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Mercury toxicokinetics—dependency on strain and gender

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ABSTRACT

Mercury (Hg) exposure from dental amalgam fillings and thimerosal in vaccines is not a major health hazard, but adverse health effects cannot be ruled out in a small and more susceptible part of the exposed population. Individual differences in toxicokinetics may explain susceptibility to mercury. Inbred, H-2-congenic A.SW and B10.S mice and their F1- and F2-hybrids were given $HgCl_2$ with 2.0 mg Hg/L drinking water and traces of ^{203}Hg. Whole-body retention (WBR) was monitored until steady state after 5 weeks, when the organ Hg content was assessed. Despite similar Hg intake, A.SW males attained a 20–30% significantly higher WBR and 2- to 5-fold higher total renal Hg retention/concentration than A.SW females and B10.S mice. A selective renal Hg accumulation but of lower magnitude was seen also in B10.S males compared with females. Differences in WBR and organ Hg accumulation are therefore regulated by non-H-2 genes and gender. Lymph nodes lacked the strain- and gender-dependent Hg accumulation profile of kidney, liver and spleen. After 15 days without Hg A.SW mice showed a 4-fold higher WBR and liver Hg concentration, but 11-fold higher renal Hg concentration, showing the key role for the kidneys in explaining the slower Hg elimination in A.SW mice. The trait causing higher mercury accumulation was not dominantly inherited in the F1 hybrids. F2 mice showed a large inter-individual variation in Hg accumulation, showing that multiple genetic factors influence the Hg toxicokinetics in the mouse. The genetically heterogeneous human population may therefore show a large variation in mercury toxicokinetics.

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Introduction

All living organisms are exposed to low levels of mercury due to its eternal presence in the environment, but exposure leading to health hazards are generally related to specific human activities and of multitude origin: from unintentional occupational exposure (Rohling and Demakis, 2006), to ingestion of Hg as an ingredient in folk remedies, religious attributes, and skin-lightening creams (Pollard and Hultman, 2007; Risher and De Rosa, 2007). However, the main forms of Hg exposure recently discussed (Clarkson and Magos, 2006) as a source of adverse health effects are amalgam fillings (inorganic Hg) (Bates, 2006; Martin and Woods, 2006), food, especially fish (methyl Hg) (Grandjean et al., 2003; Passos et al., 2007), and as a preservative in vaccines (thimerosal) (Clifton, 2007).

A number of recent studies in cohorts of humans exposed in the above ways (Bellinger et al., 2006; de Burbure et al., 2006; DeRouen et al., 2006; Passos et al., 2007; Woods et al., 2007, 2008; Barregard et al., 2008; Pichichero et al., 2008) have tried to establish association between exposure to mercury and any adverse health effects. In addition, case reports of disease conditions after various forms of Hg exposure regularly appear in the medical literature (Mahaffey, 2005; Risher and De Rosa, 2007). Furthermore, experiments in mammals (Havarinasab et al., 2007) also including non-human primates (Burbacher et al., 2005) have been used to increase the knowledge of Hg toxicokinetics and related health effects.

The majority of the studies on these forms of Hg exposure have concluded that there is no clear evidence for significant health effects except in special situations such as methyl mercury exposure due to high consumption of Hg-rich fish and seafood (Grandjean et al., 2003). However, increased urinary Hg excretion (Woods et al., 2007; Dunn et al., 2008; Ye et al., 2008) , and increased urinary protein excretion (microalbuminiuria) has been described following exposure from dental amalgam fillings (Barregard et al., 2008). However, even after studies in large exposed cohorts, uncertainty remains with regard to possible adverse health effects due to Hg exposure in susceptible individuals (Barregard, 2005; Bellinger et al., 2008). Individual susceptibility may be due to unexpected high exposure caused for example by gum chewing or bruxism in dental amalgam bearers (Sallsten et al., 1996; Isacsson et al., 1997; Barregard et al., 2008), or genetic factors causing differences in the toxicokinetics of

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mercury (Miller and Csonka, 1968). In humans large inter-individual differences exist in the elimination kinetics of Hg after chronic exposure in chloralkali workers (Ellingsen et al., 1993). Although the underlying mechanisms are largely unknown, recent studies have shown that polymorphisms in human genes regulating the production of glutathione (glutamyl-cysteine ligase) and the conjugation of Hg with glutathione (glutathione S-transferase) (Custodio et al., 2005; Schlawicke Engstrom et al., 2008) influence the tissue concentration of Hg. Finally, the main target tissues for Hg, the nervous (Counter and Buchanan, 2004) and immune system (Dourson et al., 2001), may be unexpectedly sensitive due to either genetic factors (Nielsen and Hultman, 2002) or concomitant exposure to other environmental risk factors (Abdelouahab et al., 2008).

An important factor only recently appreciated as affecting metal toxicokinetics and adverse effects is gender (Vahter et al., 2002). Studies in kidney donors (Barregard et al., 1999) showed a 3-fold higher renal Hg concentrations in females, and several studies have observed a higher urinary Hg excretion in females (Bates, 2006) when confounding factors were taken into consideration.

The aim of this study was to examine the differences in Hg toxicokinetics caused by gender and non-H-2 genes using two inbred and H-2 congenic mouse strains and their F1- and F2-hybrids. Both gender and non-H-2 genes played an important and interactive role in regulating whole body as well as renal, hepatic and splenic Hg accumulation. The F1 hybrid mice inherited the low-accumulation pattern of one of the parental strains. In contrast, the inter-individual variation Hg accumulation was large in F2 hybrids, indicating a multigenic inheritance of the factors which regulate the accumulation.

Material and methods

Mice. Male and female A.SW (*H-2*^{s}) and B10.S (*H-2*^{s}) mice, obtained from Taconic (Ry, Denmark) and Jackson (Cal Harbour, MN, USA), respectively, were used. F1-hybrids were derived by crossing female A.SW and male B10.S mice only, since the reciprocal cross was repeatedly found to be infertile, and F2-hybrids by crossing the F1 hybrids. The animals used in the study were 6–14 weeks of age at onset of the experiments. The animals were housed in straw-bedded steel-wire cages in a high-barrier unit at the Animal Facilities of the Faculty of Health Sciences, Linköping University, and kept under controlled environment (12-h dark, 12-h light cycles, temperature 21 ± 1°C). The animals were given standard mouse pellets (CRME rodent, Special Diets services, Witham, UK) and drinking water *ad libitum*. The animals were kept in groups of 5–10 mice/cage. The studies performed were approved by the Laboratory Animal Ethics Committee, Linköping, Sweden.

