (as IHg)	MeHg (alone) produced similar effect to Pb and IHg, therefore inferred	Additive	= IIB
Pb and Hg <sup>0</sup> (as IHg)	Astroglial accumulation of metals/oxidative damage/Ca homeostasis leading to neuronal damage/apoptosis	Additive	= IB
Pb and MeHg			= IB
MeHg and Hg <sup>0</sup> (as IHg)			= IB
Pb and Hg <sup>0</sup> (as IHg)	Effects on parameters of neurotransmission	Additive	= IB
Pb and MeHg			= IB
MeHg and Hg <sup>0</sup> (as IHg)			= IB
Hg <sup>0</sup> and MeHg	Neurological/behavioural and spontaneous motor activity effects in adult rats exposed in utero to Hg <sup>0</sup> and MeHg	Potentiation	> IIA

Similar to the conclusions of the ATSDR, the preponderance of the weight of available evidence suggests that the direction of the interaction would be additive for neurotoxicity for binary combinations of the mixture, that is, combinations of Pb and MeHg; Pb and Hg<sup>0</sup>; and MeHg and Hg<sup>0</sup>. However, it is noted that limited evidence was also available to suggest synergism in stimulus-evoked CNS activity and spontaneous neurotransmitter release following combined exposures to Pb and Hg<sup>0</sup> (as IHg), and potentiation of neurodevelopmental effects of MeHg by concurrent *in utero* Hg<sup>0</sup> exposure.

### **2.5.5 Epidemiology studies**

The results of several recent and sentinel epidemiology studies on neurological effects associated with Hg exposures, often including measurements of PbB, are summarized below. Although not representative of all available epidemiological studies, the weight of available evidence did not suggest additive or synergistic effects for measured neurological outcomes associated with exposures to Pb and Hg.

Evidence of behavioural teratogenicity associated with maternal exposure to MeHg was provided in several epidemiology studies reporting neurobehavioral disorders in the offspring of women consuming MeHg in fish (Minamata Bay, Japan) at concentrations which did not produce clinical symptoms of maternal toxicity; epidemiology studies have also associated deficits in intelligence, auditory perception, attention, and class-room performance with exposures to low levels of Pb during neurodevelopment (Nelson 1991).

Significant longitudinal studies of neurodevelopmental effects in the off-spring of fish-consuming populations in the Seychelles Islands and Faroe Islands were reviewed by Risher et al (2002). In summary, within the Seychelles Islands population, chronic maternal (and fetal/neonatal) exposure to low levels of MeHg through the maternal consumption of a variety of ocean fish during pregnancy and breastfeeding was observed but was not associated with adverse neurobehavioural or neurodevelopmental effects in offspring evaluated at 6, 19, 29, 66 months, and followed up at 9 years of age. The mean Hg content measured in maternal hair during pregnancy (6.8 ppm) was comparable to the mean Hg content in the hair of children at 66-months (6.5 ppm).



In contrast to the results for the Seychelles Islands population, chronic maternal MeHg-exposure was correlated to dysfunctions in language, attention, memory, and visuospatial and motor function (to a lesser extent) in 7-year old off-spring in the fish-consuming population from the Faroe Islands. Geometric mean values for Hg concentrations in maternal cord blood and hair samples were 22.9 ppm and 4.27 ppm, respectively. A significant anomaly for this population compared to the Seychelles Islands population was the regular consumption (1-2 meals/week) of pilot whale meat, in addition to ocean fish, which was considered a predominant source of MeHg exposure. Although concurrent exposure to polychlorinated biphenyls (PCB) and other persistent organic pollutants (POP) was reported for the Faroese test population, the study authors attributed the observed deficits to MeHg exposures, rather than exposures to PCB or other POP. A follow-up paper on the Faroe Islands population (Grandjean et al., 1999) suggested that Hg concentrations in cord-blood were the best predictor of language, attention, and memory dysfunctions and that Hg concentrations in maternal hair at parturition (reflecting fetal exposure during the second trimester) were best associated with decrements in fine motor coordination. The authors also suggested that the greatest susceptibility to MeHg neurotoxicity occurred during late gestation.

