

Naval Health Research Center Detachment (Toxicology)

**BIOLOGICAL AND HEALTH  
EFFECTS OF JP-8 EXPOSURE**

Glenn D. Ritchie, Ph.D.<sup>1</sup>  
Marni Y. V. Bekkedal, Ph.D.<sup>2</sup>  
LT Andrew J. Bobb (Ph.D.), MSC, USNR<sup>2</sup>  
CAPT Kenneth R. Still (Ph.D.), MSC, USN<sup>2</sup>

**REPORT NO.**

**TOXDET 01-01**

*Approved for public release; distribution is unlimited*

**NAVAL HEALTH RESEARCH CENTER DETACHMENT (TOXICOLOGY)  
2612 FIFTH STREET, BUILDING 433, AREA B  
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-7903**



20020320 074



# **BIOLOGICAL AND HEALTH EFFECTS OF JP-8 EXPOSURE**

Glenn D. Ritchie, Ph.D.

Marni Y. V. Bekkedal, Ph.D.

LT Andrew J. Bobb (Ph.D.), MSC, USNR

CAPT Kenneth R. Still (Ph.D.), MSC, USN

This work was performed under JP-8 Jet Fuels Work Units.

Animal handling procedures used in this study were subject to review and approval by the Animal Care and Use Committee located at Wright-Patterson AFB and the Airforce Surgeon General. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research, National Research Council, DHHS, National Institutes of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended.

## Table of Contents

Page

1.	Introduction .....	3
2.	Chemical and Physical Properties of JP-8 .....	3
3.	Chemical Constituents of JP-8 .....	3
4.	Self-Reported and Medically Diagnosed Health Effects in Humans Exposed to JP-8 .....	5
5.	JP-8 Exposure Scenarios .....	6
6.	Carcinogenicity or Mortality .....	7
7.	Acute JP-8 Exposure Effects .....	7
8.	Central or Peripheral Nervous System Effects .....	8
9.	Reproductive System and Developmental Effects .....	10
10.	Pulmonary System Effects .....	11
11.	Heart and Circulatory System Effects .....	12
12.	Dermal System Effects .....	13
13.	Gastrointestinal System Effects .....	14
14.	Immune System Effects .....	14
15.	Musculoskeletal System Effects .....	16
16.	Renal System Effects .....	16
17.	Hepatic System Effects .....	17
18.	Endocrine System Effects .....	18
19.	Metabolic Effects .....	18
20.	Genotoxic Effects .....	18
21.	Blood System Effects .....	19
22.	Acute Lymphocytic Leukemia (ALL), Acute Myelogenous Leukemia (AML) .....	19
23.	Human, Animal and <i>In Vitro</i> Consequences of Acute or Long-Term Exposure to Specific Chemical Constituents of JP-8: .....	20
	A. Polycyclic Aromatic Hydrocarbons (PAHs) .....	20
	B. Benzene .....	22
	C. Toluene .....	23
	D. Trimethylbenzenes .....	25
	E. Xylenes .....	26
	F. <i>n</i> -Hexane .....	26
24.	Conclusions .....	27
25.	References .....	28
26.	Report Documentation Page (SF 298) .....	45



## 1. Introduction

Approximately 60 billion gallons of military Jet Propulsion Fuel-8 (JP-8, domestic; F-34 international) and the commercial jet industry equivalents Jet A (domestic) and Jet A-1 (international flights) are consumed internationally on an annual basis (26 billion gallons in the US) [Armbrust Aviation Group, 1998; Henz, 1998]. Although JP-8, Jet A and Jet A-1 are chemically identical, except for performance additive packages, and are all distilled from de-sulfurized kerosene (for kerosene toxicity reviews, see US Dept. of Health and Human Services, 1998; Committee on Toxicology, 2001), this report is limited to discussion of JP-8 toxicity. While JP-8 (F-34) has been used since 1972 by the militaries of some North Atlantic Treaty Organization (NATO) countries, and since 1992-1996 by the US Air Force (USAF), the US Army and the Japanese Self-Defense Forces, there is remarkably little published human research investigating possible human health effects. There is, however, a wealth of recently published animal and *in vitro* studies of JP-8 toxicity. This report summarizes available human, animal and *in vitro* studies investigating biological and health effects from acute or long-term exposure to JP-8, its combustion products, and each of six major chemical constituents of JP-8 with known human toxicity potential.

## 2. Chemical and Physical Properties of JP-8

Physical State/Appearance:	Clear and bright to light amber liquid
Odor Description:	Light hydrocarbon/kerosene odor
Molecular Weight:	180 (average) [Exxon/Mobil, 1999]
Lower Explosive Limit	0.7-0.9%
Upper Explosive Limit	5-6%
Autoignition Temp.	210°C; 475°F
Freezing Point	-53°F
Flash Point Method:	TCC
Flash Point:	100-113°F
Melting Point:	-53°F
Vapor Pressure (mm Hg):	2 mm @68°F; 20 mm@158°F;
Vapor Density (Air = 1):	4.5-5
Specific Gravity:	0.8
Conversion Factors: (@25°C):	Unknown
Solubility in Water:	Negligible

[References - Current JP-8 MSDS Exxon/Mobil; Amoco; Pride Company; BP Oil; Shell Oil; Mapco Alaska Petroleum; Arco Products; Najavo Refining; Coastal; La Gloria Oil and Gas; Hunt Refining; Chevron Oil; Age Refining; Repsol Oil International, Ltd.; Diamond Shamrock Refining; Toxicological Profiles, DHHS, Aug. 1998(Ref.#179)]

## 3. Chemical Constituents of JP-8

As determined by gas chromatography (GC), JP-8 contains approximately 228 identifiable hydrocarbon constituents (C<sub>5</sub>-C<sub>17</sub>+), although this number may exceed 2,000 when all isomeric forms of these constituents are considered (Allen *et al.*, 2001; Ritchie

