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Effects of Mercury on Human Polymorphonuclear Leukocyte Function In Vitro

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A variety of heavy metals are recognized as environmental pollutants, and although a significant body of literature exists on the acute toxicity of these metals in various tissues, little is known about the effects of metals such as mercury on host defense. Therefore, the effect of mercuric chloride ($HgCl_2$) on human polymorphonuclear leukocytes (PMN) function *in vitro* was evaluated. The acute toxicity of $HgCl_2$ for human PMN was calculated initially using vital dye exclusion (trypan blue), and lactate dehydrogenase (LDH) release. Concentrations of $HgCl_2 \leq 10^{-6}$ M did not induce significant LDH release, or uptake of tryphan blue. Additionally, $HgCl_2$ at $\leq 10^{-7}$ M produced no ultrastructural alterations in the PMN. The effects of $HgCl_2$ on human PMN functions involved in host defense were evaluated next. $HgCl_2$ consistently suppressed human PMN adherence, polarization, chemotaxis, and erythrophagocytosis at concentrations between 10^{-6} and 10^{-17} M. Because of the established role of oxygen metabolites in host defense, the effects of $HgCl_2$ on human PMN chemiluminescence and H_2O_2 production were evaluated next. These studies demonstrated that low concentrations of $HgCl_2$ (ie, 10^{-9}–10^{-15} M) significantly enhanced chemiluminescence, as well as stimulated H_2O_2 production by the PMN. These studies clearly demonstrate the ability of extremely low levels of $HgCl_2$ not only to suppress various PMN functions involved in host defense, but also to stimulate oxygen metabolism. *In vivo,* these $HgCl_2$ effects would not only compromise host defense but also promote tissue injury via the local production of oxygen metabolites. (Am J Pathol 1988, 132:110–118)

NUMEROUS CHEMICALS ARE known to influence a variety of *in vitro* functions of the immune system, such as lymphocyte activation, antibody synthesis, production of regulatory and inflammatory lymphokines as well as other cell-mediated reactions.[1,2] There is a paucity of systematic studies, however, on the effects of heavy metals on the functions of polymorphonuclear leukocytes (PMN). Limited observations have demonstrated that lithium, aluminum, and zirconium increase the phagocytic activity of macrophages, whereas gold and cadmium inhibit phagocytosis by macrophages and PMN.[3–6] Recently another heavy metal, mercury, has been shown to affect lymphocyte function, and cause autoimmune responses in experimental animals and humans.[7,8] Little is known about mercuric chloride ($HgCl_2$) effects on host defense in general and human PMN functions specifically. Thus, we investigated the effects of $HgCl_2$ on human PMN function *in vitro.* Our present studies demonstrate that at extremely low doses, mercury is a potent modulator of PMN functions *in vitro,* including adherence, chemotaxis, phagocytosis, and oxygen metabolism. Further investigations into the mechanisms of mercury-induced modulation of PMN functions will provide insights not only on the influence of heavy metals on host defense and tissue injury, but also on the basic nature of PMN.

Materials and Methods

Isolation of Polymorphonuclear Leukocytes from Peripheral Human Blood

Human polymorphonuclear leukocytes (PMN) were used for all studies described in the present investigation and were isolated from 3.8% citrated venous blood of healthy donors using Ficoll–Hypaque gradient centrifugation, followed by dextran sedimenta-

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tion.[9,10] The cells were consistently >98% viable as determined by trypan blue dye exclusion. The isolated PMN were adjusted to the appropriate concentration for each assay using Hank's Balanced Salt Solution (HBSS). Microscopically, the PMN preparations used for these studies contained 82–95% neutrophils as determined by cytocentrifugation and Wright–Giemsa staining.

Preparations of $HgCl_2$ Solutions

$HgCl_2$ was obtained from Sigma Chemical Company (St. Louis, MO). Solutions of 2×10^{-4}–2×10^{-17} M $HgCl_2$ were prepared from a 0.1 M $HgCl_2$ stock solution that was prepared immediately prior to use. All $HgCl_2$ solutions were prepared in HBSS at a final pH of 7.25.

Measurement of Cell Viability

Quantitation of the toxicity of $HgCl_2$ was determined by lactate dehydrogenase (LDH) release from these cells.[11] For this assay, 0.5 ml of PMN at 5×10^6/ml were added to 0.5 ml of various $HgCl_2$ solutions to achieve final concentrations of 10^{-4}–10^{-15} M. These mixtures were then incubated for 20 minutes at room temperature or for 45 minutes at 37 C. The resulting samples were then centrifuged (500*g*) for 10 minutes at 4 C, and the presence of lactate dehydrogenase (LDH) in the resulting supernatants was determined spectrophotometrically by measuring NADH consumption during LDH catalyzed reduction of pyruvate to lactate. To determine the total LDH content, 2.5×10^6/ml PMN in Hanks Balanced Salt Solution without Ca^{++} or Mg^{++} ($HBSS^{--}$) were lysed with 0.1% Triton X-100 for 15 minutes at room temperature, followed by centrifugation at 800*g* for 10 minutes, and the presence of LDH in the cell lysate was quantitated as described above. All LDH data were expressed as percent LDH release from the $HgCl_2$-treated PMN and were compared to non-$HgCl_2$ treated controls (total LDH).

