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6. AUTHOR(S)
TERENCE H. RISBY, PH.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
THE JOHNS HOPKINS UNIVERSITY
DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES
SCHOOL OF HYGIENE AND PUBLIC HEALTH
615, NORTH WOLFE STREET
BALTIMORE, MD 21205

8. PERFORMING ORGANIZATION REPORT NUMBER

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During this past 4.5 years, research has been conducted with support from the Air Force Office of Scientific Research. This research has been focused upon assessing the health effects of exposure to jet fuel, JP-8 in military personnel. In addition, research has continued into identifying molecules in exhaled breath that serve as sentinels of exposure, susceptibility and disease. Exhaled breath is composed of many molecules in the gaseous matrix that consists of oxygen, nitrogen, water vapor, carbon dioxide and the inert gases. Endogenously produced molecules are present in concentrations that are less than 100 parts per billion (v/v) whereas the concentrations of exogenous molecules are dependent upon the exposure concentration. Another important factor is that the composition of exhaled breath changes throughout the normal breathing cycle. The sources of endogenous molecules in exhaled breath may be systemic tissues or cells found at the alveolar membrane junction, in conducting airway, or in the oral-nasal-pharyngeal cavity. The sources of exogenous molecules in exhaled breath may be from previous or current inspiratory air.

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AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
4015 Wilson Blvd., Room 713
Arlington, VA 22203

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Address	School of Hygiene and Public Health 615, North Wolfe Street Baltimore, MD. 21205
Principal Investigator:	Terence H. Risby, Ph.D. Department of Environmental Health Sciences
Telephone Number	(410) 955-0024
Fax Number	(410) 955-0027
E-mail	thrisby@jhmi.edu
Co-Investigators:	W. Michael Foster, Ph.D. James F. Burdick, M.D. Shelley S. Sehnert, Ph.D.

Summary Abstract

During this past 4.5 years, research has been conducted with support from the Air Force Office of Scientific Research. This research has been focused upon assessing the health effects of exposure to jet fuel, JP-8, in military personnel. In addition, research has continued into identifying molecules in exhaled breath that serve as sentinels of exposure, susceptibility and disease. Exhaled breath is composed of many molecules in the gaseous matrix that consists of oxygen, nitrogen, water vapor, carbon dioxide and the inert gases. Endogenously produced molecules are present in concentrations that are less than 100 parts per billion (v/v) whereas the concentrations of exogenous molecules are dependent upon the exposure concentration. Another important factor is that the composition of exhaled breath changes throughout the normal breathing cycle. The sources of endogenous molecules in exhaled breath may be systemic tissues or cells found at the alveolar membrane junction, in conducting airway, or in the oral-nasal-pharyngeal cavity. The sources of exogenous molecules in exhaled breath may be from previous or current inspiratory air.

Research conducted during this period has been focused on many aspects of breath analysis since the one of the goals of this project was to collect and analyze representative breath samples from laboratory rodents and human subjects. During the course of this research it has been established that repeatable breath samples can be collected from spontaneously breathing human subjects providing that a human subject breathes with defined tidal volume and frequency. Also extensive research was conducted to prepare novel well-defined carbonaceous adsorbents that can capture quantitatively and selectively breath molecules from this gaseous matrix at ambient temperatures and release them reproducibly and quantitatively for subsequent analysis. This method of breath sampling was developed so that breath samples could be collected and concentrated in the field, and transported back

to the laboratory for subsequent analysis. This ability has been validated in field studies conducted in Arizona, Arkansas, and Virginia. Since the concentrations of the molecules of interest in breath are low, analytical methods were developed to separate and detect breath biomarkers. Furthermore, methods were developed to remove contaminant molecules from inspiratory air and thereby prevent interference with subsequent breath analysis. These studies were necessary in order to avoid the use of compressed gases in field studies.

Additionally, real-time methods for the analysis of nitric oxide, carbon monoxide and volatile sulfur-containing compounds were developed. These methods use dedicated monitors and are based upon single breath maneuvers. Monitoring the actual concentrations of breath biomarkers in a single breath is predicated upon the ability to obtain a reproducible and repeatable single breath sample. This is achieved by exhaling through a critical orifice while maintaining a constant mouth pressure. This exhalation maneuver produces a constant flow rate and stable analytical signal. Moreover, maintaining a constant mouth pressure (> 5 cm of H_2O) blocks the velum and minimizes the concentrations of molecules generated in the nasal-pharyngeal cavity. If the source of the breath molecule is cells found in the distal lung then the analytical signal follows the profile for carbon dioxide and peaks at the end tidal portion of the breath cycle. Whereas, nitric oxide peaks earlier because its major source is airway epithelium.

An analytical method was developed during the course of this investigation to determine actual exposure to jet fuel, JP-8. Specifically this method allows exposure routes to be estimated based upon the physical properties of the aliphatic and aromatic hydrocarbons and their quantifications. This method of analysis is based upon quantifying complete breath profiles (C_1 to C_{16}), which allows endogenously produced molecules in breath to be determined concomitantly.

Quantifiable levels of reactive oxygen species (ROS) are present in cells as a result of normal physiology. However, as a direct or indirect result of exposure to pollutants the concentrations of ROS may be increased and can produce cellular injury and tissue dysfunction by damaging DNA, denaturing proteins, or modifying membrane lipids. This damage can be ameliorated by the presence of antioxidants. The dynamic balance between oxidants and antioxidant defenses is known as oxidative stress status (OSS). Increased OSS identifies populations at risk for oxidant damage and breath ethane is as a non-invasive biomarker of this status. An example of a susceptible population is human subjects who are cigarette smokers. These people have increased concentrations of breath ethane and are susceptible to infections and many pulmonary and cardiovascular diseases. These people may also be susceptible to exposure to pollutants. In studies reported herein, cigarette smoking was found to be an important confounder in personnel who were exposed to jet fuel.

Risk factors for cardiovascular diseases have been causally linked epidemiologically to increased exposure to levels of environmental or occupational pollutants. It is proposed that abnormally high levels of breath isoprene, a volatile byproduct in the pathway for the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins, can be used to identify human subjects at risk for cardiovascular disease.

Hepatic dysfunction has also been linked to exposure to pollutants, including hydrocarbon-based fuels. Human studies have resulted in the discovery of novel sulfur-containing compounds that can be used to detect and stage liver disease. A patent has been issued during the course of this study that reports these results, US 6,248,078B1 June 19, 2001 "Volatile Biomarkers for Analysis of Hepatic Disorders." Funding for this invention was provided in part by this grant.

Renal dysfunction has also been linked to increased exposure to hydrocarbon-based fuels, and therefore human subjects with chronic renal diseases have been studied. Preliminary studies have

resulted in the detection of nitrogen-containing compounds that may be biomarkers of renal disease in human subjects.

Recently it has reported that as many as 60% of the adult American public are obese (body mass index (BMI) greater than 27) and the number of these obese individuals is increasing. Obesity shows some dependence with race and gender although this rate increase is independent. The incidence of obesity is increasing in all age groups including infants and children. Concurrent with this increase in the body mass index is the increase in prevalence of fatty liver disease (FLD). Fatty liver disease has been typically associated with alcohol drinkers but it is becoming increasing apparent that a significant number of nondrinkers develop nonalcoholic fatty liver disease (NAFLD). Moreover, there is evidence that even moderate drinkers who are obese have increased risk to develop alcohol-induced fatty liver disease. Recently, breath of obese humans was found to contain increased levels of ethanol, which appeared to be endogenously produced. The source of this ethanol was shown in a genetic mouse model of obesity to be gut flora. Typically obese human subjects have reduced gut motility and are at increased risk for developing diabetes, liver, and cardiovascular diseases. The increased amounts of adipose tissue in obese subjects may pose increased risk from the effects of exposure to lipophilic pollutants.

Since many of the studies reported herein involve the collection and analysis of breath, investigations were performed to examine the variations of breath molecules during the course of the day. In general, no major diurnal variations in the concentrations of endogenous molecules were found in a study performed in 20 normal healthy human subjects. There were, however, variations in the concentrations of molecules that were derived from metabolic processes. Moreover, the concentrations of breath molecules were found to vary with minute ventilation and/or cardiac output. Therefore a study was undertaken to investigate the effects of exercise on the composition of exhaled breath. This

study was performed in collaboration with investigators from the Johns Hopkins School of Medicine and from the National Institute for Aging. This study was performed with 18 normal human subjects that ranged in age from 22 to 82 years. The results of this study advocate that exercise does not cause uncoupling of oxygen consumption in mitochondria since the concentrations of reactive oxygen species increased linearly with oxygen consumption. Also, it was found that most endogenously produced breath biomarkers are excreted under perfusion control. Breath acetone was found to be a biomarker for evidence of fat burning during exercise; this exciting observation has major relevance to weight loss and exercise.

