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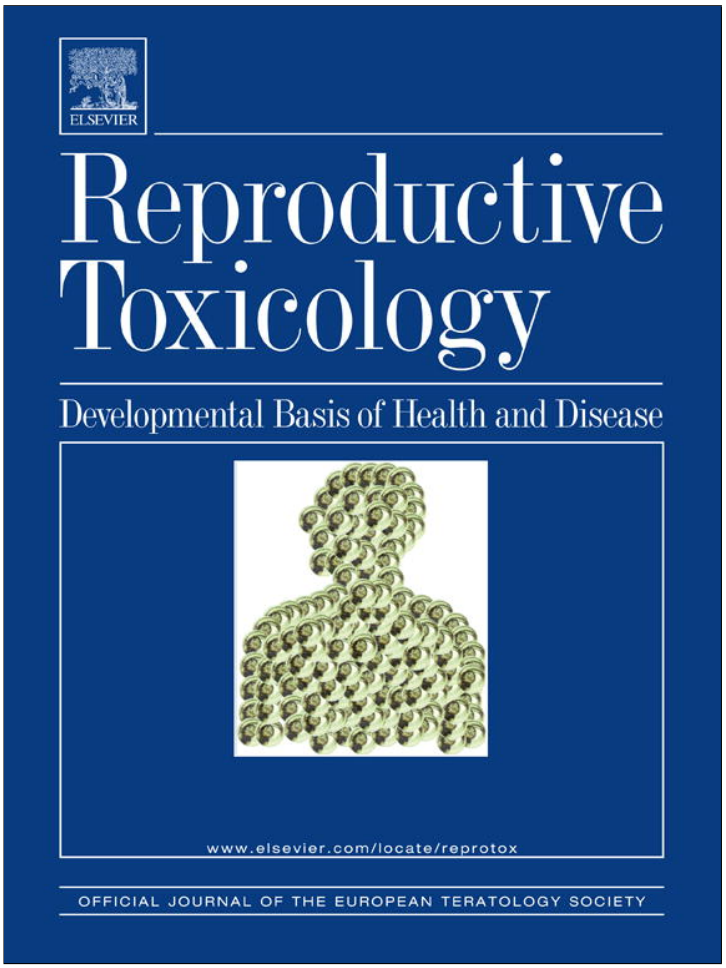

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Placental and fetal disposition of mercuric ions in rats exposed to methylmercury: Role of Mrp2

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ABSTRACT

Methylmercury is a prevalent environmental toxicant that can have deleterious effects on a developing fetus. Previous studies indicate that the multidrug resistance-associated protein 2 (Mrp2) is involved in renal and hepatic export of mercuric ions. Therefore, we hypothesize that Mrp2 is also involved in export of mercuric ions from placental trophoblasts and fetal tissues. To test this hypothesis, we assessed the disposition of mercuric ions in pregnant Wistar and TR$^-$ (Mrp2-deficient) rats exposed to a single dose of methylmercury. The amount of mercury in renal tissues (cortex and outer stripe of outer medulla), liver, blood, amniotic fluid, uterus, placentas and fetuses was significantly greater in TR$^-$ rats than in Wistar rats. Urinary and fecal elimination of mercury was greater in Wistar dams than in TR$^-$ dams. Thus, our findings suggest that Mrp2 may be involved in the export of mercuric ions from maternal and fetal organs following exposure to methylmercury.

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1. Introduction

Methylmercury (CH_3Hg^+) is a reproductive toxicant to which humans are exposed regularly [1]. One of the most common routes of exposure is consumption of various species of freshwater and saltwater fish. In particular, large, predatory species of fish often contain high levels of CH_3Hg^+ due to bioaccumulation of mercuric ions. In recent years, a great deal of emphasis has been placed on exposure of pregnant women to CH_3Hg^+ because of the potential harmful effects that this organomercurial can have on the developing fetus. Despite warnings to limit exposure to mercuric compounds during pregnancy, significant levels of mercury continue to be detected in placentas of women in the United States and throughout the world [2,3]. CH_3Hg^+ in maternal blood has been shown to readily cross the placenta and accumulate in fetal and placental tissues [4–6]. Maternal exposure to CH_3Hg^+ may lead to deleterious effects in the fetus, even at concentrations that would not result in significant negative effects in the mother [7–9]. The primary toxicological target of CH_3Hg^+ is the neurological system; therefore, prenatal exposure to this metal can have serious neurological consequences in the fetus. These include reduced cognitive function, dysarthria, strabismus, and cerebral palsy [10–13]. Because CH_3Hg^+ is a prevalent environmental toxicant which humans are exposed to frequently and owing to its propensity to negatively affect fetal health, it is important that we understand completely the handling of mercuric ions by placental and fetal tissues.

Even though understanding placental and fetal handling of mercuric ions is clinically important, few studies have been carried out to investigate the molecular mechanisms by which CH_3Hg^+ crosses the placenta. Once CH_3Hg^+ enters systemic circulation, it is bound to the reduced sulfur atom of a sulfhydryl (thiol) group present on endogenous biological compounds such as albumin, glutathione (GSH) and/or cysteine (Cys). Due to similarities between Cys-*S*-conjugates of CH_3Hg^+ (Cys-*S*-CH_3Hg) and methionine, it has been proposed that these mercuric conjugates may utilize one or more methionine transporters for uptake into target cells [14–17]. Indeed, it has been suggested that the uptake of Cys-*S*-CH_3Hg from maternal blood across placental trophoblasts is mediated by the amino acid carrier, system L [18]. Subsequent *in vitro* studies utilizing *Xenopus laevis* oocytes provided direct evidence that Cys-*S*-CH_3Hg may act as a substrate of both isoforms of human system L, LAT1 (*Slc7A5*) and LAT2 (*Slc7A8*) [19]. LAT1 and LAT2 are localized in the apical and basolateral plasma membrane, respectively, of placental trophoblasts [20] and thus may play a role in the transfer of mercuric ions to fetal tissues.

The mechanisms by which mercuric ions are eliminated from placental trophoblasts and fetal tissues have yet to be determined. Our previous *in vivo* studies using Wistar and TR$^-$ (multidrug

Abbreviations: CH_3Hg^+, methylmercury; CH_3HgCl, methylmercury chloride; $CH_3[^{203}Hg^+]$, radioactive methylmercury; Cys, cysteine; Cys-*S*-CH_3Hg, cysteine-thiol-conjugates of methylmercury; Mrp, multidrug resistance-associated transporter; OAT, organic anion transporter; DCM, dichloromethane.