Treatment and design. $HgCl_2$ (Fluka, Milwaukee, WI, USA) was mixed with the gamma-emitting ^{203}Hg isotope, obtained from Dr. Delon Barfuss, Georgia State University, USA. The amount of ^{203}Hg added to the $HgCl_2$ solution was set so that 1 mL drinking water contained 35,000–45,000 counts per minute (cpm) at onset of the specific experiment. $HgCl_2$ was given as 2.7 mg $HgCl_2$/L drinking water (2.0 mg Hg/L). Water consumption was measured by subtracting the weight of the water bottle used in the cage for 1 week from that of the same bottle just before being placed in the cage. The resulting volume was divided by the number of animals in the cage and the number of days the bottle was used. For Hg accumulation studies mice were kept on Hg treatment for 6 weeks before sacrifice. In the elimination studies the mice were treated with Hg for 5 weeks followed by 2 weeks without Hg treatment before termination of the experiment.

Blood and tissue sampling. Blood samples were collected from the retroorbital vein plexus after 2 and 6 weeks treatment following anesthesia by halothane (Forene, Abbott Scandinavia, Solna, Sweden). After 6 weeks treatment the animals were anesthetized with halothane, blood collected from the retroorbital plexa and the animals sacrificed by cervical dislocation. The left kidney, part of the liver and spleen, as well as the mesenterial lymph nodes were excised and weighed. In the elimination studies blood, kidney and a piece of the liver were obtained at sacrifice after 15 days without treatment.

Assessment of mercury accumulation in the whole body and the organs. The whole-body retention (WBR) of mercury was measured in live animals at regular intervals throughout the experimental period by placing them in a whole-body counter (NaI well crystal, diameter 50 mm, 125 mm deep). Counting was performed between 9:30 and 12:30 AM in order to reduce influence of diurnal rhythms with respect to water and food consumption. The detection limit of the counter was set to background + 3SD. Validation of the experimental model has previously been published (Nielsen, 1992). Steady state was attained after 30 days, and the steady-state WBR was defined as the mean values of WBR measurements obtained days 30–42 (2–4 measurements). In the elimination studies the whole-body counting took place during seven occasions (Table 3). The radioactivity of the tissue material obtained at sacrifice was measured using a gamma counter (Perkin Elmer, 2470 Wizard, Waltham, MA, USA). The whole body and organ counts were related to the known standard (1 mL of drinking water) included in all counting sessions.

Assessment of reduced glutathione (GSH) and glutathione S-transferase (GST)activity. Reduced glutathione (GSH) concentration and glutathione S-transferase (GST) specific activity were determined using fluorimetric GSH and GST assay kits (Sigma, Saint Louis, USA). Briefly 0.1–0.2 g of the frozen mouse kidney or 0.8–1 g mouse frozen liver were extracted using perchloric acid sedimentation. GSH values were obtained from standard curve according to the manufacturer protocol. GST specific activity were calculated according to the manufacturers recommended formula.

Assessment of metallothionein 1 (MT-1) and metallothionein 2 (MT-2) gene expression. For MT-1 and MT-2 gene expression analysis, 0.1–0.2 g of mouse liver and kidney tissue were excised and immediately put in RNALater (Ambion, Foster city, CA, USA) until analysis. The tissue pieces were transferred to the homogenization buffer of RNeasy Mini kit (Qiagen, Hilden, Germany) and total RNA was isolated according to the manufacturer's protocol. Two microgram of isolated RNNA was reversely transcribed with SuperScript (Invitrogen, Carlsbad, CA, USA) according to the manufacturers recommendation, and MT-1 and MT-2 cDNA were co-amplified with gene specific primers (available on request). PCR amplicons for respective genes were separated on 1.5% agarose gels, stained with ethidium bromide and semi-quantitative densitometry was performed in a BioRad Gel Doc imaging system (BioRad, Hercules, CA, USA). The relative expression is expressed as the ratio to the house keeping gene GAPDH.

Statistics. Differences among the means for the parental strains, the F1- and the F2-hybrids were determined using the parametric Welch analysis of variance (ANOVA) followed by Tamhane's T2 *post-hoc* test. Differences between genders within the same strain/group were determined with the Welch T-test. Differences between or among means were regarded significant at an a priori chosen level of $p<0.05$. Differences in GSH, GST, MT-1 and MT-2 between strains and gender was determined using Mann-Whitney U-test.

Results

Intake and retention of mercury

The male mice showed a 40–50% higher mean body mass than the corresponding female mice in all groups and the difference was significant (Table 1). There was no significant difference in body mass

Table 1
Intake and retention of mercury after 6 weeks exposure to 2 mg Hg/L in drinking water.

Strain	Group	Number of mice	Water intake (mL/mouse/day)	Intake of Hg (μg Hg)	WBR (μg Hg/mouse)	Retention (% of intake)	Body mass (g)	WBR (μg Hg/g b.w.)
A.SW	Male	18	3.0	252	4.35 (0.76)*	1.7	26.1 (4.0)*	0.17 (0.059)
	Female	17	2.8	235	3.40 (0.42)	1.4	19.4 (1.5)	0.18 (0.019)
B10.S	Male	20	3.2	269	3.60 (0.57)*	1.3	28.7 (4.2)*	0.13 (0.028)*
	Female	23	2.9	244	3.24 (0.50)	1.3	20.1 (2.1)	0.16 (0.028)
F1[a]	Male	19	3.2	269	3.74 (0.61)	1.4	31.6 (2.9)*	0.12 (0.020)*
	Female	20	3.0	252	3.49 (0.56)	1.4	22.2 (1.8)	0.16 (0.026)
F2	Male	154	3.1	260	3.08 (0.61)*	1.2	30.8 (4.5)*	0.10 (0.026)*
	Female	180	2.8	235	2.82 (0.56)	1.2	22.6 (3.5)	0.13 (0.031)

Values are mean, figures in parentheses denote ±SD.
b.w, body weight.
* $p<0.05$, significant difference between males and females within the same strain/group (Welch *t*-test).
[a] A.SW male×B10.S female.

between mice in the A.SW strain as compared with mice in the B10.S strain of the same gender. Male A.SW mice had a significantly lower body mass than male F1- and F2-hybrid mice which was not true for male B10.S mice. In females, both B10.S and A.SW mice showed a significant lower body mass than the F1- and F2-hybrids.

