N-Acetylcysteine as an Antidote in Methylmercury Poisoning

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Methylmercury is a ubiquitous environmental pollutant and a potent neurotoxin. Treatment of methylmercury poisoning relies almost exclusively on the use of chelating agents to accelerate excretion of the metal. The present study demonstrates that oral administration of *N*-acetylcysteine (NAC), a widely available and largely nontoxic amino acid derivative, produces a profound acceleration of urinary methylmercury excretion in mice. Mice that received NAC in the drinking water (10 mg/ml) starting at 48 hr after methylmercury administration excreted from 47 to 54% of the ^{203}Hg in urine over the subsequent 48 hr, as compared to 4–10% excretion in control animals. When NAC-containing water was given from the time of methylmercury administration, it was even more effective at enhancing urinary methylmercury excretion and at lowering tissue mercury levels. In contrast, excretion of inorganic mercury was not affected by oral NAC administration. The ability of NAC to enhance methylmercury excretion when given orally, its relatively low toxicity, and its wide availability in the clinical setting indicate that it may be an ideal therapeutic agent for use in methylmercury poisoning. *Key words:* antidote, brain, glutathione, inorganic mercury, methylmercury, *N*-acetylcysteine, poisoning. *Environ Health Perspect* 106:267–271 (1998). [Online 31 March 1998]
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Human exposure to methylmercury occurs mostly through consumption of fish and other seafood, although several major inadvertent exposures have also occurred, including epidemics in Japan (Minamata disease), Iraq, Pakistan, and Guatemala (*1–3*). Exposure may also occur in research laboratories, as evidenced by the recent fatal poisoning of a chemist at Dartmouth College (*4*).

Individuals poisoned with methylmercury exhibit severe neurological disturbances, including paresthesia, ataxia, sensory and speech impairment, and visual field constriction (*1–3*). These clinical symptoms become evident only after a relatively long latent period of 1–2 months. Because the damage to the central nervous system appears to be irreversible, treatment must be initiated promptly after exposure (*3,5*). The only way to prevent or ameliorate toxicity once methylmercury has been ingested is to accelerate its elimination from the body.

Strategies for removing methylmercury include hemodialysis, exchange transfusion, and chelation therapy, with the latter being the least invasive and most common therapeutic intervention (*3,5,6*). Although many chelating agents have been tested over the past four decades, no single best agent has yet been identified for use in methylmercury poisoning. Of the agents that have been examined, *meso*-2,3-dimercaptosuccinic acid (DMSA, succimer) and 2,3-dimercapto-1-propanesulfonic acid (DMPS, Dimaval) are the most efficient at enhancing methylmercury excretion and in preventing toxicity in both experimental animals (*7–16*) and humans (*5,6,17*). However, these agents have limited stability in solution, limited availability for human use, and a propensity to mobilize other essential and nonessential metals (*18–21*).

Surprisingly, the possible use of *N*-acetylcysteine (NAC) as a methylmercury chelating agent has not been previously evaluated. This omission is probably related to three factors. First, NAC was found to be ineffective at accelerating excretion of divalent toxic metals, including inorganic mercury, Hg^{2+} (*22–28*), and conflicting results have been reported regarding the ability of NAC to protect against methylmercury-induced embryotoxicity (*29,30*). Second, compounds that are structurally related to NAC, namely, *N*-acetyl-penicillamine (*N*-acetyl-β, β-dimethylcysteine) and penicillamine itself, are only minimally effective in accelerating methylmercury excretion (*5,15*). Third, because some NAC is converted to cysteine (*31*), an amino acid that increases methylmercury delivery to its target site, the brain (*32,33*), its use would appear to be contraindicated.

However, in two studies in which NAC was used in conjunction with hemodialysis of methylmercury-contaminated human blood, this chelator was quite effective at enhancing methylmercury clearance from blood (*17,34*). Lund and her collaborators (*17*) noted a striking increase in urinary methylmercury excretion during, as well as following, hemodialysis in a patient with acute methylmercury poisoning. These investigators suggested that NAC should be examined further as a possible methylmercury antidote. The present study supports this suggestion by demonstrating that NAC produces a dramatic acceleration of methylmercury excretion when administered orally to mice.

