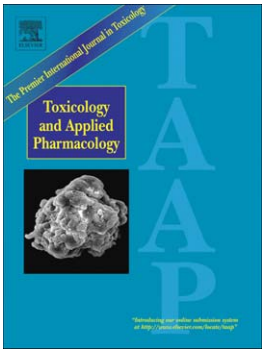

The retention time of inorganic mercury in the brain - A systematic review of the evidence

James P.K. Rooney

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Title Page

Article Title: **The retention time of inorganic mercury in the brain - a systematic review of the evidence**

Running Title: Brain Retention time of Inorganic Mercury

Author: James PK Rooney[†], MSc, MB BCh BAO

Affiliations:

[†] Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College, Dublin, Ireland.

Corresponding Address:

Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College, 152-160 Pearse Street, Dublin 2, Ireland.

Tel: +353 1 896 4497. Email: jrooney@rcsi.ie

Abstract

Reports from human case studies indicate a half-life for inorganic mercury in the brain of years - contradicting older radioisotope studies that estimated half-lives in the order of weeks to months in duration. This study systematically reviews available evidence on the retention time of inorganic mercury in humans and primates to better understand this conflicting evidence. A broad search strategy was used to capture 16,539 abstracts on the Pubmed database. Abstracts were screened to include only study types containing relevant information. 131 studies of interest were identified. Only 1 primate study made a numeric estimate for the half-life of inorganic mercury (227 – 540 days). Eighteen human mercury poisoning cases were followed up long term including autopsy. Brain inorganic mercury concentrations at death were consistent with a half-life of several years or longer. 5 radionucleotide studies were found, one of which estimated ***head*** half-life (21 days). This estimate has sometimes been misinterpreted to be equivalent to brain half-life – which ignores several confounding factors including limited radioactive half-life and radioactive decay from surrounding tissues including circulating blood. No autopsy cohort study estimated a half-life for inorganic mercury, although some noted bioaccumulation of brain mercury with age. Modeling studies provided some extreme estimates (69 days vs 22 years). Estimates from modeling studies appear sensitive to model assumptions, however predications based on a long half-life (27.4 years) are consistent with autopsy findings. In summary, shorter estimates of half-life are not supported by evidence from animal studies, human case studies, or modeling studies based on appropriate assumptions. Evidence from such studies point to a half-life of inorganic mercury in human brains of several years to several decades. This finding carries important implications for pharmcokinetic modeling of mercury and potentially for the regulatory toxicology of mercury.

Key Words: Mercury, half-life, brain, inorganic, retention, bioaccumulation

Introduction

The concept of elimination half-life is fundamental to the study of pharmacokinetics and toxicokinetics. The half-life for a xenobiotic is defined as the time taken for the xenobiotic to decrease its concentration in a given body comparment by 50% and this relationship is observed to hold true for a given xenobiotic provided the assumption of first order kinetics is valid (Flomenbaum et al., 2006). Additionally, a steady state concentration is arrived at after a time of approximately 5 times the elimination half-life for a given xenobiotic (assuming first order kinetics) - the ultimate concentration reached depending on the elimination half-life, the rate of exposure and the volume of distribution for the particular xenobiotic(Flomenbaum et al., 2006). It follows that given a longer half-life, the phenomenon of bioaccumulation may be observed – i.e. the slow increase in tissue levels of a xenobiotic with time at constant exposure – even at very low exposure levels.

Consideration of these concepts allows for modeling and analysis that can be used to address important practical issues, such as maximum safe daily exposure levels for a given toxic substance. For example, using such considerations Takeuchi et al made use of estimations of the half-life of methyl-mercury in combination with clinical observations of toxicity in Minamata Disease patients to calculate a maximum safe permissible daily intake of methyl-mercury(Takeuchi et al., 1970) (N.B. A recent follow up of Niigata Minamata Disease patients has found evidence of toxic effects at exposure levels lower than had previously been realized(Maruyama et al., 2012)). Turning our attention to inorganic mercury, it is striking that the half-life of inorganic mercury in the brain remains an undefined quantity. Inorganic mercury itself cannot access the brain, however as elemental mercury, ethyl-mercury and methyl-mercury are all metabolised to inorganic mercury within the brain(Burbacher et al., 2005; Dórea et al., 2013; Vahter et al., 1994), knowledge of it's half-life is important in the modeling of the toxicity of all forms of mercury in humans.

It is thought that the long-term storage form of inorganic mercury in the brain is mercury-selenide (Björkman et al., 1995; Clarkson & Magos, 2006; Falnoga & Tusek-Znidaric, 2007; Kosta et al., 1975; Nylander & Weiner, 1991). Based on observations in occupationally exposed cohorts (Falnoga & Tusek-Znidaric, 2007), and a very low solubility product of mercury selenide ($K_S = 10^{-58}$) (Clarkson & Magos, 2006; WHO, 1990), it has been assumed that mercury-selenide deposits in the brain are chemically inert and non-toxic. However studies in monkeys have found that persistent inorganic mercury in the brain was associated with increased count of inflammatory cells (microglia) and decreased count of astrocytes (Burbacher et al., 2005)(Charleston et al., 1994; Charleston et al., 1995; Vahter et al., 1994; Vahter et al., 1995). More recently a study by Korbas et al found evidence that

mercury-selenide may not be the only form of mercury present in people exposed to methyl-mercury over different doses and timescales (Korbas et al., 2010). Our understanding of mercuric-selenide in the human brain is therefore evolving, however this is beyond the scope of the current paper, which aims to focus on the half-life of inorganic mercury in the brain from a pharmacokinetic perspective.