Since a critical part of this research involved using breath to estimate total body exposure to JP-8, *in vivo* studies were performed in laboratory rodents (rats and mice) in collaboration with Dr. Mark Witten from the University of Arizona. The goal of these studies was to validate the analytical method and to investigate the clearance of JP-8. Rats were nose-only exposed to aerosolized JP-8 (1000 mg/m^3) for 1 hour a day for 7, 14, 21 and 58 days. A special mixture of JP-8 was used in these animal studies, which was a blend of samples of JP-8 fuel collected from sources throughout the world by the US Air Force. Two different strains of rats were used (Fischer 344 and Fischer 344/Brown Norway). Exposures were performed at different times of the year (7 and 14 day in August; 21 day exposure in December; and the 58 day exposure in July). A similar protocol was used to expose male C57BL/6 NHsd mice to aerosolized JP-8 ($1000mg/m^3$) for 1 hour a day for 7 days. These mouse studies were performed in January. In all these studies, rodents were placed in a specially designed Pyrex chamber through which ultra-pure air was flowed. The outflow gas from the chamber was collected at defined times points after the last day of the exposure protocol. The half-life of clearance of n-decane (a typical hydrocarbon found in JP-8) was approximately 150 minutes and this rate of clearance could be saturated. There did not appear to major differences between rats and mice although less time points

were obtained since multiple mice had to be sampled simultaneously in order to obtain sufficient samples for subsequent analysis.

Investigators from Johns Hopkins were members of a major task force formed from various Federal Government Agencies and other universities to investigate exposure to the jet fuel JP-8 at different U.S. Air Force Bases. This task force studied a total of 324 air force personnel at 7 bases although the Hopkins group only attended 3 bases, Davis Monthan AFB, AZ; Langley AFB, VA; and Little Rock AFB, AR. The task force selected fuel cell workers, and fuel specialists as examples of exposed study subjects. A control group of nominally non-exposed personnel were also studied. JP-8 exposure was measured both externally in the environment immediately surrounding the study subject and internally using several body burden measures. The health impact of JP-8 exposure was evaluated using a series of neurological, hormonal and immunological measures. Cytotoxic and genotoxic effects of JP-8 exposure were also evaluated. Self-reporting health problems, health care visit frequency, and early indicators of liver and kidney damage were also investigated. In general all study subjects had received measurable exposure to JP-8. However, in preliminary studies no adverse health effects could be related to this exposure. Nevertheless, personnel who were studied were young and healthy and their duration of exposure was often less than 1 year.

This critical limitation was addressed in occupational assessment of exposure to JP-8 made at Warfield Air National Guard Base near to Baltimore, MD. Full-time air guard is exposed to jet fuel for longer periods of time and guard typically perform the same task. The age of personnel at this base was 41 ±9 years (US Air Force average age 26 years) and 65 study subjects were recruited. The number of endpoints studied was less than the Air Force study. Total exposure to the jet fuel was quantified using breath analysis. Health effects associated with exposure were measured using a neurocognitive testing battery and liver and kidney function tests. Breath analysis provided an

estimate of an individual's total JP-8 exposure that occurred via inhalation and dermal routes. Moreover, breath analysis was used to evaluate normal and abnormal physiology. All individuals studied on base exhaled the aromatic and aliphatic hydrocarbons found in JP-8. The most exposed subject had a breath concentration of 11.5 mg/m^3. This breath concentration suggested that acute exposure to JP-8 at this air guard base is much less than current permissible exposure guidelines and was also 2 orders of magnitude less than the exposure found during the previous Air Force study. This reduction in acute exposure to JP-8 is attributed to the improved safety practices and standard operating procedures carried out by air guard personnel. Highest exposures to JP-8 were found in fuel cell workers, fuel specialists and in smokers, who smoked outside downwind from the flightline. Although study-day exposures appear to be much less than current guidelines, chronic exposure at these low levels affected neurocognitive functioning. JP-8 exposed individuals performed significantly poorer than non-exposed control individuals, who had no exposure to jet fuel, on 20 of 42 measures of information processing and other cognitive functions. No evidences of hepatic or renal dysfunction that could be related to JP-8 exposure were observed.

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Participating Professionals

Terence H. Risby, Ph.D. Principal Investigator
Johns Hopkins University School of Hygiene and Public Health
Johns Hopkins University School of Medicine

W. Michael Foster, Ph.D. Co-Investigator
Johns Hopkins University School of Hygiene and Public Health

James F. Burdick, M.D. Co-Investigator
Johns Hopkins University School of Medicine

Shelley S. Sehnert, Ph.D. Co-Investigator
Johns Hopkins University School of Hygiene and Public Health

Clifford S. Mitchell, M.D. Investigator
Johns Hopkins University School of Hygiene and Public Health

Keary A.Cope, Ph.D. Former graduate Student
Johns Hopkins University School of Hygiene and Public Health

Raymond Tu, Sc.M. Former graduate Student
Johns Hopkins University School of Hygiene and Public Health

Jennifer Johnson, B.S. Minority Summer Student
Johns Hopkins University School of Hygiene and Public Health

Patrick N. Breysse, Ph.D. Consultant
Johns Hopkins University School of Hygiene and Public Health

Charles A. Rohde, Ph.D. Consultant
Johns Hopkins University School of Hygiene and Public Health

Robert J. Rubin, Ph.D. Consultant
Johns Hopkins University School of Hygiene and Public Health

Andrew C. Warren, M.D. Consultant
Johns Hopkins University School of Medicine

Mark Witten, Ph.D. Collaborator
University of Arizona School of Medicine

David Harris, Ph.D. Collaborator
University of Arizona School of Medicine

Scott Young, M.S. Collaborator
University of Arizona School of Medicine

Coupling Activities

As a result of our funded research in the field of breath analysis we have developed active collaboration with the following investigators. These collaborations have been directed towards different aspects of our research program. i). Collaborations aimed at the development of commercial systems for collection and analysis of breath biomarkers:

William Betz, Ph.D.
Supelco Corporation

Andrew Tipler, Ph.D.
Perkin Elmer Corporation

ii.) The following collaborators has been supplied with information on adsorbents for the collection of breath:

Garry Handelman, Ph.D.
Tufts University College of Medicine

Michael Davis, DVM, Ph.D.
Oklahoma State University College of Veterinary Medicine

Frank Tittel, Ph.D.
Rice University College of Engineering

Jochen Schubert, MD
Rostock University and Hospital

Nandor Marczin, MD
Harefield and Royal Brompton Hospitals

iii.) We have collaborated with the following researchers by performing breath analyses in mutually interesting research projects:

Joseph Rifkind, Ph.D.
National Institute of Aging

Edgar Miller, M.D.
Johns Hopkins University School of Medicine

Anna Mae Diehl, M.D.
Johns Hopkins University School of Medicine

Jonathon Orens, M.D.
Johns Hopkins University School of Medicine

Sean Studer, M.D.
Johns Hopkins University School of Medicine

James Eshelman, M.D.
Johns Hopkins University School of Medicine

iv.) We have coordinated our research on the effects of the jet fuel JP-8 through active collaboration with the following additional investigators funded by Air Force Office of Scientific Research or by Air Force:

Carol Barnes, Ph.D.
University of Arizona College of Medicine

Frank Witzmann, Ph.D.
Indiana University

Lt. Col Roger Gibson
Lt. Col. Donald Christensen
Maj. Brian Blazicko
Maj David Sonntag
Maj Leslie Smith
US. Air Force

Joachim Pliel
US Environmental Protection Agency

Stephen Rappaport, Ph.D.
University of North Carolina Chapel Hill

Members of the US Air Force JP-8 Task Force.

Discoveries, Inventions, Patent Disclosures, and Specific Applications

A patent has been issued during the course of this study that reports these results, US 6,248,078B1 June 19, 2001 "Volatile Biomarkers for Analysis of Hepatic Disorders." Funding for this invention was provided in part by this grant.

Research Accomplishments

Objectives: Subtle alterations in normal physiologic function that may result from chronic low-dose exposure to Jet-Propulsion Fuel 8 are the focus of the studies proposed in this grant application. During the first year, collaborative studies are proposed with investigators at the University of Arizona who are conducting mechanistic toxicological studies on nose-only exposure to aerosolized JP-8 in rodents (Fischer 344 rats).

Our contribution to their studies will be to: 1.) investigate whether the breath biomarker of oxidative damage of tissue, as measured by increased levels of breath ethane, is elevated as a result of nose-only exposure to JP-8 aerosol at two concentrations (500 $mg/m^3/hr$, 1000 $mg/m^3/hr$); 2.) investigate whether breath biomarkers of liver dysfunction are elevated, as measured by increased levels of breath sulfur-containing compounds, as a result of nose-only exposure to JP-8 aerosol at two concentrations (500 $mg/m^3/hr$, 1000 $mg/m^3/hr$); 3.) investigate whether novel biomarkers of injury dysfunction are produced as a result of nose-only exposure to JP-8 aerosol at two concentrations (500 $mg/m^3/hr$, 1000 $mg/m^3/hr$); 4.) evaluate the accuracy of breath biomarkers of exposure to estimate inhalation exposure to two concentrations (500 $mg/m^3/hr$, 1000 $mg/m^3/hr$); and 5.) integrate our findings of exposure, oxidant injury, liver, and other tissue dysfunction with the concurrent mechanistic toxicological studies performed in the same rats.