Experimental Procedures

Methylmercury chloride was obtained from ICN (Plainsville, NY), and $^{203}HgCl_2$ was purchased from Amersham Corporation (Arlington Heights, IL). $CH_3{}^{203}HgCl$ was synthesized from $^{203}HgCl_2$ by the method of Toribara (*35*). We confirmed the purity of the synthesized $CH_3{}^{203}HgCl$ using cold vapor atomic absorption (*36*). The product contained less than 1% inorganic mercury impurity. NAC was purchased from Sigma Chemical Company (St. Louis, MO). It was dissolved in water, the pH adjusted to 6.7–6.8 by adding 5 M NaOH, and diluted to a final concentration of 10 mg/ml. We prepared fresh NAC solutions every 2–3 days.

Female and male C57Bl/6 mice (15–26 g, 8 weeks of age) were obtained from Jackson Laboratory, Bar Harbor, Maine, and from Baylor College of Medicine, Houston, Texas. The animals were maintained on Purina Mills laboratory chow with water *ad libitum* in a temperature-controlled room with a 12-hr alternating light cycle. Experiments were conducted in accordance with the guidelines of the National Institutes of Health for care of laboratory animals.

Mice were placed individually in stainless-steel metabolic cages (Lab Products Inc., Rochelle Park, NJ) and were allowed to acclimate to the cages for 3–4 days. Urine and feces were collected onto plastic-lined absorbent bench paper placed underneath each cage. Feces was separated from the urine-stained paper, and each was stored in a

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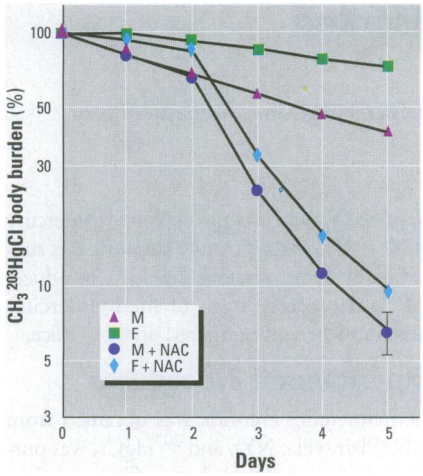

Figure 1. Body burden of ^{203}Hg-labeled methylmercury in male (M) and female (F) C57Bl/6 mice injected intraperitoneally with 0.5 µmol/kg body weight of $CH_3{}^{203}HgCl$. After 48 hr, some of the mice received *N*-acetylcysteine (NAC) in their drinking water (10 mg/ml). Values are means ± standard errors of four to five mice in each group (for most of the data points, error bars are smaller than the symbol).

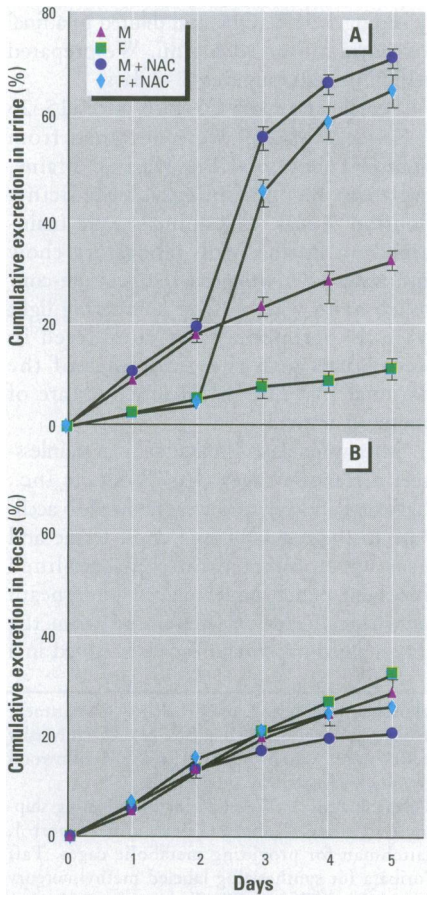

Figure 2. Cumulative (A) urinary and (B) fecal excretion of ^{203}Hg in male (M) and female (F) mice that received 0.5 µmol/kg body weight of $CH_3{}^{203}HgCl$. After 48 hr, some of the mice received *N*-acetylcysteine (NAC) in their drinking water (10 mg/ml). Values are means ± standard errors of four to five mice in each group.

small plastic bag. Some mice were given NAC in their drinking water starting 48 hr before mercury administration. Mice were injected intraperitoneally with ^{203}Hg-labeled methylmercury chloride (0.5 or 25 µmol/kg body weight) or $^{203}HgCl_2$ (0.5 µmol/kg, ip). Injection solutions were prepared in Krebs-Henseleit buffer and administered in a volume of 10 ml/kg body weight (20 µCi/kg). Body burden and urinary and fecal ^{203}Hg content were measured daily in a Packard model 3002 gamma counter. We counted ^{203}Hg standards prepared from the dosing solutions daily in the same instrument to correct for decay and counter efficiency.