The male mice consumed 3.0–3.2 mL drinking water/mouse/day, and the females 2.8–3.0 mL/mouse/day (Table 1). The variation in drinking water consumption caused a modest difference in total mercury intake among the different groups during the 6-week period, 252–269 μg Hg in the males and 235–252 μg Hg in the females (Table 1).

The different groups of male (Fig. 1A) and female (Fig. 1B) mice showed a rapid increase in the whole-body retention (WBR) of Hg during the first week, and steady state was reached within 5 weeks of treatment (Fig. 1). The WBR at steady state was 3.1–4.4 μg Hg/mouse in males and 2.8–3.5 μg Hg/mouse in females (Table 1).

Comparison of WBR in males from the different groups at steady state

The A.SW males showed a higher mean WBR of Hg than any other group, and the difference was significant compared with that in the male B10.S mice as well as the male F1- and F2- hybrid mice (Fig. 2A). The male B10.S mice and F1-hybrids showed similar WBR, while the F2-hybrids showed a significantly lower WBR ($p<0.01$) than all other male groups. Adjustment of WBR for differences in body weight did not change the differences between the male groups except for the appearance of a significant difference between male A.SW and F1 hybrid mice (Fig. 2C).

Comparison of WBR in females from the different groups at steady state

Among the females, the A.SW, B10.S and F1-hybrid mice showed similar WBR (Fig. 2B), while the female F2-hybrids showed significantly lower WBR than F1-hybrids and A.SW mice. The weight-adjusted mean WBR was, however 12% higher in female A.SW mice compared with female B10.S mice (Fig. 2D). The weight-adjusted mean WBR was significantly and 25–40% lower in the female F2-hybrids compared with that in all other female groups.

Comparison of WBR in males and females in the same group at steady state

The mean WBR uncorrected for the body mass was higher ($p<0.05$) in the males than females in all groups except the F1 hybrids (Table 1). In contrast, the weight-adjusted mean WBR was significantly higher in the females in all groups, except in the A.SW strain (Table 1).

Retention of Hg in relation to intake at steady state

The mean fraction of Hg taken up in the body at steady state in relation to the total intake (retention) was 25–40% higher in A.SW males than in all other groups including A.SW females (Table 1). The retention of consumed Hg in B10.S and F1 mice was similar (1.3–1.4%) with no difference between males and females in the different groups. The retention in F2 hybrids was marginally lower (1.2%), but still without any difference between males and females.

Organ deposition of Hg at steady state

The dominant deposition of Hg was in the order: kidneys, liver, mesenterial lymph nodes, and spleen (Table 2, Fig. 3). The absolute renal Hg retention and renal Hg concentration were 2.5–4 times higher in the male A.SW mice than in the males in the other groups (Table 2). In female A.SW mice the absolute renal Hg retention and

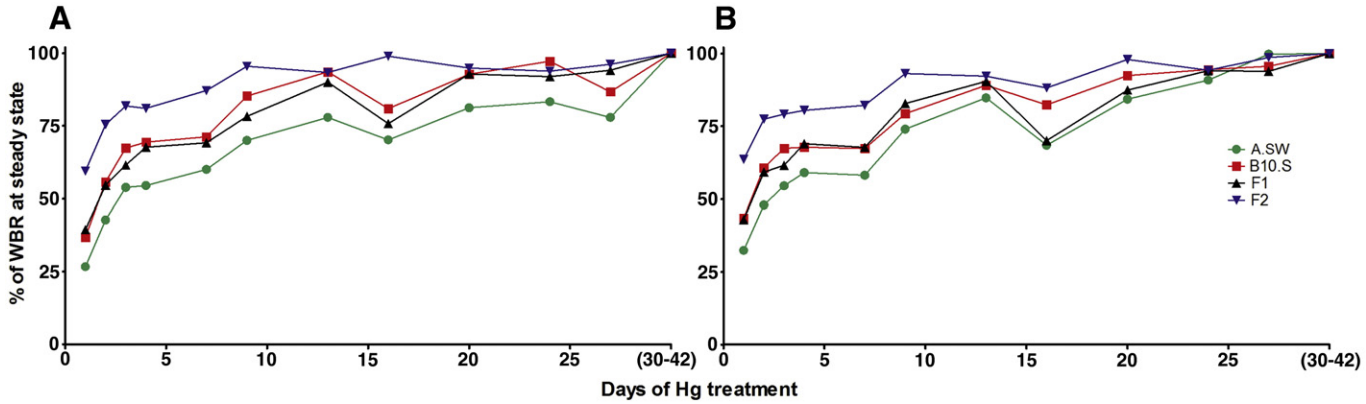

Fig. 1. Accumulation of Hg, expressed as % of steady state whole-body retention (WBR) in male (A) and female (B) mice treated with 2.0 mg Hg/L drinking water *ad libitum* for 6 weeks. The WBR at 30 days was calculated as the mean of all Hg measurements (2–4) taking place after 35–42 days of treatment.