The results from the studies in rats will determine the design of the studies proposed in years two and three in personnel who have occupational exposure to JP-8 at Dover Air Force Base (AFB) and at the Montana Air Guard Base (AGB). The goal of this aspect of research is to investigate whether assessment of the risk to human health posed by realistic occupational levels of jet-propulsion

fuel-8 can be made rapidly and non-invasively using breath biomarkers of exposure and effect. Specifically, studies are proposed to: 1.) investigate whether the breath biomarker of oxidative damage of tissue, as measured by breath ethane, is elevated as a result of an 8 hour exposure to occupational levels of JP-8; 2.) investigate whether the breath biomarker of repair of damage, as measured by breath isoprene, is induced as a result of previous exposure to occupational levels of JP-8; 3.) investigate whether CNS toxicity, as measured by changes in airway occlusion pressure, occurs as a result of an 8 hour exposure to occupational levels of JP-8; 4.) investigate whether breath biomarkers of liver dysfunction, as measured by breath sulfur-containing compounds, are elevated as result of previous occupational exposure to JP-8; 5.) investigate whether breath biomarkers of other tissue dysfunction are elevated or depressed as a result of occupational exposure to JP-8; 6.) evaluate total body exposure to JP-8 using breath biomarkers of exposure; and 7.) integrate our findings of exposure, oxidant injury and repair, dysfunction of liver and other tissues with studies performed by collaborators at the University of Arizona and at various laboratories of the US Air Force.

These objectives will be achieved by collecting and analyzing exhaled breath from rodents and Air Force (Guard) personnel and correlating them to standard clinical end-points:

Year 1 Studies are proposed in rodents. These studies will be performed at the University of Arizona.

Years 2 & 3 Studies are proposed with human volunteers. These studies will be performed at the Dover AFB in Dover, Delaware and at the Montana AGB in Great Falls, Montana.

When the research plan was originally submitted, we had commitments to study personnel from Dover AFB in Dover, Delaware and from the Montana AGB in Great Falls, Montana. This plan was changed as a result of the funding of the multi-investigator study of seven Air Force Bases coordinated by Texas Tech University. Montana AGB in Great Falls, Montana was replaced by Warfield AGB in Baltimore MD. This change was due to experience gained in the AFB study that showed occupational

assessment requires extensive time be spent on base and funds were not sufficient to support this time commitment in Montana. This problem was discussed with the AFOSR Program Manager and other US Air Force personnel and these changes were viewed to be desirable.

Accomplishments:

In the past four and a half years research has been conducted in a number of areas: breath collection; breath collection media; breath analysis; breath biomarkers of: oxidative stress status, cholesterol biosynthesis, hepatic dysfunction, renal dysfunction, and exposure assessment; and the evaluation of tests that could be used to assess changes in neuropsychological function as a result of exposure to JP-8.

Breath Collection

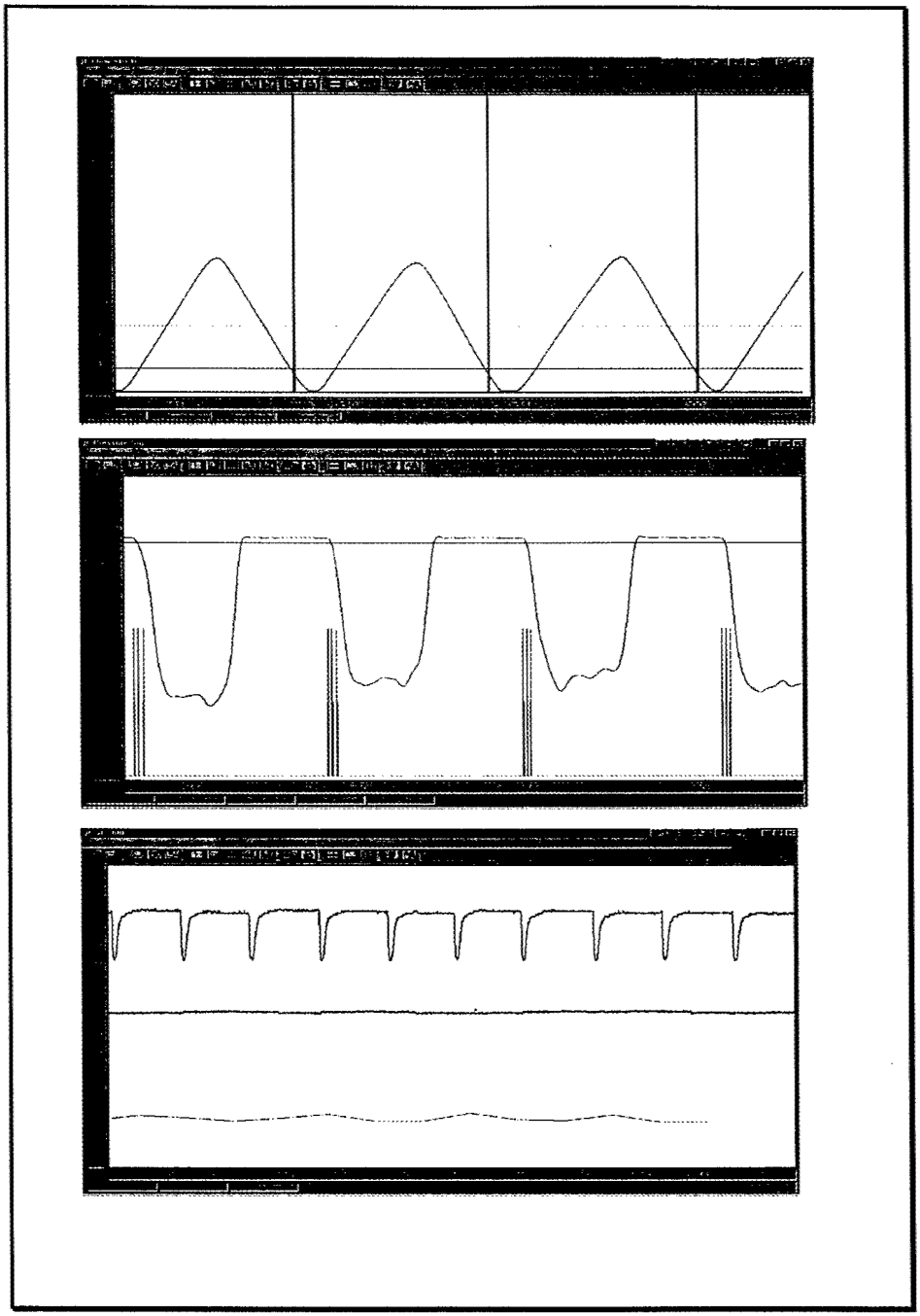
a. Laboratory Rodents:

A specially designed flow-through glass chamber (shown below) was used for all studies. This chamber enables exhaled breath from rodents to be sampled in the outflow gas from the chamber. In order to avoid stressing the rodent the concentration of carbon dioxide in the outflow gas of the chamber is monitored continuously. Additionally, turbulent flow of air through the chamber is maintained to ensure that the gas collected at the outflow is representative. In order to use this chamber for collection of respiratory gas, the rodent is initially equilibrated to hydrocarbon free air by high flow of gas through the chamber. After this period of equilibration (generally 50 volume changes) the air flow rate is reduced and the outflow gas from the chamber is collected for measurement. Typically, during sample collection the concentration of carbon dioxide in the outflow gas is maintained below 7 Torr. The ability to collect rodent breath using this chamber has been demonstrated and, in addition, physiological measurements of oxygen consumption and carbon dioxide production have agreed with published values.

b. Human Subjects:

Two approaches were developed to collect samples of breath from spontaneously breathing human subjects. The first involves the collection of breath over a period of one minute seated relaxed subject is breathing tidally. The subject receives visual aids from a computer screen that displays breathing frequency, tidal volume, inspiratory mouth pressure and carbon dioxide profiles. A respiratory workbench that is microprocessor-controlled and can be changed to meet the comfortable respiratory requirements for each study subject has been developed. Parameters such as the respiration rate, tidal volume, end-tidal carbon dioxide concentration, minute ventilation, and average carbon dioxide concentration are recorded continuously during breath collection. The ratio of average carbon dioxide concentration to end-tidal carbon dioxide concentration is used to evaluate the anatomic dead-space and evaluate the breathing of the study subject. If this ratio falls below 50% the breath collection is repeated. This system uses commercially available modules. Briefly, the study subject inspires and expires through a disposable mouthpiece and in-line biological filter into the mouth port of a non-rebreathing valve. The inspiratory port of this non-rebreathing valve contains a pressure sensor that is used to trigger the computer program to each breath cycle. The inspiratory port also includes a balloon valve that allows the inspiratory port to be occluded under computer control. During occlusion, the decrease in pressure is measured by sensor. Occlusion pressure is simply the pressure measured in the airway of a spontaneously breathing subject after the airway has been occluded just at end-expiration. The occlusion pressure has been widely used to evaluate neuronal output of the respiratory centers of the brain. The inspiratory air is filtered with a disposable charcoal respiratory cartridge. This approach was found to produce a minimum backpressure on the inspiratory port of the non-rebreathing valve. It has been established that these respiratory cartridges can remove airborne contaminants from the inspired air for at least 30 minutes although a new cartridge is used for each study subject. Study subjects have been found to require approximately 5 minutes of training to use this system and during this training time an effective washout of exogenous pollutants from ambient air is achieved.

