



**SOT** | Society of  
Toxicology

# **The Toxicologist**

Supplement to *Toxicological Sciences*

An Official Journal of the  
Society of Toxicology

*45<sup>th</sup> Annual Meeting  
and ToxExpo<sup>TM</sup>  
San Diego, California*

**OXFORD**  
UNIVERSITY PRESS

ISSN 1096-6080  
Volume 90, Number 1, March 2006

[www.toxsci.oupjournals.org](http://www.toxsci.oupjournals.org)

were observed between LP and HP condensates. Besides, no clear dose-response in MN frequency (MNF) expressed as a % of DMSO control, was observed with or without S9 activation. Overall, the CSC from HP cigarettes showed a trend (not statistically significant) of lower MNF compared to the LP cigarette condensates, which may be attributable to qualitative and/or quantitative differences in smoke chemistry, merits further investigation.

## 558 VALIDATION OF A NON-RADIOACTIVE FLOW CYTOMETRY-BASED UNSCHEDULED DNA SYNTHESIS (FL-UDS) ASSAY

C. A. Kirk, M. K. Reeder and G. L. DeGeorge. *MB Research Labs, Spinnerstown, PA.*

The possible genotoxic potential of new chemicals and drugs drives the growing need for an inexpensive, reliable genotoxicity screening assay. The Unscheduled DNA Synthesis (UDS) assay has been proven to identify and characterize genotoxic chemicals by detecting repair of damaged DNA, via measurement of the incorporation of <sup>3</sup>H-Thymidine following the induction of various types of genetic lesions. Since this DNA repair is distinct from de novo DNA synthesis observed in normally dividing cells, it is commonly referred to as "Unscheduled" DNA Synthesis. MB Research has developed and optimized, a high-throughput and non-radioactive UDS assay using flow cytometry, termed FL-UDS. This assay measures incorporation of fluorescently-labeled or conjugated thymidine analogs, rather than radioactive nucleotides. The FL-UDS assay is run on an automated microplate-driven platform, which is to be more cost-efficient, shortens study time (1 wk vs. 16 wks), can interrogate a much larger number cells (10,000 vs. 50 – 100), increasing both accuracy and throughput. Another key improvement of FL-UDS over the radioactive UDS is that FL-UDS methodology can resolve three types of genotoxic agents: activation-dependent, activation-independent and those that are detoxified by biotransformation.

We have evaluated 15 known genotoxins and non-genotoxins and successfully classified 14 of the 15 test chemicals (Accuracy = 93%). Due to its many improvements over the standard UDS assay in genotoxicity assessments (especially lower cost and faster turn-around), the FL-UDS assay is proving to be of considerable commercial value to the Pharmaceutical, Biotech, Chemical, Cosmetic and Consumer Products industries.

## 559 EVALUATION OF A NOVEL MICRONUCLEUS ASSAY USING A HUMAN 3-D SKIN MODEL, EPIDERM™

R. D. Curren<sup>1</sup>, M. Aardema<sup>2</sup>, P. J. Hayden<sup>3</sup>, G. Mun<sup>1</sup>, T. Hu<sup>2</sup>, N. Wilt<sup>1</sup> and D. Gibson<sup>2</sup>. <sup>1</sup>Institute for IN VITRO Sciences, Inc., Gaithersburg, MD, <sup>2</sup>Procter & Gamble Co., Cincinnati, OH and <sup>3</sup>MatTek Corporation, Ashland, MA.

Many chemicals and products, most notably cosmetics, have skin as a major target organ; however, very few assays are available to directly address potential genotoxicity to this tissue. Although rodent models are being developed to measure micronucleus induction in the skin, European legislation such as the 7th amendment to the Cosmetics Directive precludes the use of in vivo assays for genotoxicity assessments of cosmetic ingredients after 2009. To provide a useful alternative, we are developing an in vitro human skin micronucleus assay using the 3-D EpiDerm™ skin model (MatTek Corp, Ashland, MA). Theoretically such a model could approximate the complexities typical of in vivo exposures, e.g. absorption, tissue specificity, metabolism, etc., and at the same time reflect human-specific responses in these parameters. Our standard assay utilizes two 10 ul doses of test material applied to the surface of the EpiDerm™ tissue 24 hours apart, with harvest 24 hours after the last dose. Using this procedure we show dose related increases in both cytotoxicity and micronuclei induction for several model genotoxins including mitomycin C (maximum micronucleus response [MMR] ~8% at 0.6 ug total dose), vinblastine sulfate (MMR ~4% at 0.01 ug total dose), methylmethane sulfonate (MMR ~0.6% at 20 ug total dose), and N-methyl-N'-nitro-N-nitrosoguanidine (MMR ~0.7% at 40 ug total dose). The background frequency of micronuclei is low at <0.1% (N=20), with a positive control of 3 ug/ml or 6 ug/ml MMC resulting in 1.2% micronucleated cells/binucleated cell (N=7) and 2.3% micronucleated cells/binucleated cell (N=8), respectively. We have initiated studies to investigate whether the model will respond to genotoxins requiring metabolic activation and find that EpiDerm™ cultures from different donors express numerous genes associated with xenobiotic metabolism that are also found in normal human skin. This novel assay system appears to hold excellent promise as a human "in vivo-like" genotoxicity model.