Perilously few studies on the half-life of inorganic mercury in the human brain exist. However in the past a number of studies were carried out using radioisotopes – that is administration of small quantities of radioactive Hg^{197} & Hg^{203} to volunteers and measurement of the radiation emitted by various body parts over a follow up time(Hattula & Rahola, 1975; Rahola et al., 1973; Hursh et al., 1976). The study by Hursh and colleagues led to an estimate of the half-life of inhaled mercury in the head of 21 days(Hursh et al., 1976), and based upon Hursh's paper the figure of 20 days remains listed as the half-life of inorganic mercury in the brain in table 2.4 of the influential ATSDR toxicological profile for mercury(ATSDR, 1999) (This figure is again cited in Appendix A of the profile as supporting evidence for calculated minimum risk level's (MRL's) for exposure to mercury vapour (ATSDR, 1999)). Such low figures for the brain half-life are in sharp contrast to evidence from primate studies(Vahter et al., 1995), findings in known cases of mercury poisoning followed up over the very long term, and estimates from some kinetics modeling studies(Sugita, 1978). Numerous cases of both elemental mercury exposure and organic mercury exposure have been followed up long term, and on autopsy many years after exposure significant levels of inorganic mercury have been found in the brain(Opitz et al., 1996; Hargreaves et al., 1988; Kosta et al., 1975; Takeuchi et al., 1989; Davis et al., 1994; Eto et al., 1999). Assuming first order kinetics, these results imply a half-life in the brain of years in duration. However as we often cannot accurately determine initial dose it is not possible to calculate a value for the half-life from individual cases. The absence of an agreed figure for the half-life has led to a lack of appreciation amongst some authors for the extremely long retention time of mercury in the brain: *"Studies with radioactive tracers indicate that the rate of overall excretion of mercury from the body can be described by a single half-time of about 58 days, corresponding to an excretion rate of slightly more than 1% of the body burden per day. Most tissues have the same or shorter half-times."*(Clarkson, 2002)

Such uncertainty surrounding the half-life of inorganic mercury in the human brain is clearly problematic. Therefore this work was undertaken with the aim to perform a systematic review of the mercury literature to identify all available evidence in both primates and humans that could be used to make analytic inferences about the half-life of inorganic mercury in the brain.

Study selection

The search was limited to human and animal studies because “observed inter- and intraspecies differences in the type and severity of the toxic response to mercury may result from differences in the absorption, distribution, transformation, and end tissue concentration of the parent mercury compound.” (ATSDR, 1999). Such differences are likely to lead to differences in estimates of brain half-life between species. Initial search strategies using descriptive terms such as “brain” “half-life” and ”mercury” failed to provide useful results. For this reason it was decided to use a very broad search strategy to capture as many papers with relevant information as possible. The Pubmed database was searched (last search update on 23/04/2013) using MESH terms pertaining to mercury toxicity. (Figure 1)

This led to a very large number of hits N = 16,539. The search was restricted to English language papers in humans or mammals whose title or abstract mentioned mercury in the brain, organ measurements of mercury, autopsy studies and mercury case studies, or half-life. Review papers and studies examining samples from feotuses and children were excluded as pharmacokinetics may differ in the very young. This left 984 papers of potential interest. After a second round of screening remaining papers were categorised by species – human or primate. A limited number of additional papers not captured by the search but known by the author were also included. The full reprint of all remaining papers was then obtained where possible and reviewed. Reprints of 7 papers could not be obtained: Ando et al., 1985 – a tissue study; Carrel et al., 1979 – a cohort exposure study; Cheung & Verity, 1983 – an experimental exposure study; Fair et al., 1986 – an experimental exposure study; Kozik, 1978 – an autopsy study; Newton & Fry, 1978 – report of accidental exposure; Murai et al., 1982 – primate experimental study).

The search strategy and numbers of papers found are summarised in the flow diagram in figure 1.

Analysis strategy

Primate Studies

Primate studies were reviewed to identify papers that provided direct estimates of the mercury half-life. Effectively this meant that papers included were those where primates were exposed to some form of mercury, allowed to survive for some period of time post exposure, and at sacrifice had autopsy with measurement of inorganic mercury levels in brain tissue. The analysis strategy and results for those studies that determined the retention half-life for inorganic mercury during analysis were summarised.

Modeling studies

Modeling studies that were captured by the search strategy were identified and examined for calculations of the brain half-life. These studies were summarized in tabular form detailing modeling approach, important assumptions, and half-life findings if present.

Human Case Studies

Human case reports where one or more persons were exposed to mercury and followed up over a prolonged period before death, and where subsequent autopsy including measurement of tissue levels of mercury, were identified. These studies could not be used to calculate a precise half-life for mercury in the brain for each case, however assuming first order kinetics (implying near complete elimination of inorganic mercury after 5 half-lives), presence or absence of elevated levels of inorganic mercury at autopsy would allow the calculation of a minimum or maximum bound for half-life. Only cases with follow up post mercury exposure of one year or greater were used to estimate bounds.

Autopsy cohort studies

Autopsy studies with N >15 and where brain Hg levels were measured were summarised in tabular form. These studies are potentially useful in determining half-life as results from autopsy studies can be used to determine organ half-life using kinetics modeling approaches(Sugita, 1978). Any studies that calculated a half-life for inorganic mercury in the brain were identified. These studies were also of interest as analysis of trends of bioaccumulation of mercury in the brain over time amongst populations with relatively uniform mercury exposure may be informative regarding half-life. This is based on the observation that, assuming first order kinetics at a steady state exposure, the end organ concentration will reach a steady state in approximately 5 half-lives for a given exogenous substance(Flomenbaum et al., 2006). Assuming that the exposure over time remains relatively constant, this allows us to make predictions of what the relationship between age and brain inorganic mercury levels might look like given different half-lives.

However, in attempting to utilise such an approach to measure the half-life of mercury in humans, we must recognise the dangers of unmeasured confounders, idiosyncatic kinetics in individuals and population subgroups with altered toxicokinetics (for example – carriage of GCLM-588 T allele has been associated with elevated levels of blood, plasma and urine total mercury(Custodio et al., 2005)). Nevertheless examination of autopsy studies may provide insight regarding bioaccumulation.