*et al.*, 2001a). As a function of the fuel manufacturer, fuel lot, and targeted fuel performance objectives, the volume percentage of specific constituents may vary substantially. Additionally, JP-8 formulations developed for specific environments and fuel performance applications may include unique performance additive packages. JP-8 contains three additives: 1) the icing inhibitor diethylene glycolmonomethyl ether (DiEGME), 0.1% v/v; 2) the anti-static compound Stadis 450, 2 mg/L; and 3) the corrosion inhibitor DCI-4A, 15 mg/L (Allen *et al.*, 2001). The possible toxicity of these individual additives and possible additive or synergistic toxicity with hydrocarbon constituents of the parent fuel has been only minimally researched. JP-8 (100), a new formulation being introduced for use by the USAF, is identical to JP-8 except for the addition of three more performance additives. These additives are 1) the antioxidant butylated hydroxytoluene (BHT), 25 ppm; 2) the metal deactivator (MDA), 3 ppm; and 3) the detergent and dispersant 8Q405, 70 ppm (Kanikkannan *et al.*, 2001). JP-8 (100) is presently being manufactured by at least 5 different refining companies, with targeted performance objectives of improving JP-8 thermal stability by 100°F, improving fuel heat sink capacity by 50%, and reducing fouling in jet engine nozzles and afterburner spray assemblies. Because JP-8 (100) is used at only two air bases (Kingsley Air National Guard base and Sheppard AFB) (Wolfe *et al.*, 1997), its discussion in this report will be limited to studies containing toxicity comparisons to JP-8. While the possible toxicity of the vast majority of the hydrocarbon constituents (and particularly their isoforms or metabolites) and performance additives of JP-8 has not been researched, there is significant scientific literature describing health effects from acute or long-term exposure to several of the constituents of JP-8. (Current JP-8 MSDS Exxon/Mobil; Amoco; Pride Company; BP Oil; Shell Oil; Mapco Alaska Petroleum; Arco Products; Najavo Refining; Coastal; La Gloria Oil and Gas; Hunt Refining; Chevron Oil; Age Refining; Repsol Oil International, Ltd.; Diamond Shamrock Refining; Kannikannan *et al.* 2001):

<b>Major Chemical Constituent</b>	<b>Approximate Volume/Volume Concentration in JP-8</b>
A) Polycyclic Aromatic Hydrocarbons (including Naphthalene)	0.29-3%
B) Benzene	0.10-0.8%
C) Toluene	0.06-1%
D) 1,2,4-Trimethylbenzene	0.75-1%
E) <i>o</i> -, <i>m</i> -, <i>p</i> -Xylenes	1.00-1.23%
F) <i>n</i> -Hexane	<0.1%

Because the health effects of these individual constituents of JP-8 are relatively well known, a subsequent section (Section 23) of this report will summarize these results. It must be remembered that health consequences from exposure to higher concentrations of these constituents, as may occur in various occupational environments, do not necessarily imply the same health effects from acute or repeated exposure to the lower concentrations contained in JP-8. Further, it should be considered that hydrocarbon exposure histories (*i.e.*, Jet A, Jet A-1, JP-4, JP-5, AVGAS, MOGAS, diesel fuel, marine diesel fuel, benzene, toluene or xylene based solvents, paints or glues, *etc.*) of

personnel presently exposed to JP-8 must be considered in individual risk analyses.

#### 4. Self-Reported and Medically Diagnosed Health Effects in Humans Exposed to JP-8

Since the 1992-1996 conversion from predominant use of JP-4 jet fuel (40-50% kerosene: 50-60% unleaded gasoline) to JP-8 by the USAF and US Army, there have been increased self-reported and/or medically diagnosed complaints from exposed personnel. Medical symptoms include nausea, headaches, fatigue, blocked nasal passages, skin irritation, respiratory distress, and ear infections (Ullrich and Lyons, 2000; Ritchie *et al.*, 2001a). JP-8 was refined to exhibit a higher flash point, lower vapor pressure, and increased handling safety compared to JP-4. JP-8 necessarily vaporizes less quickly from skin, clothing, environmental surfaces, soil, and groundwater, and is more likely to be found in aerosolized versus vapor phase compared to JP-4. (Allen *et al.*, 2000). These characteristics of JP-8, compared to JP-4, may provide increased human dermal exposure to raw fuel as well as increased respiratory exposure to aerosol phase.

Repeated exposure of humans to JP-8 vapor and/or aerosol, or to JP-8 combustion by-products (elemental C, CO, NO<sub>x</sub>, SO<sub>x</sub>, formaldehyde, PAHs, *etc.*) [Childers *et al.*, 2000] is commonly self-reported to induce irritation of the mucous membranes of the respiratory system (Kobayashi and Kikukawa, 2000, Ritchie *et al.*, 2001a). The transition from JP-4 to JP-8 by the USAF and US Army may have exacerbated respiratory irritant effects, as the lower volatility of JP-8 may result in increased probability of aerosol formation. Exhaust from JP-8 combustion may contain higher concentrations of the respiratory irritant formaldehyde than comparable exhaust from aircraft previously using JP-4 (Kobayashi and Kikukawa, 2000). There has been, however, no published study of possible pulmonary toxicity in humans exposed repeatedly to JP-8 vapor, aerosol, or exhaust (US Dept. of Health and Human Services, 1998).

A very common self-reported or diagnosed human health effect of JP-8 exposure is skin irritation (Ritchie *et al.*, 2001a). JP-8 has been shown to produce skin irritation or skin sensitization in several species and strains (Kinkead *et al.*, 1992, Wolfe *et al.*, 1996). This is consistent with NIOSH statistics, indicating that skin disease is the second most common type of occupational health effect (Kanikkannan *et al.*, 2000). With repeated dermal exposure to JP-8, as occurs in a number of fuel handling and aircraft maintenance tasks, the possibility exists for severe dermal toxicity, leading to possibly increased systemic absorption of some toxic JP-8 constituents (Rosenthal *et al.*, 2001). Baker *et al.* (1999) has, indeed, shown that JP-8 is more irritating to rats than JP-4 when equal volumes are applied dermally.

The transition from use of JP-4 (a very volatile jet fuel), to predominant use of JP-8, a fuel with a lower vapor pressure, increases the probable duration of dermal exposure (skin and clothing) in at least fuel-handling and avionics maintenance personnel (Kannikannen *et al.*, 2000; McDougal *et al.*, 2000). Repeated exposure of humans to JP-8 vapor and/or aerosol, or to JP-8 combustion by-products (elemental C, CO, NO<sub>x</sub>, SO<sub>x</sub>, formaldehyde, PAHs, *etc.*) [Childers *et al.*, 2000] is commonly self-reported to induce irritation of the mucous membranes of the respiratory system



(Kobayashi and Kikukawa, 2000, Ritchie *et al.*, 2001a). The transition from JP-4 to JP-8 may have exacerbated respiratory irritant effects, as the lower volatility of JP-8 may result in increased probability of aerosol formation. Exhaust from JP-8 combustion may contain higher concentrations of the respiratory irritant formaldehyde than comparable exhaust from aircraft previously using JP-4 (Kobayashi and Kikukawa, 2000). There has been, however, no published study of possible pulmonary toxicity in humans exposed repeatedly to JP-8 vapor, aerosol, or exhaust (US Dept. of Health and Human Services, 1998).

