1751 METABOLOMIC ANALYSIS OF PLASMA AND URINE IN RATS TREATED WITH FENOFIBRATE AND PHENOBARBITAL.

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A metabolomic analysis was performed to study the effects of fenofibrate and phenobarbital, two known rodent carcinogens, in rats. Plasma and 24-hour urine samples from rats treated with fenofibrate (300 mg/kg/day), phenobarbital (50 mg/kg/day), and vehicle control were collected at day 2 and day 14. Using a non-biased global profiling technology based on LC-MS and GC-MS, 496 and 974 compounds were measured from plasma and urine, respectively. We observed significant alterations in biochemical profiles by both drugs at both time points. Fenofibrate was found to increase fatty acid β-oxidation and down regulate glycolysis, resulting in a shift in energy production homeostasis. Phenobarbital was shown to impact various biochemical pathways, including carbohydrate metabolism and ascorbic acid biosynthesis. A group of toxicology markers related to liver and renal function, oxidative stress, and cell apoptosis were regulated in similar patterns by both fenofibrate and phenobarbital. Some of the toxicology markers were detected at day 2 and became more significant at day 14. The results demonstrated that metabolomics is an effective technology for studying drug mechanism and toxicology effects.

1752 CARBON NANOTUBE-BASED ELECTROCHEMICAL SENSOR FOR CHOLINESTERASE ENZYME ACTIVITY: AN ORGANOPHOSPHORUS INSECTICIDE AND NERVE AGENT EXPOSURE MONITOR.

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Simple, rapid and sensitive analytical sensors are needed to evaluate exposures to organophosphorus insecticides and nerve agents utilizing readily obtainable biological fluids, like saliva. An electrochemical sensor coupled with a micro-flow injection system was developed to characterize cholinesterase (ChE) enzyme activity. The sensor is based on a carbon nanotube (CNT)-modified screen-printed carbon electrode (SPE) integrated into a flow cell. The electrocatalytic activity of the carbon nanotubes, enable the sensor to detect electroactive species that are produced from enzymatic reactions with high sensitivity. The electrochemical properties of ChE enzymatic products were studied using the sensor, and the operation parameters such as the applied potential and substrate concentration were optimized. ChE activity was further investigated using the CNT-based sensor with purified human acetylcholinesterase (AChE) and *in vitro* with saliva obtained from naïve rats. For purified AChE the calibration curve was linear over a range of concentrations (5 pM to 0.5 nM) and the detection limit was estimated to be ~2 pM. The dynamics of salivary ChE activity was also studied *in vitro*. Paraoxon (0.7 nM and 7 nM) was mixed with 10-fold diluted saliva (1:1 v/v) and incubated for different time periods (5 to 120 min) then 100 µL of the mixed solution was reacted with an equal volume of 5 mM acetylthiocholine (~10 min) and sequentially assayed with the sensor. A concentration-dependent inhibition of salivary ChE activity was observed. At 0.7 nM paraoxon, the sensor response indicated ~80% enzyme inhibition within 0.5 h; whereas, at 7 nM 100% inhibition was achieved. Future studies will evaluate the sensor performance following *in vivo* exposure of rats to ChE inhibiting pesticides. This new CNT-based electrochemical sensor represents a potential next generation sensor for noninvasive biomonitoring of exposure to OP insecticides and chemical nerve agents. (Supported by CDC/NIOSH grant R01 OH008173-02)