After 5 days, mice were anesthetized with sodium pentobarbital (55 mg/kg, ip), and selected tissues (liver, kidney, brain, and whole blood) were taken for glutathione (GSH) and ^{203}Hg analysis. The animals were killed between 1300 and 1600 hr. Whole blood was withdrawn from the abdominal vena cava. The median lobe of the liver, one kidney, the brain, and 0.2–0.3 ml of whole blood were each placed in tared tubes containing 5 ml of 2.5% sulfosalicylic acid/0.25 mM acivicin for subsequent GSH determination, as described previously (*37*). We counted the brain and blood samples using a Packard model 3002 gamma counter for 30 sec each and then homogenized the samples. Liver and kidney samples were homogenized immediately. The rest of the liver and the other kidney were removed, weighed, and used for ^{203}Hg determination. Urine was withdrawn from the bladders of some of the animals using a 1-ml syringe and 27-gauge needle for analysis of NAC content. Urine samples were treated with dithiothreitol to reduce disulfide linkages, derivatized with 1-fluoro-2,4-dinitrobenzene (*38*), then analyzed for NAC using an HPLC protocol previously described (*39*).

Results

Male and female C57Bl/6 mice treated acutely with $CH_3{}^{203}HgCl$ (0.5 µmol/kg body weight) excreted 59% and 28% of the dose in 5 days, respectively (Fig. 1). This corresponds to a biological half-life of approximately 4 days in males and 9 days in females, values comparable to those reported in previous studies of mice (*9,13*). Urinary excretion of ^{203}Hg was approximately three times higher in male mice (Fig. 2A), whereas fecal excretion was similar between males and females (Fig. 2B).

In contrast with this relatively slow rate of excretion in control animals, mice that received NAC in their drinking water beginning 48 hr after methylmercury administration excreted 91–94% of the dose during the same time period (Fig. 1). Most of the methylmercury was excreted in urine (Fig. 2A). NAC had no effect on fecal ^{203}Hg excretion (Fig. 2B).

NAC efficiently mobilized methylmercury from all tissues examined, including the brain (Table 1). The ^{203}Hg content of blood, kidneys, and liver in mice that received NAC was approximately 10–20% that of control animals, whereas brain levels were decreased to about one-half those of controls (Table 1).

Mice that received NAC in their drinking water from the time of methylmercury administration exhibited an even more dramatic acceleration of whole-body ^{203}Hg elimination (Fig. 3) and urinary excretion (Fig. 4A). These animals excreted 87% of the methylmercury dose in only 2 days (Fig. 3). Conversely, when NAC was removed from the drinking water, there was an abrupt decrease in methylmercury excretion (Fig. 3). The biological half-life for methylmercury after cessation of NAC administration, as assessed from the slope of the line, was similar to that in mice that did not receive NAC (Fig. 3).

Table 1. Tissue ^{203}Hg distribution 5 days after administration of 0.5 µmol/kg of either $CH_3{}^{203}HgCl$ or $^{203}HgCl_2$ to male and female mice in the presence and absence of 10 mg/ml of *N*-acetylcysteine (NAC) in the drinking water (dosing days 2–5)

		Percent of dose			
	n	Whole blood	Kidneys	Liver	Brain
$CH_3{}^{203}HgCl$					
Males					
Control	4	1.14 ± 0.11	5.25 ± 0.28	4.27 ± 0.35	0.54 ± 0.02
NAC	5	0.15 ± 0.07*	0.47 ± 0.03*	1.06 ± 0.12*	0.32 ± 0.02*
Females					
Control	4	2.28 ± 0.22	4.28 ± 0.37	10.68 ± 0.72	1.01 ± 0.12
NAC	5	0.21 ± 0.04*	0.64 ± 0.08*	2.33 ± 0.19*	0.42 ± 0.02*
$^{203}HgCl_2$					
Males					
Control	4	0.24 ± 0.03	7.08 ± 0.55	1.94 ± 0.03	0.09 ± 0.02
NAC	5	0.18 ± 0.05	3.24 ± 0.37*	2.02 ± 0.13	0.06 ± 0.01
Females					
Control	4	0.22 ± 0.05	5.35 ± 0.60	3.29 ± 0.13	0.11 ± 0.01
NAC	5	0.16 ± 0.03	3.31 ± 0.41*	4.29 ± 0.57	0.11 ± 0.01