## **5. JP-8 Exposure Scenarios**

Exposure to JP-8 occurs to military and civilian avionics, aircraft maintenance, and fuel handling personnel through dermal contact with raw fuel or with clothing/gloves saturated with fuel. Through respiratory exposure to fuel in vapor or aerosol phase, or occasionally through oral exposure to atmospheric aerosol or to fuel-contaminated food or water (Harris *et al.*, 1997, Pleil *et al.*, 2000). Exposure of military personnel to JP-8 can also occur through more atypical uses of the fuel. These uses include fueling of land vehicles and equipment, fueling of heaters, use of JP-8 as a coolant (heat sink) in aircraft, aerosolization of JP-8 for use as a combat obscurant, use of JP-8 to suppress environmental sand or dust, or use of JP-8 as a carrier for herbicide applications (Ritchie *et al.*, 2001a). Exposure of non-military, non-avionics personnel to JP-8 occurs primarily through atmospheric, soil or groundwater contamination with JP-8 or its combustion products, or through off-gassing from the skin and clothing of fuel-exposed personnel (Ritchie *et al.*, 2001a). Major identified sources of atmospheric and groundwater contamination with JP-8 include: 1) unavoidable leakage or accidental spillage of JP-8 from manufacturing facilities, transportation and storage systems (including pipelines); 2) fueling/defueling/maintenance operations, aircraft and vehicle operation (including cold start-up of engines); and 3) occasional atmospheric jettisoning (usually above 6,000 ft.) of JP-8 during emergency aircraft landing (Pfeiffer, 1994).

Carlton and Smith (2000) measured JP-8 and benzene exposures during aircraft fuel tank (foam-filled) entry and repair at twelve USAF bases. Breathing zone samples were collected on the fuel handlers during occupational assignments, while instantaneous samples were taken at various points during the procedures with SUMMA canisters and subsequent analysis by mass spectrometry. The highest 8-hr time-weighted average (TWA) was 1304 mg/m<sup>3</sup>; the highest short-term (15-min average) exposure was 10,295 mg/m<sup>3</sup>. The instantaneous sampling results indicated benzene exposures during fuel tank repair up to 49.1 mg/m<sup>3</sup>. These readings occurred within aircraft fuel tanks, from which foam blocks soaked with JP-8 were inspected and removed. In this worst case scenario, workers entering the tanks are required to wear self-contained breathing apparatus (SCBA) and chemically-resistant gloves and boots, but only cotton jumpsuits, allowing extensive dermal exposure. Personnel working outside the fuel tanks, but assisting in removal of the foam blocks, do not typically wear SCBAs, allowing both extensive dermal and respiratory exposure to JP-8 (Pleil *et al.*, 2000).

## **6. Carcinogenicity or Mortality**

There are no published reports of human death, consistent organic illness, or carcinogenicity associated with JP-8 exposure, or with repeated exposure to other similar jet fuels (Selden and Ahlborg, 1987, 1991; McDougal *et al.*, 2000). Only one study of JP-8 toxicity has indicated a significant increase in death in JP-8 exposed animals. Mattie *et al.* (1991), exposing male and female mice by whole body inhalation to JP-8 vapor (0, 500 or 1000 mg/m<sup>3</sup>) continuously for 90 days, reported a significantly increased mortality in exposed male rats (up to 9 months post-exposure) versus exposed female rats or control animals. Mattie *et al.* (1991) hypothesized that necrotizing dermatitis associated with fighting among males or a "male rodent-specific" renal disorder (see Section 16) associated with the exposures may have accounted for the increased mortality observed.

## **7. Acute JP-8 Exposure Effects**

To date, there has been only one published study exploring possible health effects in animals or humans from acute exposure to JP-8. Wolfe *et al.* (1997) exposed male or female rats or rabbits orally to 5 mg/kg, or dermally to 2 g/kg JP-8. There were no deaths or persisting signs of toxicity observed, although post-exposure lethargy and shallow breathing was commonly observed. In rabbits, 4-hr dermal exposure with JP-8 resulted in only slight erythema. Rats exposed by whole body inhalation for 4 hours to as much as 3,700 mg/m<sup>3</sup> JP-8 or 5,000 mg/m<sup>3</sup> vapor/aerosol exhibited eye and upper respiratory irritation, but no deaths. All exposed animals survived for 14 days post-exposure with no significant weight loss or obvious lesions.

There are only two published studies of acute effects in humans from exposure to any jet fuel formulation (JP-4 or JP-5). The majority of Material Safety Data Sheets (MSDSs) appear to utilize data from these studies for "Special Precautions: Signs/Symptoms of Overexposure" sections. The following symptoms are summarized from 15 different MSDSs for JP-8 and from a study (Porter, 1990) in which two Navy aviators exposed to a "high level" of jet fuel vapor/aerosol due to in-flight leakage into the aircraft cabin:

- Burning eyes (hyperemic conjunctiva)
- Nausea/vomiting
- Incoordination/Impairment of eye-hand coordination
- Anorexia
- Euphoria and laughing
- Fatigue
- Skin rash; perception of skin heat or burning
- Mild hypertension
- Apparent intoxication
- Memory impairment [*i.e.*, recalling emergency procedures, flight plan information, personal information (*i.e.*, wife's name)]

There are a number of published studies of JP-8 *in vitro* toxicity, in which cell cultures or other tissues are exposed acutely to JP-8. Each of these studies will be summarized in the section appropriate for the body organ associated with the cell or tissue culture.