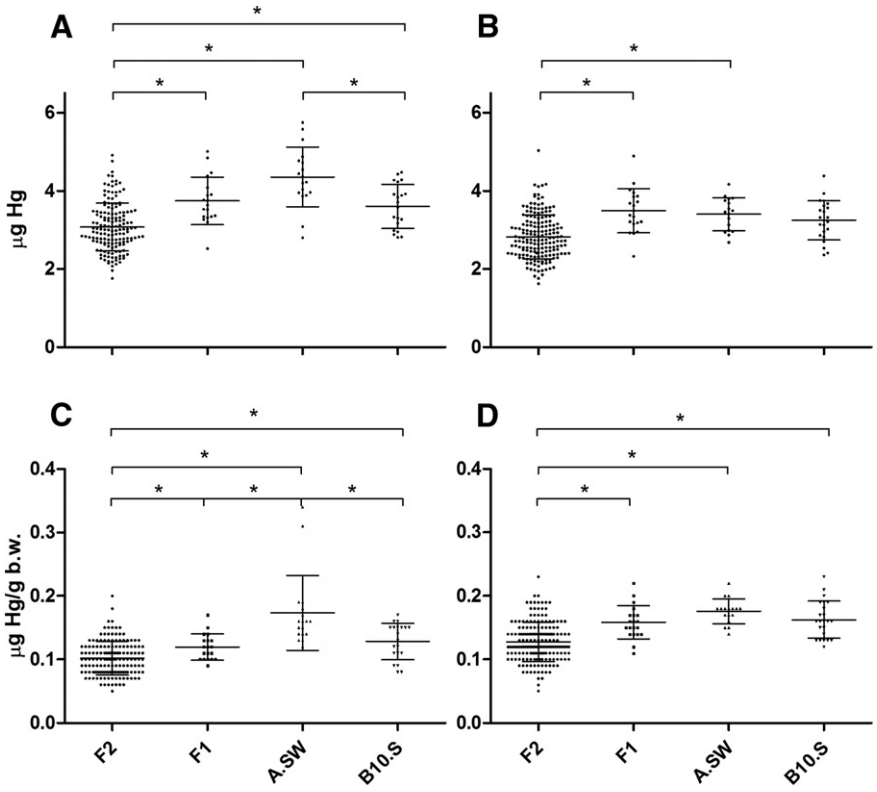

Fig. 2. Whole-body retention (WBR) of Hg, uncorrected (A, B) and corrected (C, D) for weight, in male (A, C) and female (B, D) mice treated with 2.0 mg Hg/L drinking water *ad libitum* for 6 weeks. The WBR at 30 days was calculated as the mean of all Hg measurements (2–4) taking place after 35–42 days of treatment. Differences between strains/groups:*$p<0.05$ using Welch ANOVA test followed by Tamhane's T2 *post-hoc* test.

concentration was 50–100% and 70–80% higher, respectively, than in the females from the other three groups.

Within the different strains/groups, the absolute renal Hg retention was 2.5-fold higher in male A.SW mice than in females (Table 2). Although a significantly higher Hg retention was seen in the males also in the other groups the absolute difference was smaller, in the order of 25–50% higher than in the females. Male A.SW mice showed 90% higher renal Hg concentration than the females, while in the B10.S strain the females showed a significant 20% higher Hg concentration than the males. In F1-hybrids the males showed a significant 15% higher Hg concentration than the females, while the difference was not significant in the F2 hybrids.

The fraction of the WBR deposited in the kidneys was 3.5-fold higher in male A.SW mice than in B10.S males, but only 2-fold higher than in male F1- and F2-hybrids (Table 2). Within the strains/groups, the relative renal accumulation of Hg in male A.SW mice was twice as high as in the females. There was however no significant difference between male and female B10.S mice in this respect, and only 30–40% higher fractional retention of Hg in male F1- and F2-hybrids compared with that in females. The selective renal accumulation of Hg in male A.SW mice was shown by the significantly lower liver/kidney ratio of Hg, which was also observed in the F1- and F2-hybrids (Table 2).

Accumulation of Hg in the liver and spleen largely resembled the renal Hg accumulation pattern with higher Hg concentration in the A.SW strain compared with B10.S and F1 hybrid mice, although the differences were not significant in the spleen of the females (Table 2, Fig. 3). Within the strains/groups, the hepatic Hg concentration was lower in the males than in the females ($p<0.05$), while there was no significant difference in the splenic Hg concentration between males and females.

Table 2
Organ mercury depositions after 6 weeks exposure to 2 mg Hg/L drinking water.

Strain	Group	Number of mice	WBR (µg Hg)	Kidney Hg retention[a] (ng Hg)	Fractional retention[b] (%)	[Hg] Kidney (ng/g w.w.)	[Hg] Liver (ng/g w.w.)	Liver/Kidney ratio	[Hg] Spleen (ng/g w.w.)	[Hg] Lymph nodes (ng/g w.w.)
A.SW	Male	18	4.35 (0.76)*	3814 (868)*	87.2 (8.9)*	12069 (4403)*	429 (151)*	0.036 (0.0068)*	141 (60)	395 (268)
	Female	17	3.40 (0.42)	1483 (202)	44.0 (6.3)	6406 (823)	536 (75)	0.084 (0.0089)	139 (72)	328 (247)
B10.S	Male	20	3.60 (0.57)*	913 (258)*	25.6 (7.3)	2931 (472)*	330 (59)*	0.109 (0.017)	73.5 (54)	268 (256)*
	Female	23	3.24 (0.50)	735 (175)	22.9 (5.3)	3511 (596)	402 (85)	0.112 (0.021)	86.8 (55)	515 (385)
F1[c]	Male	19	3.74 (0.61)	1505 (334)*	40.4 (7.2)*	4385 (753)*	306 (70)*	0.069 (.012)*	79.8 (32)	222 (224)
	Female	20	3.49 (0.56)	1006 (384)	28.6 (7.0)	3841 (514)	433 (98)	0.109 (0.018)	94.7 (64)	402 (348)
F2	Male	154	3.08 (0.61)*	1215 (460)*	39.1 (10.5)*	4104 (1587)	300 (100)*	0.074 (0.019)*	76.7 (34)	355 (302)*
	Female	180	2.82 (0.56)	855 (276)	30.3 (8.2)	3859 (1048)	330 (85)	0.086 (0.021)	75.9 (30)	470 (377)

Values are mean, figures in parentheses denote SD.
w.w., Wet weight.
[a] Results multiplied with 2 as only the left kidney was measured.
[b] Fractional retention, Kidney Hg retention/WBR.
[c] A.SW male × B10.S female.
* $p<0.05$, significant difference between males and females within the same strain/group (Welch *t*-test).