Several studies attempted to correlate neurofunctional deficits with fish consumption in predominately adult populations from Canada (Upper St. Lawrence river) and Brazil (Tapajós river). A dose-effect relationship was observed for total Hg content in the hair of consumers of fish from the Tapajós river and neurological deficits, including loss of fine motor capacities, coordination manual dexterity and visual functions. Hair mercury levels varied with fish intake and with the type of fish consumed. A median hair Hg concentration of 11 ppm (n=233, aged 15-81 years) and a median total Hg blood concentration of 28 µg/L (ranging 9 to 115 µg/L, n=96) were reported for the Amazon population. Although neurofunctional effects (slowed information processing) were observed in fish consumers from the Upper St. Lawrence river, no dose-effect relationship was established with total HgB (median = 1 µg/L, ranging from 0.1 to 4.8 µg/L) or PbB (3.73 µg/dL) levels in fish consumers (Mergler et al 1998; Mergler 2002).

Neuropsychological effects were evaluated in 129 adults (>17 yrs) living in fishing communities in the Pantanal region of Brazil (Yokoo et al 2003). Hg exposures were associated with fish consumption and were measured by hair Hg concentrations (ranged from 0.56 to 13.6 ppm; mean = 4.2 ppm). The study reported associations between hair mercury levels and alterations

in performance on tests of fine motor speed and dexterity, concentration, and some aspects of verbal learning and memory. The magnitude of the effects increased with hair mercury concentration

A study of cognitive and sensorimotor functions in children (n=384) aged 5 to 7 years living in three cities in Germany (Leipzig, Gardelegen, and Duisburg) associated subtle non-IQ dysfunctions (sustained attention) with background PbB levels (geo mean = 42.5 µg/L) but not with background urinary Hg levels (geo mean = 0.16 µg/24 h). No other measures of Hg exposures were reported. The authors noted that the behavioural endpoints were selected to cover Pb effects, although were not markedly different from neurobehavioral effects observed with occupational exposure to Hg<sup>0</sup> (Walkowiak et al 1998). Visual functions (visual-evoked potentials (VEPs) and contrast sensitivity (CS)) were examined in the same cohort (Altmann et al 1998). Subtle, but statistically significant changes were associated with increasing PbB concentrations (i.e., VEP latencies) and increasing HgB concentrations (i.e., reduced CS).

Blood Pb and Hg levels and auditory sensory-neural function were examined in 76 Andean children (aged 4-15 years) living in either a Pb-contaminated area of Ecuador (n=62) or in a neighboring gold mining area with no known Pb exposures (n=14) (Counter et al 1997). Median PbB levels for Pb-exposed children and unexposed children were 52.6 µg/dL (range 9.9-110.0 µg/dL) and 6.4 µg/dL (range 3.9-12.0 µg/dL), respectively. Median HgB levels for Pb-exposed children and unexposed children were 0.16 µg/dL (range 0.04-0.58 µg/dL) and 0.22 µg/dL (range 0.1-0.44 µg/dL), respectively. The authors did not find any significant effects of low or high PbB or low HgB concentrations on auditory sensory-neural function (auditory thresholds and auditory brain stem responses) in any of the children evaluated.

Recent large scale epidemiology studies conducted in the US (Boston and Maine) reported an absence of statistically significant neuropsychological or renal effects associated with increased urinary Hg levels (mean = 0.9 µg/g creatinine) in children (aged 6-10 at start of study) who were followed over a 5 year period during which they received dental amalgam fillings and compared to a similar group of children receiving composite fillings (Bellinger et al. 2007). Baseline mean PbB levels reported for the amalgam and composite groups were 2.4 µg/dL and 2.3 µg/dL, respectively (Bellinger et al 2006). However, it should be noted that the short duration of this study (5 years), a number of limitations in the statistical analyses published to date, and the

overlap of Hg exposures (as measured by urine Hg concentration) between the exposed group (those receiving amalgam fillings) and referents (those receiving composite resin fillings) may limit the reliability of these studies. These limitations are discussed in detail in Part 1 of this report.

Similar studies conducted in Portugal (Lisbon) reported no statistically significant neurological or nerve conduction differences between amalgam and composite treated children (aged 8-10 at start of study) followed over a 7 year period. Inclusion criteria for the study included baseline UHg concentrations below 10 µg/L and PbB levels below 15 µg/dL (DeRouen et al 2006; Lauterbach et al 2008). Again, however, it should be noted that the short duration of this study (7 years), a number of limitations in the statistical analyses published to date, and the overlap of Hg exposures (as measured by urine Hg concentration) between the exposed group (those receiving amalgam fillings) and referents (those receiving composite resin fillings) may limit the reliability of these studies. These limitations are discussed in detail in Part 1 of this report.