## **8. Central or Peripheral Nervous Systems Effects**

There are presently only two published neurobehavioral studies detailing long-term effects of repeated JP-8/JP-4 exposure on human central nervous system (CNS) or peripheral nervous system (PNS) function. Smith *et al.* (1997) reported the effects on postural balance of 0.8-30 yr. (mean = 4.56 yr.) exposure to JP-8, (although some subjects also reported an exposure history to JP-4). Exposed subjects and matched controls were tested before the work shift (12-24 hours rest from exposure) and again after 4-6 hours of occupational exposure to JP-8. Subjects were evaluated for the capacity to maintain postural equilibrium on a standard postural balance platform during each of four testing conditions:

- Eyes Open: Stable Platform
- Eyes Closed: Stable Platform
- Eyes Open: Standing on 4" Foam
- Eyes Closed: Standing on 4" Foam

Workers exposed for  $\geq 9$  months to JP-4/JP-8 exhibited significantly increased postural sway patterns, relative to controls, but only during the most difficult testing condition, in which eyes are closed and the subject stands on a 4" thick section of packing foam. Persisting postural equilibrium deficits are known to reflect deficits in brainstem vestibular or proprioceptive control systems, but may additionally reflect deficits in peripheral proprioceptive mechanisms (Smith *et al.*, 1997). Performance deficits were correlated with breathing space and breath levels of benzene [5.03 $\pm$ 1.4 parts per million (ppm)], toluene (6.11 $\pm$ 1.5 ppm), and xylenes (6.04 $\pm$ 1.4 ppm), but not naphthas (491.6 $\pm$ 108.9 ppm). The reported deficits were subchronic/chronic and were not significantly modulated by the "acute" 4-6 hours JP-8 occupational exposures.

Recently, McInturf *et al.* (2001) measured learning of an eyeblink classically conditioned (Pavlovian) response (EBCC) in JP-8 exposed USAF personnel ( $\geq 4$  months exposure to JP-8; n = 28) and matched controls (n = 46). Subjects learned a classically conditioned association between a 1000 Hz tone (conditioned stimulus, or CS) and a 3-5 pounds/inch<sup>2</sup> corneal airpuff (unconditioned stimulus, or US), such that the CS eventually elicited an eyeblink response (conditioned response, or CR). Subjects were trained following a 24-72 hours rest from occupational exposure, then relearned the task 30-90 min. following a 4-6 hours return to work. It was reported that JP-8 exposed personnel were significantly deficient, relative to controls, in both the acquisition (percentage of trials in which CR was elicited by the CS) of the habit and in the mean time from onset of the CS to the peak eyeblink response (CR). Deficits in EBCC acquisition are known to identify deficits in brainstem (e.g., cochlear, pontine, red nuclei) and cerebellar (e.g., nucleus interpositus) function (McInturf *et al.*, 2001).

Several animal studies of JP-8 neurotoxicity have been published. Mattie, *et al.* (1995) reported no clinical signs of neurotoxicity (Functional Observational Battery, or FOB) in female rats treated orally with 0-2,000 mg/kg/d JP-8 from gestational days 6-15. Also no histopathological changes in the brains or sciatic nerves of male rats administered 750, 1500, or 3,000 mg/kg JP-8 by gavage once daily for 90 days. Several studies (Nordholm, 1998; Rossi III *et al.*, 2001; Ritchie *et al.*, 2001b) reported the effects of repeated exposure (6 hours/day, 5 d/wk, 6 week) of adult rats to JP-8 vapor (500 or 1000 mg/m<sup>3</sup>). In these studies, it was shown that repeated JP-8 exposure modulated neurobehavioral capacity in several areas, and that this modulation persisted for up to 200+ days post-exposure. Repeated JP-8 exposure to 1000 mg/m<sup>3</sup> reduced the capacity of rats to learn highly difficult (but not simple) operant tasks, compared to low dose (500 mg/m<sup>3</sup>) JP-8 or control exposures. Further, repeated exposure to 1000-mg/m<sup>3</sup> JP-8 significantly increased approach of the exposed rats to an appetitive stimulus, as compared to controls. Examination of neurotransmitter levels in the brains and blood of JP-8 exposed rats indicated: 1) a significantly increased level of dopamine (DA) in the cortex; 2) a significantly increased level of DOPAC, (3,4-dihydroxyphenylacetic acid) the major metabolite of DA, in the brainstem; and 3) a decreased level of the serotonin (5-HT) metabolite 5-HIAA (5-hydroxyindole acetic acid) in the serum as long as 200+ days post-exposure.

Baldwin *et al.* (2001) exposed male rats to JP-8 aerosol (with or without substance P) for 1 hour/d for 28 d (1059 mg/m<sup>3</sup> for 25 days, then 2,491 mg/m<sup>3</sup> for 3 days), then tested the animals using the FOB, as well as the Morris water maze (a test of spatial discrimination). While exposed rats (with or without substance P) exhibited no deficits on the Morris water maze, relative to controls, these rats exhibited significant post-exposure weight loss, increased rearing, reduced grooming, increased open field spontaneous locomotor activity, and increased swimming speed relative to controls.

In combination, these studies of subchronic or chronic exposure of humans or animals to JP-8 would appear to indicate persisting changes in at least cortical, brainstem and cerebellar systems, as manifested by changes in neurobehavioral capacity and/or neurotransmitter levels. Baldwin *et al.* (2001) concluded that repeated exposure of rats to JP-8 aerosol results in increased arousal levels and locomotor activity akin to repeated psychostimulant administration that is mediated by the mesolimbic dopaminergic system.

McGuire *et al.* (2000) exposed mice to JP-8 aerosol at 1000 mg/m<sup>3</sup> or 2,500 mg/m<sup>3</sup> for 1hr/d for 7 d, then evaluated the retinas of exposed animals by immunohistochemical methods. The fuel exposure induced a marked increase in the immunoreactivity of anti-glutathione-S-transferase (GST) antibodies within the Muller cells, the radial glial cells of the retina. The authors hypothesized that JP-8 may act as a toxicant in the mouse retina by increasing the flux of xenobiotics across the blood-retina barrier. Mattie *et al.* (1995) exposed male Sprague-Dawley rats by oral gavage for 90 days to 750, 1500 or 3,000 mg/kg/d JP-8. In all exposure conditions, the authors reported significantly increased brain/body weight ratios,

There is one published study of the *in vitro* effects of acute exposure to JP-8 on nervous system tissue. Grant *et al.* (2000) investigated the *in vitro* cytotoxicity and electrophysiological effects of JP-8 on neuroblastoma x glioma (NG108-15) cell cultures, as well as on embryonic hippocampal neurons. Acute JP-8 (in 5% ethanol)

exposure of the hippocampal neurons proved to be highly toxic ( $IC_{50}$  of  $< 2$  micrograms ( $\mu\text{g}/\text{ml}$ ) while, in contrast, the NG108-15 cells were much less sensitive. Electrophysiological examination of NG108-15 cells showed that administration of JP-8 at  $1 \mu\text{g}/\text{ml}$  did not alter significantly any of the electrophysiological properties. However, exposure to JP-8 at  $10 \mu\text{g}/\text{ml}$  during a current stimulus of  $+46$  picoAmperes decreased the amplitude of the action potential to  $83 \pm 7\%$ , the rate of rise ( $dV/dt_{\text{MAX}}$ ) to  $50 \pm 8\%$ , and the spiking rate to  $25 \pm 11\%$  of the corresponding control levels. These results demonstrate JP-8 induced cytotoxicity varies among cell types, and for the first time that CNS neurons may alter electrophysiological function without cell death in response to JP-8 exposure.