Synthesis of Results

Primate studies

Of the 42 primate research papers identified, only five studies provided estimates or made inferences about the half-life of inorganic mercury in the brain. Table 1 displays a summary of the critical factors of these studies including exposure type, follow up time and estimated inorganic mercury brain half-life. Note that not all papers measured inorganic mercury and two studies are based on the same experimental animals. Of the five studies only one study made numeric estimates of the brain half-life of inorganic mercury. As part of a landmark series of studies(Vahter et al., 1994; Vahter et al., 1995; Charleston et al., 1994; Charleston et al., 1995; Charleston et al., 1996; Björkman et al., 1995) on the kinetics of chronic methyl-mercury exposure in monkeys Vahter et al determined the half-life of inorganic mercury in the brain of 5 Macaca Fascicularis monkeys exposed to methlymercury daily for 12 months and then sacrificed after a further 6 months for measurement of organ speciated mercury levels(Vahter et al., 1995). They determined the half-life for the cerebellum, occipital pole, pons, motor strip and frontal pole in Macaca Fascicularis monkeys to be between 227 and 540 days (mean 345 days, SD 126 days)(Vahter et al., 1995). Vahter et al identified the thalamus and the pituitary as the brain areas with longest elimination time(Vahter et al., 1995). A study by Burbacher et al that exposed Macaca Fascicularis monkeys to intermittent doses of methyl- or ethyl-mercury with sacrifice of animals at different intervals, subsequent autopsy and measurement with speciation of organ mercury levels determined that the half life of inorganic mercury in the brain was too long to be determined from the study, but was estimated to be greater than 120 days(Burbacher et al., 2005). Stinson et al studied the kinetics of methyl-mercury again in Macaa Fascicularis monkeys but did not calculate a brain half-life for inorganic mercury - however they commented that it had “an extremely long half-life”(Stinson et al., 1989). Rice et al determined a shorter half-life for mercury in the brain in methyl-mercury exposed Macaca Fascicularis monkeys of between 38 and 56 days(Rice, 1989). However this study did not perform speciated mercury analysis, therefore this half-life figure represents total mercury half-life (methyl-mercury plus inorganic mercury), which is likely to appear much shorter than the half-life of inorganic mercury alone because the half-life of methyl-mercury was seen to be short in the other animal studies(Burbacher et al., 2005; Vahter et al., 1995).

Modeling Studies

10 modeling studies were identified by the literature search and these have been summarized in table 2. Of the 10, only two provided estimates of the half-life of inorganic

mercury in the brain. The first of these by Sugita in 1978 calculated the half life of inorganic mercury in the total brain to be 22 years(Sugita, 1978). In this study, Sugita set out to determine the half-life of heavy metals in different human tissues using data from autopsy studies. In the case of mercury they modeled data from 166 specimens of human cerebrum, cerebellum and hair, by use of differential equations to model change in organ concentration with time and allowing for a short half-life compartment and a long half-life compartment in each organ. This was based on the assumption that the amount of metal within organs was proportional to food intake by age. Whilst this approach had the advantage of allowing for long half-life compartments in organs, and it is thought that mercury can bioaccumulate in the brain over time, the assumption that exposure to inorganic mercury corresponds *linearly* with age may be too strong, and may be broken by individual idiosyncratic exposures to mercury in individuals with excessive dental work or very large fish consumption. In sharp contrast Young et al calculated the half life of inorganic mercury in the brain to be 69 days(Young et al., 2001). Their analysis modeled the pharmacokinetics of methyl-mercury allowing for transformation to inorganic mercury using a physiologically based pharmacokinetic (PBPK) model. This model attempted to extrapolate a human pharmacokinetic model for methyl-mercury based on extrapolations from animal studies and limited human data including results of previous human autopsy studies and some human experimental data. Young et al made the assumption that autopsy organ measurements were 'steady state' values – implying that they were not increasing with age. The extreme difference in half-life estimation between the Young and Sugita studies is striking. These differences may be routed in the differing assumptions of steady state (Young et al) and linearly increasing with age (Sugita) organ concentrations. Since it is known from animal studies(Vahter et al., 1995) that inorganic mercury does tend to bio-accumulate in the brain over time and therefore a steady state is likely not achieved, the assumption by Sugita is arguably more appropriate than that of Young.

Vimy et al(Vimy et al., 1986) used an interesting approach to validate the model developed by Bernard & Purdue(Bernard & Purdue, 1984) which utilized a four compartment model with a long retention component of half-life 10,000 days (27.4 years). Vimy et al used estimates of mercury vapour release rates in combination with Bernard & Purdue's model to predict blood and organ mercury levels over time. They then checked their predictions against published data for in vivo blood mercury levels and post-mortem brain mercury levels from autopsy studies and found them to be in approximate agreement, although this relies on the assumption that the long retention compartment modeled by Barnard & Purdue corresponds to brain tissue.

Most of the remaining studies did not attempt to determine the half-life of mercury in the brain. In part this was because the concentrations of mercury within the brain are much lower than that of blood or elimination organs such as the liver or kidney. For example, when attempting to model the whole body kinetics of methyl-mercury Carrier et al found "*Blood-brain exchange parameters for inorganic mercury and brain metabolism rate constant {k_{BBr}, k_{BrB}, d_{BBr}} could not be determined specifically for humans for lack of time profile data.*"(Carrier, Bouchard, et al., 2001) They went on to conclude "*Since the amount of inorganic mercury in the brain is very small compared to the total inorganic mercury burden (in the rat at most 0.011 %), precise knowledge of its value was not necessary to determine the mercury kinetics in other organs, blood, hair, and excreta.*" Thus, when modeling the kinetics of mercury in the body specific modeling of a brain compartment may seem to some to unnecessarily complicate the model due to the small concentrations of mercury in the brain relative to blood. However the exclusion of a brain compartment negates the possiblity of modeling bio-accumulation of inorganic mercury therein. Therefore kinetic models for mercury (and indeed other toxins) need to be designed at outset with specific consideration of sensitive organs with long half-lives, in particular the brain, given the potential for bio-accumulation.