Spontaneous, relaxed breathing is sampled for a period of 1 or 3 minutes depending upon the method of breath collection. The coefficient of variation for breath samples has been found to be less than 5%. Examples of the system in use and the computer screens for pressure, flow and carbon dioxide are shown below. These are the displays that study subjects view as aids to control breathing patterns. This technique represents a significant advance in the science of precision breath analysis.



Breath Collection

Two methods for sampling breath have been examined during the course of this research. Each method has distinct advantages.

a. Collection on Thermal Desorption Tubes:

Collection of breath samples using commercial multibed adsorbents packed in thermal desorption tubes was then examined since this method of breath collection is ideally suited for field studies. Commercial thermal desorption tubes have unique numbers engraved on the tube that can be used to track each sample. We evaluated a number of adsorbents for their ability to collect the molecules of interest in exhaled breath from the background matrix of high concentrations of water vapor and carbon dioxide. The ideal adsorbent should allow sufficient breath to be sampled so that the signal-to-noise ratio of the analyte molecules is greater than 3:1.

Two different of adsorbent beds were developed during this research: the first design was sequential equal beds of a non-porous graphitized carbon (Carbopack X) a followed by a bed of carbon molecular sieve (Carboxen 1018). The graphitized carbon is weak adsorbent with a surface area of 240 m^2/g that adsorbs all compounds greater than about C_4 or C_5. Whereas the carbon molecular sieve has a surface area of 700 m^2/g and is a strong adsorbent with both meso- and micro-pores that adsorbs compounds in the range C_1 through C_5. The second design was identical except that it includes an additional bed of another carbon molecular sieve (Carboxen 1021). This carbon molecular sieve has a surface area of 650 m^2/g and is the strongest adsorbent developed to date and contains only micro-pores. Breath was drawn through the weak adsorbent bed followed by the strong adsorbent bed(s) packed sequentially in the same tube. Differing amounts of these adsorbent beds were examined. At the time of this report, we feel that the optimum packings are either 50% Carbopack X and 50% Carboxen 1018 or 34% Carbopack X, 33% Carboxen 1018 and 33% Carboxen 1021. The first design of

adsorbent bed has been used to collect breath samples that include JP-8 whereas the second design is the optimum for all other types of breath collection. The breath molecules collected by these adsorbent systems are subsequent thermally desorbed and analysis by capillary gas chromatography.

Other variables examined were the sampling rate and the volume sampled. Breath is drawn through the thermal desorption tubes using a battery-operated commercial two-tube sampler. This sampler consists of a rechargeable battery, vacuum pump, programmable controller that controls the sampling time. The flow at each of the two-tubes can be controlled independently. On the basis of studies to date the optimum flow rate has been found to in the range of 20 ml/min with an optimum sampling time of four minutes. However, this length of sampling for human subjects was found to be too long. Subjects were unable to maintain paced tidal breathing for four minutes and therefore breath was sampled at 80ml/min for 1 minute. In all cases duplicate samples are obtained.

After the sample has been collected, the thermal desorption tube is capped with Swagelok fittings and stored until it is analyzed. Research has shown that breath and air samples are stable in thermal desorption tubes for at least a year, although all samples are analyzed as soon as possible. Capped breath samples can be transported by commercial air carriers without any degradations of samples..

b. Sampling restricted breathing maneuvers with real-time monitors:

Additional breath markers, nitric oxide, carbon monoxide, and total volatile sulfur compounds, were quantified using real-time monitors. A restricted breath maneuver was used to prevent contamination of the breath from the oral-nasal-pharyngeal cavity. This was accomplished by having participants exhale at a rate of 50 ml/s (200 ml/min for CO) through a critical orifice while maintaining a constant mouth-pressure of 10 cm H_2O. Subjects were asked to completely exhale to their expiratory reserve volume and breath is sampled continuous by the dedicated monitor. The subject is prompted

by either a computer screen or a series of lights to maintain the required mouth pressure. The carbon dioxide profile is also monitored to ensure that the end-tidal portion of the breath cycle is sampled.

Breath analysis

a. Two Stage Thermal Desorption:

Analysis of breath samples was performed using a commercial two-stage thermal desorption system (Perkin Elmer ATD 400 or TurboMatrix) that was coupled to either a gas chromatograph with parallel flame ionization, and flame photometric detectors or to a gas chromatograph with parallel thermionic and electron impact mass spectrometric detectors.

The automated two-stage thermal desorption system can handle up to 50 samples sequentially. The process by which the gas sample that was collected in the field is analyzed by capillary gas chromatography is as follows: the long-term storage Swagelok caps that were placed on each thermal desorption tube after the sample had been collected are removed and replaced by polytetrafluoroethylene end caps. The resulting thermal desorption tubes are inserted into 50 tube carousel and the analysis is performed automatically. The two-stage thermal desorption system takes each sample tube in turn, and uncaps it in the carrier gas stream. It will perform a leak test to ensure that the tube is sealed correctly and then heats the tube to 300°C for fifteen minutes with the carrier gas flowing through the multibed adsorbents. Thermal desorption is performed with the flow in the opposite direction to the flow used originally to adsorb the sample. This reverse flow ensures quantitative thermal desorption of the adsorbed molecules. The thermally desorbed molecules are concentrated in a low thermal mass cold trap (-30°C) containing small amounts of the same carbonaceous adsorbents. The cold trap is heated rapidly to at a rate of 40°C/s to 200 °C and the thermally desorbed molecules are transferred through a heated fused silica transfer line to the head of a fused silica capillary column.

Separation of the components of breath are performed using capillary gas chromatography using 0.32 mm. 60 m fused silica capillary columns wall-coated with 5μm dimethyl silicone. The separation protocol is as follows: isothermal at 35°C for ten minutes, 35-210 at 5°C/min, isothermal at 210°C for ten minutes. A linear gas velocity of 25 cm/sec at 25°C is used. The available detectors enable all the analyte molecules of interest to be quantified. These two-stage thermal desorption capillary gas chromatographic systems are microprocessor controlled and data are stored digitally.

b. Real-time breath monitors:

Nitric oxide was analyzed *via* a chemiluminescent reaction with ozone using a dedicated monitor (280 NOA, Sievers Instruments). This monitor is under computer control and the analyte signal and the mouth pressure are displayed as a function of time. Single breath maneuvers were performed in triplicate and average of the three measurements of the nitric oxide concentration was archived. Carbon monoxide was analyzed using a membrane electrode (LR250, Logan Research Limited). This monitor is also under computer control and the analyte signal and the mouth pressure are displayed as a function of time. Single breath maneuvers were performed in triplicate and average of the three measurements of the carbon monoxide concentration was archived. Total volatile sulfur-containing compounds in breath were determined using a membrane electrode (Halimeter RH-17 Series, Interscan Corporation). This monitor was modified for these studies. A visual monitor consisting of light diodes was designed and built to allow the subject to maintain the required mouth pressure. Single breath maneuvers were performed in triplicate and average of the three measurements of the total volatile sulfur-containing compounds concentration was archived.

Breath Biomarkers

During the course of this research, breath biomarkers of exposure, susceptibility and disease have been examined in human subjects and laboratory rodents.