A recent study by Cao et al (2010) concluded that adverse effects on IQ and behavior were not detectable at background postnatal MeHg exposure levels in US children. The study examined MeHg exposure, IQ and behavior in 780 US children from Philadelphia, Newark, Cincinnati, and Baltimore, enrolled in the Treatment of Pb-exposed Children (TLC) clinical trial. Children accepted for the trial were aged 12 to 33 months with PbB levels ranging from 20 to 44 µg/dL; approximately half the cohort (n=396) received succimer treatment. Baseline blood MeHg levels and post-succimer treatment blood MeHg levels were similar for the placebo and succimer groups (ranging from 0.48 to 0.54 µg/L) and therefore the groups were combined and IQ and neurobehavioral performance were tested at 2, 5 and 7 years of age. No significant associations were observed for blood MeHg and IQ test scores or tests of cognition or behaviour. As cited by the authors, cordblood or childrens blood MeHg concentrations were higher in studies reporting developmental effects (from 0.88 to 22.9 µg/L) compared to the levels observed in this study. It is noted that the conclusion for MeHg and IQ scores in this study was not altered when multivariable models were adjusted for PbB levels, but confirmed that concurrent PbB was strongly associated with IQ and behavior in TLC children.

Ha et al (2009) examined the relationship between attention-deficit hyperactivity disorder (ADHD) symptoms and lead and mercury levels in blood in the first Children's Health and

Environment Research (CHEER) survey, conducted annually, in Korea. The study included 1778 children (6-10 years of age) from 10 elementary schools in 6 Korean cities. Significant associations were reported for PbB concentrations (geo mean = 1.8 µg/dL) and symptoms of ADHD, however no correlation was observed between HgB (geomean = 2.4 µg/L) and ADHD symptoms. As noted by the authors, PbB concentrations in the study ranged from 0.1 to 10.1 µg/dL with a geometric mean (1.8 µg/dL) well below the action limit of 10 µg/dL issued by the Korean Ministry of the Environment.

In summary, the current weight of evidence from epidemiological studies, while supporting associations for adverse effects occurring from individual chemical (i.e., lead or MeHg) exposures, does not support the occurrence of joint toxic effects between Pb and Hg as a result of ambient or, in some cases elevated exposures to Pb, MeHg or Hg<sup>0</sup>. One exception was the observation of subtle, but statistically significant, changes in visual functions in the cohort of German children with background PbB and HgU levels, where increasing PbB concentrations were associated with VEP latencies and increasing HgB concentrations were associated with reduced CS (Altmann et al 1998). When considering mechanistic effects of Pb and Hg species, there appears to be a strong potential for joint toxicity.

The lack of an effect by Hg, in the presence of an association of an effect with PbB levels, may be related to uncertainty regarding how (analytical methods), when (during exposure or post-exposure) and where (urine, hair, blood) concentrations of Hg should be measured to ascertain exposure for the purpose of association with adverse effects. Furthermore, the evidence for delayed neurotoxicity in monkeys 6 years after Hg exposures had stopped, and ~6 years after HgB levels dropped below detection (Rice 1996), raises concerns over the validity of attempting to associate “measured” Hg exposures with the occurrence of adverse effects and introduces uncertainty as to the timing of measurements and adverse effects associated with Hg exposures.

### **3. DISCUSSION/CONCLUSIONS**

As previously described, there is ample evidence to suggest the potential for co-exposure to Pb, MeHg and Hg<sup>0</sup> within the US population. Neurological effects, particularly in the nervous system of the developing fetus/neonate/child, represent the most sensitive endpoint for exposures to neurotoxic substances, such as Pb, MeHg, and Hg<sup>0</sup>. The USATSDR (2006) identified the critical effect of concern for combined exposure to Pb and MeHg to be neurological, with children (i.e., developing nervous system) representing the sensitive subpopulation of concern.

Recent literature on neurological effects following individual and combined exposures to Pb, MeHg, and Hg<sup>0</sup> was obtained. As per ASTDR and US EPA guidance, potential similarities in toxicokinetic and toxicodynamic data for individual and combined exposures were reviewed and potential interactions identified. The available toxicokinetic data for Pb, MeHg, and Hg<sup>0</sup> supports the potential for toxicodynamic interactions on neurological effects following combined exposures to these chemicals. This is based on similarities in distribution to the brain, including the developing brain of the fetus, which can accumulate Pb and Hg via the placenta, and the neonate/child exposed to Pb and Hg in breast milk, foods, household contamination and other sources.