Additionally, there is one published study of the effects of hydrocarbon fuels exhaust on the rat brain. Microinjection of exhaust emissions, containing polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs into the hippocampus or striatum induced significant lesions, with tissue loss and disappearance of immunoreactivity for glial fibrillary acidic protein (GFAP), tyrosine hydroxylase, and acetylcholinesterase (AChE) [Andersson *et al.*, 1998].

A comprehensive review of the neurotoxicity of acute and repeated exposure to various hydrocarbon fuels and solvents has been recently published (Ritchie *et al.*, 2001a).

## **9. Reproductive System and Developmental Effects**

There is only one published study of possible JP-8 exposure effects on the human reproductive system. In this study, Lemasters *et al.* (1999) examined a number of sperm parameters (sperm concentration, sperm motion, viability, morphology, morphometrics, and stability of sperm chromatin) in USAF personnel exposed repeatedly to JP-8/JP-4 and/or hydrocarbon solvents. A comparison of sperm parameters pre-exposure versus after 15-30 weeks of occupational responsibility indicated no significant differences for any measure.

Two recent animal studies similarly examined the effects of 91 days of exposure (6 hours/d, 7 d/wk) to JP-8 vapor (0, 250, 500 or  $1000 \text{ mg}/\text{m}^3$ ) or exposure for 6 hours/d, 5 d/wk, for 6 weeks to  $1000 \text{ mg}/\text{m}^3$  JP-8 vapor on the reproductive systems of adult male Sprague-Dawley rats (Briggs *et al.*, 1999, 2001). No significant effects on sperm morphology, quality, concentration, or motility were reported for any exposure concentration. Through proteomic analysis approximately 50 proteins specific to the testes were found overexpressed or underexpressed. For example, significantly overexpressed proteins included tubulin, the A1 or A2 isoform of vacuolar  $\text{H}^+$ -ATPase subunit A, heat shock protein 70 (hsp70), aldehyde dehydrogenase, T-complex polypeptide 1, and GTP-binding nuclear protein RAN (Briggs *et al.*, 2001).

Because approximately 14.6% of active duty Navy, Air Force and Army personnel are women of childbearing age (Military Personnel Statistics: May 2001), there is increasing interest in possible reproductive or developmental effects of JP-8 exposure. Mattie and Cooper (1996) reported that JP-8 did not cause fetal malformation after oral exposure (0, 500, 1000, 1500 or  $2,000 \text{ mg}/\text{kg}/\text{d}$ ) exposure of pregnant rat dams during gestation days 6-15. Dams in the 1000, 1500 and  $2,000 \text{ mg}/\text{kg}/\text{d}$  groups gained significantly less body weight during pregnancy than did control dams. Embryo

toxicity was indicated by a significant reduction in fetal body weight (13-15%) in the 1500 and 2,000 mg/kg/d dose groups. Mattie *et al.* (2001) exposed female rats by oral gavage to 0, 325, 750, or 1500 mg/kg/d JP-8 for 21 weeks, including 90 days prior to gestation and lactation. Body weights from the high dose group were significantly decreased, relative to controls, on postnatal days 4-21. There were no differences among exposure groups and controls for surface righting and negative geotaxis. For swimming ability, however, there was a significant dose-related reduction in exposed versus control animals, leading the authors to hypothesize a delay in development of coordinated motor movements related to the swimming task.

Finally, Harris *et al.* (2000) hypothesized that reduced natural killer (NK) cell function, an effect of brief exposure of mice to JP-8 aerosol. Lanier (1999) hypothesized that reduced NK function may result in reduced placentation during gestation and, thus, impaired reproductive ability.

## **10. Pulmonary System Effects**

A number of animal studies have been published examining pulmonary toxicity from JP-8 exposures. Mattie *et al.* (1995) reported no histopathological changes in the lungs or nasal turbinates of male rats administered up to 3,000 mg/kg/d JP-8 by oral gavage once daily for 90 days. Fischer-344 rats exposed to 497 or 520 mg/m<sup>3</sup> (vapor/aerosol) JP-8 for 1 hour/d for 7 or 28 d exhibited significant increases in pulmonary resistance, increased alveolar clearance of a radiolabeled compound (99mTc-labeled diethylenetriaminepentaacetic acid), and a decrease in bronchoalveolar lavage fluid (BALF) concentration of the neuropeptide substance P (Chen *et al.*, 1992).

Hays *et al.* (1995) exposed male rats for 1 hour/d for 7, 28, or 56 days to 500 (low dose) or 813-1094 mg/m<sup>3</sup> (high dose) JP-8 aerosol. Rats in all groups experienced perivascular and interstitial edema as well as thickening of the alveolar septa, accompanied by leukocytic infiltration. Morphological changes induced by JP-8 peaked at 28 days of exposure. Alveolar permeability generally increased with increasing JP-8 exposure, in a dose-related manner. Similarly, Pfaff *et al.* (1995) reported significant changes in terminal bronchiolar airways accompanied by subendothelial edema in rats exposed for 28 d to 500 - 1000 mg/m<sup>3</sup> JP-8. Pfaff *et al.* (1996) exposed Fischer-344 rats to an aerosol/vapor mix of JP-8 (7, 28, or 56 d at 469-520 mg/m<sup>3</sup>/hr or 814-1263 mg/m<sup>3</sup>/hr). In response to JP-8 inhalation, exposed animals exhibited a dose-dependent as well as duration-determined reduction in BALF substance P concentration, consistent with significant histopathological changes in lower pulmonary structures.

