M E M O R A N D U M

TO: Val F. Siebal
Chief Deputy Director

VIA: George V. Alexeeff, Ph.D., D.A.B.T.
Deputy Director for Scientific Affairs

VIA: Anna M. Fan, Ph.D., Chief
Pesticide and Environmental Toxicology Section

VIA: Robert A. Howd, Ph.D., Chief
Water Toxicology Unit
Pesticide and Environmental Toxicology Section

FROM: Lubow Jowa, Ph.D., Staff Toxicologist
Water Toxicology Unit
Pesticide and Environmental Toxicology Section

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SUBJECT: Update of PHG for Inorganic Mercury

Under the Calderon-Sher California Safe Drinking Water Act of 1996, the Office of Environmental Health Hazard Assessment (OEHHA) develops public health goals (PHGs) for regulated chemicals in drinking water and reviews and updates the risk assessments every five years (Health and Safety Code Section 116365(e)(1). This memorandum represents an update of the literature review and reevaluation of the existing PHG for inorganic mercury (OEHHA, 1999). Our re-evaluation supports the previous PHG derivation in 1999. We conclude that the PHG for inorganic mercury should remain at 1.2 ppb.

Summary of review

We have surveyed the scientific literature for recently published inorganic mercury research studies to determine if there are new studies on inorganic mercury toxicity that would warrant revising the PHG of 1.2 ppb or making substantive changes to the PHG support document. We searched for new risk assessments of inorganic mercury compounds since the publication of the PHG document in 1999, including U.S. EPA reviews and new risk assessment methods that might be applied to evaluation of inorganic mercury. Finally, the NTP (1993) report was reviewed again for additional insights.

No new studies were found that affect the choice of the critical study used as the basis for the existing PHG value, nor any of its assumptions. New data were found that can provide some further insight on the toxic effects of inorganic mercury. Although there is no basis for proposing a change in the inorganic mercury PHG, a few recent studies that provide additional perspectives are described.

Literature review

The few recent studies on inorganic mercury focus on specific aspects of mercury toxicity and mode of action. These studies are summarized below:

Reproductive or developmental toxicity:

In the first of two studies by this group, Atkinson *et al*. (2001) investigated the reproductive toxicity of mercuric chloride administered in drinking water to male and female rats from age 30 days through delivery, for two generations. The doses administered were 0, 0.5, 1,0, 1.5 mg/kg-day for males and 0, 0.75, 1.5 and 2.5 mg/kg-day for females of the F_0 generation; doses were the same for the F_1 generation, except that the highest dose was omitted. Fertility was affected in a dose-related fashion for the treated F_0 groups, with reduction by a third from the control population at the lowest dose. However, in the F_1 generation no decrease in fertility rates was noted. Live births/litter were reduced in a dose-related fashion for the F_1 but not the F_2 generation. There was also a dose-related decrease in body weight and the 4-day survival rate in the F_1 generation.

In the more recent study, Khan *et al*. (2004) exposed mice to mercuric chloride for one generation. Male and female mice were given 0, 0.25, 0.5 or 1.0 mg/kg-day of mercuric chloride in their drinking water. All treated groups had a decrease in fertility index of about 60 percent. There were four live litters for 25 cohabited pairs, compared to 11 for 25 pairs in the control group, which is unusually low. There were significant decreases in live pups/litter and live birth index at the highest dose only. All other measured parameters were within a normal range.

Both of these studies have serious experimental and data-reporting deficiencies. In the rat study, the tabulated implant efficiencies are not supported by the data provided, and the offspring sex ratios vary widely without being noted; in both studies, the reported mean live pups/litter are inconsistent with the data provided. At the highest dose in the rat study, the stated number of F_1 males is equal to the total number of live offspring, which contradicts the reported sex ratio. OEHHA concludes that these reproductive studies are not suitable for quantitative risk assessment.

Two studies by another group on certain parameters relating to the reproductive process were also found. Ramalingam *et al*. (2002) orally administered 1 mg/kg mercuric chloride to male rats for 30 days. Four products related to sperm maturation were evaluated in their spermatozoa at the end of that period: glyceryl phosphoryl choline, sialic acid, carnitine and

acetylcarnitine. Another group of rats was exposed and then withdrawn from treatment for thirty days, upon which their spermatozoa were collected and these indices measured. The first group showed significantly decreased levels of all the markers compared to untreated rats. For the second group, the marker levels were not significantly different from untreated animals.

In the second study, Ramalingam *et al*. (2003) exposed groups of rats for thirty days to mercuric chloride at 1.0 and 2.0 mg/kg-day (stated in the abstract as 0.5 and 1.0 mg-kg-day). They measured the circulating hormones testosterone, luteinizing hormone, prolactin, and follicle-stimulating hormone. Testosterone and luteinizing hormone were significantly depressed at both doses, and the other two only at the highest dose.