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resistance-associated protein 2 (Mrp2)-deficient) rats have implicated Mrp2 (*Abcc2*) in the renal elimination of mercuric conjugates [21,22]. In placenta, Mrp2 is localized in the apical (maternal-facing) membrane [23] where it is thought to mediate the export of metabolic wastes from fetal and placental tissues [24]. Based on the role of Mrp2 in the renal export of mercuric ions and its localization in placental trophoblasts, we hypothesize that this carrier may also play a role in the export of thiol-*S*-conjugates of CH_3Hg^+ from within placental and fetal tissues. Therefore, the purpose of the current study was to test the hypothesis that Mrp2 is involved in the extraction of mercuric ions from placental and fetal tissues of rats exposed to a non-toxic dose of CH_3HgCl.

2. Materials and methods

2.1. Animals

Male and female TR⁻ rats were obtained from the laboratory of Dr. Kim Brouwer at the University of North Carolina. TR⁻ rats possess a spontaneous mutation which results in a non-functional Mrp2 protein [25,26]. Male and female Wistar rats were obtained from Harlan (Indianapolis, IN). Female Wistar and TR⁻ rats, weighing 200–235 g, were mated with males of each respective strain in our facility for 36 h in order to obtain pregnant dams. All animals were provided a commercial laboratory diet (Tekland 6% rat diet, Harlan Laboratories) and water *ad libitum* throughout all aspects of experimentation. The animal protocol for the current study was reviewed and approved by the Institutional Animal Care and Use Committee. Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

2.2. Manufacture of radioactive methylmercury ($CH_3[^{203}Hg^+]$)

The protocol for manufacturing radioactive mercury ($[^{203}Hg^{2+}]$) has been described previously [27,28]. Briefly, three milligrams of mercuric oxide were sealed in quartz tubing and were irradiated by neutron activation for 4 weeks at the Missouri University Research Reactor (MURR) facility. After irradiation, the mercuric oxide was dissolved in 1 N HCl. The radioactivity of the solution was determined using a Victoreen Ion Chamber Survey Meter (Fluke Biomedical, Cleaveland, OH). The specific activities of the $[^{203}Hg^{2+}]$ ranged from 6 to 12 mCi/mg.

$CH_3[^{203}Hg^+]$ was generated according to a previously published protocol [29] adapted from Rouleau and Block [30]. Briefly, 2 mCi of $[^{203}Hg^{2+}]$ (1.25 μmol) were added to sodium acetate (2 M in acetic acid) and 2 mL of 1.55 mM methylcobalamin (3.1 mmol), which served as the donor of methyl groups. Following a 24-h incubation, potassium chloride (30% in 4% hydrochloric acid) was added to the solution. $CH_3[^{203}Hg^+]$ was extracted with five washes of dichloromethane (DCM). DCM was evaporated by bubbling nitrogen gas into the solution and remaining $CH_3[^{203}Hg^+]$ was collected. The specific activity was calculated to be approximately 5 mCi/mg. The purity of the extracted $CH_3[^{203}Hg^+]$ has been confirmed previously by thin layer chromatography [30].

2.3. Intravenous injections

Pregnant rats were injected intravenously (i.v.), on day 17 of pregnancy, with a non-nephrotoxic dose of CH_3HgCl (5 mg/kg in 2 mL normal saline containing 1 μCi of $CH_3[^{203}Hg^+]$ per rat) according to our previously published protocol [21,29,31]. Animals at this stage of pregnancy were chosen in order to examine the disposition of mercuric ions in fetuses near the end of development, which is approximately 21 days for rats. Each animal was anesthetized with isoflurane and a small incision was made in the skin in the mid-ventral region of the thigh to expose the femoral vein and artery. The dose of CH_3HgCl was administered into the vein and the wound was closed with two 9-mm wound clips.

Following injection with CH_3HgCl, animals were placed individually in plastic metabolic cages. Forty-eight hours after the initial injection with CH_3HgCl, rats were sacrificed and fetuses (embryonic day 19) were harvested.

2.4. Collection of fetuses, tissues, organs, urine and feces

At the time of sacrifice, pregnant rats were anesthetized with an intraperitoneal overdose of ketamine and xylazine (70/30 mg/kg in 2 mL saline). A 1-mL sample of blood was obtained from the inferior vena cava and placed in a polystyrene tube for estimation of $[^{203}Hg]$ content. A small sample of blood was placed in blood separation tubes in order to separate plasma from the cellular contents of blood. Total blood volume was estimated to be 6% of body weight.

The uterus of each anesthetized animal was removed and each fetus and placenta was extracted. The number of fetuses and placentas harvested from each dam was 10–17. Each placenta was weighed and placed in a polystyrene tube for estimation of $[^{203}Hg]$ content. In addition, each fetus was weighed, decapitated, placed in 3 mL of 80% ethanol in a glass scintillation vial. After determining the total amount of $[^{203}Hg]$ in each fetus, brain, kidneys and liver were removed. Each organ was weighed and placed in separate polystyrene tubes for the determination of $[^{203}Hg]$ content.

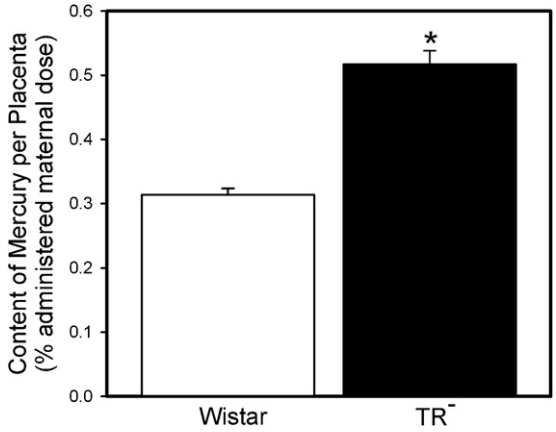

Fig. 1. Content of mercury in placenta. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Placentas were harvested 48 h after injection with CH_3HgCl. Data represent mean ± SE of placentas from three dams. *Significantly different from corresponding Wistar dams ($p < 0.05$).

Following removal of the uterus from the dam, right and left kidneys were also removed from the pregnant rats. Each kidney was weighed and cut in half along a transverse plane. A 3-mm transverse slice of the left kidney was utilized to obtain samples of cortex, outer stripe of outer medulla, inner stripe of outer medulla and inner medulla. Each zone of the kidney was weighed and placed in a separate polystyrene tube for estimation of $[^{203}Hg]$ content. The liver was then excised, weighed, and a 1-g section of liver was removed for determination of $[^{203}Hg]$ content.