Values are means ± standard errors. Blood ^{203}Hg content was calculated assuming a blood volume of 55 ml/kg body weight.
*Significantly different from control ($p<0.05$) using Student's *t*-test.

NAC was also effective in mice given a 50-fold larger dose of methylmercury chloride, 25 µmol/kg body weight (Fig. 5). This dose is at the threshold of toxicity in mice (*16*), but no clinical signs of toxicity were noted in the present study. The results at this higher dose were similar to those at the lower dose, except that the biological half-life was somewhat longer (compare Figs. 3 and 5). Control animals excreted 16% of the methylmercury dose during the first 2 days (Fig. 5). In contrast, mice that received NAC in the drinking water excreted 75% of the dose in the first 2 days (Fig. 5). When NAC was provided 2 days after methylmercury administration, there was an abrupt increase in ^{203}Hg excretion: the body burden decreased from 84% to 16% of the original dose in 3 days (Fig. 5). Withdrawal of NAC once again reverted the biological half-life of methylmercury to that seen in control animals (Fig. 5).

In contrast to methylmercury, excretion of inorganic mercury was faster (a $t_{1/2}$ of <2 days) and was not stimulated by NAC (Fig. 6). However, there was a significant tissue redistribution of ^{203}Hg in animals that received NAC (Table 1), which may contribute to the lack of effect on whole-body elimination of inorganic mercury. Kidney levels of ^{203}Hg were lower in mice that received NAC (Table 1). This redistribution away from kidneys (which contain approximately one-third of the body burden) may limit the amount of mercury that can be excreted in urine.

Glutathione levels in liver, kidney, brain, and whole blood were also measured at necropsy; no differences between control and NAC-treated mice were seen (data not shown). Thus, although some of the NAC may be converted to cysteine (*31*), this additional cysteine does not increase tissue GSH levels in mice fed *ad libitum*.

Analysis of urine samples collected from the urinary bladder at necropsy indicated high concentrations of NAC: 10.5 ± 1.5 mM (range of 6–18 mM, n = 7) from mice that received NAC in their drinking water.

Discussion

At present, no effective strategy exists to promote the rapid clearance of methylmercury from poisoned individuals. A number of sulfhydryl-containing complexing agents have been evaluated extensively and found to be only marginally effective (*7–17*). In contrast, the present study demonstrates that NAC may be an ideal therapeutic agent for use in methylmercury poisoning. NAC is an amino acid derivative that is currently in use in clinical medicine; it is not toxic to humans, and it is rapidly excreted in urine. This rapid urinary excretion of NAC most likely explains its ability to enhance urinary methylmercury excretion.

Another cysteine derivative, D-penicillamine, was one of the earliest agents found to enhance urinary excretion of methylmercury (*7*). However, subsequent studies indicated that compounds containing two thiol groups, such as DMSA and DMPS, are more effective than D-penicillamine in mobilizing methylmercury from various tissue

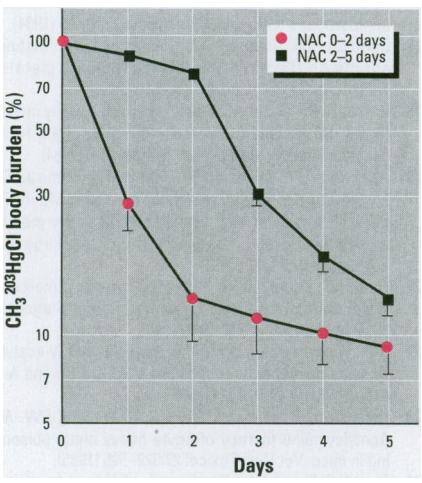

Figure 3. Body burden of ^{203}Hg-labeled methylmercury in female mice injected intraperitoneally with 0.5 µmol/kg body weight of $CH_3{}^{203}HgCl$. One group of mice received NAC in the drinking water (10 mg/ml) for the first 48 hr after dosing with methylmercury, whereas the other group received *N*-acetylcysteine (NAC) in the drinking water from days 2 to 5. Values are means ± standard errors of five mice in each group.

