

areas. In spite of the high cost associated with transportation of the contaminated material and the building and maintenance of incinerators, the most common method of remediation is incineration. Bioremediation has been shown to be a promising, less costly method of treatment. Aerobic and non-aerobic bacteria, as well as fungi, are being studied to find strains suitable for bioremediation. We are currently working with three different aerobic bacteria that have varying abilities to degrade TNT. The work reported here is the purification of a TNT-reducing enzyme isolated from a *Bacillus sp.* This enzyme shows a TNT-dependent NADPH oxidation. Kinetic studies have shown this to be a first order reaction for both TNT and NADPH. The enzyme has a relatively small  $K_m$ , and has much higher activity with NADPH than NADH. Based on enzymatic activities using TNT metabolites as substrates, the enzyme appears to preferentially reduce the  $\text{NO}_2$  group at the 4 position on the ring.

**33** RATIONAL ENZYME DESIGN: COMPUTER MODELING AND SITE-DIRECTED MUTAGENESIS AS TOOLS TO IMPROVE CATALYTIC ACTIVITY AND SPECIFICITY OF ORGANOPHOSPHORUS HYDROLASE

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Organophosphorus hydrolase (OPH) is a bacterial metalloenzyme which performs the first cleavage step in the bioremediation of neurotoxic organophosphate compounds. Significant improvement of OPH catalytic activity (kcat) and substrate specificity (kcat/ $K_m$ ) for some chemical warfare agents (CWAs) and their analogues has been achieved through site-directed mutagenesis of histidiny residues 254 and 257. Individual mutants show up to 18-fold improvement in activity against demeton-S, 4-fold improvement in activity against VX, and 33-fold improvement in activity against soman. Many mutants retain excellent catalytic activity and specificity for the wild-type enzyme's preferred substrate, paraoxon, despite the loss of one of the two moles of metal present in each mole of native enzyme. E-ray crystallographic coordinates of the native wild-type apoenzyme and modeling of important features at the active site suggest critical attributes that can be further modified to continue the rational design of this enzyme for bioremediation of pesticide contaminants and CWA stockpiles. (We acknowledge H Holden, The Enzyme Center, University of Wisconsin for providing x-ray coordinates to enhance this study.)

**34** ENZYME BASED BIOSENSOR FOR DIRECT DETECTION OF ORGANOPHOSPHOROUS NEUROTOXINS

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The widespread use of organophosphorous (OP) compounds in agricultural and industrial applications and the demand for chemical nerve agent destruction pose an environmental challenge for detection of these OP neurotoxins. In order to ensure that these substances are not present at hazardous levels in food, ground water, and soil, analytical methods have been developed and modified for many years. A new biosensor for the direct detection of OP neurotoxins has been developed. Immobilized organophosphate hydrolase (OPH) or cryoimmobilized recombinant *Escherichia coli*, capable of hydrolyzing a wide spectrum of OP neurotoxins were used as biological transducers. The pH-based assay was evaluated as a direct function of the concentration of organophosphates. A dynamic range of paraoxon determination between 0.25 ppm to 250 ppm ( $10^{-6}$  -  $10^{-3}$  M) was observed. The response time for the flow-through system was 2 minutes for enzyme based biosensor and 20 minutes for the cell sensor. Immobilized OPH retained activity for over two month at room temperature. Cryoimmobilized *E. coli* exhibited a stable hydrolytic activity for over four months under storage in K-phosphate buffer +4°C.

**35** COMPARISON OF HUMAN HEALTH AND ECOLOGICALLY-BASED REMEDIATION GOALS FOR NITROAROMATIC EXPLOSIVES IN SOIL

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A remedial investigation conducted at a former U.S. Army explosives manu-

facturing site delineated the nature and extent of contamination, and established clean-up remediation goals that would be protective of both human health and the environment. The contaminants of primary concern in surface soil were the nitroaromatic compounds 2,4-dinitrotoluene, 2,6-dinitrotoluene, and 2,4,6-trinitrotoluene. Dermal contact and incidental ingestion exposure scenarios were used to establish human health risk-based clean-up criteria for these nitroaromatic compounds in soil. Earthworm 14-day acute toxicity test results were used in combination with food web exposure models to establish ecologically-based clean-up criteria for soils.

Soil clean-up remediation goals were comparable (less than an order of magnitude difference) for both human health-based and ecologically-based criteria at this site.

**36** ADVANTAGES OF DERMAL EXPOSURE ASSESSMENT FOR CHARACTERIZING OCCUPATIONAL RISKS TO SOIL AND WATER CONTAMINANTS

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A risk screening protocol was required to evaluate potential occupational exposure to environmental media (soil, surface water) using a single exposure route/pathway to characterize and compare health risks/hazards at each of more than 60 sites. Hypothetical scenarios for occupational exposure to soil or water by dermal contact or incidental ingestion were evaluated to aid in selection of a realistic and conservative risk screening protocol. Dermal contact was selected as a better overall measure of occupational exposure to environmental media. The dermal exposure algorithms provided comparable or more conservative estimates of exposure (i.e., larger daily dose rates) than incidental ingestion algorithms for these media. The use of a fixed body surface area (25%) allowed comparison of Chronic Daily Intake (CDI) and calculated risks/hazards of constituents for dermal exposure to soil or water using uniform and defensible default values.

**37** THE Cr(VI) REDUCTIVE CAPACITY OF HOUSEHOLD BEVERAGES: IMPLICATIONS FOR RISK ASSESSMENT

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Laboratory studies were conducted to determine how rapidly and completely hexavalent chromium [Cr(VI)] is reduced to non-toxic trivalent chromium [Cr(III)] upon contact with common beverages mixed with tapwater. Studies were performed with five common beverages (coffee, tea, orange juice, Kool-Aid, and powdered lemonade) spiked with either 10 or 50 mg Cr(VI)/L. The concentrations of Cr(VI) were measured at several time intervals for up to 4 hours. It was demonstrated that each of these beverages had the capacity to reduce a concentration  $\geq 8$  mg Cr(VI)/L within 15 minutes, with orange juice and coffee having the capacity to instantly reduce  $> 50$  and 25 mg Cr(VI)/L, respectively. These findings indicate that many common household beverages are capable of reducing substantial quantities of Cr(VI) to Cr(III). When considered in conjunction with the fact that the reductive capacity of the gastric juice may exceed 50 mg Cr(VI) daily, it is likely that little or no Cr(VI) is systemically absorbed upon daily ingestion of household tapwater containing up to 5 - 10 mg Cr(VI)/L (a concentration at which the water is bright yellow). These findings have significant implications for risk assessments involving ingestion of household tapwater containing Cr(VI).

**38** EFFECTS OF CHEMICAL INTERACTIONS ON BACTERIAL MUTAGENICITY

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Standard methodology for human health risk assessment assumes additive interactions among the contaminants of a complex mixture. However, chemical interactions may occur which may result in synergistic or antagonistic effects. The mutagenic response of three model compounds, benzo(a)pyrene (B(a)P), pentachlorophenol (PCP) and 2,4,6-trinitrotoluene (TNT), were tested individually and in binary and tertiary solutions, using the *Salmonella* microsome assay with each of three bacterial tester strains (TA97a, TA98 and TA100). For all strains, B(a)P was mutagenic with metabolic activation. TNT was mutagenic without metabolic activation, and pentachlorophenol was inactive. In binary and tertiary solutions, pentachlorophenol had no effect on the mutagenicity of B(a)P or TNT, with or without metabolic activation. For strain TA97a, the mutagenicity of B(a)P with activation was slightly decreased in the presence of TNT; the mutagenicity of TNT without metabolic