## 560 VITOTOX™ ASSAY DETECTS CHEMICALS WITH A WIDE RANGE OF GENOTOXIC MECHANISMS

C. A. Hendricks<sup>1</sup>, J. Aubrecht<sup>2</sup> and K. Lam<sup>1</sup>. <sup>1</sup>Pfizer Global Research and Development, Cambridge, MA and <sup>2</sup>Pfizer Worldwide Safety Sciences, Groton, CT.

Improved synthesis strategies and the introduction of automated biomolecular screening methods have led to an increase in the number of compounds entering early drug discovery. Thus, the development and application of high throughput

genotoxicity screening approaches capable of detecting compounds with a broad range of genotoxic mechanisms are expected to facilitate lead generation. The VITOTOX™ assay is based on a strain of *Salmonella* that contains the bacterial luciferase operon (*luxCDABE*) under the transcriptional control of a modified *recN* promoter. Incubation of these cells with a genotoxic compound causes induction of the bacterial SOS-response and results in derepression of the *recN* promoter and expression of the *lux* operon. Thus, genotoxicity can be measured as a function of light production. In this study, we have evaluated the VITOTOX™ assay as a sensor for genotoxicity using a set of 50 model agents with diverse mechanisms of action. To date, more than half of these literature compounds have been tested and preliminary results suggest that the VITOTOX™ assay can be used to detect both mutagenic and clastogenic compounds.

## 561 AN INTEGRATED TOXICOKINETIC MODEL FOR ESTIMATING CHILDHOOD BODY BURDENS OF DIOXINS BASED ON VARIOUS STUDIES

H. Leung<sup>2</sup>, B. D. Kerger<sup>1</sup>, P. Scott<sup>3</sup> and D. J. Paustenbach<sup>4</sup>. <sup>1</sup>HSRI, Inc., Tallahassee, FL, <sup>2</sup>Consultant, Danbury, CT, <sup>3</sup>ChemRisk, Pittsburgh, PA and <sup>4</sup>ChemRisk, San Francisco, CA.

A one-compartment toxicokinetic model was developed to examine the range of childhood (ages 0-7) body burdens of dioxin-like polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs). The model is predicated on the distribution of PCDD/Fs solely in the total body adipose volume. We have significantly refined the model by replacing certain model parameter assumptions with empirical data. These included: (1) incorporating some recently observed human age-dependent half life data for PCDD/Fs, (2) updating the childhood body mass index to more accurately reflect contemporary trends; and (3) utilizing the latest intake data on 2,3,7,8-tetra-, penta-, and hexa-CDD/Fs by children. Childhood exposure scenarios examined included breast or formula feeding for 6 months, followed by other dioxin uptake from foods and ingestion/dermal contact with residential soils. The temporal trends of blood lipid TEQ for the children were compared to the background blood levels and selected benchmarks from human and animal toxicity observations. The model predicted that breast milk intake during the first six months of life corresponded to peak blood lipid TEQ followed by a generally flat or decreasing body burden trend from ages 2 through 6 when contact with contaminated soil is generally thought to be greatest. Breastfed and formula-fed children showed similar body burdens after age 3, and average soil ingestion and dermal contact increased time-weighted average TEQ in blood lipid by < 2 ppt for soil dioxin TEQ concentrations up to 1,000 ppt. This model, especially after further validation, should be helpful when attempting to estimate the contribution of PCDD/F contaminated soil on body burdens and potential health risks in children.

## 562 DEVELOPMENTAL AGE EFFECTS ON TISSUE DISPOSITION OF BDE 47 IN MICE

J. J. Diliberto<sup>1</sup>, D. F. Staskal<sup>2</sup> and L. S. Birnbaum<sup>1</sup>. <sup>1</sup>NHEERL ORD, USEPA, Research Triangle Park, NC and <sup>2</sup>Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC.

Because of reported adverse developmental effects in rodent studies following exposure to polybrominated diphenyl ethers (PBDEs), these chemicals are potentially hazardous to infants and young children. This study investigated distribution and excretion of BDE 47, most dominant PBDE congener found in USA biotic samples, at different times during development in mice. C57BL/6 mice were orally exposed to 1 mg/kg [<sup>14</sup>C]BDE 47 on postnatal day (PND) 22, 28 or 40. Tissue and urine samples were collected 24 hr after dosing; carcass analyses were used as an indirect measure of excretion. In all age groups, BDE 47 distributed to lipophilic tissues; most tissue concentrations of BDE 47 were highest in PND 22 mice, and by PND 40 closely mimicked adult tissue concentrations, except in blood and liver. Developmental changes were seen in ratios of blood:brain (0.24, PND 22 to 0.04, adult) and liver:fat (0.06, PND 22 to 0.63, adult). Previous toxicokinetic studies in adult mice have shown a rapid urinary excretion of BDE 47 in mice. Results of this study suggest that young, developing mice have an impaired ability to excrete BDE 47 based on lower concentrations of BDE 47 in urine as compared to adults and a larger % dose remaining in the body 24 hr following exposure in younger mice. The carcass residual % dose decreased from 59%, PND 22 to 34%, PND 40. Because active renal transport has been suggested to be involved with rapid excretion of BDE 47, excretion patterns in mice of increasing age (PND 22 to 40) were compared to ontological expression of renal transport proteins in this species in an attempt to further identify the mechanism(s) responsible for rapid urinary excretion. These developmental differences led to higher concentrations of BDE 47 at target tissues during critical windows of development, which may ultimately explain the sensitivity of developing systems to adverse effects of BDE 47. This abstract does not reflect EPA policy. Work partially funded by EPA DESE CT 826513.