Biomarkers of Susceptibility and Disease

a. Oxidative Stress Status

Oxidative stress status was investigated since antioxidant deficiency is a determinant of susceptibility to damage by reactive oxygen species (ROS). Increased concentrations of ROS can be produced directly or indirectly as a result of exposure to pollutants. Also increased levels of ROS are involved in the pathogenesis of most disease states. The following are some results that were obtained during the course of our studies:

Levels of breath ethane were statistically higher in smokers than non-smokers demonstrating the pro-oxidant effects of the xenobiotic cigarette smoke. Also increased levels of breath ethane in smokers may signal antioxidant deficiency and be a biomarker of susceptibility to additional oxidant damage;

Levels of breath ethane can be correlated to the time between smoking a cigarette and of breath collection. Ideally smokers should be excluded from studies that quantify breath ethane since smoking has such a major effect on breath ethane;

Increased levels of cellular antioxidants can be produced by taking vitamin pills or by eating increased fruits and vegetables. Increasing cellular antioxidants statistically reduced the levels of breath ethane in the same individual.

b. Cholesterol Biosynthesis

Breath isoprene is biomarker of the biosynthesis of cholesterol. Cholesterol biosynthesis is induced as part of the physiological response to oxidant damage. The following is a summary of our results to date:

Levels of breath isoprene show a quadratic dependence with age in all human subjects *i.e.*, they increase from below detectable at birth, to detectable levels at about six months, reach a plateau in the mid-forties and decline after 50 years of age;

Males have higher levels of breath isoprene compared to age-matched females, this difference is less pronounced after 50 years of age;

Levels of breath isoprene are increased in response to damage.

c. Hepatic Dysfunction

The liver is often the target organ of damage by exposure to pollutants. As a result of research that has been supported by this study we have identified biomarkers of hepatic dysfunction. Briefly, the results of our studies are as follows:

Breath carbonyl sulfide is a biomarker of liver dysfunction and can also be used to stage liver disease;

Breath dimethyl sulfide is a biomarker of liver dysfunction and can also be used to stage liver disease;

Breath ethane is elevated as a result of pathophysiology that accompanies liver disease.

d. Renal Dysfunction

The kidney is often the target organ of damage by exposure to pollutants. As a result of research that has been supported by this study we have identified biomarkers of renal dysfunction. Currently there are no analytical methods that are suitable for the determination of ammonia or volatile organic amines. This limitation is due to the reactivity of these species. Plans are in progress to develop real-time monitors based upon laser spectroscopy. However, to date we have evidence that:

Breath ammonia and volatile organic amines are biomarkers of renal dysfunction;

Breath acetone is a biomarker of renal dysfunction;

Breath ethane is elevated as a result of pathophysiology that accompanies renal disease.

e. Obesity.

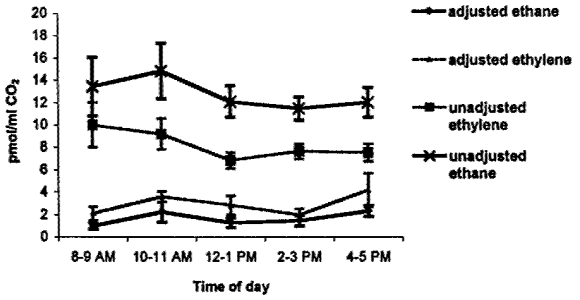
Studies performed in genetically obese mice and their lean littermates have shown that obese mice have statistically significant more ethanol in their breath than their lean littermates. Additionally, this concentration of ethanol can be reduced by treatment with poorly absorbed antibiotics. These results suggest that intestinal flora is the source of this ethanol. Another major that may play a role with gut bacteria is gut motility. Since obesity has been shown to be associated with decreased gut motility the combination of gut motility and intestinal flora may be significant to the genesis of obesity-related fatty liver. These results in laboratory rodents have been confirmed in studies of obese humans. Obese human subjects were found to have statistically more breath ethanol than age matched controls. Moreover, females were found to have more breath ethanol than age-matched males. The basis for this gender difference is currently unknown. The significance of these results is that obesity may represent a significant risk factor in the development of liver disease. Obese individuals may be susceptible population to the effects of exposure to pollutants.

f. Diurnal variations of breath biomarkers.

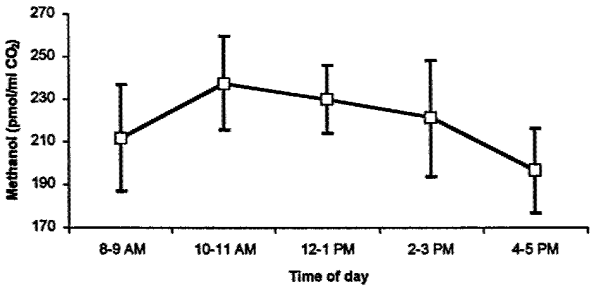
Repeated breath samples were collected from a group of non-diseased males and females to investigate whether breath profiles are different due to gender, or due to diurnal variation. The major difference for this study *versus* all previous studies was that breath samples were collected while each subject was maintaining paced tidal breathing. The following figures shown that the only breath biomarker that varies diurnally was ethanol and the source of this molecule has been shown in earlier studies to be intestinal bacteria. The maximum signal was observed after lunch. This was the first meal that some of the study subjects had eaten the day of study. There was a gender variation in the breath

isoprene and total breath sulfur-containing compounds. The major conclusion of this study was that breath samples collected at various times of the day could be compared for a given individual.

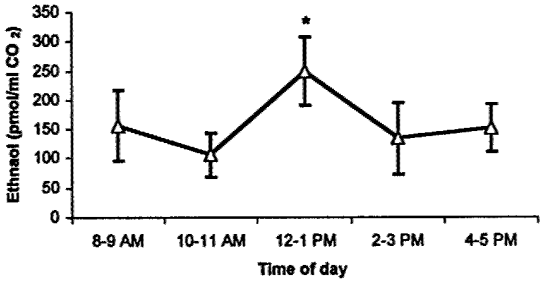
Diurnal variations in breath ethane and ethylene



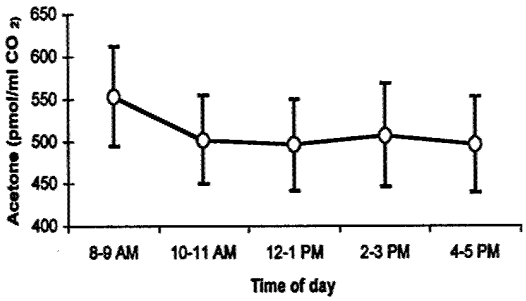
Diurnal variation in breath methanol



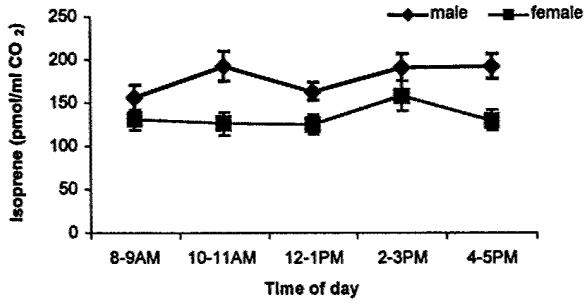
Diurnal variation in breath ethanol



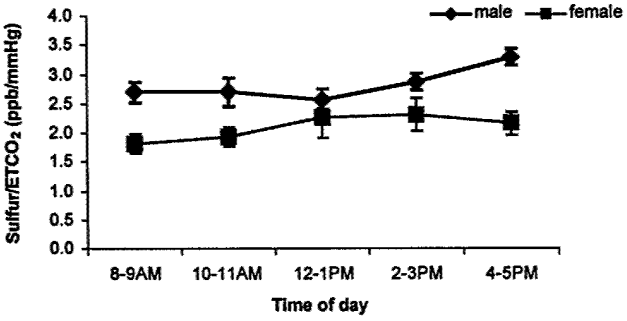
Diurnal variation in breath acetone



Diurnal variation in breath isoprene



Diurnal variation in total breath sulfur–containing compounds



e. Effects of exercise on breath biomarkers.

Healthy human subjects were recruited to undergo maximal upright bicycle exercise using an electronically controlled cycle ergometer at increasing levels of resistance. The initial workload was set at 25 watts and was increased by 25 watts every 3 minutes. During exercise the following parameters were recorded continuously: electrocardiograms, cardiovascular variables including cardiac output (using gated radionuclide ventriculography), oxygen consumption, carbon dioxide production, minute ventilation (using a metabolic cart), heart rate, and respiratory exchange ratios. Blood pressure was

monitored every 90 seconds. Breath was sampled for a one-minute interval for the last minute of each exercise stage.

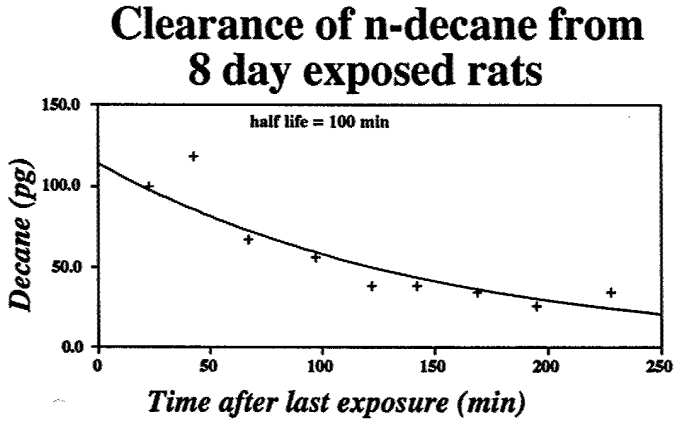
Exhaled breath biomarkers were examined as a function of exercise. Breath biomarkers were also examined as a function of normalization. Breath biomarkers increased with exercise if expressed in units of concentration, if expressed as a function of the minute ventilation rate or if expressed as a function of cardiac output. However, if breath biomarkers were normalized to oxygen consumption or carbon dioxide production then the effects of exercise were negated. Breath acetone biomarker was the only exception to this observation. Breath acetone was found to show a quadratic dependence and increased when the subject went anaerobic. This result suggests that breath acetone could be used to signal the point in an exercise protocol when a subject is burning fat. The main conclusion from this study is that breath should be normalized to oxygen consumption or to carbon dioxide if exercise is involved.

