



SOT | Society of
Toxicology

The Toxicologist

Supplement to *Toxicological Sciences*

An Official Journal of the
Society of Toxicology

*45th Annual Meeting
and ToxExpoTM
San Diego, California*

OXFORD
UNIVERSITY PRESS

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

www.toxsci.oupjournals.org

DETERMINATION OF ACESULFAME-K
CONCENTRATIONS AND PRELIMINARY
PHARMACOKINETICS IN C57BL MOUSE PLASMA AND
URINE

J. Lodge¹, B. Fletcher¹, D. Brine¹, J. Pittman¹, C. Harris¹, S. Cooper¹, S. Anderson¹, B. Collins² and C. Garner¹. ¹RTI International, Research Triangle Park, NC and ²National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The purpose of this work was to develop, validate, and apply liquid chromatographic methods for the determination of acesulfame-K (AcK) in C57BL mouse plasma and urine. These methods were subsequently used to quantitate AcK in plasma and urine from mice dosed in a preliminary toxicokinetic study. Male and female animals were dosed orally (gavage) and intravenously at 10 mg/kg and then plasma and urine collected at up to 24 hours post dose. Plasma samples were analyzed with an HPLC method using a C18 reverse phase column validated in the range of 0.750 to 300 ug/mL. Urine was analyzed with a second HPLC method using an ODS-3 column and a Novapak Phenyl 60Å in tandem validated in the range of 25.0 to 400 ug/mL. Both methods used saccharin as internal standard with detection by UV absorbance at 230 nm. Following IV administration, plasma AcK concentrations declined rapidly and linearly within 120 min and a second AcK peak was observed at 240 minutes. Half-life was estimated to be 11-15 minutes. Plasma concentrations of AcK reached maximal levels within 45 minutes and rapidly declined following oral doses. Plasma AcK were below limits of detection by 480 minutes post dose. A second peak was also observed following oral administration, suggesting enterohepatic recirculation. Following IV and PO administration, 45% (males) and 70% (females) of the dose was excreted in urine by 24 hours. Oral bioavailability was estimated to be 90-100% based on urinary data.

OXIDATIVE AND HYDROLYTIC METABOLISM OF
TYPE I PYRETHROIDS IN RAT LIVER MICROSOMES

E. J. Scollon¹, J. M. Starr², M. E. Hughes¹ and M. J. Devito¹.
¹ORD/NHEERL/ETD, USEPA, Research Triangle Park, NC and
²ORD/NERL/ETD, USEPA, Research Triangle Park, NC.

Pyrethroids are a class of neurotoxic insecticides used in a variety of agricultural and household activities. Increased potential for human exposure to pyrethroids has prompted pharmacokinetic research. To that end, our lab has determined the in vitro clearance of the Type I pyrethroids permethrin, bifenthrin, and resmethrin in male rat hepatic microsomes. The aim of this study was to determine the fraction of clearance due to NADPH-dependent oxidative metabolism and NADPH-independent hydrolytic metabolism. 0.5 µM solutions of cis-permethrin, trans-permethrin, bifenthrin and resmethrin were incubated in hepatic microsomes in the presence or absence of NADPH. Metabolism was measured using a parent depletion approach. Parent compound concentrations were determined by LC/MS. Clearance rates for individual chemicals incubated with NADPH (1mg/ml) were 4.93E-02, 2.00E-02, 1.25E-02, and 3.8E-02 L/hr per mg protein for cis-permethrin, trans-permethrin, bifenthrin, and resmethrin. In the absence of NADPH, trans-permethrin (1.22E-02 L/hr) and resmethrin (9.67E-03 L/hr) were metabolized at reduced but significant rates while cis-permethrin and bifenthrin were not appreciably cleared. The clearance rates for the cis- and trans- isomers in a commercial mixture of permethrin (21:79 cis:trans) were similar to the rates obtained for the pure isomers. Clearance for cis-, trans- and total permethrin were 5.99E-02, 3.19E-02 and 4.37E-02 L/hr per mg protein when incubated with NADPH. In the absence of NADPH, cis- did not degrade significantly but trans- and total permethrin were cleared at 1.9E-02 and 1.62E-02 L/hr per mg protein. It appears that at concentrations below Km, chemical interactions are not significant between cis- and trans-permethrin. (This abstract does not represent US EPA policy.)