Urine and feces were collected at 24- and 48-h time points after injection with CH_3HgCl. Urine from each animal was mixed and a 1-mL sample was weighed and placed in a polystyrene tube for estimation of $[^{203}Hg]$ content. All of the feces excreted by each animal during each 24-h period were counted to determine accurately the total fecal content of $[^{203}Hg]$. The content of $[^{203}Hg]$ in each sample was determined by counting in a Wallac Wizard 3 automatic gamma counter (Perkin Elmer, Boston, MA).

2.5. Data analyses

Data were analyzed first with the Kolmogorov–Smirnov test for normality and then with Levene's test for homogeneity of variances. Data were then analyzed using an unpaired, two-tailed, Student's *t*-test to assess differences among the means. It should be noted that statistical analyses were not used to compare samples from cortex with samples from the outer stripe of the outer medulla. A *p*-value of <0.05 was chosen *a priori* to represent statistical significance. Each group of animals contained three rats, with 10–17 fetuses per rat. All data are expressed as mean of percent administered maternal dose ±SE or mean of percent administered maternal dose per gram ±SE to correct for potential differences in body mass between animals.

3. Results

3.1. Content of mercury in placenta, amniotic fluid, and fetal tissues

3.1.1. Amount of mercury in placentas

Fig. 1 shows the amount of mercury present in placentas of Wistar and TR⁻ dams exposed to 5 mg/kg/2 mL CH_3HgCl. The amount of mercury (% administered dose per placenta) present in placentas from TR⁻ dams (0.517 ± 0.02) was higher and significantly different ($p = <0.001$) from that of corresponding Wistar dams (0.314 ± 0.01). The weight of Wistar placentas was not significantly different from that of corresponding TR⁻ placentas (data not shown).

3.1.2. Amount of mercury in whole fetuses

The burden of mercury was measured in whole fetuses isolated from Wistar and TR⁻ dams exposed to 5 mg/kg/2 mL CH_3HgCl (Fig. 2). The fetal burden of mercury (% administered dose per fetus) was significantly greater ($p = <0.001$) in fetuses harvested from TR⁻ dams (1.5 ± 0.04) than those from corresponding Wistar dams (1.1 ± 0.04; Fig. 2). In addition, the percentage of

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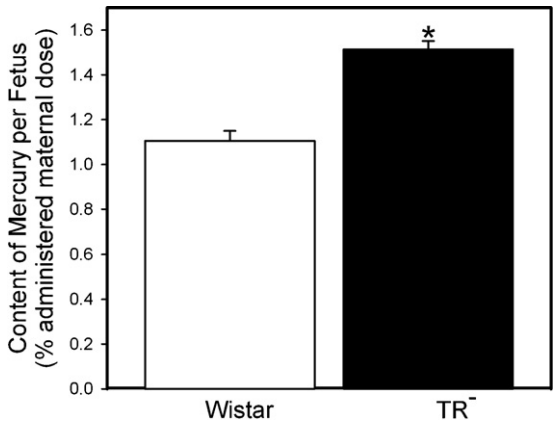

Fig. 2. Content of mercury in fetuses. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Fetuses were harvested 48 h after injection with CH_3HgCl. Data represent mean ± SE of fetuses from three dams. *Significantly different from fetuses from corresponding Wistar dams ($p < 0.05$).

administered maternal dose of mercury was greater in fetuses (Fig. 2) from both strains of rat than that detected in placentas from corresponding rats (Fig. 1). The weight of Wistar fetuses was not significantly different from that of corresponding TR⁻ fetuses (data not shown).

3.1.3. Disposition of mercury in fetal tissues

The brain, liver, and kidneys were removed from each fetus in order to determine the organ-specific burden of mercury. Of the organs examined, the liver (Wistar: 0.143 ± 0.021; TR⁻: 0.182 ± 0.008; $p = <0.001$; Fig. 3A) contained the greatest fraction of maternal dose of mercury, followed by brain (Wistar: 0.047 ± 0.007; TR⁻: 0.054 ± 0.002; $p = <0.002$; Fig. 3B), and total renal mass (Wistar: 0.009 ± 0.001; TR⁻: 0.011 ± 0.001; $p = <0.001$; Fig. 3C). When the burden of mercury was factored by the weight of the organ (% administered dose/g), the greatest amount of mercury was found in the liver (Wistar: 0.467 ± 0.018; TR⁻: 0.522 ± 0.010), followed by total renal mass (Wistar: 0.398 ± 0.097; TR⁻: 0.359 ± 0.012) and brain (Wistar: 0.229 ± 0.067; TR⁻: 0.277 ± 0.008). Regardless of the manner in which the burden of mercury was expressed, significant differences were identified between Wistar and TR⁻ rats. In liver and brain, particularly, the burden of mercury was significantly greater in fetuses from TR⁻ dams than in fetuses from corresponding Wistar dams (Fig. 3A–C).