Human Case studies

The majority of cases identified were not informative regarding brain half-life as the patient either survived, brain tissue levels were not measured at death or survival was less than one year. 18 cases from 6 papers were identified that met the criteria of having follow up post exposure of >1 year, and of having autopsy performed with measurement of mercury levels in brain tissue. The critical factors for these cases and estimates of half-life bounds are shown in table 3. Follow up times ranged up to 26 years. Exposure sources were varied with a number of cases of acute and chronic Minamata Disease included as well as cases from mercury miners with chronic mercury exposure. In all cases significant levels of inorganic mercury were found in the brain at autopsy allowing estimation of *minimum* bounds for half-life, assuming first order kinetics, of 0.27 to 5.2 years (mean 2.8 years). As no case was identified where mercury had returned to background levels no estimate of an upper bound could be obtained. It is therefore important to state that these estimated minimum bounds are likely extremely conservative as half-life estimates, depending primarily on the chance occurence of the person dying. The presence of mercury at autopsy indictates we are within 5 elimination half-lives at death allowing the minimum estimate for half-life. Therefore a conservative interpretation of these results implies that the half-life of mercury in the brains of humans is at a minimum several years, and possibly 5.2 years or greater.

Radionucleotide studies & other experimental studies

A small number of radionucleotide studies (n = 6) were identified. Miettinen et al orally administered ^{203}Hg labelled methyl-mercury to 15 volunteers (Miettinen et al., 1971). Whole body counting, stool, urine and blood Hg measurements were used to establish a whole body half life of 76 ± 3 days for methyl-mercury. In 1973 Rahola et al(Rahola et al., 1973) orally administered inorganic ^{203}Hg (half-life of ^{203}Hg = 46.6 days) to ten volunteers. They utilized a whole body counting technique, urine, faecal and blood samples to determine a whole body half-life of 42.3 days, however they did not attempt to quantify a brain half-life. A follow up study by Hattula et al(Hattula & Rahola, 1975) administered radiolabelled (^{203}Hg) methyl-mercury to 15 volunteers and inorganic mercury to 8 volunteers and then used lead shielding to isolate body parts to determine radioactivity levels in a given body compartment (e.g. the head). They estimated a biological half-time for methyl-mercury in the head of 62 – 400 days. Hattula et al noted however that ^{203}Hg could only be measured up to 120 days as afterwards statistically significant results were not obtainable and this may have affected half-life estimates. Hursh et al(Hursh et al., 1976) used a similar method to isolate body compartments in 5 human subjects exposed to ^{197}Hg/^{203}Hg vapour and calculated an average half-life of 21 days in the head as mentioned in the introduction.

Smith et al employed a different radiolabelling strategy, having administered IV radiolabelled methy-mercury, measured speciated mercury in blood and excreta over 70 days and then applied multicompartment modeling(Smith et al., 1994). Whilst they recognised a long half-life for inorganic mercury they did not quantify this and did not model specific organs. Moving away from radiolabelling a study by af Geijersstam et al asked volunteers to swallow dental amalgam and applied two compartment modeling to plasma mercury readings over 90 days leading to a slow compartment half-life estimate of 37 days - a brain compartment was not included in the model(af Geijersstam et al., 2001).

Autopsy studies

24 autopsy studies detailing inorganic or total mercury levels in the brains of deceased individuals from different exposure populations were found. Population mercury exposures ranged from background exposure in individuals with no known exposure, exposure in patients with dental amalgams, methyl-mercury exposure due to fish consumption, exposure to elemental mercury in dentists, to very high exposure to elemental mercury in gold minors. The exposures, number of autopsies and other critical factors are summarized in table 4. None of the studies attempted to determine brain mercury half-life, however some attempted to identify bio-accumulation by examining the relationship between mercury levels and age (or exposure time) and these findings have been given in the table where appropriate.

11 studies including a total of 460 individuals examined the relationship between brain inorganic mercury and age. A 1974 study by Mottet et al measured brain total mercury from 61 autopsied urban and rural individuals and found a bivariate linear correlation between decade of age and brain total mercury in selected brain regions of R = 0.436 – 0.961 (brain regions not specified)(Mottet & Body, 1974). In 1981 a study by Tucek et al determined brain total mercury levels in samples from 82 residents and found peak brain (cerebrum) levels in those aged 50-59 (Tucek & Tucek, 1981). However it should be noted that of those 82 people only 2 were aged under 40 years. A similar result was seen in a study of 46 people by Hac et al with peak brain total mercury in the 41-60 age group(Hać et al., 2000). However in this study the more elderly were under-sampled with only 6 measurements in those aged 61-90. A series of studies on Swedish people eventually including up to 44 individual autopsy samples, including some occupational exposed to elemental mercury through dental work, were published from 1987 to 1993 by Nylander, Weiner and other coauthors(Nylander et al., 1987; Nylander et al., 1989; Nylander & Weiner, 1991; Weiner & Nylander, 1993). Innovatively, these studies attempted to quantify mercury exposure by counting dental amalgam surfaces at the time of autopsy as a proxy for long-term exposure. This was based on the observation from a Swedish longitudinal study that "Amongst adults only slight changes were observed over the period both for the number of remaining teeth and the number of filled teeth"(Weiner & Nylander, 1993)(Lavstedt et al., 1987). These papers reported a relationship between number of amalgam surfaces and both occipital lobe total mercury level(Nylander et al., 1987) and pituitary total mercury level(Nylander et al., 1989). Age and an interaction between age and number of amalgam surfaces were seen to have significant but small associations with brain total mercury in multivariable linear analysis(Weiner & Nylander, 1993). Samples from 42 Tokyo residents with no known mercury exposures were examined by Matsuo et al who found that the log of cerebrum inorganic mercury was correlated with age ($r = 0.402$, $p < 0.05$)(Matsuo et al., 1989). A 1996 paper by Schumacher et al determined total mercury levels in the brains of 60 urban and rural non occupationally exposed fish-eating individuals(Schuhmacher & Corbella, 1996). On analysis they included age in a multivariable model of brain total Hg, however the coefficient for age was not significant. In a 1999 paper analysing samples from 17 Greenlanders with high methyl-mercury intake and 12 Danes with low methyl-mercury intake, Pedersen et al found a correlation between age and total CNS mercury in Greenlanders and also an inverse correlation with % organic mercury suggesting bio-accumulation of inorganic mercury in the CNS with age (Pedersen et al., 1999). In 2006 Guzzi et al reported on analysis of organ levels of mercury from 18 individuals with no known occupational or accidental exposures who underwent routine autopsy in Milan, Italy(Guzzi et al., 2006). Number of amalgam surfaces was seen to correlate with brain levels of total mercury

