NHEERL-H/NTD/NBTB/PJB/05-002-001

Neurotoxicity and Immunotoxicity of Sub-Chronic Exposure to Volatile Organic Compounds

Author: Philip J. Bushnell Date: April 12, 2006
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IS THIS A MULTILABORATORY (NERL, NRMRL) OR INTERAGENCY STUDY No
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APPROVAL TO CONDUCT:

Branch Chief: G Moser Date: 4-13-06
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Neurotoxicity and Immunotoxicity of Sub-Chronic Exposure to Volatile Organic Compounds

Author: Philip J. Bushnell Date: Nov. 18, 2005
Philip J. Bushnell (Signature/Position)

QA Category of Research: 4 (1, 2, 3, or 4, as defined in the 2005 NHEERL Quality Management Plan)

GPRA 2002 Subobjective Code Clean Air- Goal 1.02.01
GPRA 2002 Research Area Code 1-024

IS THIS A MULTILABORATORY (NERL, NRMRL) OR INTERAGENCY STUDY No
IF YES, SPECIFY

APPROVAL TO CONDUCT:

Branch Chief: _____ Date: _____
(Signature)

OPTIONAL REVIEWERS: (Advisory Review [**ADR**] or Approval Review [**APR**])

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Title: Neurotoxicity and Immunotoxicity of Sub-Chronic Exposure to Volatile Organic Compounds

Authors (NTD): Philip Bushnell, William Boyes, Tim Shafer, Virginia Moser, Christopher Gordon, Mary Gilbert, David Herr, Prasad Kodavanti, Wendy Oshiro, Tracey Samsam
ETD: Bob Luebke, Paul Evansky

Date of Protocol: November 14, 2005

Date of Revision:

INTRODUCTION:

Volatile organic compounds (VOCs) are prevalent in urban air and are generated by several types of regulated sources (defined by the Office of Air and Radiation as "area", "stationary" and "mobile" sources). In addition, many commercial products contain VOCs in the form of organic solvents, including paints, waxes, inks, cleaning solutions, and pesticides. The nature and use of these products may involve both acute and extended exposure to one or more VOCs. Inhalation of VOCs can affect attention, reaction time and cause sensory dysfunction in humans. Repeated high-level exposure to some VOCs can also cause birth defects and developmental disorders; recently, occupational exposure of pregnant women has been associated with visual disturbances in their children. Thus, the potential long-term effects of repeated exposure on the nervous system and developing fetus are of concern. In addition, there is concern that acute and long-term exposure to VOCs may be associated with suppression of immune system function.

RATIONALE, OBJECTIVE AND APPROACHES:

The overall goal of this project is to develop an animal model of the neurotoxicity of long-term exposure to VOCs that can be used to predict the effects of chronic exposure to VOCs on public health. Because studies of potential long-term effects of VOCs cannot be conducted in humans, animal models have been developed to explore the effects and mode of action of these compounds experimentally. Results of these studies are mixed: some studies report changes in behavior and neurochemical endpoints in rats, while others find no effect.

Specifially, this project will determine whether persistent effects of repeated inhalation of VOCs leads to changes in functions of the nervous or immune systems that can be quantified in rats. This project extends ongoing work on the acute effects of VOCs on the nervous system to effects of long-term, low-level exposure, and thus complements current efforts to develop an acute exposure-dose-response (EDR) model for VOCs. For this work, rats are exposed to VOCs via inhalation in a sub-chronic time frame (90 days $\approx$ 13 weeks) and observed for effects on various measures of function of the nervous and immune systems. Approaches involve the following:

- Use of toluene as a model VOC because of its extensive knowledge base.
- Use of other solvents to explore the generality of potential effects of toluene
- Use of physiologically-based toxicokinetic (PBTK) models of VOC exposure to determine the dosimetry of the compounds.
- Exposure of adult male rats to study effects in mature and aging animals.