Transmission Electron Microscopy

To analyze whether $HgCl_2$ caused ultrastructural changes, control and $HgCl_2$ treated PMN, were submitted for transmission electron microscopy (TEM). For this ultrastructural analysis, 0.5 ml of PMN (5×10^6/ml) were incubated for 20 minutes at room temperature, or 45 minutes at 37 C, with various $HgCl_2$ solutions (10^{-7}–10^{-17} M). Following incubation, the samples were centrifuged for 10 minutes at 500*g*, and the resulting pellet washed 3 times with $HBSS^{--}$. The PMN were then fixed with 4% cacodylate buffered glutaraldehyde at room temperature for 1 hour, and postfixed with 2% osmium tetroxide for 30 minutes. The cells were dehydrated through a graded series of alcohol and propylene oxide, then embedded in an epon-araldite mixture. One micron thick sections were cut and stained with toluidine blue and several blocks were selected for thin sectioning (60 μ). Thin sections were stained with uranyl acetate and lead citrate. Electron micrographs were taken on a Philips EM 300.

Adherence and Polarization Assays

When PMN come in contact with surfaces, they undergo morphologic changes as they adhere, spread, and polarize.[12,13] Thus, we next evaluated the effect of $HgCl_2$ on PMN adherence and polarization on tissue culture plastic plates. For this assay, 0.5 ml of PMN (2.5×10^6/ml) were exposed to 0.5 ml $HgCl_2$ solutions to achieve final concentrations of 10^{-6}–10^{-14} M. Next 250 μl of each sample was placed in separate wells of a 48-well tissue culture plate (Costar, Cambridge, MA). All reaction mixtures were incubated for 45 minutes at 37 C and the cells were then fixed with 300 μl of 10% buffered formalin. The control for this study consisted of 200 μl of 1.25×10^6/ml PMN in HBSS that were not exposed to $HgCl_2$. For each sample at least 300 cells were evaluated for the total number of adhered as well as polarized PMN. Polarization was determined by evaluation of round vs asymmetric PMN under the various experimental conditions. The resulting data were expressed as the number of adherent or polarized PMN in a total of 200 PMN.

Chemotaxis Assay

The effects of $HgCl_2$ on human PMN movement (random migration and chemotaxis) were evaluated using a modified Boyden Chamber technique.[10,14] For this assay, 0.5 ml of PMN (5×10^6/ml) was incubated for 20 minutes at room temperature with 0.5 ml of various $HgCl_2$ solutions to a final concentration of 10^{-17} M–10^{-4} M. After incubation, BSA was added to the samples to a final concentration of 0.5%, and 150 μl of the PMN suspension was placed in the top compartment of each Boyden chamber. Eight-micron Millipore filters were used in all chemotaxis studies. C5 fragment or HBSS was used in the bottom chamber to determine maximum chemotactic response or random migration. For all migration studies, the Boyden chambers were then incubated for 45 minutes at 37 C with 5% CO_2, to expedite cell migration through the membrane. After incubation, filters were fixed, stained with hemotoxylin, and allowed to air dry. The resulting filters were then mounted on glass slides and

the number of cells at each 10 μ level beneath the monolayer was counted using an image analyzer. The PMN movement was quantitated as Chemotactic Index (CI), ie, the summation of the distance traveled by each cell in the filter, multiplied by the cell number.[15] Cell migration was expressed as % maximum chemotactic response (% MCR).[15]

$$\% \text{ MCR} = \frac{\text{CI}_{\text{Experimental}} - \text{CI}_{\text{HBSS}}}{\text{CI}_{\text{C5 fragment}} - \text{CI}_{\text{HBSS}}} \times 100$$

Phagocytosis Assay

Erythrophagocytosis was performed to evaluate whether $HgCl_2$ suppressed the ability of PMN to phagocytize opsonized red blood cells (RBC).[16,17] For this study, 0.5 ml of PMN (5×10^6/ml) was added to various $HgCl_2$ solutions (10^{-7}–10^{-15} M). The resulting samples were then incubated for 20 minutes at 37 C. Following incubation each sample was divided into 2 0.5 ml aliquots. Next 0.5 ml of sheep red blood cells opsonized with rabbit anti-sheep IgG (Sigma Chemical Co., St. Louis, MO) was added to each aliquot to a final ratio of 1:10 (PMN:RBC). The samples were then incubated in a rotating tube rack for 30 minutes at 37 C. After incubation, the samples were placed on ice for 15 minutes to arrest phagocytosis, followed by centrifugation (300*g*) for 15 minutes at 4 C. To eliminate extracellular red blood cells, which were not phagocytized or were adhering externally to the PMN, the resulting cell pellet was then resuspended in 4 ml of cold red blood cell lysing solution (0.15 M NH_4Cl; 0.01 M $KHCO_3$; 0.001 EDTA; pH, 7.2). At this point the samples were centrifuged (300*g*) for 15 minutes at 4 C and the resulting pellet was washed twice in $HBSS^{--}$. After the final washing, the pellet was resuspended in 1 ml of HBSS containing 0.1% BSA. For each sample, duplicate smears were prepared by using a cytocentrifuge (500 rpm for 5 minutes) and stained with Wright's-Giemsa (Sigma Chemical Co., St. Louis, MO). The smears were examined using light microscopy (×100) and the number of red blood cells ingested per 200 PMN was determined.