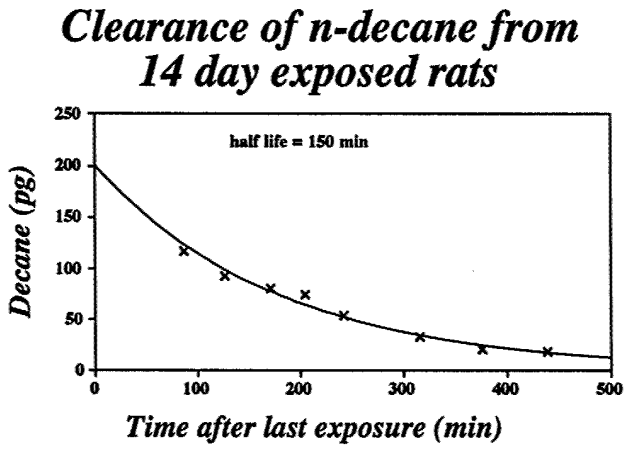
Biomarkers of exposure

Exposure assessment to JP-8.

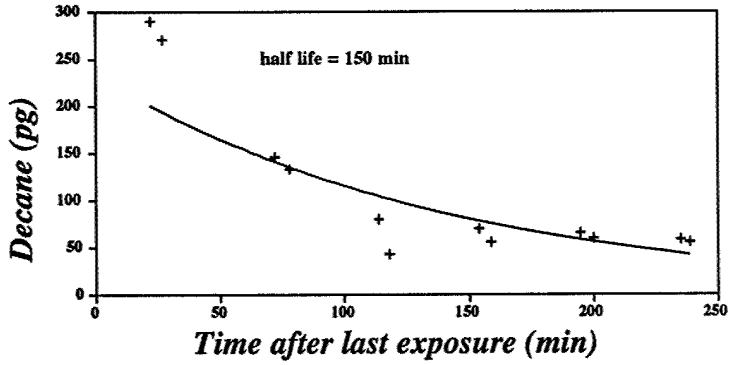
***In vivo* exposure to laboratory rodents to jet fuel:** The results of the exposure studies in rats are extremely interesting and were not as predicted. The data obtained for the rats exposed for 21 days to JP-8 are completely different from the data obtained for all the other exposure studies. The JP-8 vapor/aerosol collected during the last day of exposure of the 21-day exposure contained no light hydrocarbons whereas for comparable samples collected during all other exposures the chromatographic fingerprints of JP-8 were similar and contained the expected light hydrocarbons. The exhaled breath collected from the 21day exposed rats was the same as the JP-8 used in exposure but similarly different from the JP-8 used on the other exposures. Extensive discussions with Dr. Witten of the University of Arizona have taken place aimed at explaining these differences but no

satisfactory explanations have been proposed. Humidity is known to vary between winter and summer. The 21-day exposure was performed during the winter whereas all the other studies were performed during the summer. Clearance data shown below suggest that the excretion of the light hydrocarbons is saturated sometime between 8 and 14 days. Clearance remains constant after 14 days. The rate of clearance of light hydrocarbons ($< C_{11}$) is very different from the rate of clearance of the non-light hydrocarbons ($> C_{11}$). The latter molecules remain in the exhaled breath at relatively constant amounts post-exposure. Therefore estimating exposure based upon volatile versus non-volatile hydrocarbons may be different.





Clearance of n-decane from 58 day exposed rats

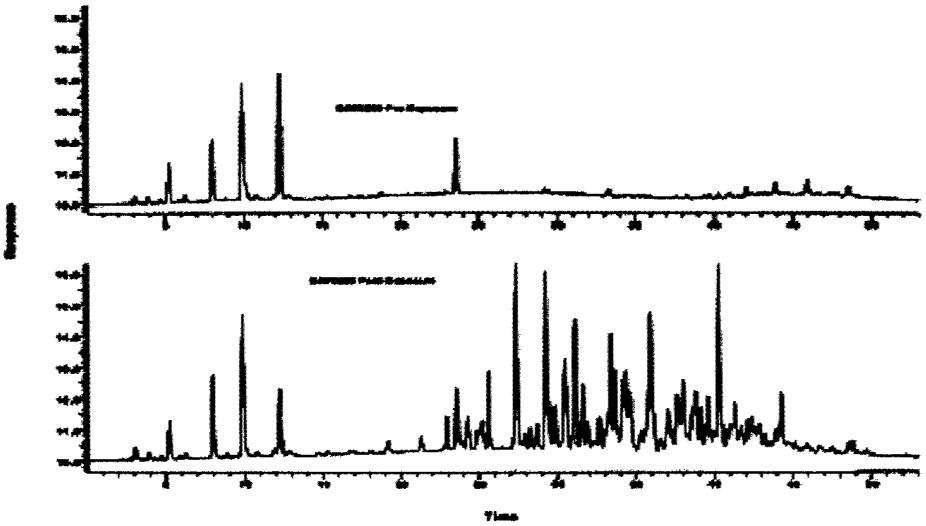


No evidence of liver or renal dysfunction was observed from exhaled breath measurements. However, evidence of lipid peroxidation was observed for 21 and 58 days exposures. The extent of lipid peroxidation was correlated to the amount of JP-8 being excreted in the breath.

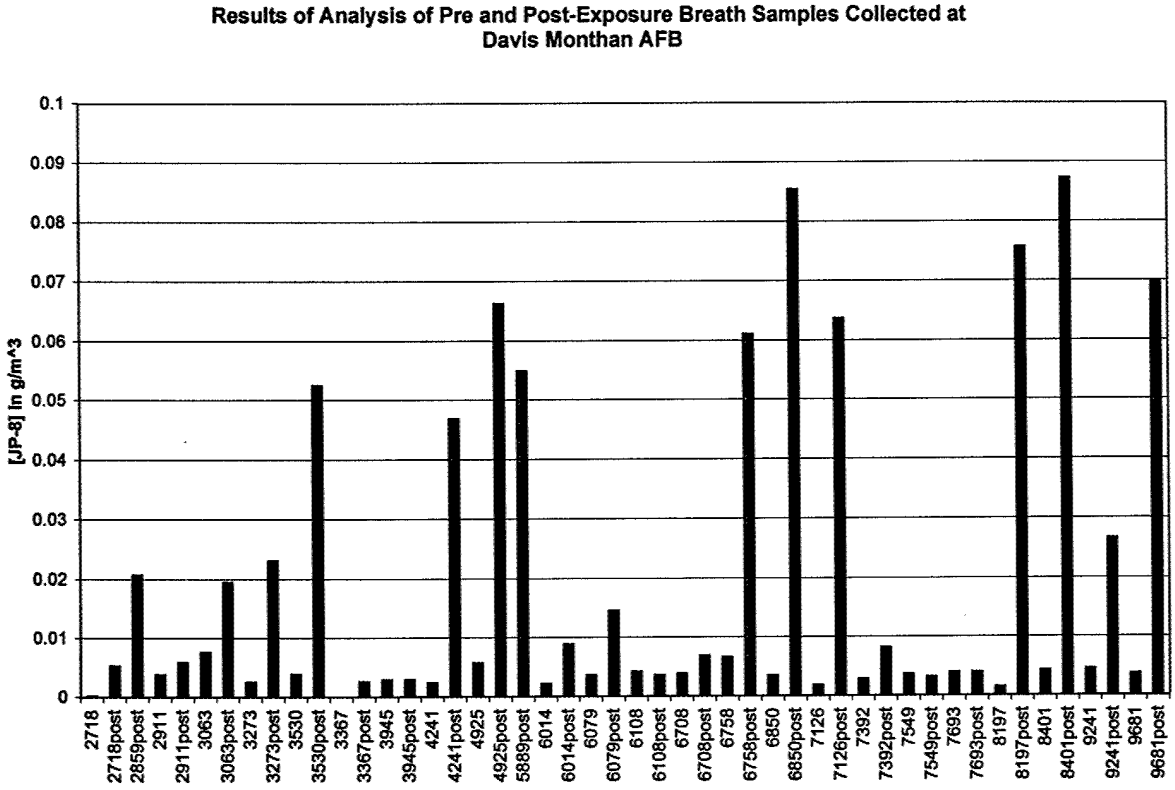
Human exposure to jet fuel: a: the Air Force acute study directed by Lt. Col. Roger Gibson. JHU investigators collaborated with the Air Force acute study by sampling breath from study subjects at three Air Force Bases. Breath samples were collected from study subjects at Davis-Monthan (n=25), Langley (n=50), and Little Rock (n=42) AFBs. The breath of nominally unexposed subjects at the end of the study period at Davis-Monthan and Langley AFB had very similar concentrations of JP-8 profiles (mean 3.7 ± 1.8 mg/m^3). The breath of nominally unexposed subjects at Little Rock AFB had higher concentrations of JP-8 profiles (mean 5.6 ± 3.5 mg/m^3). The mean JP-8 concentration of all study subjects at the start of the study period at Davis-Monthan was 3.7 ± 1.2 mg/m^3. In all these subjects, the majority of the JP-8 profile is derived from the least volatile components of JP-8. Since the concentrations of JP-8 in breath samples collected pre-and post-exposure from "nominally unexposed subjects" were similar at Davis-Monthan AFB only post-exposure samples were collected at Langley AFB, and Little Rock AFB. The mean breath