Witten (1992, 1993) and Robledo and Witten (1998) reported more mild pulmonary toxicity in mice exposed to JP-8 vapor concentrations as low as 50 mg/m<sup>3</sup> for as little as 1 h/d for 7 days. This response targeted the bronchiolar epithelium, leading to significantly increased respiratory permeability, peribronchiolar edema, and mild cellular necrosis. Robledo *et al.* (1999a) hypothesized that even non-cytotoxic exposures to JP-8 aerosol may exert a noxious effect on bronchial epithelial barrier function that may preclude pathological lung injury. Such effects may, then, modulate the protective barrier provided by the lung against absorption of potential toxicants resulting possibly in both systemic toxicity and subsequent cytotoxic lung injury.

In mice, exposure to 48 or 118 mg/m<sup>3</sup> JP-8 aerosol for 1 h/d for 7 d resulted in: 1) increased lung permeability; 2) perivascular edema; 3) Clara cell vacuolization; 4) intra-alveolar hemorrhage; 5) alterations in type II epithelial cells, including necrosis 6) BALF increases in total protein and lactic dehydrogenase (LDH); 7) reduced N-acetyl-beta-D-glucosaminidase (NAG) levels; and 8) reduced alveolar macrophage counts (Robledo *et al.*, 2000).

Robledo and Witten (1999b) exposed mice to JP-8 aerosol concentrations of 50 mg/m<sup>3</sup>, resulting in enhanced respiratory permeability to 99mTc-labeled diethylenetriaminepentaacetic acid, alveolar macrophage toxicity, and bronchiolar epithelial damage. Mice administered the neurokinin (NK) 1-receptor agonist substance P after each JP-8 exposure, however, exhibited the appearance of normal pulmonary values and tissue morphology. In contrast, endogenous NK1-receptor antagonism by CP-96345 administration exacerbated JP-8-enhanced permeability, alveolar macrophage toxicity, and bronchiolar epithelial injury. These data indicate that NK1-receptor activation, at least by the neuropeptide substance P, may have a protective role in preventing the development of hydrocarbon-induced lung injury, possibly through the modulation of bronchiolar epithelial function.

Witzmann *et al.* (1999) examined protein expression in whole lung tissue from male mice exposed for 1 h/d for 7 d to 1000 or 2,500 mg/m<sup>3</sup> JP-8 aerosol. Of 42 proteins upregulated (up to +94%) or downregulated (to -30%), 13 were identified as most impacted by JP-8 aerosol exposure. These proteins are involved in four functional areas: 1) protein synthesis; 2)-toxic/metabolic stress and detoxification; 3)-lung ultrastructure; or 4) functional responses to CO<sub>2</sub> handling, acid-base homeostasis, and fluid secretion.

Most recently, Stoica *et al.* (2001) reported that JP-8 (80 micrograms ( $\mu$ g)/ml) induced apoptosis, but not necrosis, in a rat lung alveolar type II epithelial cell line (RLE-6TN). It was shown that soon after JP-8 exposure, RLE-6TN cells exhibited markers of apoptotic cell death: caspase-3 activation, poly (ADP-ribose) polymerase (PARP) cleavage, chromatin condensation, cytochrome c release from the mitochondria, and genomic DNA cleavage into both oligonucleosomal (DNA ladder) and high-molecular weight (HMW) fragments. It was hypothesized that JP-8 exposure, at least at low levels, damaged the mitochondrial mechanism sufficiently to induce release of cytochrome c and initiate the caspase cascade, but not sufficiently to completely compromise mitochondrial ATP function, resulting in cell necrosis. It was shown further that modulations resulting in overexpression of antiapoptotic proteins (*i.e.*, Bcl-x<sub>L</sub> or Bcl-2) in the culture reduced apoptosis in response to JP-8 exposure, while overexpression of proapoptotic proteins (*i.e.*, Bax or Bad) enhanced the apoptotic response. Higher concentrations of JP-8, perhaps sufficient to induce necrosis, were not evaluated in this study.

## **11. Heart and Circulatory System Effects**

Mattie *et al.* (1995) reported no histopathological changes in the hearts of male rats administered 750, 1500, or 3,000 mg/kg JP-8 by gavage once daily for 90 days.

## 12. Dermal System Effects

Kanikkannen *et al.* (2001) examined percutaneous absorption of JP-8 across pig ear skin and human skin. In general, tested chemical constituents of JP-8 (*i.e.*, tridecane, nonane, naphthalene, and toluene) permeated through both human and porcine skin at rates proportional to their composition in JP-8. Transepidermal water loss (TEWL), skin capacitance (moisture content) and skin irritation (erythema and edema) were evaluated before treatment and at 1, 2 and 24 hours after a 24-hr exposure to JP-8. Application of toluene, nonane or JP-8 increased the TEWL; JP-8 being the highest (3.5 times higher at 24 hours compared to baseline level). JP-8 caused a moderate erythema and a moderate to severe edema that was greater than with exposure to toluene or nonane. Though the edema decreased after 24 hours, the degree of erythema remained the same until 24-h. Exposure of JP-8 caused significant changes in the barrier function of the skin as indicated by an increase in TEWL. The disruption of barrier function of skin, as indicated by increased TEWL after exposure to JP-8 was hypothesized to possibly increase permeation of its own components and/or other chemicals or infectious agents exposed to skin. Riviere *et al.* (1999) studied the absorption and cutaneous disposition of naphthalene, dodecane and hexadecane from topically applied (25 microliter ( $\mu\text{L}$ )/5  $\text{cm}^2$ ) aged JP-8, Jet-A, and JP-8 (100) jet fuels. Naphthalene absorption (1.17% of dose) had a clear peak absorptive flux at less than 1 hour, while dodecane (0.63%) and hexadecane (0.18%) had prolonged and significantly lower, absorption flux profiles. Parameters for each different fuel could be differentiated, possibly indicating the effects of different additive packages on dermal absorption.

McDougal *et al.* (1999, 2000) used diffusion cells to measure both the flux of JP-8 and components across rodent skin (2-3 times more permeable than human skin), and the kinetics of absorption into the skin. Total summed flux of the hydrocarbon components was 20.3 micrograms ( $\mu\text{g}$ )/ $\text{cm}^2/\text{hr}$  (excluding the additive DiEGME). Thirteen individual components of JP-8 penetrated into the receptor solution (DiEGME > decane > methyl naphthalenes > trimethyl benzene > undecane > naphthalene > xylenes > dimethyl naphthalenes > toluene > dodecane > nonane > ethyl benzene > tridecane) ranging from a high flux of 51.5  $\mu\text{g}/\text{cm}^2/\text{h}$  for the additive DiEGME (only 0.08% w/w of JP-8) to a low of 0.334  $\mu\text{g}/\text{cm}^2/\text{h}$  for tridecane (2.7% w/w of JP-8). There was a substantial difference in penetration times, ranging from 30 min. with DiEGME, to 120 min. for tridecane. Aromatic components penetrated most rapidly. Six aliphatic components (decane > dodecane > decane > tridecane > tetradecane > nonane) were identified in the skin. These authors suggested that the rate of dermal penetration of aromatics, *etc.* might be too low to induce acute systemic toxicity with typical exposures, although the absorption of aliphatic components into the skin may be sufficient to induce dermal irritation.