Chemiluminescence

Chemiluminescence was performed to evaluate the effect of $HgCl_2$ on PMN oxidative metabolism.[18,19] For this assay, a 5.5 mM stock solution of luminol in dimethyl sulfoxide was prepared and diluted 1:200 in HBSS. Next, 5.2 ml of HBSS, and 0.2 ml of luminol solution (final concentration, 1.0 μ) were added to 10 ml plastic scintillation vials. All vials were then dark adapted for 20 minutes in a β-counter shifted to one photomultiplier (^{3}H channel). Next 0.5 ml of PMN (5×10^6/ml) were added to various $HgCl_2$ solutions resulting in final concentrations of 10^{-7}–10^{-17} M and incubated for 1 minute at room temperature. At 1 minute intervals, 0.1 ml of each $HgCl_2$-PMN sample was added sequentially to each vial. The vials were counted at 1 minute intervals for 1 hour. The stimulating effect of $HgCl_2$ on PMN chemiluminescence was compared to control chemiluminescence, ie, PMN that were not treated with $HgCl_2$. The positive chemiluminescence control consisted of non-$HgCl_2$ PMN stimulated with opsonized zymosan.

H_2O_2 Production

The effect of $HgCl_2$ on H_2O_2 production by PMN was evaluated using a simple colorimetric assay based on the horseradish peroxidase mediated oxidation of phenol red by H_2O_2, which results in the formation of a compound demonstrating increased absorbance at 610 nm.[20,21] Generally for this assay, 0.5 ml of 2 $\times 10^7$/ml PMN was added to various $HgCl_2$ solutions (10^{-7}–10^{-18} M) containing 10 μl of 0.028 M phenol red (in HBSS; pH, 7.0) and 10 μl of horseradish peroxidase (14 mg/ml in HBSS; pH, 7.0). The various samples were then incubated for 1 hour at 37 C. At the completion of incubation, the samples were centrifuged (600*g*) for 10 minutes and the resulting cell-free supernatant was transferred to a fresh test tube. This was followed by the addition of 10 μl of 10 N NaOH to each sample. The resulting reactions were read at 610 nm against a blank which contained 1 ml of HBSS (pH, 7.0), 10 μl of 0.028 M phenol red, 10 μl of horseradish peroxidase, and 10 μl 10 N NaOH. Standard curves were prepared with known H_2O_2 concentrations (0.78–50 μ). Controls consisted of both untreated PMN and phorbol-12-myristate-13-acetate (2 $\times 10^{-7}$ M) treated PMN. All reactions were performed in duplicate. All data were expressed as the mean O.D. (610_{nm}) of 4 independent experiments.

Statistical Analysis

All data for these studies were expressed as mean ± SEM and analyzed using paired-t analysis.[27]

Results

Cell Viability and Ultrastructure

Initially the toxicity of $HgCl_2$ on human PMN was determined using cell lysis, LDH release, and trypan blue exclusion. $HgCl_2$ at concentrations $\geq 10^{-5}$ M caused significant trypan blue uptake by the PMN, and induced significant PMN lysis after incubation at 37 C for 20 minutes. Our studies also indicated that