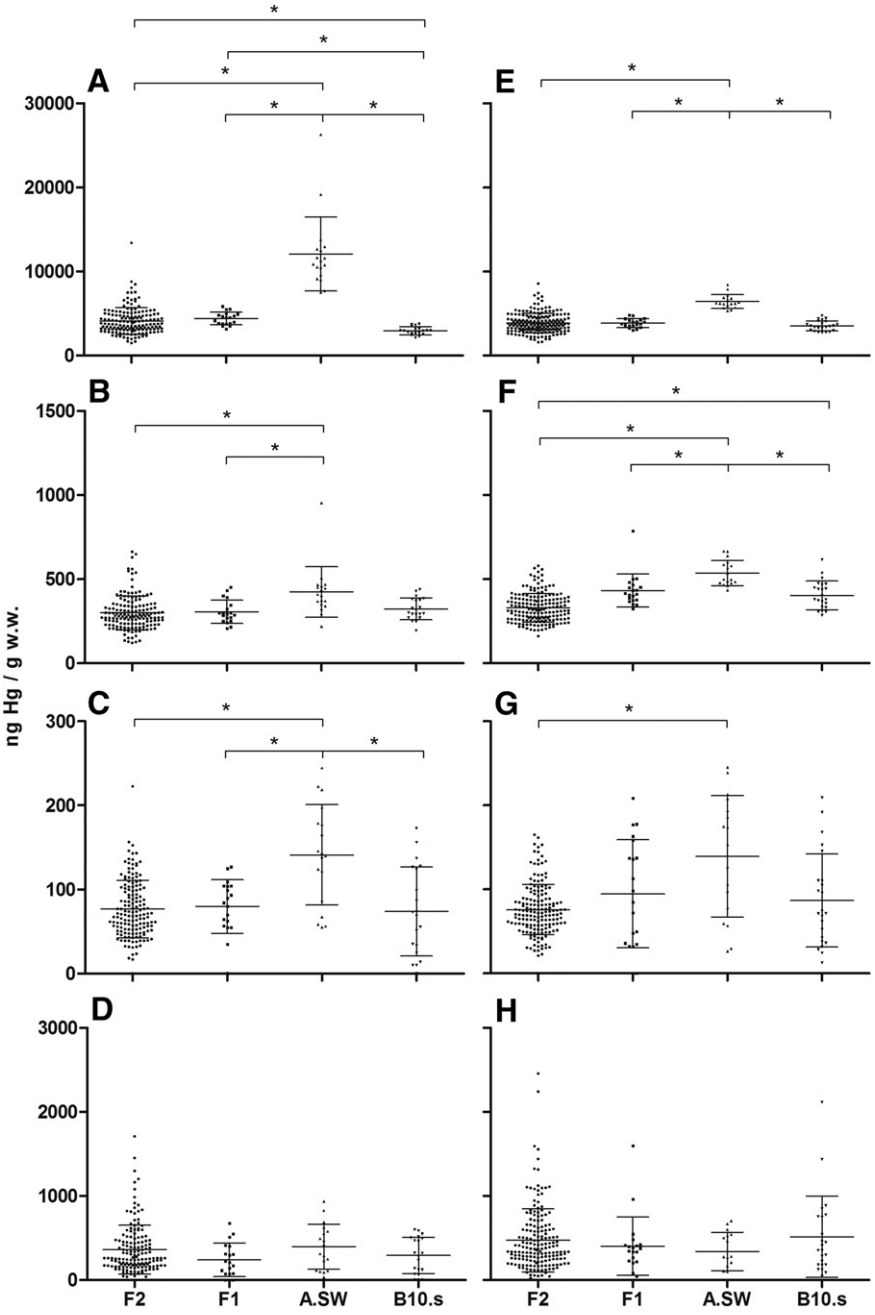

Fig. 3. Deposition of Hg in kidney (A, E), Liver (B, F), spleen (C, G), and mesenterial lymph nodes (D, H) in male (upper row) and female (lower row) mice treated with 2 mg Hg/L drinking water *ad libitum* for 6 weeks. Differences between strains/groups: *$p<0.05$ using Welch ANOVA test followed by Tamhane's T2 *post-hoc* test.

The mesenterial lymph nodes showed a Hg concentration similar to that in the liver, but substantially higher than in the spleen. The lymph nodes lacked the strain- and gender-dependent pattern of Hg accumulation seen in the other organs, with no significant difference in Hg concentration between the mice in the different groups of the same gender. However, the Hg concentration was lower in male B10.S and F1 males compared with females. (Fig. 3, Table 2).

Elimination of Hg after 5 weeks treatment

After 5 weeks Hg treatment, at steady state, the WBR was distinctly higher in the A.SW strain (Table 3). The elimination of Hg after stopping treatment was slower in the A.SW strain than in the B10.S strain throughout the 15-day study period (Fig. 4; Table 3). The B10.S and F1-hybrid mice, which showed similar WBR after 5 weeks treatment (Table 3) also displayed similar whole-body elimination curves. The F2-hybrids showed during the first 8 days an elimination curve similar to that in B10.S and F1 mice. However, after 7 days the elimination appeared to slow down in the F2 hybrids, the WBR approaching that of the A.SW strain at day 15 when the observation period was terminated.

The elimination curves showed at least two distinct phases: a rapid phase that lasted during the initial 24 hrs after treatment ended, and which most likely is due to the elimination of unabsorbed Hg in the gastrointestinal tract. The elimination half-time) ($t_{½}$) of this phase was significantly longer in the A.SW strain than in all other strains/groups. The $t_{½}$ was similar in F1 and B10.S mice, but significantly longer in the F2 mice (Table 4). Days 2–12 represented a second phase with a $t_{½}$ varying from 5.4 days in A.SW mice to 4.0 days in B10.S mice. It was significantly longer in the A.SW mice

Table 3
WBR during the first 15 days of elimination without treatment.