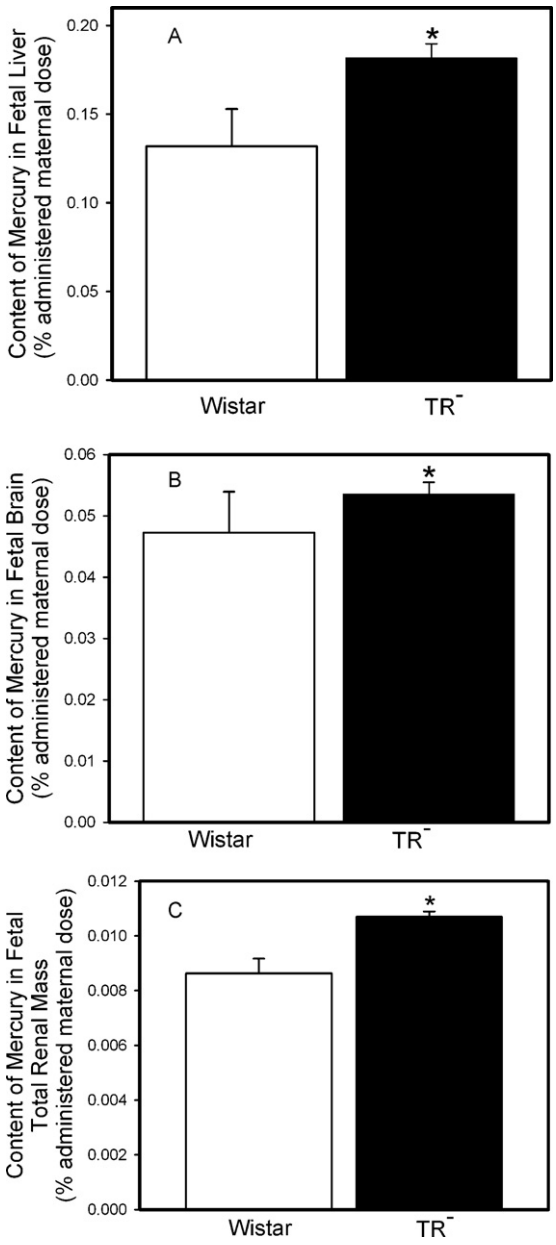

Fig. 3. Content of mercury in fetal organs (% administered maternal dose). Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Fetal livers (A), brains (B), and kidneys (C) were harvested 48 h after injection with CH_3HgCl. Data represent mean ± SE of organs of fetuses from three dams. *Significantly different from fetal organs from corresponding Wistar dams ($p < 0.05$).

3.1.4. Amount of mercury in amniotic fluid

The amniotic fluid associated with each fetus was collected at the time of sacrifice. The amount of mercury (% administered dose) detected in amniotic fluid associated with TR⁻ fetuses (0.299 ± 0.029) was nearly twofold greater than that of Wistar fetuses (0.188 ± 0.011; $p = <0.001$; Fig. 4).

3.2. Content of mercury in maternal tissues

3.2.1. Amount of mercury in uterus

The amount of mercury detected in the uterus of Wistar and TR⁻ rats is shown in Fig. 5. The uterine burden of mercury (% administered dose) was slightly greater ($p = <0.158$) in TR⁻ rats (2.45 ± 0.01) than in corresponding Wistar rats (2.14 ± 0.18).

3.2.2. Renal concentration of mercury

The concentration of mercury detected in the renal cortex of Wistar and TR⁻ dams 48 h after exposure to 5 mg/kg/2 mL CH_3HgCl is shown in Fig. 6. The cortical concentration of mercury (% administered dose per g) was significantly greater ($p = 0.004$) in TR⁻ dams (1.912 ± 0.001) than in Wistar dams (1.61 ± 0.11). In addition, the concentration of mercury in the outer stripe of the outer medulla of kidneys isolated from Wistar and TR⁻ dams is also shown in Fig. 6. The concentration of mercury (% administered dose per g) in the outer stripe of the outer medulla of TR⁻ dams (2.96 ± 0.01) was significantly greater ($p = 0.01$) than that of corresponding Wistar dams (1.99 ± 0.21). The concentration of mercury in the inner stripe of the outer medulla was 0.66 ± 0.01% of the administered dose in TR⁻ rats and 0.47 ± 0.04 in Wistar rats. In the inner medulla, the concentration of mercury was 0.16 ± 0.01 for TR⁻ rats and 0.2 ± 0.03 for Wistar rats.

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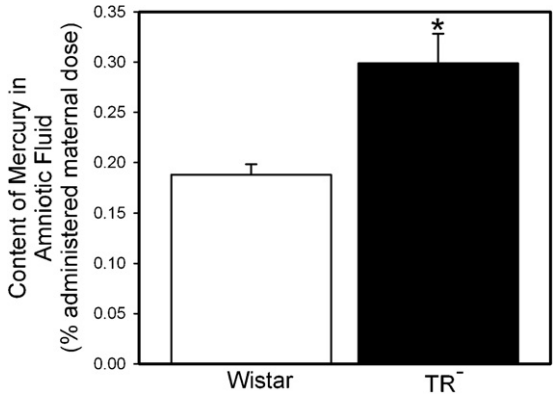

Fig. 4. Content of mercury in amniotic fluid. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Fetuses were harvested 48 h after injection with CH_3HgCl and amniotic fluid was collected on Whatman paper. Data represent mean ± SE of amniotic fluid associated with fetuses from three dams. *Significantly different from amniotic fluid from corresponding Wistar dams ($p < 0.05$).

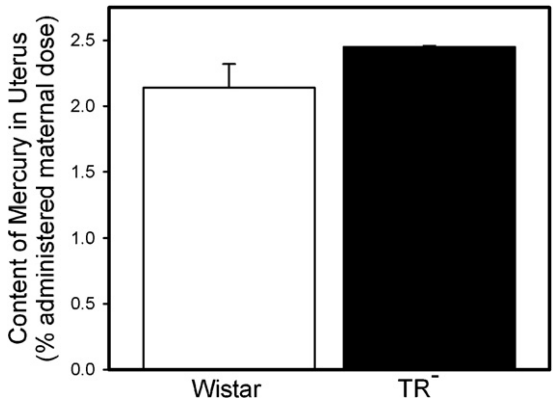

Fig. 5. Content of mercury in uterus. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Uterus was harvested 48 h after injection with CH_3HgCl. Data represent mean ± SE of three dams. Error bars, although present, may not be visible due to small standard error value. *Significantly different from corresponding Wistar dams ($p < 0.05$).

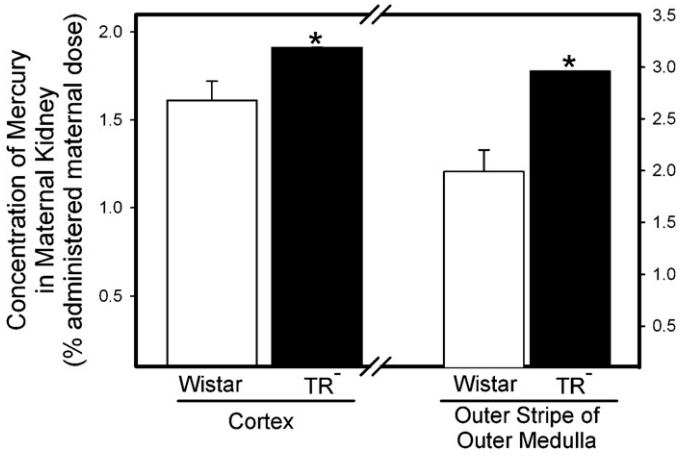

Fig. 6. Concentration of mercury in renal cortex and outer stripe of outer medulla of kidneys from dams. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Cortex and outer stripe of outer medulla were dissected from kidneys harvested 48 h after injection with CH_3HgCl. Data represent mean ± SE of three dams. Error bars, although present, may not be visible due to small error. Statistical analyses were conducted to assess differences between Wistar and TR⁻ rats. Statistical differences between samples from cortex and outer stripe of the outer medulla were not assessed. *Significantly different from corresponding Wistar dams ($p < 0.05$).