IMPLICATIONS OF AGE-DEPENDENT HALF LIVES OF
DIOXINS ON ASSESSMENT OF BREAST MILK DOSE
AND BODY BURDEN

R. O. Richter², B. D. Kerger¹, H. Leung⁴ and D. J. Paustenbach³. ¹HSRI, Inc., Tallahassee, FL, ²Exponent, Irvine, CA, ³ChemRisk, San Francisco, CA and ⁴Consultant, Danbury, CT.

Compared to typical adult dietary background doses, breast-fed infants receive daily doses (pg /kg bw/day) of dioxin-like polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) that can be one to two orders of magnitude higher. Although background PCDD/Fs in breast milk decreased several-fold during the last two decades, this source remains a potential public health concern. Recent data showing relatively short PCDD/F half lives in breast-fed infants (0.3 to 0.6 yr) combined with age-dependent rates of increase in congenere-specific half life (0.05 to 0.2 yr/yr) allow for a more rigorous examination of maternal and infant body

burdens of dioxins relating to breast milk ingestion. In reproductive age females (e.g., age 16 to 35), the age-dependent half life trends equate with much lower cumulative adipose tissue PCDD/F levels in the mother before pregnancy, and much lower peak and time-weighted average adipose tissue levels in the breast-fed infant compared to an assumption of constant half life (e.g., 7 years for TCDD). Women having their first child at a younger age exhibit lower body burdens and lower breast milk transfer of dioxins. Multiparous mothers exhibit lower body burdens and lower breast milk transfer of dioxins to second-born and subsequent infants. We believe that these factors equate with a generational effect that will continue to reduce PCDD/F body burdens in mothers and breastfed children in the future. These age-dependent half life trends suggest that steady state accumulation models should not be used to determine maternal body burden or breast milk levels, and that the impact of predominant dietary sources as well as specific site-related contributions are probably overstated by previous breast milk models adopted by USEPA.

THE PHARMACOKINETICS OF DEET IN THE MOUSE

W. McGuinn¹, M. Peden-Adams², J. EuDaly², G. Gilkeson² and D. Keil³. ¹US-FDA, Silver Spring, MD, ²MUSC, Charleston, SC and ³UNLV, Las Vegas, NV.

Previous studies in our laboratory identified that exposure to DEET (N,N-diethyl-meta-toluamide) suppresses humoral immunity. In this follow-up study, we report the subcutaneous bioavailability of DEET relative to intravenous (IV) administration using B6C3F1 female mice dosed with 15.5 mg/kg (81-umol/kg) of DEET. One mouse per time point was dosed for a total of 16 time points to 7 hours intravenously (IV) and 6 hours SC. The mice were exsanguinated at the predetermined time. The serum was separated by centrifugation and extracted with chloroform. Two microliters of the chloroform layer was injected Hewlett-Packard 5890 gas chromatograph to determine the concentration of DEET. We analyzed this plasma concentration vs. time data using a non-compartmental model with WinNonLin Professional 4.1 (Pharsight Corp.). After IV administration, T_{max} did not occur until 6 minutes after dosing probably because of slow circulation through the vascular bed in the mouse tail-vein. After IV administration, DEET was very widely and rapidly distributed to a volume, V_z, of 3569 L/kg. The clearance was 20 L/min/kg and the AUC_∞ was 3983 nmol*min/L. The terminal elimination half-life was 121 minutes, based on λ_z determined from the last 10 time points. Parameters determined after SC dosing were comparable. The V_z/F was 2640 L/kg, clearance was 21 L/min/kg and the half-life was 85 minutes, based on 4 points. AUC_∞ was 3786 nmol*min/L giving a SC bioavailability (F) of 95%. The shapes of the elimination curves were comparable. Thus, administration of DEET via the SC route provides exposures comparable to those obtained after IV administration.

AGE- AND DOSE-DEPENDENT TISSUE DISTRIBUTION
OF DELTAMETHRIN (DLM) IN MALE SPRAGUE-
DAWLEY (S-D) RATS

K. Kim^{1,2}, J. V. Bruckner¹ and H. Kim¹. ¹Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA and ²Pharmacology Department, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, South Korea.