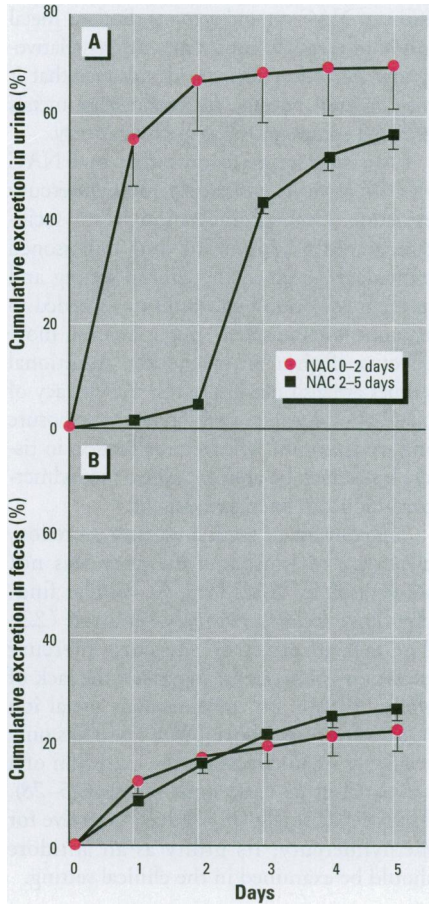

Figure 4. Cumulative (A) urinary and (B) fecal excretion of ^{203}Hg in mice that received 0.5 µmol/kg body weight of $CH_3{}^{203}HgCl$. One group of mice received NAC in the drinking water (10 mg/ml) for the first 48 hr after dosing with methylmercury, whereas the other group received *N*-acetylcysteine (NAC) in the drinking water from days 2 to 5. Values are means ± standard errors of five mice in each group.

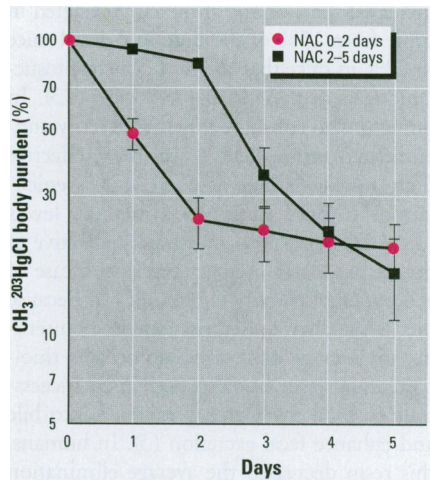

Figure 5. Body burden of methylmercury in female mice injected intraperitoneally with 25 µmol/kg body weight of $CH_3{}^{203}HgCl$. One group of mice received *N*-acetylcysteine (NAC) in their drinking water (10 mg/ml) for the first 48 hr after dosing with methylmercury, whereas the other group received NAC in the drinking water from days 2 to 5. Values are means ± standard errors of five mice in each group.

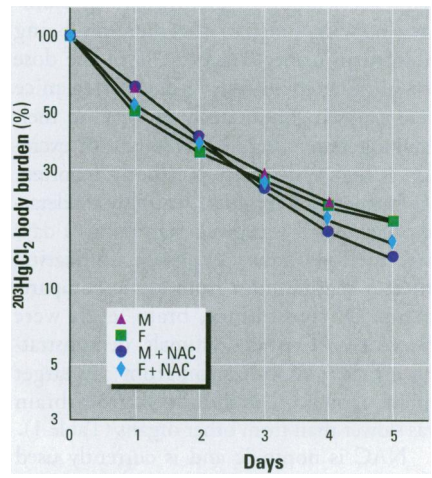

Figure 6. Body burden of inorganic mercury in male (M) and female (F) mice injected intraperitoneally with 0.5 µmol/kg body weight of $^{203}HgCl_2$. After 48 hr some of the mice received *N*-acetylcysteine (NAC) in their drinking water (10 mg/ml). Values are means ± standard errors of four to five mice in each group.