1753 QUANTUM-DOTS-BASED ELECTROCHEMICAL IMMUNOASSAY FOR THE CHLORPYRIFOS AND TRICHLOPYR METABOLITE TRICHLOROPYRIDINOL.

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Chlorpyfios and trichlopyr are two routinely utilized pesticides that share a common urinary metabolite, trichloropyridinol (TCPy), which can be used to biomonitor for exposure. To facilitate biomonitoring there is a need to develop simple, rapid and sensitive sensors to evaluate exposures utilizing readily obtainable biological fluids, like saliva. A quantum-dot (QD)-based electrochemical immunoassay was developed to detect TCPy. QD conjugated with TCP derivative, triclopyr, was used as a label in the competitive immunorecognition event. TCP and the QD-labeled triclopyr competitively bind with the limited TCP antibodies on the magnetic beads (TCP-Ab-MB). The captured QD labels on the magnetic beads were determined by highly sensitive stripping voltammetric measurement of the dissolved metallic component (cadmium) with a disposable-screen-printed electrode. The parameters (e.g., amount of triclopyr-QD, amount of TCP Ab-MB and immunoreaction time) that govern the sensitivity and reproducibility of the electrochemical immunoassay were optimized. The voltammetric response is inversely proportional to the TCP concentration in the sample solution and is linear over the range of 0.05 to 1 ng mL^{-1} TCP. The limit of detection is estimated to be 0.02 ng mL^{-1}, which is 10 times lower than that of commercial enzyme linked immunosorbance assay kit (0.25 ng mL^{-1}). The performance of the developed immunoassay system was successfully evaluated on rat saliva samples spiked with TCP. This technique could be readily used for detecting other environmental contaminants by developing specific antibodies against contaminants and is expected to open new opportunities for biological monitoring. This new QD-based electrochemical sensor represents a potential next generation sensor for noninvasive biomonitoring of exposure to commercially important insecticides. (Supported by CDC/NIOSH grant R01 OH008173-02)

1754 MOLECULAR DOSIMETRY OF THE VINYL CHLORIDE-INDUCED DNA ADDUCT, 7-OXOETHYLGUANINE.

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Vinyl Chloride (VC) is an industrial chemical and known animal and human carcinogen which acts by a genotoxic mechanism. In the 1970s, it was shown that high level exposure of VC caused angiosarcoma of the liver (ASL) in VC workers. Early reports suggested that exposure to VC may also be associated with brain tumors. Using exposure to [$^{13}C_2$]-VC, we demonstrated in adult rats that neither N^2,3-ethenoguanine (EG), nor 7-oxoethylguanine (7-OEG) was detectable in brains of rats exposed to 1100 ppm. We continue this research by developing new LC-MS/MS methods for 7-OEG and applying these methods to analyze tissues from both adult and weanling rats exposed to 1100 ppm [$^{13}C_2$]-VC for 5 days. The 7-OEG was derivatized with O-t-butylhydroxylamine to an oxime adduct. Isotopically-labeled 7-OEG was synthesized by the reaction of chloroethylene oxide with dG. Following HPLC clean-up, it was characterized by MS and NMR and quantified by fluoresence. The level of 7-OEG in liver DNA was 28±6 adducts/10^6 nucleotides for adult rats exposed to [$^{13}C_2$]-VC (1100 ppm, 6hrs/day, 5 days) and 127±33 adducts/10^6 nucleotides for weanling rats exposed to [$^{13}C_2$]-VC (1100 ppm, 6hrs/day, 5 days). The method gave a detection limit of 15±2 adducts /10^8nucleotides in 5 ug DNA. To determine the persistence of 7-OEG, we found 74±19 adducts/10^8 nucleotides for animals sacrificed 2 wks post exposure, 17±2 adducts/10^8 nucleotides for 4 wks post exposure, and no adducts were found for 8 wks post exposure for adults rats exposed to [^{13}C2]-VC (1100 ppm, 6hrs/day, 5 days). Additionally, we will explore the formation of 7-OEG in the brains of VC exposed weanling and adult rats.