Strain	Number of mice	Time after stopping treatment (days)							
		0	1	2	3	4	7	12	15
A.SW	10	5.42 (0.99)	3.83 (0.68)	3.53 (0.62)	3.27 (0.66)	3.09 (0.75)	1.97 (0.50)	0.97 (0.27)	0.74 (0.19)
B10.S	10	3.25 (0.49)	1.58 (0.15)	1.35 (0.13)	1.28 (0.10)	1.23 (0.12)	0.71 (0.08)	0.23 (0.05)	0.20 (0.03)
F1[a]	20	3.35 (0.39)	1.66 (0.14)	1.47 (0.14)	1.33 (0.11)	1.26 (0.11)	0.74 (0.05)	0.25 (0.03)	0.21 (0.02)
F2	105	3.76 (1.09)	1.94 (0.59)	1.65 (0.50)	1.48 (0.47)	1.31 (0.45)	0.83 (0.29)	0.44 (0.16)	0.39 (0.15)

Values in mean, parentheses denote ±SD (μg Hg/mouse).
[a] A.SW male × B10.S female.

than in the B10.S and F1 mice. The elimination time of the second phase was significantly longer in the F2-hybrids than in B10.S and F1hybrid mice (Table 4). There was an indication of a third phase on days 12–15 with a slower elimination, especially in F2 mice (Fig. 4).

The renal and hepatic Hg concentration was assessed after 15 days of elimination without treatment (Table 4). The A.SW strain showed a 3.5-fold higher mean WBR, but an 11-fold higher absolute renal Hg retention and concentration compared with those in the B10.S strain. In contrast, the hepatic Hg concentration was only 4-fold higher in the A.SW strain than in the B10.S strain, causing a significantly reduced liver/kidney ratio in A.SW mice, indicating that the elimination from the liver was substantially slower than from the kidney. The F1 mice showed significantly higher renal Hg retention and liver Hg concentration than the B10.S mice, significantly lower than in the A. SW strain. The renal and hepatic Hg concentration in the F2 hybrids was only 30% and 70%, respectively, of that in the A.SW mice but 3- to 4-fold higher than that in the B10.S mice and F1 hybrids (Table 4).

Glutathione and glutathione S-transferase activity in kidney and liver in A.SW and B10.S parental strains

Since the tissue concentration of Hg may be influenced by GSH production and conjugation with GSH via GST, we assessed the tissue levels of GSH and GST in the parental strains.

There are only minor differences between the parental strains and sexes in the levels of GSH and GST in kidney and liver. Significant differences were only found in GSH levels, where B10.S male mice have 20% lower kidney levels compared to B10.S females. A similar 20% difference can be seen between the kidney GSH levels between B10.S females and ASW females (Table 5). The GST-activity seems not to be different on strain or gender in kidney or liver (Table 5).

Relative expression of MT-1 and MT-2 in kidney and liver in A.SW and B10.S parental strains

The mRNA expression of Hg-binding metallothionein proteins (MT-1 and MT-2) were assessed in both liver and kidney tissue in both males and females of both parental strains. MT-1 relative expression in kidney was lower in B10.S female mice when compared to males. A.SW males had a slightly higher relative expression compared to B10.S males. B10.S female mice had a higher relative expression of MT-2 in kidneys but a lower expression in liver when compared to A.SW female mice. A.SW male had a higher MT-2 relative expression in liver compared to B10.S males (Table 5).

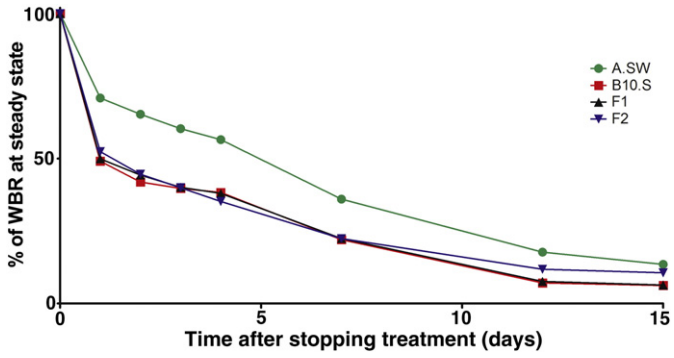

Fig. 4. Elimination of Hg expressed as % of steady state whole-body retention (WBR) during the first 15 days after terminating treatment. Steady state WBR was defined as the WBR of Hg just prior to withdrawal of treatment with 2 mg Hg/L drinking water for 5 weeks in males.

Discussion

Despite a low variation in drinking water consumption, and hence intake of mercury among the groups, male A.SW mice attained a substantially higher WBR than male B10.S mice, which was true also after adjustment for weight. The high accumulation in A.SW males was also reflected in the highest retention of mercury in relation to intake, which is a proxy for the difference between the intestinal absorption and excretion of Hg, i. e., the amount of the exposed Hg that is being deposited in the body. Significant differences in accumulation of Hg between inbred mouse strains (Nielsen, 1992; Griem et al., 1997) suggest a potential and substantial inter-individual variation in genetically heterogeneous population such as the human. Different toxicokinetics for Hg between inbred strains was first observed (Miller and Csonka, 1968) using male mice of the strains A and C57. These strains bear a close relationship to the strains used in the present study, the A strain having the same non-H-2 genes as the A.SW mice, and the C57 strain a common ancestor to the background strain (C57BL/10) in the B10.S mice. The A strain retained more Hg than the C57 strain during the 8-day-long observation period after a single intramuscular injection of $HgCl_2$. Doi et al. (1983) found that polymorphism in the hemoglobin b locus of inbred mouse strains affected the blood mercury level after a single injection of methyl Hg.

In the present study, a significant genderwise higher concentration of Hg was seen in the kidney, liver, and spleen (males only) in A.SW mice compared with B10.S mice. Kidney as the dominating organ for Hg accumulation, followed by the liver as the second highest accumulating organ is in agreement with previous findings in animals and humans exposed to inorganic Hg (Berlin et al., 2007). The 4 times higher renal Hg retention and tissue concentration and the 3-fold higher fraction of the WBR in the kidneys in the A.SW males as compared with the B10.S males is in view of a mere 50% increase in WBR in A.SW males, evidence for a selective renal accumulation of Hg in male A.SW mice. This selective renal Hg accumulation was, however, not simply an effect of the male gender. This was shown by the doubling of the renal Hg retention, the fraction of the WBR accumulated in the kidneys, and the renal Hg concentration in A.SW females as compared with those in B10.S females despite a mere 5–12% higher WBR. We therefore conclude that both gender factors and non-H-2 genes in A.SW mice contributed to the high Hg accumulation in the kidneys of A.SW males.