compartments and in enhancing its excretion from the body (*8–17*). Gabard (*11*) and Magos (*9*) reported that DMSA is effective in decreasing the body burden and the concentration of mercury in brain, blood, liver, and kidneys of rats and mice treated with methylmercury chloride. Aaseth and Friedheim (*13*) compared the effects of five chelating agents in methylmercury-poisoned mice (DMSA, DMPS, *N*-acetyl-D,L-penicillamine, mercaptosuccinic acid, and mercaptostarch). They also found that DMSA was superior in accelerating whole-body elimination and decreasing tissue levels. DMSA increased the amount of mercury excreted in urine from 15% of the dose in control mice to 62% in mice that received dimercaptosuccinic acid in their food for 8 days (*13*). In humans who consumed methylmercury-contaminated bread, DMPS was more effective than D-penicillamine or *N*-acetyl-D,L-penicillamine in decreasing blood mercury levels (*5*). DMPS was also moderately effective at decreasing blood mercury levels in a case of acute methylmercury ingestion (*17*). Because methylmercury undergoes extensive enterohepatic recirculation, a nonabsorbable thiol-containing resin has also been used successfully to bind the mercury excreted into bile and enhance fecal excretion (*5*). In humans, this resin decreased the average elimination half-time of methylmercury in blood from 65 to 20 days (*5*). Because of these early findings with dithiol and polythiol compounds, most subsequent efforts to identify methylmercury antidotes have focused on this class of chelating agents.

The present study demonstrates that NAC may be superior to previously tested complexing agents. NAC produced a dramatic acceleration of methylmercury elimination, with most of the mercury being excreted in urine. Nearly 90% of the dose was eliminated in only 2 days when mice were exposed continuously to NAC in their drinking water (Fig. 3). This rate of excretion is higher than any previously reported. Methylmercury excretion was also accelerated when NAC was given starting at 2 days after methylmercury exposure, indicating efficient mobilization from tissue compartments. Of significance, brain levels were lower in NAC-treated animals, demonstrating efficient mobilization from the target organ. However, mobilization from brain was slower than from other organs (Table 1).

NAC is nontoxic and is currently used clinically as a mucolytic agent and in the treatment of acetaminophen overdose (*40,41*). It is widely available, relatively inexpensive, easily administered, and well tolerated by patients. The clinical pharmacology of NAC is well described (*42–45*). It has a volume of distribution of 0.33 l/kg, indicating distribution mainly to extracellular water. It is rapidly eliminated in urine, with approximately one-third excreted in urine during the first 12 hr after administration (*42*). In urine, it is present mostly as the symmetrical disulfide and as the mixed disulfide with cysteine, although it is excreted as the free thiol as well (*46,47*). In humans the $t_{1/2}$ for NAC in blood plasma is approximately 2 hr (*42–45*). This short half-life is consistent with the rapid acceleration of methylmercury excretion observed during NAC administration and with the rapid deceleration in methylmercury excretion after NAC withdrawal (Figs. 1, 3, and 5).

In contrast to some dithiol-complexing agents (*18–21*), NAC does not alter tissue distribution of essential metals (*48*). Hjortso et al. (*48*) measured plasma and urinary concentrations of trace metals (Ca, Mg, Fe, Zn, and Cu) in healthy human volunteers treated with NAC for 2 weeks and found no significant change in plasma concentration or excretion of these essential metals. This lack of effect of NAC on endogenous divalent metal ion homeostasis is consistent with its relatively low intrinsic toxicity and indicates that it may be more selective for monovalent metals or metal compounds like methylmercury.

It is important to note that oral NAC was effective in enhancing methylmercury excretion. Oral administration greatly facilitates administration of the drug to poisoned individuals, both in the clinical setting and in the field. Additional studies are needed to examine whether NAC might be even more effective if given intravenously. Additional studies are also needed to test the efficacy of NAC after chronic methylmercury exposure and to determine whether a reduction in tissue levels may be able to reverse methylmercury-induced functional deficits.

In contrast to methylmercury excretion, clearance of inorganic mercury was not accelerated by NAC (Fig. 6). Similar findings have been previously reported (*22*). The lack of effect on mercuric mercury excretion is not surprising given the lack of effect of NAC on trace divalent metal ion homeostasis (see above). NAC also has only relatively small effects on the excretion of a variety of other exogenous metals (*25–28*). Thus, NAC might be relatively selective for methylmercury. Its utility as an antidote should be examined in the clinical setting.

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