concentrations of JP-8 for the "nominally exposed subjects" at Davis-Monthan, Langley AFB, and Little Rock AFB were 40.1 ± 29.0 mg/m^3, 13.5 ± 18.6 mg/m^3 and 17.1 ± 13.7 mg/m^3 respectively. The ranges of levels of the concentration of JP-8 varied significantly between "nominally exposed subjects" at each AFB. Moreover, the route of exposure appeared to be greater from inhalation at Davis-Monthan and Little Rock AFB as compared to Langley AFB. The results from the analyses of exhaled breath collected during this study demonstrate conclusively that Air Force personnel are exposed to the jet fuel, JP-8. Moreover, even the samples of breath collected from "nominally unexposed subjects" were found to contain quantifiable levels of JP-8. Additionally, the exhaled breath of nominally exposed subjects who were performing similar duties had widely varying concentrations of JP-8. These significant differences may be a reflection of the actual duties performed during the study period or the effective use of protection devices by some of the study subjects.

The following figure is an example of exposure that was observed in a subject during air force study. This exposure was typical for exposed subjects.



The following figure shows the amounts of JP-8 in pre- and post-breath samples collected at the Davis Monthan AFB.



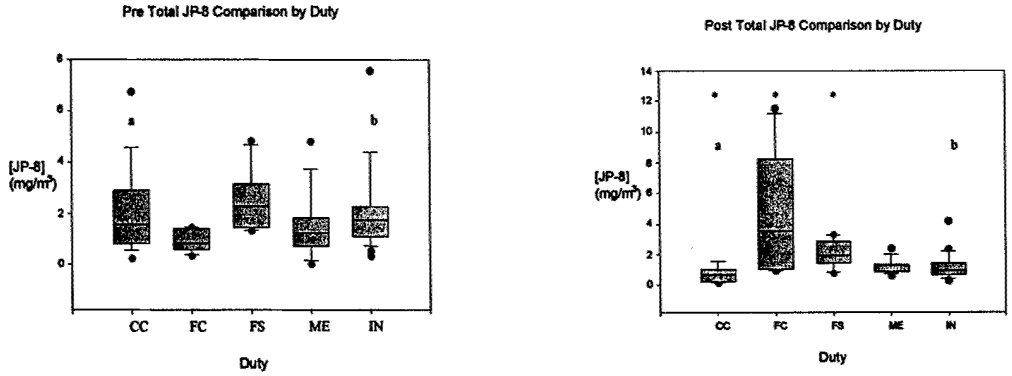
b: the Air Guard Study at Warfield AGB in Baltimore Maryland. Sixty- three completed the one-day study. The requirements for participation were to:

1. Provide breath samples before work (pre-sample)
2. Provide breath samples after work (post-sample)
3. Provide a blood sample
4. Provide a urine sample
5. Take a computerized test of neurocognitive functions
6. Complete and return a questionnaire that documented personal activities including tobacco and alcohol use and self-reporting exposure-related symptoms.

Study subjects were grouped by the duty performed on base. Subjects in direct contact with fuel during normal operations were fuel cell workers (n=6), fuel specialists (n=6), aircraft/ground

equipment mechanics (n=10), and crew chiefs (n=13). Incidental workers (n=28), i.e., individuals less likely to be in direct contact with fuel, were supply workers, environmental officers, and people working with non-fuel aspects of the aircraft like structural maintenance, ejection systems, and electricians. Most of the subjects were observed during their study-day while performing their normal duties to document the tasks they performed and to record safety practices and equipment used.

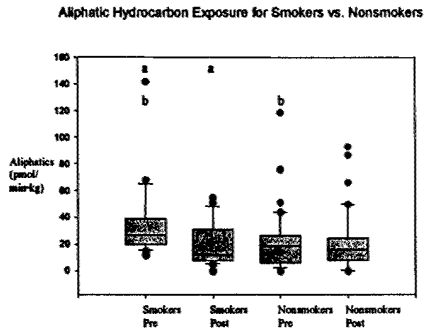
Total JP-8 exposure was determined by analysis of pre- and post- breath samples collected from each subject shown below:

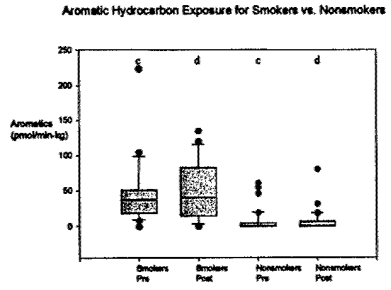


Crew chiefs, fuel cell workers, fuel specialists, and mechanics all had comparable pre-concentrations of total JP-8. Crew chiefs and incidental subjects showed significant decreases from their pre- to post-breath concentrations. Fuel specialists and fuel cell workers had significantly higher post-breath concentrations of total JP-8 than did the crew chiefs.

To determine the effect of smoking on exposure levels, results for smokers were compared to nonsmokers. Smokers were present in all five duty-categories and when grouped together, they showed significantly greater exposure to total JP-8 and JP-8 volatiles in both their pre- and post-breath samples compared to nonsmokers. Smokers and nonsmokers had comparable concentrations of

aliphatic hydrocarbons, whereas, smokers had three to four times more aromatic hydrocarbons in exhaled breath than did nonsmokers see below.





Exhaled breath concentrations of ethane, isoprene, carbon monoxide, nitric oxide, and total sulfur compounds were compared as a function of duty. There were no significant differences for these breath biomarkers between the five duty groups. When smokers were grouped and compared to nonsmokers, there were several significant differences in these breath biomarkers. Smokers had significantly more ethane in exhaled breath in their pre-breath samples than did nonsmokers. Smokers had a three to five times higher concentration of carbon monoxide in exhaled breath than nonsmokers. Smokers as a group had a lower level of nitric oxide in exhaled breath than nonsmokers and also had a significantly higher concentration of total sulfur-containing compounds.

Although there was some variability in liver function and urinalysis tests, these were not statistically related to JP-8 exposure. Neurocognitive functioning of subjects exposed to JP-8 were characterized at the end of the workday using a commercially available, computer-administered, standardized test battery, CogScreen-Aeromedical Edition (AE). This test battery is used to detect subtle changes in cognitive functioning that are predictive of changes in 'real-world' functioning. This test is composed of 12 different subtests and generally takes about one hour to complete. Data

collected from these subtests include measures of response speed, accuracy, efficiency and other test-specific metrics: RTC (response time for correct responses)=speed; ACC=accuracy; PUT=throughput; ERR=error; PRE=number of premature responses during visual monitoring task; RUL=ability to identify rules in a reasoning task and HIT=number of boundary hits in a tracking test. Practice sessions were provided within the tests to ensure that subjects understood directions. CogScreen data for a non-JP-8 exposed age- and education-matched control sample were used as the comparison standard for evaluating the CogScreen results obtained from the study subjects. A brief description of the subtests is shown below:

CogScreen AE subtest	Description of task	Abilities Measured
Backward digit span (BDS)	Reproduce sequence of visually presented numbers in reverse order	Visual attention, working memory, and verbal-sequential processing
Math	Multistep problem solving on a 10^{th} grade difficulty	Computation math skills, concentration, reading comprehension, logical reasoning
Visual sequence comparison (VSC)	Compare two simultaneously presented series of letters and numbers to determine if identical or not	Verbal-sequential processing, visual-perceptual speed, visual attention
Symbol digit coding (SDC)	Digits are substituted for symbols using a key and subjects perform immediate and delayed recall of pairs	Memory, visual-scanning, attention
Matching-to-sample (MTS)	Determine if two 4-by-4 pattern of colored squares match	Spatial processing, visual-perceptual speed, visual-working memory
Manikin (MAN)	Determine in which hand a rotated human figure is holding a flag	Spatial orientation, visual-spatial perception
Divided attention test (DAT)	React when vertically moving bar crosses either an upper or lower boundary	Reaction time, impulsivity
Multitask exercise with VSC and DAT		
Auditory sequence comparison (ASC)	Compare two series of tone sequences	Auditory attention, working memory, sound pattern discrimination
Pathfinder (PF)	Sequence numbers, letters, and alternating sequence of numbers and letters in alphabetical or numerical order	Motor coordination, visual scanning, sequencing skills
Shifting attention test (SAT)	Test-taker learns 3 rules individually and must use deductive reasoning to identify rule and apply it until feedback indicates rule has changed	Concept formation, deductive reasoning, mental flexibility
Dual task (DTT) First performed separately then together	1^{st} is visual motor tracking test and 2^{nd} is recalling a previously viewed number	Sustained attention, visual-motor tracking, working memory