DLM is a widely-used type II pyrethroid insecticide. The parent compound is a relatively potent neurotoxicant. Previous studies have shown that immature rats are more susceptible to acute DLM neurotoxicity than adults. The disposition of DLM was evaluated in the brain and fat of male Sprague-Dawley (S-D) rats to determine whether the immature rats' greater susceptibility is due to age-dependent target tissue deposition of DLM. Ten-, 21- and 40-day-old (immature) and 90-day-old (adult) S-D rats received 0.4, 2 and 10 mg DLM/kg by gavage in glycerol formal. Serial plasma, brain, and fat samples were collected over 96 hr and analyzed for DLM content by high performance liquid chromatography. While salivation and tremor were transient in 40- and 90-day-old rats, the signs were severe in 10- and 21-day-old animals, leading to their death by 6 and 12 h, respectively, at 10 mg/kg. In the 2 mg/kg group, all the animals lived up to the 96-hr time point, but 10- and 21-day-old rats showed neurotoxic signs. In each age group, DLM concentrations vs. time curves showed dose-dependent kinetics in plasma, whole brain, and fat. The area under the DLM concentrations vs. time curves (AUCs) for plasma, brain, and fat each progressively decreased with increasing age (for example, AUC_{0-96hr} values for brain isolated from rats given 2 mg/kg were: 0.46 (10-day-old), 0.26 (21-day-old), 0.11 (40-day-old) and 0.04 (90-day-old) mg•hr/L, respectively). In a related study (Anand et al., 2006), we reported that DLM metabolism in liver and plasma steadily increased during maturation. The marked differences in target organ (brain) and depot (fat) disposition of DLM in immature and mature rats indicate that toxicokinetics plays an important role in the susceptibility of immature rats to acute DLM neurotoxicity. (Supported by EPA STAR Grant R830800)

573 EFFECTS OF PERFLUOROOCCTANESULFONATE ON ¹²⁵I ELIMINATION IN RATS AFTER A SINGLE INTRAVENOUS DOSE OF ¹²⁵I-LABELED THYROXINE

S. Tanaka¹, M. Eastvold², E. Foshay¹, J. Hart¹ and J. Butenhof¹. ¹3M Company, St. Paul, MN and ²Mayo Medical Laboratories, Rochester, MN.

Perfluorooctanesulfonate (PFOS) has been found widely distributed in humans and wildlife. Prior studies have observed decreased total and free thyroid hormones (TH) in serum without a major compensatory rise in thyrotropin (TSH) or altered histology of the thyroid. Using a reference method (equilibrium dialysis-RIA) for free thyroxine (FT₄), we have found that analog methods in serum containing PFOS are prone to artificially low FT₄ measurements due to binding competition between PFOS and thyroxine (T₄). In fact, after short-term dosing with PFOS, FT₄ is transiently increased, as well as the hepatic response (malic enzyme) to TH. Because the diagnosis of hypothyroidism is based on elevated TSH and decreased FT₄, our data suggested a lack of hypothyroid state in rats; however, it remained unclear why there was a reduction of serum total thyroxine (TT₄) (hypothyroxinemia). We hypothesized that, in the presence of PFOS, increased serum free TH can increase TH availability to peripheral tissues and result in enhanced utilization and excretion. The effects of PFOS on serum TT₄, ¹²⁵I tissue distribution, and ¹²⁵I elimination in urine and feces after a single intravenous injection of ~10 µCi ¹²⁵I-T₄ followed by an oral dose of either vehicle (controls) or 15 mg PFOS/kg (treated) were investigated in male and female rats over a 24-hour period. Statistically significant changes from control included: decreased serum TT₄, and serum and liver ¹²⁵I; increased fecal and urinary (males only) ¹²⁵I. Urinary ¹²⁵I was increased in females without statistical significance. The increases in fecal and urinary elimination of ¹²⁵I and the corresponding decreases in serum and liver ¹²⁵I and serum TT₄ suggest greater turnover of T₄, possibly due to increased FT₄ leading to increased peripheral tissue uptake, metabolism, and elimination. This would explain the reduced serum TT₄ observed after dosing with PFOS.