- Exposure of pregnant female rats to study effects in offspring.
- Endpoints to be measured include:
  - Unconditioned behavior using the Functional Observational Battery and Motor Activity (FOB/MA) screen.
  - Learning and attention with operant signal detection and trace fear conditioning.
  - Emotional behavior with the elevated plus maze and open field.
  - Visual function assessed electrophysiologically with visual evoked potentials, electroretinograms and optokinetic measurements.
  - Dopamine neurochemistry
  - Immune system functions by measuring antibody responses in rats sensitized with SRBC antigen.
  - mRNA expression patterns in cortex and nucleus accumbens, regions receiving dopaminergic input and important for the behavioral endpoints to be measured.
  - Indicators of oxidative stress in cortex and nucleus accumbens.
  - Cardiovascular and thermoregulatory responses measured by radiotelemetry.

TEST MATERIALS:

VOCs include:

Toluene (Aldrich Chemical Co., Milwaukee, WI): 0 - 1000 ppm;
Perchloroethylene (Aldrich Chemical Co., Milwaukee, WI): 0 - 2000 ppm;
Trichloroethylene (Aldrich Chemical Co., Milwaukee, WI): 0 - 1000 ppm;
1,1,1-trichloroethane (Aldrich Chemical Co., Milwaukee, WI): 0 - 3000 ppm.

LOCATION AND DESIGNATION OF OPERATING PROCEDURES:

Operating Procedures (OPs) refer to documents written either for the Neurotoxicology Division (NRD) or the Experimental Toxicology Division (ETD). NTD OPs are maintained on the NHEERL server at nheerl_ntd on 'ORD-RTP File/Print Server One (v2626umcec001)', known in NTD as W:\Neurotoxicology Division QA Documents. Folders for the Neurophysiological Toxicology, Cellular and Molecular, and Neurobehavioral Toxicology Branches each contain folders called \Branch Documents\PI Documents. Each PI's documents are found in folders under their names as described below.

OPs for exposure and animal care during exposure to toluene

Paul Evansky, Exposure Engineer / Operator

All Operating Procedure Titles have: OP-NHEERL-H/ETD, before /IEG...

OP Title	OP Number
Operating Procedure for Handling Dead and Moribund Animals	/IEG/97- 7-00
Handling Animals	/IEG/97- 21-00
OP for Emergency Response to Sentry Watch Call-In	/IEG/97- 36-00
Operation of Automatic Watering System in J216A	/IEG/98- 38-00
DAILY MAINTENANCE OF HAZELTON CHAMBERS	/IEG/98- 39-00
Animal Inhalation Exposures to Gases and Vapors	/IEG/01- 64-00

Operation and Calibration of FMI Pump	/IEG/01- 65-00
Operation and Calibration of Mass Flow Controllers	/IEG/01- 66-00
Operation and Calibration of Rotronics I - 200 Temperature and Humidity Sensors	/IEG/01- 67-01
Calibration of Chamber Airflow and Static Pressures with Magnehlic Guages and Validyne Pressure Transducer	/IEG/01- 69-03
Operation and Calibration of Harvard Syringe Pump	/IEG/01- 70-03
Set-Up and Operation of Data Acquisiton Systems (DAS)	/IEG/01- 81-00
Set-Up, Adjustment and Calibration of Miran 1A Infrared Gas Analyzer	/IEG/01- 84-00
Calculation of Test Agent Required for Gas and Vapor Exposures	/IEG/01- 85-00

OPs for assessments after exposure to toluene

Signal detection behavior and elevated plus-maze: Bushnell's documents are found at W:\Neurotoxicology Division QA Documents\ Neurobehavioral Toxicology Branch\Philip Bushnell\Operating Procedures\ROPXnumber.

Functional Observational Battery / Motor Activity (FOB / MA): Moser's documents are found at W:\Neurotoxicology Division QA Documents\ Neurobehavioral Toxicology Branch\Ginger Moser\OPs on that drive.

Fear conditioning: Gilbert's documents are found at W:\Neurotoxicology Division QA Documents\ Neurophysiological Toxicology Branch\Mary Gilbert\ on that drive. MEG/2005-018-000 applies to the fear conditioning studies.

Autonomic functions (telemetered body temperature and heart rate): CHRIS

Electrophysiology of vision in vivo: Boyes's documents are found in W:\Neurotoxicology Division QA Documents\Neurophysiological Toxicology Branch\Branch Documents\PI Documents\William Boyes\SOPs

Neurotransmitter Assays: Herr's documents are found in W:\Neurotoxicology Division QA Documents\Neurophysiological Toxicology Branch\Branch Documents\PI Documents\David Herr\SOPs.