In contrast to the kidney, liver and spleen, the accumulation of Hg in the mesenterial lymph nodes was not significantly different between any of the groups of the same gender, or between A.SW males and females. However, the concentration of Hg was significantly lower in B10.S males compared with females. The reason for the different pattern of Hg accumulation in the lymph nodes versus

Table 4
Hg toxicokinetics after 15 days of elimination without treatment.

Strain	Number of mice	WBR (μg Hg/mouse)	$t_{½}$—k1 (days)	$t_{½}$—k2 (days)	Kidney Hg retention (ng Hg)	[Hg] Kidney (ng Hg/g w.w.)	[Hg] Liver (ng Hg/g w.w.)	Liver/kidney ratio
A.SW	10	0.74 (0.19)	2.04 (0.24)	5.39 (0.44)	366.4 (141.7)	1905.3 (686.6)	36.0 (11.3)	0.019 (0.003)
B10.S	10	0.20 (0.03)*,**	0.98 (0.11)*,**	3.97 (0.28)*,**	34.0 (13.0)*,**	177.9 (42.8)*,**	8.4 (1.8)*,**	0.048 (0.007)*
F1[a]	20	0.21 (0.02)*,**	1.01 (0.14)*,**	4.02 (0.25)*,**	38.0 (6.7)*,**	245.3 (52.9)*,**,***	8.1 (1.6)*,**	0.034 (0.007)*,**,***
F2	105	0.39 (0.15)*	1.15 (0.58)*	5.10 (0.65)	117.8 (82.6)*	713.3 (480.9)*	26.7 (13.3)	0.046 (0.024)*

Mice were treated 5 weeks with 2 mg Hg/L in drinking water *ad libitum* followed by 15 days without treatment.
w.w, wet weight.
[a] A.SW male × B10.S female.
* Significantly different from the A.SW strain, $p<0.05$ (Welch ANOVA + Tamhane's T2).
** Significantly different from the F2 hybrids, $p<0.05$ (Welch ANOVA + Tamhane's T2).
*** Significantly different from the B10.S strain, $p<0.05$ (Welch ANOVA + Tamhane's T2).

the other organs is unknown. Mercury is however differently handled in lymph nodes. For example, the lymphatic tissue accumulates more inorganic Hg than other organs following exposure to methyl Hg (Havarinasab et al., 2007), probably due to strong tissue demethylation caused by their high content of macrophages.

Differences in the toxicokinetics of Hg between males and females have recently been highlighted in a study on Hg exposure due to amalgam fillings in children (Woods et al., 2007), where it was concluded likely that the higher urinary Hg concentration in females was due to an effect *per se* of the gender rather than confounding factors. Akesson et al. (1991) found higher excretion of Hg in the urine of females as compared with males bearing a similar amount of dental amalgam fillings. Recently, a study on blood levels of mercury in young people living in Britain (Bates, 2006) also showed that females exhibited significantly higher Hg levels than males. Finally, the kidneys belonging to female Swedish kidney donors had a 3-fold higher mean renal Hg concentration compared with that in males (Barregard et al., 1999), for which no explanation was found. In the present study, the gender differences in toxicokinetics were distinct, but also strain dependent (see above). While B10.S males showed a modestly higher accumulation of Hg in the kidney than females, renal accumulation of Hg in A.SW males was much greater than that in the females. Therefore, gender factors as well as non-H-2 genes influence target organ accumulation of Hg.

How can the observed differences in Hg toxicokinetics be explained?

Toxicokinetics of Hg was dynamic in these mice with a daily intake of approximately 6 μg, and a daily absorption and excretion of around 1 μg at steady state. Since the intake of Hg was similar, the differences observed in toxicokinetics must be due either to differences in the intestinal uptake or elimination of mercury. With regard to intestinal uptake, the possible existence of specific carriers in the enterocytes has been discussed, but there is presently no evidence for their importance with regard to mercury (Berlin et al., 2007). Therefore, the absorption may be ascribed to a passive intracellular, transmembrane, and/or transcellular diffusion according to Fick's law. At steady state the differences observed in Hg accumulation in the present study will be due to differences in elimination kinetics.

We therefore assessed the elimination of Hg at steady state after 5 weeks continuous Hg exposure. The whole-body elimination after discontinuation of the treatment occurred in at least two phases: the initial phase was well defined with a $t_{½}$ of 0.98 and 2 days in B10.S and A.SW mice, respectively, and has been ascribed to the elimination of unabsorbed Hg form the gastrointestinal tract (Nielsen and Hultman, 1998). The second phase seemed to last for 10 days with a $t_{½}$ of 4.0 and 5.4 days, respectively. The elimination curves indicated the possibility of a third phase after 12 days with a slower elimination ($t_{½}$ 33 and 11 days, respectively), but the observation time of 15 days was too short to allow definite conclusions. In general, the slower elimination in A.SW mice compared with all other groups tallies with the elimination profile demonstrated in female H-2^{s} mice treated for 12 weeks with a 2.5-fold higher dose (Nielsen and Hultman, 1998). The trait of rapid elimination in the B10.S mice was dominantly inherited by the F1-hybrids, while the F2-hybrids showed an elimination in-between A.SW and B10.S mice.

The slower elimination of Hg in A.SW mice explains at least part of the higher Hg accumulation in the whole body, kidney and liver of A.SW mice compared to B10.S mice. This was reflected in the elimination study by an almost 4-fold higher WBR, but 11-fold higher renal Hg deposition and concentration in the A.SW strain. Since the liver Hg concentration was only 4-fold higher in the A.SW mice, renal elimination seems to be the main rate-limiting step for elimination of inorganic Hg in A.SW mice, although some reduction in the ability of the liver to transfer recently absorbed Hg to the kidney may have contributed.