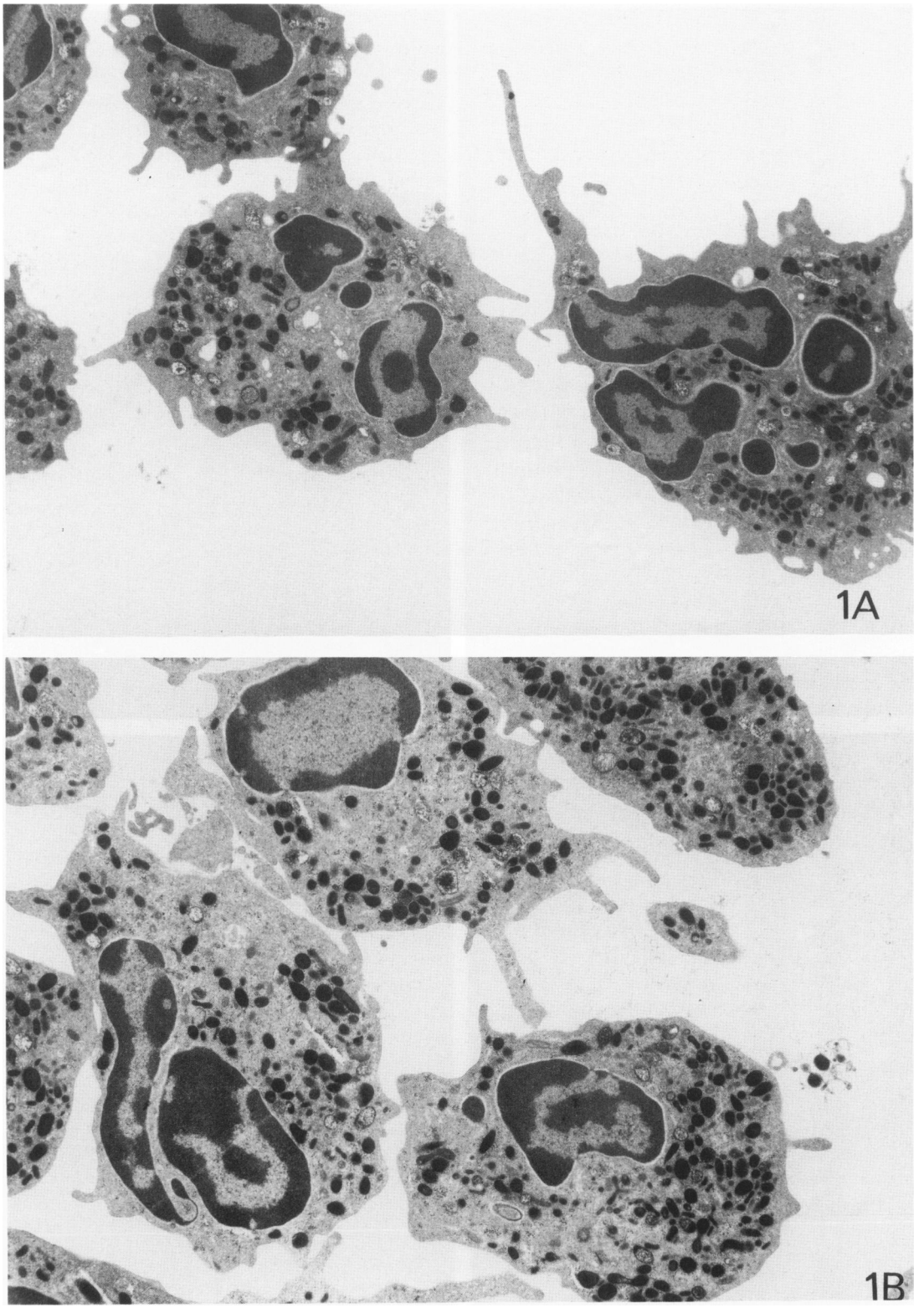

Figure 1—Ultrastructural Analysis of Human PMN Exposed to 10^{-7} M $HgCl_2$. **A**—A photomicrograph (×9100) of human peripheral PMN exposed to buffer for 15 minutes at 37 C. **B**—is a photomicrograph (×9100) of human peripheral PMN exposed to 10^{-7} M $HgCl_2$ for 15 minutes at 37 C. No major ultrastructural changes were seen in PMN exposed to $HgCl_2$ at concentrations $\leq 10^{-7}$ M.