Transcriptional analysis of CNS tissue ex vivo: Shafer's documents are found in W:\Neurotoxicology Division QA Documents\Neurophysiological Toxicology Branch\Branch Documents\Tim Shafer\SOPs

Measures of oxidative stress: Oxidative stress measures include both measurements of free radical production and antioxidants in different brain regions. The OPs for these oxidative stress measures are located in the common drive for Experimental Toxicology Division.

Glutathione peroxidase - SOP-NHEERL-H/ETD/PTB/JR/GPX-99-15-000
Alpha –1 Antiproteinase - SOP-NHEERL-H/ETD/PTB/JR/APA-05-32-000
Glutathione Reductase - SOP-NHEERL-H/ETD/PTB/JR/GRD--99-33-001
Gamma Glutamylcysteine Synthetase - SOP-NHEERL-H/ETD/PTB/JR/gGCS--05-34-000

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Glutathione transferase – SOP-NHEERL-H/ETD/PTB/JR/GTR-99-25-001
Superoxide dismutase - SOP-NHEERL-H/ETD/PTB/JR/SOD-98-31-001
NADPH Quinone oxidoreductase-1 - SOP-NHEERL-H/ETD/PTB/JR/NQ01-05-30-000
Aconitase - SOP-NHEERL-H/ETD/PTB/JR/ACON-03-23-000
Glutamate Dehydrogenase - SOP-NHEERL-H/ETD/PTB/JR/GLD-05-27-000
Pyruvate Dehydrogenase - SOP-NHEERL-H/ETD/PTB/05-29-000
alpha Ketoglutarate Dehydrogenase -SOP-NHEERL-H/ETD/PTB/JR/05-28-000.
NADH-Ubiquinone Reductase - SOP-NHEERL-H/ETD/PTB/JR/UBIQ--05-36-000
Protein determination - SOP-NHEERL-H/ETD/PTB/JR/Protein-98-04-001
Glutathione – SOP-NHEERL-H/ETD/PTB/JR (need to develop this)
Ascorbic acid - SOP-NHEERL-H/ETD/PTB/JR(need to develop this)

Immune function: Luebke's documents are maintained on file in his office (B486).

TEST ANIMALS AND CARE:

Adult male Long-Evans rats (Charles River) are singly housed in the RTP animal colony in plastic cages with hardwood chip bedding. Animals are provided water ad libitum during the 90 day exposure period.

Some animals (for VCM and PJB) are maintained at a constant body weight (Ali et al., 1992; OPs PJB/95-101-006, PJB/95-102-009, PJB/98-105-007, PJB/05-106-001) for motivational purposes after the exposure period ends. Free-feeding body weights for each rat are determined at the end of exposure, and each animal is gradually reduced to,a nd maintained at, 80% of this weight for the remainder of the study. Rats so maintained remain healthy, non-obese, and motivated for behavior analysis.

For some studies, timed pregnant female Long-Evans rats will be received on Day 1 of gestation (GD1) and exposed to a VOC from GD6 – GD10 and GD13 – GD18. They will be allowed to give birth in plastic cages with bedding (GD21); the behavior and physiology of their offspring will be assessed during development and adulthood.

EXPERIMENTAL METHODS:

Exposure. Adult male animals will be exposed to toluene vapor 6 hr/day, 5 days/week for 3 months (13-week, or 90-day subchronic scenario) in Hazelton 2000 exposure chambers in A599K. Four chambers will be used, for air (control), low, medium, and high VOC concentrations (concs to be determined). The animals will be weighed each weekend and placed in the exposure chambers Monday morning. Exposure Monday through Friday as shown in Appendix *Schedule*. Water will be available to all rats *ad libitum*, including duri ng exposures. The rats will be given food *ad libitum* after each daily exposure. All rats will be removed from the exposure chambers Friday afternoon and will be placed on wood chip bedding in plastic cages in the animal colony over the weekend.

Pregnant female rats will be exposed similarly, except that the duration of exposure will be between GD6 and GD18, as described above.

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