however age did not significantly modify this relationship. Finally, in 2007 Bjorkman et al determined speciated brain mercury levels in 30 routine autopsy cadavers with no know occupational exposure (Björkman et al., 2007). Data was collected on number of amalgam surfaces, history of alcohol abuse and other confounders. A significant correlation was found between number of amalgam surfaces and occipital cortex and pituitary inorganic mercury levels (Björkman et al., 2007). Subsequent multivariable modeling failed to show a significant effect of age however Bjorkman commented 'the statistical power was too low to exclude a minor effect from age (Björkman et al., 2007).

Despite having identified a large number of autopsy studies, only three (Matsuo et al(Matsuo et al., 1989), Pedersen et al(Pedersen et al., 1999), Bjorkman et al(Björkman et al., 2007)) reported on the relationship of speciated mercury (i.e. separately measured organic and inorganic mercury) with age, and of those only Matsuo report a clear linear relationship between log of brain inorganic mercury and age (although other studies did report positive associations between brain total mercury concentration and age). Results from animal studies(Charleston et al., 1994; Charleston et al., 1995; Vahter et al., 1995; Björkman et al., 1995) demonstrate accumulation of inorganic mercury with constant exposure and human cases summarized in table 3 point towards a very long retention time of inorganic mercury (at least at higher exposure levels) - so it is perhaps surprising not to see more striking bio-accumulation in autopsy studies. However, it is clear from examining graphs of brain mercury concentrations in many autopsy studies that there is a high level of variability of brain mercury levels(Mottet & Body, 1974; Nylander et al., 1987; Nylander et al., 1989; Matsuo et al., 1989; Weiner & Nylander, 1993; Uchino et al., 1995; Drasch et al., 1994; Pedersen et al., 1999; Falnoga et al., 2006; Lech & Sadlik, 2004; Björkman et al., 2007) – likely due to measured and unmeasured confounders such as dental history, dietary habits, accidental/environmental exposures and genetics. It was also noted that some of the studies had very sparse sampling of certain age groups(Tucek & Tucek, 1981; Hać et al., 2000), making interpretation of age effects very difficult. Therefore it is unlikely that small studies would detect a small effect of age on brain concentration. Unfortunately many of the larger autopsy studies did not report on relationships between brain concentration and age.

Discussion

Despite a broad and extensive search strategy this review has identified only a handful of studies providing evidence on the retention time of inorganic mercury in the primate and human brain. Whilst several studies have noted the half life to be very long, only one animal study and two modeling studies put figures on a half-life estimate specifically for inorganic mercury, with Vahter et al(Vahter et al., 1995) arriving at a figure of 227- 540 days (0.62 – 1.48 years) in macaca fascicularis monkeys, and with the modelling study by Sugita arriving at a half-life of 22 years in humans lying in sharp contrast to the estimate by Young of 69 days(Young et al., 2001).

In comparing these estimates to the results found from 18 human cases of mercury toxicity with long term and subsequent autopsy, it is noteworthy that in all of the cases excess mercury levels were found in the brain at autopsy. This points to a very long half-life – i.e. *evidence found in human case studies indicate that the brain half-life of inorganic mercury was likely at least several years, and indeed may exceed 5.2 years*. This may indicate that the half-life in humans is substantially longer than that of primates. However, caution must be applied here lest there is a publication bias at play in the reporting of human case studies – perhaps investigators have performed autopsies in mercury poisoned individuals and failed to find appreciable mercury levels in the brain and simply not reported the findings. This highlights the continuing importance of life long follow up of clinical cases of mercury toxicology with autopsy and measurement of tissue levels where possible and the consistent reporting of those results even if negative.

These findings stand in stark contrast to the shorter estimates of half-life obtained from basic radionucleotide studies e.g. 21 days as estimated by Hursh et al(Hursh et al., 1976). It is critically important to recognise that in addition to the limitations imposed by the relatively short half-lives of the radioactive isotopes, such approaches measure half-life of mercury in <u>all tissues of the head including circulating blood</u>, not solely that of the brain. And, as noted in the discussion on modeling studies, the levels of mercury in the brain are typically much smaller than that of other tissues such as blood(Carrier, Bouchard, et al., 2001). Furthermore, this approach cannot be used to determine the quantity of inhaled elemental mercury or ingested methyl-mercury that is known to be metabolised to inorganic mercury once inside the blood brain barrier(Dórea et al., 2013; Rooney, 2007; Burbacher et al., 2005). Therefore such approaches *cannot be used to accurately determine the half-life of inorganic mercury in the brain.* A more sophisticated radionucleotide study by Smith et al which speciated mercury after exposure to radiolabelled methyl-mercury was able to detect a long retention time for inorganic Hg although could not enumerate it. Authors and agencies

need to be aware of the shortcomings of these studies in determining brain inorganic mercury half-life.