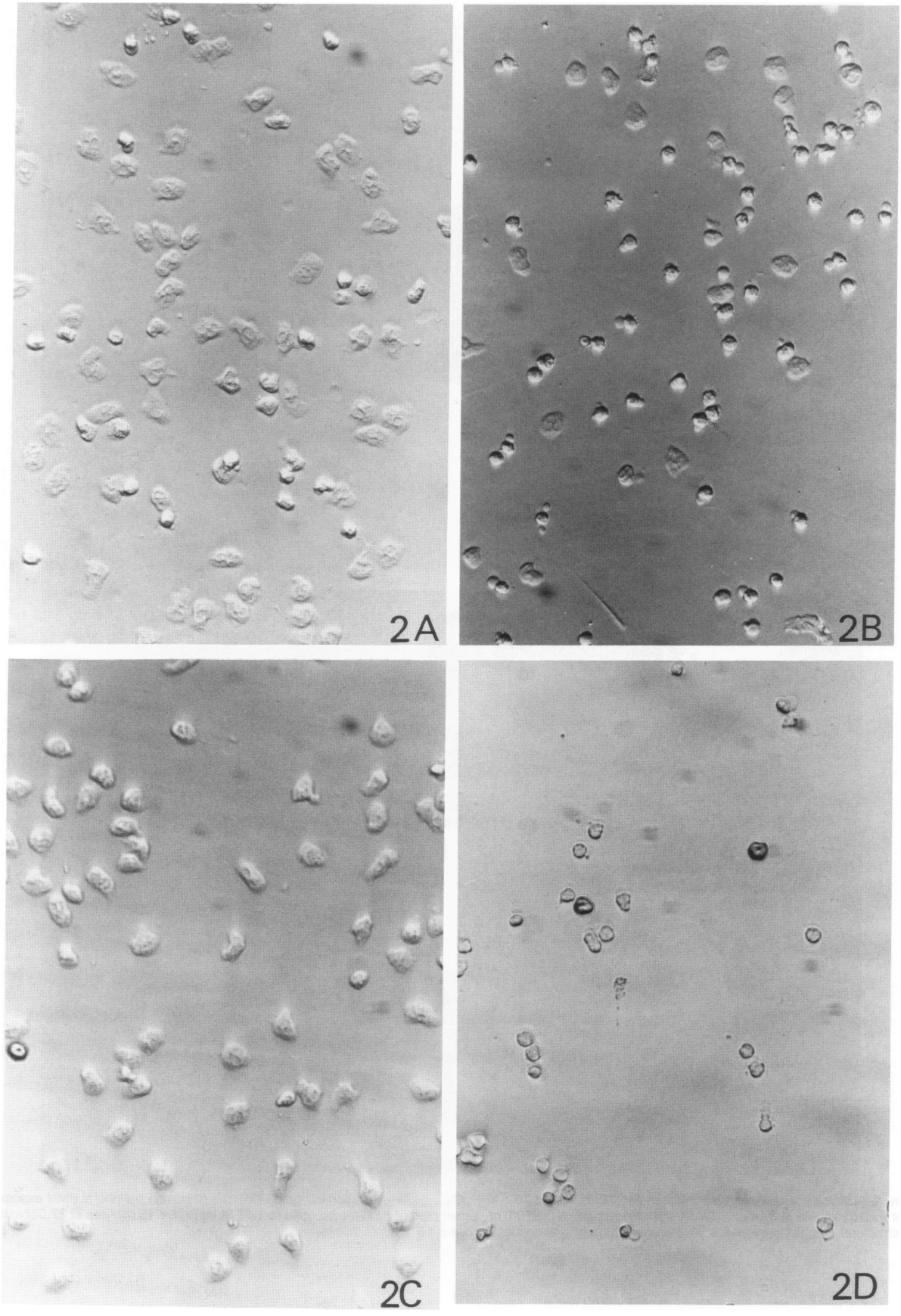
2A
2B
2C
2D

Figure 2—Effect of Mercuric Chloride on Human PMN Morphology. **A** and **C** are phase contrast photomicrographs (×40) of human PMN incubated with buffer for 10 minutes (A) or 25 minutes (C) at 37 C. Presented in **B** and **D** are photomicrographs of the effect of 10^{-7} M $HgCl_2$ on human PMN after 10 minute (B) or 25 minute (D) incubation at 37 C. $HgCl_2$ induced significant inhibition of the adherence and spreading of PMN after 10 minutes in culture and its effect was enhanced significantly at 25 minutes.

$HgCl_2$ at concentrations $\geq 10^{-5}$ M interfered with the lactate dehydrogenase assay (data not shown). At concentrations of $HgCl_2 \leq 10^{-6}$ M, there were no interference with the lactate dehydrogenase assay, or significant LDH release detected (ie, control LDH release 9%; 10^{-6} M $HgCl_2$ LDH release: 8%). These studies thus indicate clearly that $HgCl_2$ at concentrations $\geq 10^{-5}$ M caused significant toxicity to PMN, but at concentrations $\leq 10^{-6}$ M caused no significant LDH release, and was not acutely toxic. Having established the concentration of $HgCl_2$ that induces no significant LDH release, we next determined the effect of $HgCl_2$ on PMN ultrastructure. As can be seen in Figure 1, 10^{-7} M $HgCl_2$ caused no detectable ultrastructural changes in human PMN as evaluated by transmission electron microscopy when compared with untreated PMN. These data were similar whether PMN were incubated at room temperature or at 37 C for varying times (20–45 minutes).

Effect of $HgCl_2$ on PMN Adherence and Polarization

After establishing concentrations of $HgCl_2$ that did not induce toxicity or ultrastructural changes in the PMN, we next evaluated the influence of $HgCl_2$ on PMN adherence (Figure 2). As can be seen in Figure 3, $HgCl_2$ induced significant inhibition of leukocyte adherence at concentrations between 10^{-6} and 10^{-7} M. No significant effects on PMN adherence were detected at concentrations $< 10^{-8}$ M. The adherence ID_{50} for $HgCl_2$ on the PMN was 7×10^{-6} M. After establishing that $HgCl_2$ suppressed PMN adherence, we next investigated the effect of $HgCl_2$ on leukocyte polarization. As can be seen in Figure 4, $HgCl_2$ induced significant suppression of PMN polarization at concentrations as low as 10^{-16} M, with a polarization ID_{50} of 10^{-12} M.

Effect of $HgCl_2$ on PMN Chemotaxis

Because adherence and polarization are closely associated with cell movement, we next evaluated the effects of $HgCl_2$ on PMN chemotaxis. As can be seen in Figure 5, extremely low concentrations of $HgCl_2$ induced significant inhibition of PMN chemotaxis. The $HgCl_2$ induced inhibition displayed dose-response characteristics, with a chemotaxis ID_{50} of 10^{-14} M (see Figure 5). $HgCl_2$ at concentrations between 10^{-4} and 10^{-8} M totally abolished PMN migration. At $HgCl_2$ concentrations between 10^{-8} and 10^{-13} M approximately 30–40% of the control chemotactic response (ie, nontreated PMN) was seen. Only at concentrations of 10^{-17} M was chemotactic activity in $HgCl_2$ treated PMN similar to that seen in untreated controls.

Effect of $HgCl_2$ on PMN Phagocytosis

Using erythrophagocytosis of opsonized sheep red blood cells, we determined the effects of $HgCl_2$ on hu-

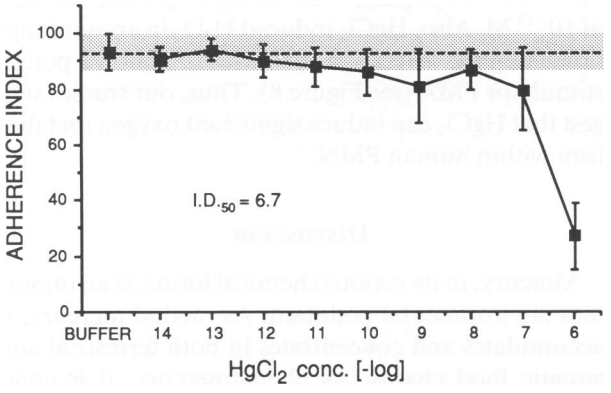

Figure 3—Effect of $HgCl_2$ on Human PMN Adherence. The data represent the mean of 5 independent studies ± SEM. The data are expressed as the number of adherent PMN in a total of 200 PMN. The results for the buffer treated PMN adherence is designated by the shaded line and represents 100% adherence of non-$HgCl_2$ treated cells. $HgCl_2$ induced a significant suppression of PMN adherence at 10^{-6} M, treated with a $P \leq 0.0005$. The ID_{50} for the effect of $HgCl_2$ on adherence of human PMN was 7×10^{-6} M.

