and monomethylarsonous acid (MMA^{III}) are formed during the metabolism of inorganic arsenic *in vivo*. It is possible that the mixture of arsenicals in the urine of arsenic-treated rats plays a role the urothelial cytotoxicity. The objective of this study was to determine if combining arsenicals increased the cytotoxicity of the individual arsenicals in a rat urothelial cell line. The MYP3 cell line, an immortalized but not transformed rat urinary bladder epithelial cell line, was seeded into appropriate culture wells. Treatment with the combined arsenicals was begun 24 hours after seeding and continued for 3 days. Combinations of arsenicals used were DMA^{III} with arsenite, dimethylarsinic acid (DMA^{V}) or trimethylarsine oxide (TMAO). Combinations of concentrations used were the LC_{50}, one-quarter or one-half the LC_{50} of one arsenical with one-quarter or one-half the LC_{50} of the other arsenical. When cells were treated with one-quarter or one-half the LC_{50} concentration of both arsenicals, the cytotoxicity was approximately the same as when cells were treated with half the LC_{50} concentration or the LC_{50} concentration, respectively, of either arsenical. Treatment with one-quarter the LC_{50} concentration of one arsenical plus the LC_{50} concentration of a second arsenical had similar cytotoxicity as treatment with the LC_{50} concentration of either of the arsenicals. The effect on the cytotoxicity of arsenicals in combination was additive rather than synergistic toward a rat urothelial cell line.

1625 ARSENIC EXPOSURE DECREASES BETA DEFENSIN-1 EXPRESSION.

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Arsenic (As) exposure has been associated with various cancers, including those of the kidney and bladder. Proteomic analysis of biological samples from two human population studies identified the presence of lower levels of human β-defensin-1 (HBD-1) peptides in the urine of individuals exposed to arsenic in drinking water. To further validate these findings, we examined the effect of As exposure upon *HBD1* gene expression *in vitro*. mRNA levels of *HBD1* were quantified in two cell lines following exposure to sodium arsenite (iAs^{III}) and its methylated metabolite, $MAs^{III}O$. Dose-dependent decreases in *HBD1* gene expression were observed in HeLa cells and in the human embryonic kidney derived 293T cell line following treatment with iAs^{III} and $MAs^{III}O$ for 48h. $MAs^{III}O$ was more potent than iAs^{III} in decreasing *HBD1* expression, in agreement with its postulated role as the toxic metabolite of As. These effects on *HBD1* expression occurred at As doses which did not cause an overall suppression of gene expression. HBD-1 is a cationic antimicrobial peptide constitutively expressed in multiple tissues including epithelial cells of the respiratory tract and urogenital system. Renal cell carcinomas have been shown to have decreased HBD-1 expression both at the mRNA and protein levels, suggesting that *HBD-1* may be a tumor suppressor gene for urological cancers. It is therefore plausible that decreased HBD-1 levels play a role in the development of associated urogenital and/or kidney cancers arising from As exposure. *Supported by NIH grant P42ES04705.*

1626 ARSENIC INDUCED AN INCREASE IN ENDOTHELIAL CELL PERMEABILITY: INVOLVEMENT OF CYTOSKELETON SIGNALING.

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Although it has been shown that human exposure to arsenic is related to an increase in inflammation, cardiovascular diseases, and cancers, the molecular mechanisms of its action remains to be elucidated. In this study, we demonstrated that exposure to arsenic caused an increase in cell permeability in human microvascular endothelial cells (HMEC), which is correlated with the remodeling of actin, microtubule cytoskeleton, and the activation of Erk1/2 signaling by the production of reactive oxygen species (ROS). Inhibition of Erk1/2 activities, blockage of actin filament remodeling, or removal of ROS partially suppressed the arsenic-induced permeability increase, indicating the involvement of cytoskeleton, Erk1/2, and ROS signaling pathways in arsenic-induced endothelial cell morphological changes. Furthermore, we found that arsenic treatment disrupted the localization of VE-cadherin at adherens junctions in HMEC and induced its translocation into the nucleus. Moreover, it was found that arsenic-induced VE-cadherin nuclear translocation was inhibited by treatments with ROS scavengers, and actin filament and microtubule inhibitors. Taken together, this study demonstrates that arsenic stimulation induces an increase in cell permeability in HMEC, which involves actin and microtubule cytoskeleton signaling. The results from this study may contribute to dissecting the molecular mechanisms involved in arsenic toxicity.