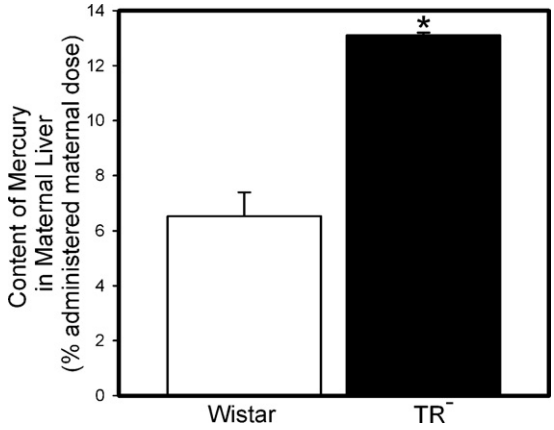

Fig. 7. Content of mercury in liver of dams. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Liver was harvested 48 h after injection with CH_3HgCl. Data represent mean ± SE of three dams. *Significantly different from corresponding Wistar dams ($p < 0.05$).

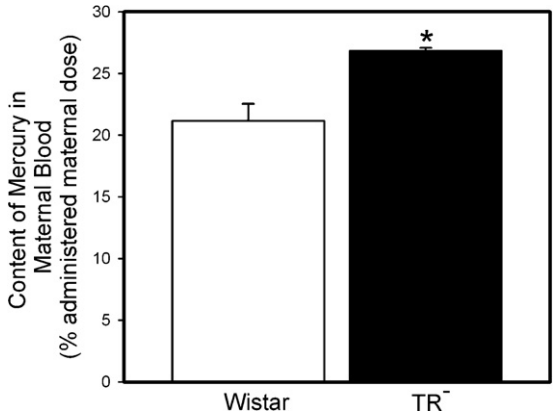

Fig. 8. Content of mercury in blood of dams. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Blood was collected 48 h after injection with CH_3HgCl. Data represent mean ± SE of three dams. *Significantly different from corresponding Wistar dams ($p < 0.05$).

3.2.3. Hepatic and hematologic burden of mercury

The hepatic burden of mercury in TR⁻ dams exposed to 5 mg/kg/2 mL CH_3HgCl was 13.1 ± 0.01% of the administered dose (Fig. 7). This amount was significantly greater ($p = <0.002$) than that in liver of corresponding Wistar dams (6.52 ± 0.87).

The content of mercury in the total volume of blood of Wistar dams exposed to 5 mg/kg/2 mL CH_3HgCl was 21.16 ± 1.38% of the administered dose (Fig. 8). The amount of mercury in blood of corresponding TR⁻ dams was significantly greater ($p = 0.015$) at 26.82 ± 0.25% of the dose.

3.2.4. Urinary and fecal excretion of mercury

The urinary excretion of mercury in pregnant Wistar and TR⁻ dams exposed to 5 mg/kg/2 mL CH_3HgCl is shown in Fig. 9A. The amount of mercury (% administered dose) excreted in urine of pregnant Wistar dams (0.79 ± 0.17) was significantly greater ($p = <0.001$) than that detected in urine of TR⁻ dams (0.37 ± 0.01).

The pattern of fecal excretion of mercury was similar to that of urinary excretion. The amount of mercury excreted in feces by Wistar dams was 4.22 ± 0.27% of the administered maternal dose and was significantly greater ($p = 0.011$) than that of TR⁻ dams (3.50 ± 0.05; Fig. 9B).

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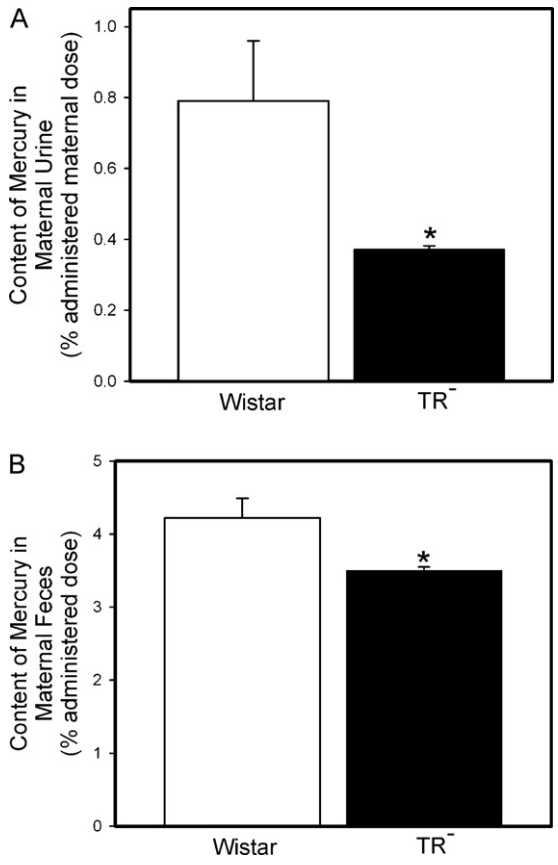

Fig. 9. Content of mercury in urine and feces of dams. Pregnant Wistar and TR⁻ rats were exposed intravenously to 5 mg/kg/2 mL CH_3HgCl. Urine (A) and feces (B) were collected 24 and 48 h after injection with CH_3HgCl. Data represent mean ± SE of three dams. *Significantly different from corresponding Wistar dams ($p < 0.05$).