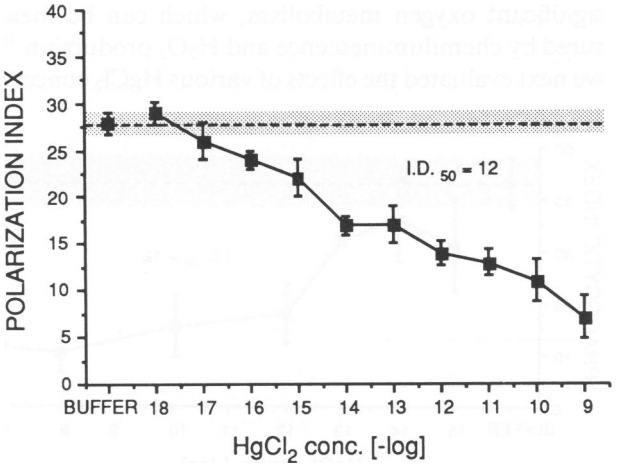

Figure 4—Effect of $HgCl_2$ on Polarization. The effect of $HgCl_2$ on human PMN polarization is demonstrated. The data presented represent the mean of 5 independent experiments ± SEM. All data are expressed as the number of polarized PMN in a total of 200 PMN. As compared to the buffer treated PMN (shaded line), $HgCl_2$ induced a dose dependent suppression of PMN polarization at concentrations between 10^{-17} and 10^{-9} M. The ID_{50} for $HgCl_2$ in this assay was 10^{-12} M. All concentrations of $HgCl_2 \geq 10^{-15}$ M significantly suppressed PMN polarization and had a $P \leq 0.005$.

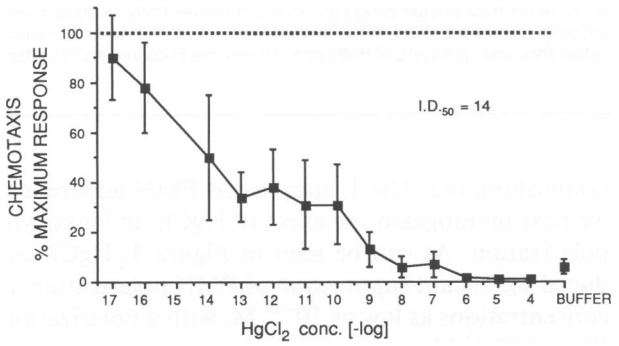

Figure 5—Effect of $HgCl_2$ on PMN Chemotaxis. The data are the mean of 5 independent experiments ± SEM. All data are presented as maximum chemotactic response as compared to the known chemotactic factor C5 Fragment:

$$MCR = \frac{CI_{Experimental} - CI_{HBSS}}{CI_{C5\ Fragment} - CI_{HBSS}} \times 100.$$

The dotted line represents maximum (100%) chemotactic response of non-$HgCl_2$ treated cells. $HgCl_2$ induced a dose-dependent inhibition of human PMN chemotaxis at concentrations between 10^{-6} and 10^{-17} M $HgCl_2$ with an ID_{50} of 10^{-14} M. All concentration of $HgCl_2 > 10^{-14}$ suppressed PMN chemotaxis with a $P < 0.0005$.

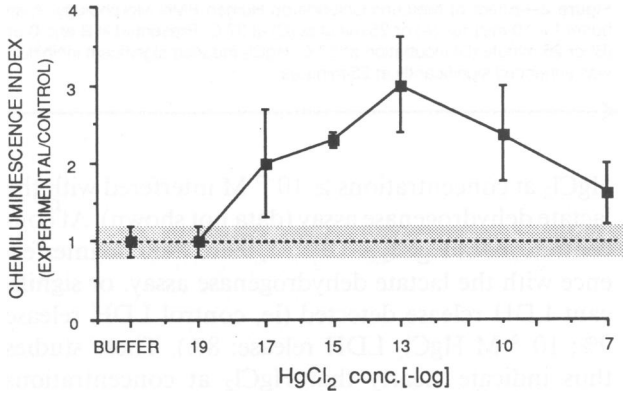

Figure 7—Effect of $HgCl_2$ on Human PMN Chemiluminescence. The data presented are the mean of 4 independent experiments ± SEM. The effect of $HgCl_2$ on human PMN chemiluminescence was determined by fluorometric detection using luminol as an enhancer and a β-counter (^3H channel). As compared to the buffer treated PMN (shaded line), $HgCl_2$ at concentration between 10^{-19} and 10^{-13} M induced a dose dependence stimulation of chemiluminescence. Higher concentrations of $HgCl_2$ (10^{-10}–10^{-7} M $HgCl_2$) consistently suppressed chemiluminescence relative to the peak chemiluminescence response (10^{-13} M $HgCl_2$). The SI_{50} for $HgCl_2$ induced chemiluminescence was calculated to be 10^{-17} M. The significance value for the peak $HgCl_2$ induced chemiluminescence (ie, 10^{-13} M) was $P \leq 0.005$.