1755 A NOVEL APPROACH FOR ANALYSIS OF N-TERMINAL VALINE ADDUCTS AS BIOMARKERS FOR EXPOSURE TO ALKYLATING COMPOUNDS.

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Protein adducts are well established biomarkers for alkylating agents and are used to study environmental or occupational exposures. Thus far, these adducts have been measured by modified Edman degradation, immunohistochemistry or by analysis of the tryptic fragments after immunoaffinity chromatography (IA). However, these highly specific and sensitive methods usually allow only quantitation of a single adduct. To overcome this limitation we present here the proof-of-concept for simultaneous analysis of multiple adducts. Rather than using antibodies specific to the adduct of interest, antibodies were raised against the C-terminus of the rat N-terminal tryptic peptide, and 'selection' immunoaffinity columns were prepared. This antibody has high specificity towards the rat peptide whether its N-terminal valine is alkylated or not. The recovery of diepoxybutane (DEB) derived N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-Val) was ~ 93%. Analysis of peptide standards or globin treated in vitro with 1,3-epoxy-3-butene (EB), DEB or EB plus DEB demonstrate the suitability of the IA columns to enrich N-terminal peptides. However, since these IA columns also enrich normal N-terminal peptides, interference and poor recovery was observed for low level pyr-Val standard peptides (2 pmol) in presence of larger amounts (5mg) of globin. Subsequently, antibodies were raised against the unmodified N-terminal peptide and 'depletion' immunoaffinity columns were prepared. The recoveries of pyr-Val and the EB derived 2-hydroxy-3-butenyl-valine peptide standards in globin after trypsin hydrolysis, de-

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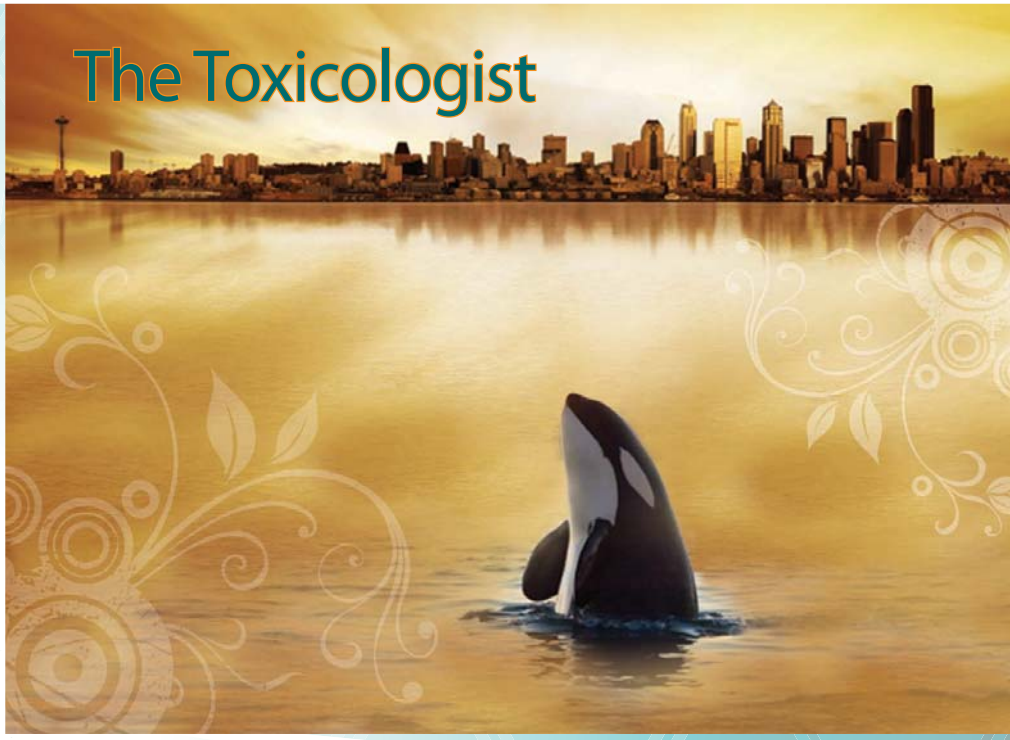
The Toxicologist

March 2008

The Toxicologist



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