The studies of Ramalingam *et al.* (2002, 2003) provide evidence that mercuric chloride may be a reproductive toxin, but have limitations for defining a critical effect for risk assessment. These studies focused only on assessment of the specific parameters without providing any clinical information about the state of the animals, or gross or histological evaluation of the testes or its constituents. Moreover, there is some uncertainty regarding the dosages for the second study. However, if a health-protective concentration in drinking water were derived from these studies, it would be higher than that provided by the NTP (1993) study.

Endocrine Function

Tiwari and Bhattacharya (2004) evaluated the effect of mercuric chloride on thyroid function by measuring thyroid peroxidase and thyroxine, as well as serum cholesterol, in a study of the protective effects of phospholipids on mercury toxicity. Groups of mice were given approximately 10 mg/kg-day of mercuric chloride for 7, 14, or 21 days. The thyroxine and peroxidase levels were depressed by nearly one half compared to that of controls, but the cholesterol levels were only marginally depressed. Daily co-administration of an “essential phospholipid” preparation antagonized these effects of mercury. These data suggest mercuric chloride impacts thyroid function; however, the dose used is rather high compared to other critical effect doses.

Immunotoxicity

Recent research on inorganic mercury-associated immunotoxicity is less intense than it was during the 1990s, but work continues along several promising mechanistic lines using sensitive strains of rats, mice and other animals. In particular, specific strains of mice have been developed which show mercury-induced autoimmunity, in which the onset of autoimmunity seems to be linked with the MHC (major histocompatibility complex) H-2 genes. Thus the strains of mice with specific H-2 haplotypes *s* and *q* are the most likely to manifest mercury-induced autoimmunity, in which antibodies are produced against the nucleolar protein fibrillarin (Nielsen and Hultman, 2002).

In a study comparing eight different strains of autoimmune-susceptible strains of mice, Nielsen and Hultman (2002) exposed groups of male and female mice to radioactively-labeled

mercuric chloride in their drinking water at doses from 0.5 to 1.0 mg/kg-day for ten weeks before sacrifice. Mercury-containing antibodies were monitored. In addition, serum IgE was monitored to demonstrate the onset of autoimmunity. The authors found an increase in antibody titer associated with mercury dose and exposure duration, which reached a steady-state over time. Female mice tended to respond to mercury with larger, earlier-increasing, antibody titers than males.

In another study, Via *et al.* (2003) studied whether low doses of inorganic mercury promoted autoimmunity in a mouse lupus model (splenocyte-induced graft versus host disease). Groups of five BDF_1 mice of a non-autoimmune-susceptible strain were injected sc with 0, 20 or 200 μg/kg mercuric chloride every other day for 15 days; five days thereafter they received mouse splenocytes intravenously to produce a mild graft vs. host disease. Surviving mice were terminated at four months, based on morbidity of the animals. The mercury pretreatment apparently resulted in increased mortality, glomerular nephritis, and proteinuria, with greater effects at 20 than at 200 μg/kg mercuric chloride. At sacrifice, there was only one mouse remaining in the low-dose group and three in the high-dose group, while all five survived in the control group. The authors conclude that the mercury pretreatment increased the susceptibility of the mice to graft vs. host disease, and hypothesized that "the two doses of mercuric chloride may have different effects on autoimmunity." The authors acknowledge that neither mercury dose alone results in immunotoxicity in this strain. Considering the small number of mice per group, lack of a dose-response, the non-oral route of administration, and lack of effects of mercury alone, the results do not appear usable for quantitative risk assessment of inorganic mercury.

Mellergard *et al.* (2004) studied the systemic autoimmune response in H-2 mice, a condition with antinucleolar antibodies targeting the nucleolar protein fibrillarin, transient polyclonal B-cell activation, hyperimmunoglobinemia, and systemic immunoglobulin deposits. Mice were given 6 mg/kg-day of mercuric chloride in drinking water for 22 weeks. Pretreatment with several immune response-inhibiting agents suppressed the formation of antinucleolar antibodies with mercuric chloride. Mercuric chloride induced a strong systemic autoimmune response, including renal IgG deposits in tight skin mice (Tsk/+). However, inorganic mercury did not cause dermal fibrosis, the precursor to skin thickening (Hansson and Abedi-Valugerdi, 2004). The tight skin mouse was developed to have a propensity toward skin thickening, to experimentally mimic human scleroderma.

It was reported that mercuric chloride inhibits nitric oxide production in cultured murine macrophages, while modulating p38 cytokine expression by mitogen active protein kinase (Kim *et al.*, 2002). In a more recent study, Kim and Sharma (2003) explored the effect of mercuric chloride on murine T and B lymphoma cell lines and found an increase in reactive oxygen generation with an increase in necrosis and apoptosis.