man PMN phagocytosis. These studies found that extremely low levels of $HgCl_2$ (ie, 10^{-12} M) induced significant suppression of the phagocytosis of opsonized sheep red blood cells. As can be seen in Figure 6, $HgCl_2$ induced dramatic suppression of phagocytosis at concentrations between 10^{-10} and 10^{-7} M and only at concentrations of 10^{-13} M and less was there a significant phagocytosis.

Effect of $HgCl_2$ on PMN Chemiluminescence and H_2O_2 Production by PMN

Because phagocytosis by PMN generally induces significant oxygen metabolism, which can be measured by chemiluminescence and H_2O_2 production,[18] we next evaluated the effects of various $HgCl_2$ concentrations on PMN chemiluminescence and H_2O_2 production. As can be seen in Figure 7, extremely low concentrations of $HgCl_2$ (between 10^{-19} and 10^{-15} M $HgCl_2$) induced significant chemiluminescence in resting (nonstimulated) PMN. Peak chemiluminescence activity was seen at 10^{-13} M $HgCl_2$, followed by a dose-dependent suppression of chemiluminescence at concentrations $> 10^{-13}$ M. The SI_{50} for the $HgCl_2$-induced chemiluminescence was approximately 10^{-17} M. Since $HgCl_2$-induced chemiluminescence in human PMN is linked to H_2O_2 production, we next determined whether or not $HgCl_2$ could induce H_2O_2 production in PMN. As can be seen in Figure 8, $HgCl_2$ induced a consistent dose-dependent increase in H_2O_2 production by PMN at concentrations ranging from 10^{-19}–10^{-7} M $HgCl_2$, with an H_2O_2 production SI_{50} of 10^{-12} M. Also, $HgCl_2$ induced H_2O_2 in amounts approaching the levels obtained with the known potent stimulator PMA (see Figure 8). Thus, our studies suggest that $HgCl_2$ can induce significant oxygen metabolism within human PMN.

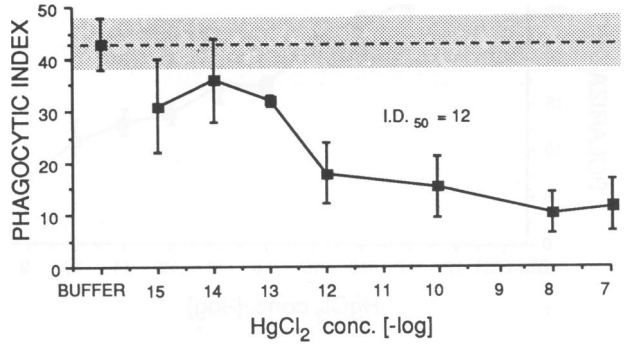

Figure 6—Effects of $HgCl_2$ on PMN Phagocytosis. The data presented are the mean of 3 independent experiments ± SEM. The data are expressed as phagocytic index (ie, number of red blood cells ingested in a total of 200 PMN). As compared to the buffer treated PMN (shaded line), $HgCl_2$ exposed PMN induced a dose-dependent inhibition of PMN phagocytosis at concentrations of 10^{-7}–10^{-14} M, with an ID_{50} of 10^{-12} M. All concentrations of $HgCl_2 \geq 10^{-14}$ M significantly suppressed PMN phagocytosis and had a $P \leq 0.01$.

Discussion

Mercury, in its various chemical forms, is an important environmental pollutant. As methyl mercury, it accumulates and concentrates in both terrestrial and aquatic food chains. As phenylmercury, it is commonly used as a preservative and may also be a food contaminant. Finally, in its elemental form, mercury is used in amalgams, lamps, electrodes, and other products, resulting in an occupational hazard for industrial workers. Quantitative estimates of the dose-

response relationship of mercury in humans are fraught with many difficulties, caused by the variety of compounds (elemental mercury vapour, methylmercury, ethylmercury, inorganic mercury), different routes of exposure, exposure range, and size of population exposed to the chemical.[28] It has been estimated that levels of mercury as low as 20–48 μg/100 ml of blood and a body burden of 0.5–0.8 mg/kg are associated with effects (usually paraesthesia) in the most sensitive group of the population.[28] Higher levels result in the well-known acute or chronic toxic effects of mercury. Thus, there is a need for both *in vitro* and *in vivo* studies of the effects of mercury on various human cells, including those involved in host defense. Our present *in vitro* investigations demonstrate that mercuric chloride is capable of influencing various PMN functions, including the adherence, polarization, chemotaxis, and phagocytosis. We also demonstrated that mercury causes a significant increase in human PMN oxygen metabolism (H_2O_2 and chemiluminescence). Interestingly, at high concentrations of $HgCl_2$, chemiluminescence was inhibited while H_2O_2 production remained unchanged. This dichotomy may be the result of the fact that chemiluminescence is dependent on both myeloperoxidase (MPO) and H_2O_2 concentrations within the PMN, while the H_2O_2 levels in the PMN are measured directly. Thus, the higher concentrations of $HgCl_2$ may be inhibiting either the MPO enzyme activity directly or degranulation of the MPO into the phagolysosome. Future studies to dissect these possibilities will be useful.