Kanikkannan *et al.* (2000) evaluated JP-8, JP-8 (100), and Jet A as possible skin sensitizers in female mice, using the murine local lymph node assay (LLNA). It was reported that JP-8, but not Jet A or JP-8 (100), was a mild skin sensitizer. In fact, it was shown that the addition of the antioxidant performance additive, butylated



hydroxytoluene (BHT), to the JP-8 (100) formulation appeared to reduce its dermal toxicity, as compared to the JP-8 formulation. Freeman *et al.* (1993) and Broddle *et al.* (1996), however, reported that middle distillate petroleum (MDP) streams, similar to JP-8 without performance additives, increased the incidence of skin cancer in mice treated dermally for  $\geq 24$  months.

Allen *et al.* (2000, 2001) examined the capacity of acute JP-8, JP-8 (100) or Jet A exposure to induce or suppress cytokine release in *in vitro* preparations. Primary porcine keratinocytes (PKC) or immortalized porcine keratinocyte cell lines (MSK3877) were exposed for up to 8 hours to 0.1% jet fuel. In the PKC culture, fuel exposure [JP-8, Jet A or JP-8 (100)] induced a slight upregulation of tumor necrosis factor-alpha (TNF-alpha), although there was a significant decrease in the proinflammatory cytokine interleukin-8 (IL-8) after 8-hours of exposure.

Most recently, Rosenthal *et al.* (2001b) reported that JP-8 exposure of skin fibroblast or human keratinocyte cell cultures or grafted human keratinocytes, in a dose-related manner, resulted in necrosis, but not apoptotic responses. Exposure to levels of JP-8 (80  $\mu\text{g}/\text{ml}$ ) sufficient to induce apoptosis in lung or immune system cultures (Stoica *et al.*, 2001) induced neither apoptosis nor necrosis in skin cell cultures. Exposure to higher levels of JP-8 ( $> 200 \mu\text{g}/\text{ml}$ ), however, resulted in morphological and metabolic changes typical of necrotic changes (no caspase cascade), although certain proapoptotic proteins were still upregulated and antiapoptotic proteins were downregulated in effected cells. It was hypothesized that, although different cell types exhibit differing sensitivity to JP-8, interference with mitochondrial function may be common to both necrotic and apoptotic outcomes.

### **13. Gastrointestinal System Effects**

Mattie *et al.* (1995) reported gastritis and hyperplasia (stratum corneum of squamous portion) of the stomach, as well as anal dermatitis and hyperplasia in male rats administered 750, 1500, or 3,000 mg/kg JP-8 by gavage once daily for 90 days.

### **14. Immune System Effects**

There is no published research examining effects of JP-8 exposure on immune system function in humans.

Several recent studies (Harris *et al.*, 1997, 2000; Ullrich, 1999; 2000) have indicated severe immunosuppression in rodents exposed dermally to raw JP-8 or by inhalation to JP-8 aerosol. Major alterations in immune system function can: 1) increase susceptibility to infectious agents; 2) increase the probability of development of cancers (Freeman *et al.*, 1993; Broddle *et al.*, 1996); 3) increase the probability of development of autoimmune diseases; or 4) increase the toxicity potential of exposure to chemicals and stressors. Mattie *et al.* (1995) reported a significantly increased spleen/body weight ratio in male rats administered 3,000 mg/kg/d (but not 750 or 1,500 mg/kg/d) JP-8 by oral gavage for 90 days. It should be noted, however, that these exposures also induced significant reductions in mean body weight, as compared to vehicle controls. Significant decreases in lymphocytes were observed in male rats treated with 750, 1500

or 3,000 mg/kg JP-8. In the same study, there were no histopathological changes detected in the lymph nodes.

Dudley *et al.* (2001) reported that oral gavage exposure of mice to 2,000 mg/kg/d JP-8 for 7 d resulted in significant decreases in thymus weight and cellularity (mean = -37 to -40%). Similarly, exposure to 1000 or 2,000 mg/kg/d JP-8 resulted in significantly reduced plaque-forming cell (PFC) response to sheep red-blood-cell suspension injection, a sensitive measure of immunological disruption. Further, Dudley *et al.* (2001) tested the hypothesis that JP-8 induced immunosuppression in mice may occur through a mechanism related to the aryl (aromatic) hydrocarbon receptor (AhR). To test this hypothesis, an Ah-responsive mouse strain (B6C3F1) and a classically non-responsive mouse strain (DBA/2) bearing a lower affinity AhR were gavaged with JP-8 for 7 days. The results suggest that both mouse strains were equally sensitive to JP-8 toxicity at several endpoints, including thymus weight and cellularity, liver weight, and specific IgM antibody responses. These results suggested that JP-8 might exert its toxicity via an AhR-independent mechanism.

Harris *et al.* (2000) exposed female C57Bl/6 mice by nose-only exposure to 1000 mg/m<sup>3</sup> JP-8 aerosol for 1 hour/d for 7 d. Mice were sacrificed 1 hour following the final exposure and were assayed for spleen natural killer (NK) and lymphokine activated killer (LAK) cell activity. NK cells are known to be involved in immune surveillance against newly developed malignancies, in defense against viral infections, and in control of immune B cell function. It was shown that JP-8 exposed mice were significantly deficient in both NK and LAK activity (during incubation with IL-2) in response to challenge with prototypical tumor cell lines (*i.e.*, YAC-1). Additionally, JP-8 aerosol exposure significantly reduced cytotoxic T lymphocyte precursor (CTLp) activity and significantly impacted helper T cell function, as measured by proliferation in response to a variety of stimuli, in the absence of exogenous cytokines. In previously published research, Harris *et al.* (1997a, b) reported that brief exposure of mice to JP-8 aerosol (as low as 100 mg/m<sup>3</sup>) for 1 hour/d for 7 d resulted, as soon as 2-4 d post-exposure, in reduced immune system organ weights, loss of viable immune cell numbers (T-cells, B cells, monocytes/macrophages), and suppression of a number of immune functions (*i.e.*, T cell mitogenesis) for up to 28 d following the brief exposures.