1627 ROLE OF NA/PHOSPHATE COTRANSPORTERS IN THE CELL MEMBRANE TRANSPORT OF ARSENATE.

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Arsenate (AsV) is the commonest form of the metalloid As in nature. After exposure and absorption, mainly through the intestine, it is distributed to periphery tissues and reduced to arsenite. In spite of the extensive toxicokinetic studies, the molecular mechanisms of AsV translocation through the cell plasma membranes are still unknown, but it is generally accepted that the phosphate transporters are involved. We have analyzed the role of Na-coupled inorganic phosphate (Pi) transporters in the transport of AsV by performing inhibition kinetics in Xenopus laevis oocytes. Rat NaPi2a, b, and c, as well as Pit1 and Pit2 were expressed in X. laevis oocytes by injection of in vitro transcribed cRNA. Competition kinetics of 32Pi uptake revealed that NaPi2a and NaPi2c (renal isoforms) were inhibited by arsenate with a Ki ten times higher (about 1 mM) than the affinity for phosphate, while Pit1 and Pit2 (ubiquitous transporters) were inhibited with a Ki of 3.3 mM. Because the plasma phosphate concentration is 1-2 mM, the Pi transporters are completely saturated (with Km of 50-100 μM), and therefore the actual uptake of AsV through these transporters is negligible. The intestinal NaPi2b transporter was, however, inhibited by AsV with a Ki of only 40 μM. As the concentration of Pi in the intestine is very variable but generally very low, NaPi2b is therefore a good candidate as a major carrier involved in the absorption of AsV through the intestine. In addition, phosphonoformic acid, a classical inhibitor of renal Pi reabsorption, inhibited all transporters with the same intensity as AsV did, but no one was inhibited with dimethyl-arsenate (cacodylic acid). These findings were confirmed using brush-border membrane vesicles of rat small intestine and kidney cortex, and with rat vascular smooth muscle cells using similar radiotracer assays.

1628 TISSUE DISTRIBUTION OF ARSENIC SPECIES IN MICE CHRONICALLY EXPOSED TO METHYLARSONOUS ACID.

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The metabolism of inorganic arsenic (iAs) in humans yields toxic and carcinogenic methyl-As (MAs) and dimethyl-As (DMAs) intermediates. Methylarsonous acid (MAsIII) is the most acutely toxic species of characterized iAs metabolites. Here, we examined the concentrations of As species in urinary bladder, brain, heart, kidney, liver, lung, pancreas, adipose tissue, skeletal muscle, and spleen of male C57BL/6 mice exposed for 8 weeks to MAsIII (0.1, 1.0, 2.5 5.0 ppm As) in drinking water and in control mice drinking tap water. Mice were fed a Purina Rodent Diet (5058) with the total As content of 24 to 66 ng/g. Arsenic speciation analysis was carried out in digested tissue homogenates by hydride generation-atomic absorption spectrometry with cryotrapping for separation of arsines. Exposure to MAs did not affect water consumption or body weights and did not produce any overt signs of toxicity. DMAs was the major metabolite of MAs in all tissues except for the kidney, which retained mostly MAs. Highest concentrations of DMAs were found in urinary bladder. The presence of iAs in tissues of mice varied according to the lot of diet consumed. iAs derived from diet was retained mainly in kidney, heart, lung, and brain. Concentrations of iAs in some tissues decreased with exposure to MAsIII. Regardless of exposure level, the lowest concentrations of all As species were found in adipose tissue. These data suggest that exposures to MAsIII result in accumulation of MAs in the kidney and DMAs in urinary bladder. Thus, interactions of MAs and DMAs with specific molecular targets in these two tissues may represent common mechanisms for adverse effects associated with chronic exposures to both iAs and MAsIII. (This abstract does not reflect U.S. EPA policy.)