The elimination of inorganic Hg in humans has mostly been studied in chloralkali workers. One study found an initial phase with a half-time for urinary Hg excretion of 3 days, followed by a slow excretion phase with the elimination constant rate of 0.001 day^{-1}

Table 5
Glutathione S-transferase (GST), glutathione (GSH), metallothionein 1 and 2 (MT-1, MT-2) in B10.S and A.SW mouse tissues.

Strain	Gender	N	GSH[a]		GST[b]		MT-1[c]		MT-2[c]	
			Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
B10.S	Female	9	2.29 (0.16)	2.05 (0.33)*	43 (14)	42 (10)	1.07 (0.144)	1.15 (0.057)*	1.40 (0.157)	1.60 (0.223)
	Male	5	2.32 (0.23)	1.63 (0.19)	50 (7)	41 (8)	1.30 (0.54)	1.33 (0.135)	1.65 (0.382)	1.39 (0.454)
A.SW	Female	8	2.26 (0.17)	1.62 (0.16)**	53 (11)	44 (13)	1.32 (0.278)**	1.25 (0.375)	2.13 (0.682)**	0.923 (0.182)**
	Male	8	2.35 (0.20)	1.48 (0.16)	48 (15)	43 (8)	1.48 (0.517)	1.13 (0.136)**	2.38 (0.669)**	1.20 (0.182)*

Values are mean, figures in parentheses denote SD.
[a] GSH concentration (mmol/g ww).
[b] GST specific activity (mmol/mL/min).
[c] Relative expression (ratio to the house keeping gene GAPDH).
* $p<0.05$, significant difference between males and females within the same strain (Mann–Whitney *U*-test).
** $p<0.05$, significant difference compared to B10.S mice of the same gender (Mann–Whitney *U*-test).

(Piotrowski et al., 1975). In a group with 17 workers exposed for several years, the mean half-time was 75 days (range 27–96 days) (Ellingsen et al., 1993). In workers exposed for 10 years (median) two phases were described with a blood Hg concentration half-time of 0.1–7.9 days and 14–77 days (Sallsten et al., 1993). The large variations may at least partly be due to genetic differences. Although a number of models have been used for describing the elimination of inorganic Hg from humans and animals (Cember, 1969; Nordberg and Skerfving, 1972; Bernard and Purdue, 1984; Carrier et al., 2001a, 2001b) there is at present no accepted model (Berlin et al., 2007).

Increased transfer of Hg from liver to kidney in male mice will decrease liver/kidney ratio and might be related to increased hepatic levels of GSH or GST in males. Likewise, differences in the ability to express MT1 and MT2 could potentially influence disposition of mercury and eventually toxicity.

A gender difference in liver/kidney ratio is seen in A.SW mice only. However, in A.SW mice, there do not appear to be any prominent differences between gene expression of MT1 or MT2, or levels of GSH and GSR in males and females. Thus, neither MT1, MT2 expression, GSH levels, nor GST activity seem to explain the gender related differences in mercury disposition or higher fractional renal deposition observed in A.SW and B10.S mice.

The hepatic MT-2 gene expression is significantly higher in A.SW mice as compared to B10.S mice, but in the kidneys the MT-2 gene expression is slightly higher in the B10.S mice as compared to A.SW mice. A fractional renal mercury deposition in female A.SW mice twice as high as in B10.S mice might correspond to a comparable increased gene expression of MT-2 in female B10.S mice. It is, however, difficult to explain the 3-fold lower renal deposition of mercury observed in B10.S males with a less than 20% higher renal MT-2 gene expression in male B10.S mice.

The significant differences in elimination kinetics observed between A.SW and B10.S strains exemplified through a 4- and 10-fold higher hepatic and renal deposition following 15 days elimination is also not explained by the relatively minor differences found in levels of GSH or GST or gene expression of MT-1 or MT-2.

Thus, neither minor differences in the background levels of GSH or GST nor in the ability to express MT-1 and MT-2 alone offers obvious mechanistic explanations for the observed differences in the gender and strain dependent toxicokinetics of inorganic mercury between B.10,S and A.SW mice. Future studies will therefore have to look for genes outside the MHC region potentially in combination with GSH and genes coding for the expression of metallothionein to explain deposition and elimination kinetics of inorganic mercury.

The present study included F1- and F-2 hybrids between the toxicokinetically different A.SW and B10.S strains in order to describe the effect of genetic factors. The high accumulation of Hg in male A.SW mice was not inherited by the F1 hybrid, which had a WBR after weight adjustment very similar to that of B10.S mice. In fact, with exception of the renal Hg concentration in males, which was slightly but significantly higher in F1 hybrids as compared with B10.S mice, the organ Hg concentration did not differ between F1 hybrid and B10.S mice, irrespectively of gender, but was significantly lower in the F1 hybrids compared with the A.SW mice. Also, the variation in Hg accumulation in the F1 hybrids was not larger than in the parental strains, indicating that the sum of the genetic factors governing increased Hg accumulation in the A.SW strain was not dominantly inherited.

The mean WBR was significantly lower in the F2 mice than in the parental strains and the F1 hybrids. In the organs, there was generally no significant difference in Hg concentration between F2 hybrids and the B10.S mice, while the concentration was significantly lower than in the A.SW strain. However, the genetically heterogeneous F2-hybrids showed a larger variation in WBR and organ accumulation than the F1-hybrids and the parental strains. Individual F2 hybrids showed even lower Hg accumulation than the least accumulating B10.S mice, while other F2-hybrids accumulated as much mercury as the top accumulating mice in the A.SW strain. This indicates that multiple genetic factors are of importance for determining the toxicokinetics in the mouse, but also that a genetically heterogeneous population, like humans, may be subject to large variations in mercury toxicokinetics. This is in accordance with findings in elimination studies of long-term exposed chloralkali workers. Further studies on the genetic factors regulating Hg toxicokinetics in humans, and in the present model, should help improve the assessment of possible health hazards with low dose Hg exposure to larger and heterogenic populations.

Conflict of interest statement

None of the authors or their institutions have had a financial or other relationship with other people or organizations during the last 3 years that could inappropriately influence this work.

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