On consideration of the contrasting estimates from modeling studies (i.e. 22 years (Sugita, 1978) vs 69 days (Young et al., 2001)), it seems likely initial assumptions may have large effects on estimates of half-life. In general modeling studies did not estimate the half-life of inorganic mercury in the brain either because the amount of mercury in the brain was very small relative to the blood or other organs, or it was not recognized that bio-accumulation may occur, thereby illustrating the need to consider such eventualities at outset when model building. This comparison of modeling studies highlighted the importance of model assumptions and may hold lessons for kinetic modeling of toxins beyond mercury. To allow for potential bioaccumulation in models, particular caution should be taken to explicitly model sensitive organs with long half-lives, even if concentrations of the toxin are low in that organ over shorter timescales.

Although a large number of autopsy studies were identified, these were not informative regarding the half-life of inorganic mercury. The need for further studies measuring speciated human organ levels of mercury (i.e. organic and inorganic mercury) in deceased individuals with well-characterized mercury exposures and confounders(e.g. dental history, dietary exposure, accidental exposure) is apparent – much larger studies may be needed to detect small bio-accumulation effects or estimate half-life. Genetics may also be a factor and the existence of subpopulations with altered kinetics should be born in mind.

While the search strategy was broad and identified several different forms of evidence, the search was limited to English language studies and it is likely that there are relevant papers in other languages. The use of a systematic search strategy proved useful in identifying varied sources of evidence in an objective non-biased manner, and this approach may be of use in other areas of toxicology where evidence on a particular topic is scarce.

Conclusions

In conclusion, the body of evidence points towards inorganic mercury in humans having a very long half-life in the brain – likely years or decades long. Evidence from cases of mercury poisoning indicates it is likely at least several years and possibly over 5 years. Probably the best estimate of half-life in humans remains the 1978 estimate by Sugita(Sugita, 1978) of 22 years although this is based on a strong assumption of a linear relationship between food consumption by age and organ mercury level. Nevertheless a predictive model with half-life of 27.4 years was found to produce mercury organ concentration estimates in close

agreement with post-mortem studies (Bernard & Purdue, 1984; Vimy et al., 1986). The combination of human cases and modeling studies raises the prospect that the human half-life may be much greater than that of primates – estimated by Vahter et al as 227 – 540 days (Vahter et al., 1995). Finally, there is no convincing evidence from primate studies, human case studies, modeling studies or well-designed experimental studies to support estimates for the half-life of inorganic mercury in the brain as low as 20 days. These findings carry important implications for pharmacokinetic modeling of mercury toxicity that may in turn have consequences for determining regulatory exposure measures for mercury exposure, such as minimum risk levels (MRL's).

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Conflict of Interest Statement

I have no conflicts of interest to declare.

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Table 1 Estimates of brain half-lie of inorganic mercury in primate research

First Author	Year	Study Type	Primate Species	N	Mercury exposure type	Exposure/Follow-up times/Hg measurement method	Inorganic mercury brain Half-life estimate
Burbacher	2005	In vivo	Macaca Fascicularis	41	Methyl-mercury or thimerosal	49 days maximum exposure + follow up. Intermittent sacrifices from different exposure groups. Cold vapor atomic absorption at 254nm.	>120 days
Rice	1989	In vivo	Macaca Fascicularis	12	Methyl-mercury	At least 1.7year exposure – 230days of follow up. Flameless atomic absorption spectrometry.	*
Stinson	1989	Reanalysis of in vivo studies	Macaca Fascicularis	45	Methyl-mercury	Up to 1143 days/ up to 668 days of follow up. Cold vapour flameless spectrophotometry.	‡ "a form of mercury with an extremely long half life"
Vahter	1994	In vivo	Macaca Fascicularis	27	Methyl-mercury	12 -18 months exposure. 6 months follow up in 12 month exposed. Cold vapour atomic absorption spectrophotometry.	† "On the order of years"
Vahter	1995	In vivo	Macaca Fascicularis	27	Methyl-mercury	12 -18 months exposure. 6 months follow up in 12 month exposed. Cold vapour atomic absorption spectrophotometry.	† 227 – 540 days thalamus pituitary longer

*Whilst this study estimated half lives of total mercury in the brain as being between 38 and 56 days – they did not speciate mercury into organic and inorganic – therefore these estimates are based on total mercury level which is influenced by more rapid decline of methyl-mercury levels

†These studies are based on analysis of the same experimental subjects

‡ This study did not measure speciated mercury levels – however the authors concluded "Therefore it appears the concentration of some form of mercury is slowly increasing in some brain regions. It is unclear whether this is a form of mercury with an extremely long half-life or whether some form of mercury is being irrevocably deposited in the brain"

Table 2 Summary of modeling studies meeting inclusion criteria

First Author	Year	Modeling approach	Estimated brain half-life for inorganic Hg
Willes	1977	Bioexponential model derived from methyl-^{203}Hg experiment in cats adapted to human model. Compared brain:blood ratios in cats and humans but did not consider metabolism to inorganic Hg	Not determined
Sugita	1978	Used differential equations to model data from autopsy studies to model organ half-lives of inorganic Hg, including brain, assuming a blood compartment and a two compartment model of each organ – a short half-life compartment and a long half-life compartment. Assumed constant background exposure with time for autopsied individuals of different ages with no known acute exposure	22 years (total brain) 18 years (cerebrum)
Bernard	1984	Assumed a four compartment model with a long retention compartment of half-life 10,000 days (27.4 years). Separate models for methyl and inorganic mercury	Long retention compartment fixed by model assumptions
Vimy	1986	Applied model by Bernard to model bioaccumulation given typical daily exposures and compared to in vivo blood measurements and results of autopsy studies	Long retention compartment fixed by model assumptions
Jonsson	1999	Applied Bayesian modeling technique to excretion data from human inhalation of mercury vapour studies. Allowed for long retention compartments of lung and kidneys but not brain.	Not determined
Carrier Carrier,	2001 2001a	Used differential equations to model multi-compartment metabolism of methyl-mercury in humans (allowing for conversion to inorganic Hg) based on animal data and cross checked against available human data	Unable to quantify brain to blood transfer rate for inorganic Hg*
Young	2001	Cross species physiologically based pharmacokinetic (PBPK) model of kinetics of methyl-mercury allowing for transformation to inorganic mercury.	69 days**
Hashemi	2003	Artificial neural networks and rough sets methodology used to predict half-life of methyl-mercury in humans. Did not allow for brain compartment or for transformation to inorganic Hg	Not determined
Verger	2007	Piecewise deterministic Markov process applied to pharmacokinetics of methyl-mercury in humans. Did not allow for brain compartment or for transformation to inorganic Hg	Not determined