4. Discussion

Since maternal exposure to CH_3Hg^+ can have detrimental effects on fetal development, understanding the mechanisms involved in placental transfer of mercuric ions is clinically relevant. Unfortunately, little is known about the transport mechanisms that are involved in the uptake and elimination of mercuric ions by placental trophoblasts and fetal tissues. A preponderance of data indicates that the most likely species of CH_3Hg^+ taken up by target cells in various organs are conjugates of non-protein thiols (*S*) [32]. Therefore, we suggest that the most likely form of CH_3Hg^+ transported across placental trophoblasts into fetal tissues is an *S*-conjugate of CH_3Hg^+ (such as Cys-*S*-CH_3Hg). Indirect evidence from *in vivo* studies, in which CH_3HgCl was administered to pregnant rats, suggests that *S*-conjugates of CH_3Hg^+ are transported across the placenta [18,33] into fetal tissues. It was suggested that this transport occurred at the site of the amino acid transporter, system L. Indeed, Cys-*S*-conjugates of CH_3Hg^+ have been shown to act as substrates of system L, specifically the human isoforms LAT1 and LAT2 [19], which have been localized to the apical and basolateral membranes of placental syncytiotrophoblasts, respectively [20]. Based on these studies, we suggest that *S*-conjugates of CH_3Hg^+ may gain access to the cytoplasmic compartment of placental syncytiotrophoblasts *via* LAT1 in the apical membrane, following which, these conjugates exit the trophoblasts and enter fetal circulation *via* LAT2 in the basolateral membrane.

The mechanisms by which mercuric ions are removed from fetal tissues and placental trophoblasts are less clear. We have hypothesized that the organic anion transporter (Oat) 4, localized in the basolateral (fetal-facing) membrane of syncytiotrophoblasts, may play a role in the transport of mercuric conjugates from fetal tissues and circulation into the intracellular compartment of trophoblasts [29]. Given that other members of the Oat family (*i.e.*, Oat1 and Oat3) have been shown to participate in the transport of S-conjugates of mercury, we can speculate that Oat4 may participate in the transport of one or more mercuric species. However, the ability of Oat4 to transport any form of mercury has not been demonstrated. We have also hypothesized that Mrp2, which is localized in the apical membrane of placental trophoblasts, may participate in the export of mercuric conjugates from within trophoblasts into maternal circulation [29]. Our previous findings have shown that mercuric ions can be extracted from placental and fetal tissues. However, the mechanisms involved in this extraction were not identified. Therefore, the current study was designed not only to examine the disposition of mercuric ions in placental and fetal tissues, but also to determine if Mrp2 plays a role in the placental and fetal export of mercuric species following maternal exposure to CH_3Hg^+. Given the broad substrate specificity of Mrp2, its presumed role in the renal and hepatic elimination of mercuric ions [21,22,31,34,35], and its localization in the apical (maternal-facing) membrane of placental trophoblasts [36], this carrier is a candidate for the export of mercuric species from placental trophoblasts and fetal tissues.

Our present findings demonstrate that placentas and whole fetuses harvested from TR⁻ dams injected with CH_3HgCl contain greater amounts of mercury than those from corresponding Wistar dams, suggesting that the absence of Mrp2 in TR⁻ rats significantly affects the handling of mercuric ions. It should be noted that although the same dose of CH_3HgCl was administered to Wistar and TR⁻ rats, the amount of mercury deposited/retained in organs and blood of TR⁻ dams was greater than that of Wistar dams. Therefore, we must consider the possibility that the increased fetal and placental burden of mercury in TR⁻ rats may be due, in part, to the retention of mercury in TR⁻ dams. This possibility may be examined by measuring embryonic exposures directly using embryonic cultures, which would eliminate maternal disposition issues. Alternatively, a pharmacokinetic model in which multiple doses of CH_3HgCl are administered to dams could be used in order to substantiate differences in the burden of mercury between TR⁻ and Wistar rats. These approaches, however, are outside the scope of the current study but are important experiments to consider for future studies. Despite this issue, we suggest that the present findings not only confirm the findings of previous reports indicating that mercuric ions can cross the placental barrier and gain access to fetal tissues [4–6,37–39], but also, they suggest that Mrp2 may be involved in the cellular elimination of mercuric ions from fetal and placental tissues. Within each fetus, the content of mercury in liver, brain and kidneys was measured. The accumulation of mercuric ions was significantly greater in organs from corresponding TR⁻ fetuses than those from Wistar fetuses, possibly due to the absence of Mrp2 and a reduced ability to export mercuric ions. Similarly, the amount of mercury in amniotic fluid was greater in TR⁻ rats than in Wistar rats. Taken together, these findings lead us to suggest that Mrp2 may be involved in the export and elimination of mercuric ions from placental trophoblasts and fetal tissues. Clearly, more definitive studies are necessary to provide additional support for our hypothesis.

We also assessed the maternal disposition of mercuric ions in uterus, kidneys, liver, blood, urine and feces of pregnant Wistar and TR⁻ dams. Within the kidneys of dams, the concentration of mercuric ions in the renal cortex and outer stripe of the outer medulla was greater in TR⁻ dams than in Wistar dams. In the kidneys, Mrp2 is localized exclusively in the proximal tubule, which is present in these two regions of the kidney. Therefore, the finding that the concentration of mercury in these zones was greater in TR⁻ rats than in Wistar rats supports the theory that Mrp2 is involved in

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the luminal elimination of mercuric ions from proximal tubular cells. Only a small amount of mercury was detected in the inner stripe of the outer medulla and the inner medulla. This finding is most likely due to the lack of transport proteins capable of transporting mercuric ions in the regions of the nephron that are found in these two zones. As expected, urinary excretion of mercury in TR$^-$ dams was less than that in Wistar dams. This finding corresponds to the findings that the concentrations of mercury in the renal zones were greater in TR$^-$ dams than in Wistar dams. Consequently, we conclude that Mrp2 likely plays a role in the proximal tubular elimination of mercuric ions in pregnant Wistar rats.

The disposition of mercuric ions in the liver of Wistar and TR$^-$ dams also supports a role for Mrp2 in the export of mercuric ions from hepatocytes. The hepatic burden of mercury was significantly greater in TR$^-$ rats than in Wistar rats, which suggests that the absence of Mrp2 in TR$^-$ rats reduces the hepatobiliary (and therefore, fecal) elimination of mercuric ions. Increased hepatic retention of mercuric ions by TR$^-$ rats exposed to inorganic and organic forms of mercury has been reported previously by our laboratory [21,29,31,35]. Similarly, Sugawara et al. have reported that hepatobiliary secretion of mercuric ions in Mrp2-deficient, Eisai hyperbilirubinemic rats is decreased following exposure to inorganic mercury [40]. The aforementioned findings correlate with the present findings showing that 1) the levels of hepatic mercury are greater in TR$^-$ dams than in Wistar dams and 2) the fecal elimination of mercury was lower in TR$^-$ dams than in Wistar dams.