In earlier studies, mercuric chloride administration to the Brown Norway rat had been shown to result in Th2-dominated autoimmunity with increased immunoglobulin E

concentrations and gut vasculitis, with the involvement of mast cells in the early phase. Viven *et al.* (2004) investigated the development of caecum vasculitis upon injection of mercuric chloride into Brown Norway rats, and found that mast cells do play a key role. In this case the autoimmunity induced by mercuric chloride apparently resolved itself after two weeks, and then provided resistance to further challenges by mercuric chloride. This resistance seemed to be due at least in part to decreased numbers of mast cells in the gastric mucosa. None of the aforementioned studies is suitable for derivation of a critical dose for risk assessment; they either used higher doses than those used to derive the PHG, or involved mercuric chloride directly injected into the animals, which is an inappropriate administration route for drinking water risk assessment. However, it is important to note that these studies indicate that critical doses for inorganic mercury effects on sensitive strains of experimental animals are about 1 mg/kg, and thus support the critical dose selection for the existing PHG. As shown below, the critical effects used for the existing PHG development were decreases in rat body weight gains and increases in relative and absolute kidney weights observed in the subchronic (6 month) study at doses of 0.46 mg/kg-day and greater, with a NOAEL of 0.23 mg/kg-day.

Human Population Studies

Silva *et al.* (2004) investigated the potential association of mercury exposure with indicators of autoimmune disease in persons exposed to mercury in the Brazilian Amazon area. One group (n = 98) was exposed to mercury (probably metallic) via gold mining, and another group (n = 140) was exposed primarily by eating fish contaminated by methylmercury); controls (n = 98) were presumed to have low exposures to mercury, although the median of their hair mercury levels was similar to the second group. The gold miners had a substantially higher rate of elevated antibodies to nuclear and nucleolar protein than the other two groups, and also a much higher prevalence of active malaria. The population presumed to have higher exposure to methylmercury had higher antibody levels than the control population. The authors state that "This is the first study to report immunologic changes, indicative of autoimmune dysfunction in persons exposed to mercury, which may also reflect interactions with infectious disease and other factors." We conclude that the confounding factors are too strong to show that mercury induces autoimmune disease in humans. Overall, this study provides no information relevant to derivation of a specific PHG value.

Review of the existing PHG value

As stated in the 1999 support document for the inorganic mercury PHG, defining suitable critical values and risk assessment approaches for deriving the inorganic mercury PHG has been particularly difficult. The basis for the current federal MCL is immunotoxicity produced by mercuric chloride in the Norway rat at LOAELs of 0.23 to 0.63 mg/kg-day (U.S. EPA, 2004). About twenty years ago, U.S. EPA assembled a committee to derive a Drinking Water Equivalent Level (DWEL) for inorganic mercury. The committee examined several Norway rat

studies and concluded that none of them was suitable alone for calculating a DWEL, but that taken together, it appeared that a number of 10 μg/L would be appropriate. The MCL that resulted from this was 2 μg/L (2 ppb), and it has been unchanged to date. However, we chose to use the findings of the NTP (1993) toxicity studies, at similar doses, as the basis for the 1999 PHG. As stated in the PHG support document, the critical effects used for PHG development were decreases in rat body weight gains and increases in relative and absolute kidney weights observed in the subchronic (6 month) study at doses of 0.46 mg/kg-day and greater, with a NOAEL of 0.23 mg/kg-day. These effects were chosen over the effects seen in the chronic study because significant lethality was observed in both doses (1.8 and 3.7 mg/kg-day) employed in the chronic study.

The NTP (1993) report was reviewed again, in an attempt to glean new insights or identify new critical effect possibilities. We again concluded that rats appear to be more sensitive than mice to the toxic effects of mercuric chloride, and that the rat subchronic study provides a better basis for the critical effect level than the chronic study. It can be argued that the effects are minor (weight gain was depressed less than 10 percent), and certainly NTP thought so, since they selected higher doses for the chronic study. However, the results of the chronic rat study proved their decision was flawed. Male rats have a propensity towards kidney disease as they age, and mercury appeared to accentuate this process, killing them prematurely. Thus there was no NOAEL in the NTP (1993) chronic study. After reevaluating the kidney effects in the subchronic study, interim sacrifices of the chronic study and the chronic study itself, OEHHA finds no appropriate alternative risk assessment endpoints, and concludes that the PHG calculation method is appropriate. OEHHA also concludes that the PHG of 1.2 ppb is adequate to protect sensitive subpopulations, including pregnant women and their fetuses, infants, and the elderly.

Other positions on inorganic mercury:

The current U.S. EPA MCL of 2 ppb, established in 1977, is based on Hg-induced autoimmune glomerulonephritis in the Brown Norway rat (U.S. EPA, 2004a,b). U.S. EPA also concluded in their review of inorganic mercury for the National Primary Drinking Water Regulations (U.S. EPA, 2003) that the current regulation "remains appropriate after data/information review."

The California Department of Health Services (DHS) reviewed the 1999 PHG for inorganic mercury (DHS, 2004). DHS reported that mercury is found infrequently in drinking water supplies, with only five detections greater than the PHG and only one greater than the MCL from 2000 through 2003. Since there were neither changes in treatment technologies nor new evidence regarding risks to public health, considering the relatively few detections of mercury and that the MCL is just 1.7 times the PHG, DHS concluded that no change or further review of the mercury MCL was needed.

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