*This was in part due to lack of data and in part due to the small concentration of inorganic mercury found in the brain in comparison to blood – i.e. when attempting to model whole body kinetics, the concentration of mercury found in the brain is small relative to blood and some other organs, thus leading to much larger errors in estimates of brain kinetics

** This was a simulated half-life predicted by a PKBK model with assumed steady state organ levels

Table 3 Summary of human cases of accidental mercury exposure

First author	Year	Mercury exposure / Hg measurement method	n	Follow-up time	I-Hg present in brain tissue at follow up ?	Lower bound for I-Hg half-life (follow-up time / 5)
Kosta	1975	Mercury Miners BP** – 33 years TA** – 29 years Neutron activating and volatilisation technique	7	 16years 16years	 Yes Yes	 ≥ 3.2yrs ≥ 3.2 yrs
Takeuchi	1989	Index case: Methylmercury, chronic over 6 years. Reference cases: Methylmercury. Unknown duration Total Hg – atomic absorption spectrophotometry Me-Hg – Paper chromoatogrphy	1 2 2 1 1 2	26 yrs 1.33 yrs 2 – 2.58 yrs 7 yrs 14 yrs 17 – 18yrs	Yes Yes Yes Yes Yes Yes	≥ 5.2 yrs ≥ 0.27 yrs ≥ 0.4 – 0.52 yrs ≥ 1.4yrs ≥ 2.8yrs ≥ 3.4 – 3.6yrs
Davis	1994	Methylmercury, 3 months exposure. Samples prepared by acid digestion – unspecified Hg measurement method.	1	22 yrs	Yes	≥ 4.4yrs
Eto	1999	HU594 – chronic I-Hg, 10 yr exposure * HU602 – chronic I-Hg, 9 yr exposure * KU6383 – Me-Hg, acute exposure KU7903 – Me-Hg, chronic exposure Total Hg – flameless atomic absorption spectrophotometry Me-Hg – Gas Chromatography	1 1 1 1	10yrs 10yrs 18yrs 25yrs	Yes Yes Yes Yes	≥ 2 yrs ≥ 2 yrs ≥ 3.6yrs ≥ 5yrs
Opitz	1996	I-Hg. Acute exposure (possible background chronic exposure) Flameless atomic absorption spectrophotometry	1	17yrs	Yes	≥ 3.4yrs
Hargreaves	1988	Elemental Hg. Chronic exposure over 18 months. Histological staining method of Danscher and Schroeder	1	16 yrs	Yes	≥ 3.2yrs

*Previously reported by Takahata et al(Takahata et al., 1970)
** Patients identifiers as per Kosta et al, 1975

Table 4 Summary of human autopsy cohort studies with N>15 and where brain inorganic mercury was measured

First Author	Year	Cohort	N (Num with brain measurements)	Exposure groups / Hg Measurement method	Age range	Relationship of brain inorganic Hg with age
Mottet	1974	Urban and rural residents autopsied at University of Washington. United States	113 (61)	Medical, occupational and social histories studied. Only 1 patient reported exposed to mercury based medication. Flameless atomic absorption spectrophotometry	Premature babies – 88 yrs	Correlation brain total Hg and age grouped by decade: R= 0.436 – 0.961 for central nervous system regions.
Gabica	1975	Autopsy cases from 6 hospitals in Idaho. United States	242	Area of natural cinnabar ore occurrence and extensive mercury mining history. Cold vapour spectrophotometry	0 – 86 yrs	Descriptive statistics only – correlation with age not calculated. Elevated levels in elderly women noted but not in men.
Sumino	1975	Cadavers examined at Koba University School of Medicine, Japan	30 (21)	Unknown exposures Total Hg: Flameless atomic absorption Me-Hg: Gas chromatography	10 – 60+ yrs	Relationship with age not reported
Kitamura	1976	Autopsy samples, Department of Legal Medicine of Kobe University, Japan	30	Residents of Hyogo Prefecture, Japan – known area of high fish consumption Total Hg: Flameless atomic absorption Me-Hg: Gas chromatography	0 – 60+ yrs	Relationship with age not reported
Tucek	1981	Predominantly residents of Klatovy, Plzeň & Domažlice, Czech Republic	87 (82)	No details given Atomic absorption spectrophotometry	0 – 90+ yrs	Peak brain total mercury in age group 50 – 59
Eggleston	1987	Tissue specimens from County Coroner's Office, Los Angeles, United States	150 (only 83 included in statistics)	Number of dental amalgam surfaces counted at autopsy Atomic absorption spectrophotometry and neutron activation analysis.	13 – 59 yrs	Age not examined. Higher levels of Hg seen in those with higher numbers of amalgams.
**Nylander	1987	Autopsy samples from County Coroner's Office, Stockholm,	34	Number of dental amalgam surfaces counted at autopsy. Occupation	16 – 80 yrs	No significant age related relationship found. Correlation found between no.