It should be noted that recently, hepatic levels of breast cancer resistance protein (Bcrp; *Abcg2*) were found to be significantly lower in TR$^-$ rats than in control Wistar rats [41]. Bcrp is a member of the ATP-binding cassette superfamily and is thought to play an important role in the elimination of metabolic wastes and environmental and dietary toxicants [42]. Although Bcrp has not been implicated in the elimination of mercuric ions, one must consider the possibility that the increased accumulation of mercuric ions in TR$^-$ rats may be due to both, the reduced expression of Bcrp and the absence of Mrp2.

It is important to note that TR$^-$ rats, despite the lack of functional Mrp2, were capable of eliminating mercuric ions from renal and hepatic tissues. Based on these data, we conclude that one or more transport mechanisms, in addition to Mrp2, must be involved in the renal and hepatic elimination of mercuric ions. This conclusion is supported by our previous findings showing that mercuric ions can be extracted from TR$^-$ rats by selected metal chelating agents [21,29,31]. Currently, the identity of additional transport proteins that may be involved in this process is not known.

Following administration of CH_3Hg^+, a large fraction of the administered dose of mercuric ions was detected in maternal blood. Of this pool, approximately 99% was associated with cellular components and the remaining 1% was found to be contained within plasma, probably conjugated to albumin and other plasma thiols. Some of the mercuric ions associated with the cellular components of blood are likely bound to the surface of erythrocytes and leukocytes. Indeed, findings from studies in BALB/c mice injected with $HgCl_2$ indicate that within minutes of injection, mercuric ions can be found bound to the surface of erythrocytes [43,44]. Additionally, some mercuric ions may be endocytosed by neutrophils [45]. In BALB/c mice injected with $HgCl_2$, approximately 50% of the neutrophils examined after 2–4 min were associated with mercuric ions [45]. In the current study, it is interesting to note that the hematologic burden of mercury was greater in TR$^-$ rats than in Wistar rats. Although the reason for this finding is unclear at present, one possible explanation is that upregulation of basolateral transporters in TR$^-$ rats may facilitate or enhance movement of mercuric ions from within target cells into blood. Alterations in the expression of selected transporters, such as Mrp3, have been shown to occur in TR$^-$ rats [46–48]; however, none of the transport proteins that have been shown to be affected in TR$^-$ rats have been identified as carriers of mercuric species. Additional studies are clearly needed to elucidate additional mechanisms involved in the transport of mercury and to understand changes in the expression of these transport proteins.

In summary, our current data support the hypothesis that Mrp2 participates in the renal and hepatic elimination of mercuric ions in adult rats. The current data also suggest that Mrp2 may play a role in the elimination of mercuric species from placental trophoblasts and fetal tissues. To our knowledge, these studies are the first to suggest that Mrp2 may be involved in the placental and fetal handling of mercury.

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References

[1] ATSDR, U.S. Department of Health and Human Services PHS. Toxicological profile for mercury. Atlanta, GA: Centers for Disease Control; 2008.
[2] Al-Saleh I, Shinwari N, Mashhour A, Mohamed Gel D, Rabah A. Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women. International Journal of Hygiene and Environmental Health 2011;214:79–101.
[3] Jones L, Parker JD, Mendola P. Blood lead and mercury levels in pregnant women in the United States, 2003–2008. NCHS Data Brief 2010:1–8.
[4] Ask K, Akesson A, Berglund M, Vahter M. Inorganic mercury and methylmercury in placentas of Swedish women. Environmental Health Perspectives 2002;110:523–6.
[5] Inouye M, Kajiwara Y. Developmental disturbances of the fetal brain in guinea-pigs caused by methylmercury. Archives of Toxicology 1988;62:15–21.
[6] Inouye M, Murao K, Kajiwara Y. Behavioral and neuropathological effects of prenatal methylmercury exposure in mice. Neurobehavioral Toxicology and Teratology 1985;7:227–32.
[7] George GN, Prince RC, Gailer J, Buttigieg GA, Denton MB, Harris HH, et al. Mercury binding to the chelation therapy agents DMSA and DMPS and the rational design of custom chelators for mercury. Chemical Research in Toxicology 2004;17:999–1006.
[8] Spyker DA, Spyker JM. Response model analysis for cross-fostering studies: prenatal versus postnatal effects on offspring exposed to methylmercury dicyandiamide. Toxicology and Applied Pharmacology 1977;40:511–27.
[9] Su MQ, Okita GT. Embryocidal and teratogenic effects of methylmercury in mice. Toxicology and Applied Pharmacology 1976;38:207–16.
[10] Davidson PW, Myers GJ, Weiss B. Mercury exposure and child development outcomes. Pediatrics 2004;113:1023–9.
[11] Eto K. Minamata disease. Neuropathology: Official Journal of the Japanese Society of Neuropathology 2000;20(Suppl.):S14–9.
[12] Kazantzis G. Mercury exposure and early effects: an overview. La Medicina del lavoro 2002;93:139–47.
[13] Fu J, Hoffmeyer RE, Pushie MJ, Singh SP, Pickering IJ, George GN. Towards a custom chelator for mercury: evaluation of coordination environments by molecular modeling. Journal of Biological Inorganic Chemistry: JBIC: A Publication of the Society of Biological Inorganic Chemistry 2011;16:15–24.
[14] Aschner M, Clarkson TW. Uptake of methylmercury in the rat brain: effects of amino acids. Brain Research 1988;462:31–9.
[15] Aschner M, Clarkson TW. Methyl mercury uptake across bovine brain capillary endothelial cells in vitro: the role of amino acids. Pharmacology & Toxicology 1989;64:293–7.
[16] Aschner M, Eberle NB, Goderie S, Kimelberg HK. Methylmercury uptake in rat primary astrocyte cultures: the role of the neutral amino acid transport system. Brain Research 1990;521:221–8.
[17] Clarkson TW, Vyas JB, Ballatori N. Mechanisms of mercury disposition in the body. American Journal of Industrial Medicine 2007;50:757–64.
[18] Kajiwara Y, Yasutake A, Adachi T, Hirayama K. Methylmercury transport across the placenta via neutral amino acid carrier. Archives of Toxicology 1996;70:310–4.
[19] Simmons-Willis TA, Koh AS, Clarkson TW, Ballatori N. Transport of a neurotoxicant by molecular mimicry: the methylmercury-L-cysteine complex is a substrate for human L-type large neutral amino acid transporter (LAT) 1 and LAT2. Biochemical Journal 2002;367:239–46.