1629 TISSUE DISTRIBUTION AND URINARY EXCRETION OF INORGANIC ARSENIC AND ITS METHYLATED METABOLITES IN C57BL/6 MICE FOLLOWING SUBCHRONIC EXPOSURE TO ARSENATE (AS^{V}) IN DRINKING WATER.

E. M. Kenyon[1], M. F. Hughes[1], B. Adair[1], J. Highfill[1], E. A. Crecelius[2], H. J. Clewell[3] and J. W. Yager[4]. [1]*ORD/NHEERL/ETD, U.S. EPA, Research Triangle Park, NC,* [2]*Battelle Marine Sciences Laboratory, Sequim, WA,* [3]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [4]*EPRI, Palo Alto, CA.*

The relationship of exposure and tissue concentration of parent chemical and metabolites over prolonged exposure is a critical issue for chronic toxicities mediated by metabolite(s) rather than parent chemical alone. This is an issue for As^{V} be-

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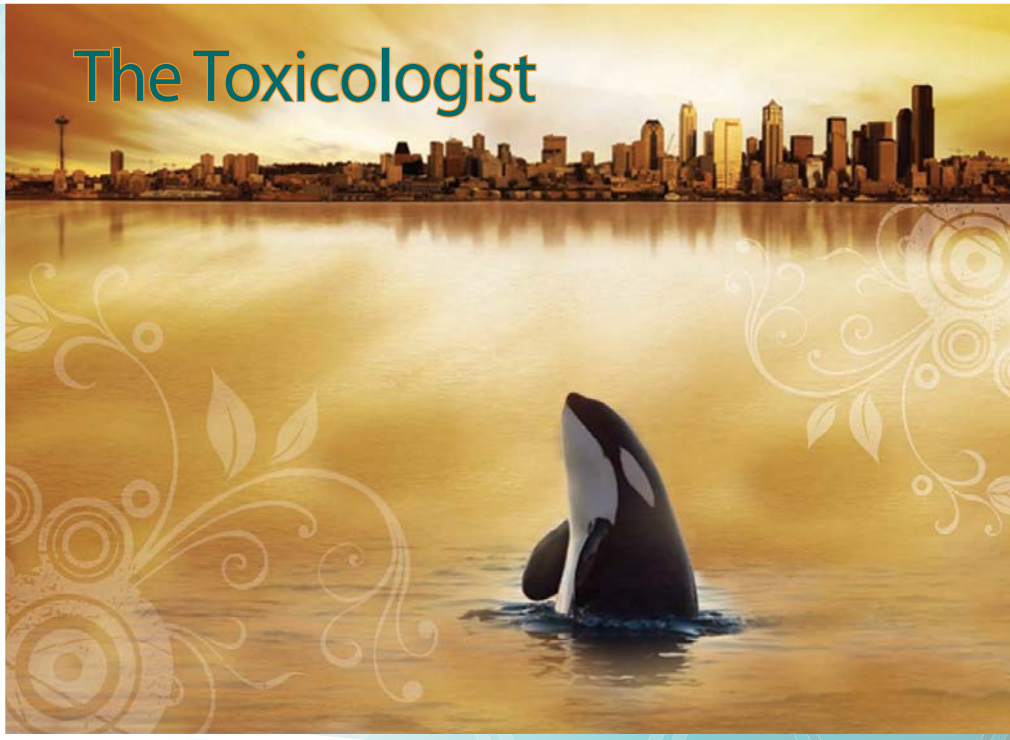
The Toxicologist

March 2008

The Toxicologist



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OXFORD UNIVERSITY PRESS ISSN 1096-6080 Volume 102, Number 1, March 2008