		Sweden		determined. Atomic absorption spectrophotometry and neutron activation analysis.		of amalgam surfaces and occipital lobe total-Hg ($r = 0.54$, $p<0.001$)
Matsuo	1989	University of Tokyo, residents of Tokyo metropolitan area, Japan	46 (28)	No known mercury exposures. Total-Hg & I-Hg: Flameless atomic absorption spectrophotometry. Me-Hg: Gas chromatography	4 months – 82 yrs	Log of Cerebrum I-Hg significantly correlated with age ($r =0.402$, $p<0.05$) %I-Hg ($r = 0.574$, $p<0.01$)
**Nylander Nylander & Weiner	1989 1991	Coroner's Office, Stockholm, Sweden	35 (28)	8 dental workers and 27 non-occupationally exposed controls. Dental surfaces counted at autopsy. Atomic absorption spectrophotometry and neutron activation analysis.	16 – 88 yrs	No comments on age(Nylander et al., 1989). Correlation found between num amalgam surfaces and pituitary total Hg($R = 0.53$, $p<0.01$ or excluding outliers $R = 0.65$, $p<0.01$) (Nylander et al., 1989) Age seen to confound regression between brain Hg and Selenium levels in dental staff but not controls(Nylander & Weiner, 1991)
Weiner	1993	Cadaver samples from general population, National Institute of Forensic Medicine, Stockholm, Sweden	44	Dental surfaces counted at autopsy. 2 cases eliminated due to prior occupational exposure. Neutron activation analysis	16 – 88 yrs	Linear model of occipital Hg level fit including age and number of amalgam surfaces found. Interaction term also found to be significant. The relationship between pituitary Hg level and age fit a linear model when outliers removed.
Bush	1995	Protocol approved by Mayo Foundation Institution Review board, Mayo Clinic, Rochester, United States.	30	Subjects were either health at prior to death or had diseases not related to metal toxicity. Cold vapour atomic absorption	18 – 85 yrs	Did not report relationships with age

				spectrophotometry		
Uchino	1995	Certified Minamata Disease patients, Japan	77	Minamata disease patients certified at autopsy. Me-Hg: Gas chromatography	36 – 96 yrs	Did not report relationships with age
Schuhmacher	1996	Urban and rural residents from Tarragona province, Spain.	60	Non-occupational exposed residents from a fish consuming area. Cold vapour atomic absorption spectrophotometry.	18 – 96 yrs	Brain total mercury correlation with age in multivariable model (r = 0.4089) – but not significant
Drasch	1997	Samples from Cadavers at Institute of Forensic Medicine, University of Munich, Germany	150	No evidence of specific heavy metal exposure in biographies of patients. Cold vapour atomic absorption spectophometry.	16 – 93 yrs	Did not report relationship with age.
Fung	1997	Samples obtained from cadavers who suffered Alzheimers or Multiple sclerosis in life or controls, National Neurological Research Specimen Bank, Los Angeles & McLean Hospital Brain Tissue Resource Centre, Belmont. United States.	34	Dental and employment histories of deceased subjects was unavailable. Neutron activation analysis.		Did not report relationship between mercury and age.
Cornett	1998	Specimens from University of Kentucky Alzheimer's Disease Research Center, United States.	79	58 Alzheimer Disease subjects & 21 controls. Neutron activation analysis.	59 – 98 yrs	Did not report relationship with age.
Pedersen	1999	Specimens from Greenlanders and Danes.	29	17 Greenlanders with high methyl-mercury intakes. 12 Danes with low methyl-mercury intake.	41 – 83 yrs	Correlation between age & log total-brain Hg in Greenlanders. %organic mercury negatively correlated with age

				Total Hg: Atomic absorption spectrophotometry. Me-Hg: AAS after extraction of Me-Hg from tissues.		suggesting bioaccumulation of I-Hg with age
Falnoga Falnoga	2000 2006	Postmortem samples from Institute of Forensic Medicine, University of Ljubljana and General Hospital of Slovenja Gradec, Slovenia	35	Control group (n = 22), residents living in contaminated area (n = 9), retired mercury miners (n = 4). Neutron activation analysis and cold vapours atomic absorption spectrophotometry	33 – 99 yrs	Did not report relationship with age.
Hac	2000	Samples from Gdańsk residents, Department of Forensic Medicine, Medical University of Gdańsk, Poland	46	No details given on Hg exposure risks Cold vapour atomic absorption spectrophotometry.	17 – 90 yrs	Highest total mercury values in 41 – 60 age group
Lech	2004	Polish residents autopsied at the Institute of Forensic Research, Kraków, Poland	75	Accidental deaths – no details given on Hg exposure risks. Cold vapour atomic absorption spectrophotometry	17 – 56 yrs	Did not report relationship with age.
Guzzi	2006	Samples from routine autopsy cases, Institute of Legal Medicine, Milan, Italy	18	No accidental or occupational exposure, not from polluted areas. Dental amalgams counted. Cold vapour atomic absorption spectrophotometry.	24 – 71 yrs	Associations found between number of amalgam surface and total mercury in cerebral cortex and pituitary ($P<0.001$ for both). Not significantly changed by including age in model
Bjorkman	2007	Samples from routine autopsy, Dept. of Pathology and Dept. of Forensic Medicine, the Gade Institute, Haukeland University Hospital, Bergen, Norway.	30	Non-occupationally exposed. Dental amalgams surfaces counted. Cold vapour atomic fluorescence spectrophotometry and sector field inductively coupled plasma-mass spectrometry	47 – 91 yrs	Correlation between no. of amalgam surfaces & I-Hg in occipital cortex ($r = 0.55$, $P = 0.002$) and pituitary ($r = 0.54$, $P=0.002$). Age had no significant effect in multivariable models but a small effect could not be out-ruled

** A number of patients from these studies are the same patients as described in 1993 by Weiner & Nylander(Weiner & Nylander, 1993)

Figure 1 Search Strategy Flow Diagram