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[20] Kudo Y, Boyd CA. Characterisation of L-tryptophan transporters in human placenta: a comparison of brush border and basal membrane vesicles. Journal of Physiology 2001;531:405–16.
[21] Bridges CC, Joshee L, Zalups RK. MRP2 and the DMPS- and DMSA-mediated elimination of mercury in TR(−) and control rats exposed to thiol S-conjugates of inorganic mercury. Toxicological Sciences 2008;105:211–20.
[22] Bridges CC, Joshee L, Zalups RK. MRP2 and the handling of mercuric ions in rats exposed acutely to inorganic and organic species of mercury. Toxicology and Applied Pharmacology 2011;251:50–8.
[23] Aye IL, Paxton JW, Evseenko DA, Keelan JA. Expression, localisation and activity of ATP binding cassette (ABC) family of drug transporters in human amnion membranes. Placenta 2007;28:868–77.
[24] Vahakangas K, Myllynen P. Drug transporters in the human blood-placental barrier. British Journal of Pharmacology 2009;158:665–78.
[25] Mayer R, Kartenbeck J, Buchler M, Jedlitschky G, Leier I, Keppler D. Expression of the MRP gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. Journal of Cell Biology 1995;131:137–50.
[26] Paulusma CC, Bosma PJ, Zaman GJ, Bakker CT, Otter M, Scheffer GL, et al. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. Science 1996;271:1126–8.
[27] Belanger M, Westin A, Barfuss DW. Some health physics aspects of working with 203 Hg in university research. Health Physics 2001;80:S28–30.
[28] Bridges CC, Bauch C, Verrey F, Zalups RK. Mercuric conjugates of cysteine are transported by the amino acid transporter system b(0,+): implications of molecular mimicry. Journal of the American Society of Nephrology 2004;15:663–73.
[29] Bridges CC, Joshee L, Zalups RK. Effect of DMPS and DMSA on the placental and fetal disposition of methylmercury. Placenta 2009;30:800–5.
[30] Rouleau C, Block M. Fast and high yield synthesisi of radioactive $CH_3{}^{203}Hg(II)$. Applied Organometallic Chemistry 1997;11:751–3.
[31] Bridges CC, Joshee L, Zalups RK. Multidrug resistance proteins and the renal elimination of inorganic mercury mediated by 2,3-dimercaptopropane-1-sulfonic acid and meso-2,3-dimercaptosuccinic acid. Journal of Pharmacology and Experimental Therapeutics 2008;324:383–90.
[32] Bridges CC, Zalups RK. Transport of inorganic mercury and methylmercury in target tissues and organs. Journal of Toxicology and Environmental Health Part B, Critical Reviews 2010;13:385–410.
[33] Aschner M, Clarkson TW. Distribution of mercury 203 in pregnant rats and their fetuses following systemic infusions with thiol-containing amino acids and glutathione during late gestation. Teratology 1988;38:145–55.
[34] Madejczyk MS, Aremu DA, Simmons-Willis TA, Clarkson TW, Ballatori N. Accelerated urinary excretion of methylmercury following administration of its antidote N-acetylcysteine requires Mrp2/Abcc2, the apical multidrug resistance-associated protein. Journal of Pharmacology and Experimental Therapeutics 2007;322:378–84.
[35] Zalups RK, Bridges CC. MRP2 involvement in renal proximal tubular elimination of methylmercury mediated by DMPS or DMSA. Toxicology and Applied Pharmacology 2009;235:10–7.
[36] St-Pierre MV, Serrano MA, Macias RI, Dubs U, Hoechli M, Lauper U, et al. Expression of members of the multidrug resistance protein family in human term placenta. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 2000;279:R1495–503.
[37] Ong CN, Chia SE, Foo SC, Ong HY, Tsakok M, Liouw P. Concentrations of heavy metals in maternal and umbilical cord blood. Biometals 1993;6:61–6.
[38] Suzuki T, Yonemoto J, Satoh H, Naganuma A, Imura N, Kigawa T. Normal organic and inorganic mercury levels in the human feto-placental system. Journal of Applied Toxicology 1984;4:249–52.
[39] Yang J, Jiang Z, Wang Y, Qureshi IA, Wu XD. Maternal-fetal transfer of metallic mercury via the placenta and milk. Annals of Clinical and Laboratory Science 1997;27:135–41.
[40] Sugawara N, Lai YR, Sugaware C, Arizono K. Decreased hepatobiliary secretion of inorganic mercury, its deposition and toxicity in the Eisai hyperbilirubinemic rat with no hepatic canalicular organic anion transporter. Toxicology 1998;126:23–31.
[41] Yue W, Lee JK, Abe K, Sugiyama Y, Brouwer KL. Decreased hepatic breast cancer resistance protein expression and function in multidrug resistance-associated protein 2-deficient (TR(−)) rats. Drug Metabolism and Disposition 2011;39:441–7.
[42] Leslie EM, Deeley RG, Cole SP. Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicology and Applied Pharmacology 2005;204:216–37.
[43] Cherdwongcharoensuk D, Oliveira MJ, Aguas AP. In vivo formation and binding of SeHg complexes to the erythrocyte surface. Biological Trace Element Research 2010;136:197–203.
[44] Cunha EM, Cherdwongcharoensuk D, Aguas AP. Quantification of particles of lethal mercury in mouse viscera: high-resolution study of mercury in cells and tissues. Toxicology and Industrial Health 2003;19:55–61.
[45] Cunha EM, Oliveira MJ, Ferreira PG, Aguas AP. Mercury intake by inflammatory phagocytes: in vivo cytology of mouse macrophages and neutrophils by X-ray elemental microanalysis coupled with scanning electron microscopy. Human & Experimental Toxicology 2004;23:447–53.
[46] Chen C, Slitt AL, Dieter MZ, Tanaka Y, Scheffer GL, Klaassen CD. Up-regulation of Mrp4 expression in kidney of Mrp2-deficient TR- rats. Biochemical Pharmacology 2005;70:1088–95.
[47] Johnson BM, Zhang P, Schuetz JD, Brouwer KL. Characterization of transport protein expression in multidrug resistance-associated protein (Mrp) 2-deficient rats. Drug Metabolism and Disposition 2006;34:556–62.
[48] Xiong H, Suzuki H, Sugiyama Y, Meier PJ, Pollack GM, Brouwer KL. Mechanisms of impaired biliary excretion of acetaminophen glucuronide after acute phenobarbital treatment or phenobarbital pretreatment. Drug Metabolism and Disposition 2002;30:962–9.