CogScreen performance was compared across duty groups and to a group of age- and education-matched control subjects. When the study subjects were compared to controls, individuals exposed to JP-8 performed worse on 20 of 42 tests. Reaction times were faster on two of the measures for the study group, though the faster reaction times were associated with greater impulsivity. In addition, the study group was more accurate than the controls on a symbol substitution test, apparently because of a slower rate of task performance. In general, exposed workers had slower reaction times than controls, no matter which duty group was tested. The duty groups were also compared to each other in order to determine if one particular duty group performed poorer than another. Fuel cell workers and fuel specialists were combined into the duty group "fuel workers" to increase the statistical power of our analysis. Crew chiefs did worse on 2 tests, fuel workers did worse on 4 tests, mechanics did worse on 7 tests, and incidental workers did worse on 1 test when compared to the other duty groups. These tests included measures of speed, accuracy, efficiency, and other test-specific metrics like premature responses and visual tracking errors. A summary of the results is shown below.

Comparisons of performance on CogScreen subtests among the various groups

Test	Crew chiefs	Fuel workers	Mechanics	Incidental	Warfield	Controls
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
MATHRTC	34.5 (15.1)	35.2 (15.7)	34.1 (12.9)	29.5 (11.5)	32.3† (13.3)	24.2 (6.0)
VSCRTC	2.64 (.71)	2.91 (.61)	**3.16*** (.91)	2.57* (.68)	2.74† (.73)	2.27 (.67)
MTSRTC	1.45 (.28)	1.43 (.25)	1.40 (.23)	1.44 (.27)	1.43† (.26)	1.29 (.23)
MANRTC	2.05 (.41)	2.12 (.51)	1.92 (.56)	1.92 (.58)	1.98 (.53)	1.84 (.42)
DATSCRTC	2.49 (.97)	**2.81*** (.84)	2.38 (.68)	2.21* (.59)	2.41 (.76)	2.13 (.49)
DATIRTC	**.38*** (.05)	.34 (.09)	.36 (.08)	.31* (.08)	.37 (.19)	.47† (.11)
DATDRTC	.70 (.19)	.82 (.24)	.84 (.50)	.69 (.35)	.74 (.33)	.81† (.20)
ASCRTC	.72* (.17)	.77 (.16)	**.88*** (.12)	.78 (.78)	.78 (.16)	.74 (.24)
PFNMRTC	.97 (.15)	1.03 (.25)	1.01 ((.23)	1.01 (.27)	1.07† (.58)	.72 (.18)
PFLMRTC	.87 (.14)	.94 (.23)	.93 (.22)	.92 (.21)	.92† (.20)	.64 (.18)
PFCMRTC	1.21 (.27)	1.93 (1.57)	1.59 (.65)	1.42 (.45)	1.50† (.81)	1.08 (.32)

SATADRTC	.70 (.16)	.69 (.10)	.70 (.08)	.68 (.10)	.69[T] (.11)	.55 (.1)
SATACRTC	.77 (.11)	.72 (.07)	.78 (.14)	.74 (.12)	.75[T] (.12)	.53 (.09)
SATINRTC	.92 (.16)	.89* (.20)	**1.11*** (.47)	.93* (.18)	.95[T] (.25)	.71 (.12)
SATDIRTC	1.18 (.49)	1.24 (.46)	1.13 (.19)	1.12 (.23)	1.16[T] (.34)	.78 (.2)
DTTPNRTC	.59* (.28)	.76 (.37)	**.99*** (.59)	.58* (.34)	.68[T] (.40)	.47 (.24)
DTTPNDRTC	.67 (.33)	.80 (.24)	.82 (.45)	.72 (.34)	.74 (.34)	.67 (.24)
DTTAAERR	16 (12)	14 (13)	23 (16)	25 (19)	21 (16)	23 (17)
DTTDAERR	70 (25)	62 (24)	73 (24)	69 (26)	69[T] (25)	51 (23)
BDSACC	68 (19)	76 (20)	72 (19)	72 (20)	72[T] (20)	79 (21)
MATHACC	49 (31)	48 (38)	64 (34)	65 (25)	58[T] (31)	81 (22)
VSCACC	97 (5)	96 (4)	97 (4)	96 (5)	96 (5)	98 (3)
SDCACC	99 (2)	**98*** (3)	100 (1)	99* (1)	99 (2)	98[T] (4)
SDCIRACC	86 (23)	72 (35)	75 (28)	73 (30)	76 (29)	82 (25)
MTSACC	93 (8)	93 (10)	94 (6)	93 (6)	93 (7)	94 (5)
MANACC	89 (14)	92 (9)	82 (20)	85 (21)	87 (18)	89 (13)
DATSCACC	90 (15)	94 (9)	86 (15)	88 (11)	89 (12)	89 (7))
ASCACC	92 (7)	93 (8)	90 (9)	89 (10)	90 (9)	88 (8)
PFNACC	**94*** (14)	100* (1)	100 (1)	100* (0)	99 (7)	100 (1)
PFLACC	99 (2)	99 (2)	99 (2)	99 (1)	99 (2)	100 (2)
PFCACC	97* (8)	**88*** (16)	91 (16)	96* (5)	94 (11)	98 (3)
SDCDRACC	73 (34)	58 (39)	65 (36)	64 (33)	65 (34)	75 (2)
SATADACC	97 (4)	99 (3)	98 (3)	98 (5)	98 (4)	99 (3)
SATACACC	99 (5)	99 (2)	99 (3)	100 (0)	99 (3)	99 (3)
SATINACC	96 (4)	91 (19)	87 (15)	93 (11)	92 (13)	96 (8)
SATDIACC	54 (12)	57 (20)	58 (11)	54 (15)	55[T] (15)	66 (14)
DTTPNAACC	**88*** (14)	**87*** (13)	**72*** (20)	90* (12)	86[T] (15)	92 (9)
DTTPNDACC	79 (11)	74 (17)	67 (29)	79 (19)	76[T] (20)	84 (13)
DATIPRE	4* (2)	4* (2)	4* (3)	7* (4)	5[T] (3)	3 (2)
SATDIRUL	3 (3)	5 (3)	5 (3)	4 (3)	4[T] (3)	7 (3)
DTTTAHIT	1 (2)	2 (2)	2 (2)	3 (4)	2 (3)	1 (2)
DTTIDHIT	6 (5)	5 (5)	6 (6)	6 (5)	6[T] (5)	3 (4)
DATSCPUT	24.4 (9.1)	21.4 (6.0)	22.4 (4.4)	25.3 (6.5)	23.9 (6.8)	26.2 (5.9)

ASCPUT	80.4* (18.6)	75.3 (16.7)	**62.6*** (13.4)	71.5 (19.4)	72.6 (18.3)	78.9 (27.8)
PFNPUT	62.3 (9.6)	61.1 (13.0)	61.3 (10.9)	63.2 (14.8)	61.4 (14.5)	89.2 (23.5)
PFCPUT	50.7* (13.4)	**36.8*** (17.3)	39.1 (14.7)	44.4 (13.0)	43.5 (14.5)	59.6 (18.4)
DTTPNAPUT	112.4 (52.6)	82.8 (32.6)	**73.7*** (67.2)	123.5* (72.3)	105.6 (64.3)	145.7 (74.7)

* - statistically significant at $p < .05$, bold number indicates group that performed poorer/poorest on that particular subtest

† - poorer score between the Warfield and Control Groups that is statistically significant at $p < .05$

In conclusion Air National Guard personnel were exposed to concentrations of JP-8 that were orders of magnitude lower than current permissible exposure levels. Comparing JP-8 exposure at Warfield Air National Guard Base (pre-work ranged from 0-7.6 mg/m^3, post-work ranged from 0.2-11.5 mg/m^3) to other military bases shows that individuals working directly with fuel can minimize JP-8 exposure by adhering to safety guidelines outlined in standard operating procedures and by taking additional steps to protect themselves, for example, changing clothing contaminated with jet fuel can reduce dermal exposure. Moreover, the use of double gloving at Warfield reduced dermal exposure when gloves were removed. Furthermore, limiting outdoor activities like cigarette smoking can also reduce JP-8 exposure. Nevertheless, even this low-level of chronic JP-8 exposure found at Warfield Air National Guard Base appears to interfere with neurocognitive functioning. Additional studies should be performed at other Air National Guard Bases to determine if these results are typical. If it is established that low-level chronic JP-8 exposure interferes with neurocognitive functioning, then additional steps must be taken to reduce exposure and the current permissible exposure guidelines lowered.