The weight of available evidence suggests that risks posed by concurrent exposure to combinations of these 3 substances should be assessed as additive. In keeping with ATSDR guidance, the calculation of a Hazard Index (sum of Hazard Quotients) is recommended where individual HQ values  $\geq 0.1$  are determined for Pb, MeHg and/or Hg<sup>0</sup>. The Hazard Index should not exceed a value of 1.0 to be reasonably assured of public health .

As discussed in Section 1 of this report, a large number of Americans exceed appropriate reference doses for Pb, MeHg and Hg<sup>0</sup>, or in other words, have Hazard Quotient values  $> 1.0$ . Based on a review of 2003-04 NHANES data, the number of Americans in whom exposure exceeds 1/10<sup>th</sup> of the appropriate reference exposure level (i.e., where HQ $>0.1$ ) are as follows:

- Pb (reference level = 10 ug Pb/L blood; CalOEHHA, 2009): 121,744,106
- MeHg (reference level = 8 ug/L blood; Legrand et al. 2010): 62,150,604

- Hg<sup>0</sup> (reference level = 0.3 ug/L urine; derived from Richardson et al. 2009): 121,677,708

Based on these results, the assessment of risks posed by concurrent exposure to these 3 substances in the US population requires determination of the Hazard Index, that combines (sums) the Hazard Quotients of the 3 substances. The Hazard Index should not exceed a value of 1.0 to be reasonably guaranteed of public health protection. From the 2003-2004 NHANES data, the number of Americans with a Hazard Index > 1 for Pb, MeHg and Hg<sup>0</sup> combined, is 121,677,708.

### **3.1 Knowledge Gaps/Uncertainty**

The focus of the current review was on the neurological effects of combined exposures to Pb, MeHg, and Hg<sup>0</sup>. Numerous studies were identified which evaluated the effects of combinations of IHg and Pb, or MeHg and Pb, but much fewer studies included the contribution of Hg<sup>0</sup> to the effects of MeHg, IHg or Pb. Studies of particular interest for follow-up include the potential for Hg<sup>0</sup> potentiation of MeHg, to confirm joint toxicity following *in utero* exposures. Data are also required to better quantify the mechanisms involved in long-latency delayed neurotoxicity observed following early development exposures to MeHg.

The occurrence of joint neurotoxic effects *in vitro* is likely the result of interactions at more than one biological site. Neurological effects observed *in vitro* might not occur *in vivo* (in post-natal and adult animals) due to conjugation/metabolism reactions not yet developed in the fetus. Adverse effects observed following acute exposures may not occur with chronic exposures due to adaptive/compensatory reactions, conversely, alterations in neurotransmission may not occur without chronic exposure. The behaviors affected by neurochemical changes require further study using solid hypotheses, good rationales and correlations of behavioral and physiological functions (Wennberg 1994; Costa 1988).

The commonalities of target organ, toxicokinetics/dynamics and mechanisms of toxic action suggest that Pb, Hg<sup>0</sup> and MeHg should be routinely assessed as a mixture within human health risk assessments of environmental contamination. To this end, we recommend that toxicity equivalency factors, akin to those for PAHs and dioxins/furans, be developed for these 3

substances to enable the more easy quantitative assessment of risks, and to emphasize the need for routine assessment of their concurrent exposures.

Although not reviewed herein, high fructose corn syrups have recently been found to contain high levels of inorganic Hg, ranging up to 0.570 ug/g (Dufault et al 2009). Average daily consumption of high fructose corn syrup is estimated at 50 grams per person per day in the United States, due to its widespread use in numerous commercial prepared foods and sweetened beverages. As a result, this potential source of inorganic Hg exposure may contribute up to 28 µg/day and exceed other major sources of dietary mercury, especially in high-end consumers of beverages sweetened with HFCS. This source of Hg exposure should be further investigated to better quantify the extent of exposure in the US population. If significant, inorganic Hg should be combined with MeHg, Hg<sup>0</sup> and Pb when population-based risk assessments of the sort presented herein are undertaken.

#### **4. DISCLAIMER**

The statements made in this report are based solely on the information obtained to date as part of the above referenced study. SNC-Lavalin Environment, Division of SNC-Lavalin Inc. (SLE) has used its professional judgement in assessing this information and formulating its opinion and recommendations. New information may result in a change in this opinion. The mandate at SLE is to perform the tasks prescribed by the Client with the due diligence of the profession. No other warranty or representation, expressed or implied, as to the accuracy of the information or recommendations is included or intended in this report.

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