574 ACCUMULATION OF NEUROTOXIC METABOLITES OF 3,4-(±) METHYLENEDIOXYMETHAMPHETAMINE IN RAT BRAIN FOLLOWING MULTIPLE DOSING

G. V. Erives, S. S. Lau and T. J. Monks. *Pharmacology and Toxicology, University of Arizona Health Science Center, Tucson, AZ.*

3,4-(±)Methylenedioxyamphetamine (MDMA, ecstasy) is a serotonergic neurotoxicant. The neurotoxicity appears dependent upon systemic metabolism of MDMA since direct injection of this amphetamine analog into the brain fails to reproduce the toxicity. MDMA is demethylated by cytochrome(s) P450 to the catechol metabolite N-methyl-α-methyl-dopamine (N-Me-α-MeDA). We have shown that thioether (glutathione [GSyl] and N-acetylcysteine [NACSyl]) metabolites of N-Me-α-MeDA are neurotoxic, and present in rat brain following subcutaneous injection of MDMA. The present study was designed to determine the effects of multiple doses of MDMA (4 X 20 mg/kg at 12h intervals) on the concentration of MDMA metabolites in rat brain striatal dialysate, since multi-dose MDMA administration is typical of drug intake during rave parties. Concentrations of 5-GSyl-N-Me-α-MeDA after each dose of MDMA were maximum between 30-60 min after drug injection. Although the C_{max} values for 5-GSyl-N-Me-α-MeDA were similar after each dose (22.5-27.0 pmol/10µl), the AUC increased after the third and fourth injections. Peak concentrations of 5-NACSyl-N-Me-α-MeDA and 2,5-bis-NACSyl-N-Me-α-MeDA occurred between 120-180 min after drug injections. The time to reach 1/2 C_{max} for 5-NACSyl-N-Me-α-MeDA decreased by 45% between the first (124 min) and fourth (68 min) injections, and the AUC for both 5-NACSyl-N-Me-α-MeDA and 2,5-bis-NACSyl-N-Me-α-MeDA increased between the first and fourth doses. The time to reach 1/2 C_{max} for 5-NACSyl-N-Me-α-MeDA decreased by 80% between the first (85 min) and fourth (17.5 min) injections, concomitant with increases in the C_{max} from 33.0 pmol/10 µl to 49.5 pmol/10µl, possibly because processes for its elimination became saturated. The data indicate that neurotoxic metabolites of MDMA may accumulate in brain following multiple dosing. (P30 ES 06694)

575 BRAIN METABOLISM OF ACRYLONITRILE TO CYANIDE: IN VITRO STUDIES

O. S. El-Tawil¹, A. M. Mohamadin², A. B. Abdel-Naim³ and A. H. Abou-Hadeed⁴. ¹Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt, ²Tumor Marker Oncology Research Unit, Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, ³Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt and ⁴Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

Acrylonitrile (ACN) is a widely used industrial chemical. It is used in the manufacture of acrylics, plastics and synthetic rubber. It has been known to cause extensive brain tumors in rats. ACN requires metabolic activation to reactive epoxide that

can bind to macromolecules to initiate a series of events that lead to tumors formation. The brain can metabolize a variety of substances and play a role in the activation of many xenobiotics. The present study was designed to assess the ability of brain tissue to activate ACN to cyanide (CN⁻) in vitro. In brain centrifugal fractions from male Sprague-Dawley rats, the majority of the metabolic activity was localized in the microsomal fraction. Pretreatment of rats with cytochrome P450 inducers (clofibrate, phenobarbital, β-naphthoflavone, or ethanol) significantly enhanced brain microsomal metabolism of VCN to CN⁻. Addition of cytochrome P450 inhibitors; quinine, metyrapone, dimethyl sulfoxide or α-naphthoflavone to the brain microsomal incubation mixtures resulted in a significant inhibition of microsomal metabolism of ACN to CN⁻. Furthermore, the rate of ACN metabolism to CN⁻ was enhanced significantly by the addition of sulphydryl compounds to the incubation mixtures such as glutathione, dithiothreitol, L-cysteine, D-penicillamine, 2-mercaptoethanol. In conclusion, the present results indicate that extra hepatic tissues such as the brain microsomes are capable of metabolizing ACN to CN⁻ via cytochrome P450-dependent mixed function oxidase system. In addition, most sulphydryl compounds enhance the release rate of CN⁻ from ACN.