A variety of *in vitro* PMN functions are modulated (inhibited or stimulated) by extremely low levels of mercuric chloride (see Table 1). If $HgCl_2$ is similarly capable of suppressing PMN functions (adherence, polarization, chemotaxis, and phagocytosis) *in vivo,* such an effect would clearly result in compromised host defense to infections. In the literature, there is a paucity of data regarding the prevalence of infectious disease in subjects exposed to mercury.[28,29] However, Russian investigators have reported that the population of the Gorno-Altay Autonomous region, where extensive extraction of mercury from ores results in high levels of this metal in soil, water, and atmosphere, suffers from a high morbidity in infectious diseases.[30] According to these reports, indicators such as infectious diseases and ear, nose and throat diseases were significantly higher (from 10–27%) in people who had lived a long time within this region than in persons living in control regions. Alternatively, the consequences of $HgCl_2$-induced stimulation of oxygen metabolism, with production of oxygen metabolites, would promote tissue injury. These are novel findings, because previous studies that demonstrated inhibition of phagocytosis and/or chemotaxis by various metals (gold, cadmium) did not show effects on other functions of PMN. Our investigations did not address the mechanism(s) of action of mercury on PMN. It has been suggested that mercury may damage epithelial cells by acting as a calcium-mimetic agent, stimulating phospholipase activity and promoting deacylation.[22] Mercury might also block reacylation by interaction with the responsible enzymes,[23] or cause loss of reacylation and accumulation of unesterified fatty acids due to mitochondrial energy depletion.[24,25] Recent studies of renal epithelial cell injury by mercuric chloride have shown accumulation of both lysophospholipids and unesterified fatty acids.[26] Alternative explanations for the effects of $HgCl_2$ on PMN function may involve the reaction of mercury with sulfhydryl groups on the cell membrane, increasing membrane permeability, and inhibiting ATPase-dependent transport.[16] Our chemiluminescence and H_2O_2 results also suggest that the *in vitro*

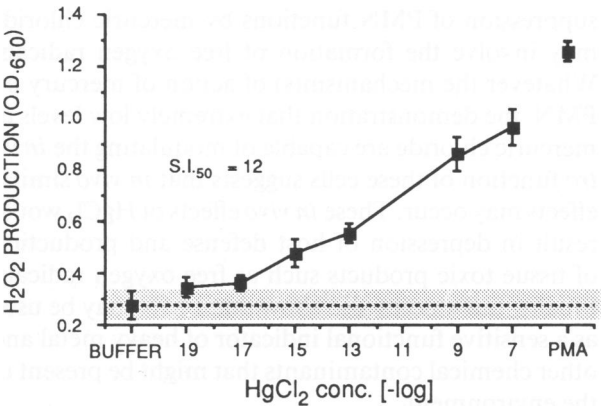

Figure 8—Effect of $HgCl_2$ on H_2O_2 Production by Human PMN. The data presented here are the mean of 4 independent experiments ± SEM. For this assay, H_2O_2 production by PMN was determined by a simple colorimetric assay based on the horseradish peroxidase mediated oxidation of phenol red by H_2O_2, which results in the formation of a compound demonstrating increased absorbance at 610 nm. To determine maximum responsiveness of the cells, non-$HgCl_2$-treated PMN were exposed to PMA and the H_2O_2 production evaluated. As compared with the buffer treated PMN (shaded line), $HgCl_2$ induced significant H_2O_2 production by PMN. The SI_{50} for $HgCl_2$ induced H_2O_2 production by human PMN was calculated to be 10^{-12} M. All concentrations of $HgCl_2 \geq 10^{-17}$ M significantly enhanced H_2O_2 production by PMN and had a $P \leq 0.005$.

Table 1—Summary of the Effect of $HgCl_2$ on Human Polymorphonuclear Leukocyte Function *In Vitro*

	Response	
	ID_{50}	SI_{50}
Adherence	7×10^{-6}	—
Polarization	1×10^{-12}	—
Chemotaxis	1×10^{-14}	—
Phagocytosis	1×10^{-12}	—
Chemiluminescence	—	1×10^{-17}
H_2O_2 production	—	1×10^{-12}

ID_{50}, Concentration of $HgCl_2$ that suppresses PMN function by 50%.
SI_{50}, Concentration of $HgCl_2$ that stimulates PMN function by 50%.

suppression of PMN functions by mercuric chloride may involve the formation of free oxygen radicals. Whatever the mechanism(s) of action of mercury on PMN, the demonstration that extremely low levels of mercuric chloride are capable of modulating the *in vitro* function of these cells suggests that *in vivo* similar effects may occur. These *in vivo* effects of $HgCl_2$ would result in depression of host defense and production of tissue toxic products such as free oxygen radicals. Finally, our studies also show that PMN may be used as a sensitive functional indicator of heavy metal and other chemical contaminants that might be present in the environment.

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