576 EVALUATION OF PERFLUOROOCCTANE SULFONATE IN THE RAT BRAIN

C. Lau¹, J. R. Thibodeaux¹, K. Das¹, D. J. Ehresman², S. Tanaka², J. Froehlich³ and J. L. Butenhof². ¹Reproductive Toxicology Division, NHEERL, ORD, US Environmental Protection Agency, Research Triangle Park, NC, ²Medical Department, 3M Company, St. Paul, MN and ³Department of Chemistry, University of California, Davis, CA.

Perfluorooctane Sulfonate (PFOS) is an environmentally persistent chemical that has been detected in humans and wildlife. PFOS is primarily distributed in liver and blood. The current study evaluated the level of PFOS in the adult and neonatal rat brain and determined whether there was a differential distribution among brain regions. Sprague-Dawley rats (60-70 day old) were given 3 mg/kg PFOS/K² by oral gavage daily for 14 days and sacrificed 24 h after the last treatment. A blood sample was obtained from the tail vein prior to sacrifice, and the brain was perfused with isotonic saline. Five brain regions: cerebral cortex, hippocampus, cerebellum, hypothalamus and brainstem, were dissected immediately and stored frozen at -80°C until analysis. For the developmental study, timed-pregnant rats were given PFOS (3 mg/kg) daily from gestational day 2 to 21 and allowed to deliver litters. Pups were sacrificed on postnatal day 7, and their brains were perfused and dissected similarly to the adults. Trunk blood was obtained from littermates. Extraction of brain homogenate employed a base digestion followed by a solid phase extraction at an acidic pH for optimal recovery. PFOS was determined by HPLC-MS-MS. Mean serum PFOS was 123 µg/ml for the adults and 52 µg/ml for the pups. Mean concentrations of PFOS among adult brain regions ranged from 5-8 µg/g, or 4.5-6.6% of the serum level. In contrast, PFOS in neonatal brain ranged from 16-29 µg/g, or 29-55% of serum level. These data indicated a limited presence of PFOS in the adult rat brain after subchronic exposure, with no differential distribution among regions. However, compared to the adults, substantially higher concentrations of PFOS were detected in the neonatal rat brain, likely due to incomplete formation of the blood-brain barrier at that developmental stage. This abstract does not necessarily reflect EPA policy.

577 AHR MEDIATED HEMATOTOXICITY IS INDUCED AT THE SITE OF BONE MARROW WHERE CONSEQUENT CYP2E1-DERIVED BENZENE METABOLITES LOCALLY INDUCE THEIR TOXICITY

Y. Hirabayashi¹, B. Yoon¹, G. Li¹, Y. Fujii-Kuriyama², T. Kaneko¹, J. Kanno¹ and T. Inoue³. ¹Cell & Mol Toxicology Division, NIHS, Tokyo, Japan, ²TARA, University of Tsukuba, Tsukuba, Japan and ³CBSR, NIHS, Tokyo, Japan.

We have reported that benzene-induced hemopoietic toxicity is transmitted by AhR. We also found that cytochrome P450 2E1 (CYP2E1) related to benzene metabolism is also up regulated in the bone marrow (BM) by benzene exposure in BM. Therefore, it is of interest to hypothesize a greater role of BM cells in hemopoietic toxicities rather than the hepatic metabolism. Accordingly, in the present study, benzene-induced hemopoietic toxicity was evaluated in wild type (Wt) mice after a lethal dose of whole-body irradiation followed by repopulation of BM cells that lack AhR or, vice versa, in AhR-KO (AhR^{-/-}) mice after repopulation of Wt BM cells. As results, benzene-induced hemopoietic toxicity seems to have been transmitted through AhR, and benzene was transformed by de novo metabolism with CYP2E1 in the BM. The establishment of homozygous AhR-KO mice is described elsewhere. The BM repopulation assay was performed similarly to the assay of CFU-S, except that 10⁶ BM cells were injected into lethally irradiated mice. One month after the transfusion of BM cells, the repopulated mice were used in the experiment. The benzene atmospheres were generated by heating liquid benzene to 16°C to form a vapor, and then the benzene-laden air was directed into 1.3 m³ in-