Harris *et al.* (1997) found that administration of aerosolized substance P (SP) [15 min. after each JP-8 exposure, at 1 micromolar or 1 nanomolar concentration] could protect JP-8 exposed animals from losses of viable immune cell numbers, but not losses in immune organ weights. Further, exposure of animals to SP inhibitors generally increased the immunotoxicity of JP-8 exposure. SP appeared to act on all immune cell populations equally as analyzed by flow cytometry, as no one immune cell population appeared to be preferentially protected by SP. Also, SP administration was capable of protecting JP-8 exposed animals from loss of immune function at all aerosol concentrations of JP-8 utilized (250-2,500 mg/m<sup>3</sup>).

Ullrich (1999) found that dermal exposure of female mice to JP-8, either multiple small exposures [50 microliter ( $\mu$ L)/d for 5 d] or a single large dose (250-300  $\mu$ L) resulted in immune suppression. The induction of contact hypersensitivity was impaired in a dose-dependent manner regardless of whether the contact allergen was applied directly to the JP-8-treated skin or at a distant, previously untreated dermal site. In addition, the generation of a classic delayed-type hypersensitivity reaction to a bacterial

antigen (*Borellia burgdorferi*) injected into the subcutaneous space was suppressed by dermal application of JP-8 at a distant site. The ability of splenic T lymphocytes from JP-8-treated mice to proliferate in response to plate-bound monoclonal anti-CD3 was also significantly suppressed. Interleukin-10 (IL-10) a cytokine with potent immune suppressive activity, was found to be upregulated in the serum of JP-8-treated mice, suggesting that the mechanism of systemic immune suppression may involve the upregulation of cytokine release by JP-8. JP-8 induced immunosuppressive effects were found to occur 24-48 hours post-exposure.

Finally, Ullrich and Lyons (2000) conducted follow-up studies in an effort to elucidate the mechanisms underlying JP-8 induced immunosuppression in mice. Again, it was shown that JP-8 exposure has a highly selective effect on immune function. T helper-1 cell-driven cell-mediated immune reactions (*i.e.*, delayed-type hypersensitivity and immunity to intracellular microorganisms) and (CD3 driven) T-cell proliferation were (up to 100% suppression) modulated by JP-8, while antibody formation was not influenced. It is noteworthy to recognize that nearly identical suppression of T helper-1 cell function occurs following exposure to ultraviolet radiation (*i.e.*, sunlight) [Brown *et al.*, 1995], a possible co-factor during typical military JP-8 exposures. Further, it was shown that administration of interleukin-12 (IL-12), monoclonal anti-IL-10, and the selective cyclooxygenase-2 (COX-2) inhibitor SC 236 (all known to suppress release of IL-10) blocked JP-8 induced immunosuppression. It was hypothesized that JP-8 exposure (at least dermal or respiratory exposure) may induce release of prostoglandin E<sub>2</sub> (PGE<sub>2</sub>), initiating a cascade of events involving IL-4 and IL-10 that ultimately results in the specific immunosuppression previously described. Again, administration of IL-12, monoclonal anti-IL-10 or COX-2 blocked JP-8 induced immunosuppression.

### **15. Musculoskeletal System Effects**

Mattie *et al.* (1995) reported no histopathological changes in the sternum or skeletal muscle of male rats administered 750, 1500, or 3,000 mg/kg JP-8 by gavage once daily for 90 days. There are no published studies of JP-8 induced deficits on the musculoskeletal systems of humans.

### **16. Renal System Effects**

There are no published studies of JP-8 induced deficits in the renal systems of humans. There are, however, a number of studies indicating severe renal complications in male rodents exposed repeatedly to JP-8. It is likely that of the small percentage of male rodents experiencing death during low- or moderate-level jet fuel exposure studies the majority exhibited fatal renal complications (Alden, 1986).

Mattie *et al.* (1991) exposed Fischer-344 rats and C57BL/6 mice of both sexes to JP-8 vapors at 0, 500, and 1000 mg/m<sup>3</sup> on a continuous basis for 90 days, then allowed recovery until approximately 24 months of age. In a number of male rats, the kidneys developed a reversible ultrastructural increase in size and propensity for crystalloid changes of phagolysosomal proteinic reabsorption droplets in the proximal convoluted tubular epithelium. A specific triad of persisting light microscopic renal lesions occurred, but functional change was limited to a decrease in urine concentration compared to

controls that persisted throughout the recovery period. Specifically, hyaline droplets were formed in the cytoplasm of the proximal tubule cells of the renal cortex. The hyaline droplets contained high concentrations of the protein  $\alpha_2$ -globulin, a protein not found in humans. It is hypothesized that the protein accumulates in the cytoplasm of the tubules, as binding slows the normal degradation of the protein with chemical constituents of JP-8 or their metabolites. The tubules near the corticomedullary junction became dilated and became filled with coarsely granular casts and necrotic debris, resulting in nephron obstruction and chronic necrosis (US Dept. of Health and Human Services, 1998). This type of hydrocarbon exposure (*i.e.*, jet fuels, decalin, gasoline, *etc.*) toxicity has been shown to progress to kidney cancer in the male rat (Bruner, 1984), but, again, is not considered relevant to humans (Flann and Lehman-McKeeman, 1991).

In a follow-up study, Mattie *et al.* (1995) exposed male Sprague-Dawley rats by oral gavage for 90 days to 750, 1500 or 3,000 mg/kg/d JP-8. As with inhalation exposures (Mattie *et al.*, 1991), significant quantities of hyaline droplets were detected in the kidneys of male rats in all exposure groups. Urine samples were collected within 24 hours post-exposure and were analyzed for protein, creatine, total volume, and metabolite content. Although observed results were not necessarily dose-related, the following significant differences, compared to controls, were reported for at least one of the three exposure groups:

- Sodium (increased)
- Chloride (increased)
- Glucose (decreased, all groups)
- Total bilirubin (increased, all groups)
- Creatine (increased)
- Total triglycerides (decreased)
- Aspartate aminotransferase (increased, all groups)
- Alanine aminotransferase (increased, all groups)

Additionally, four metabolites (retention times, 11.84, 12.82, 13.68, 1605 min.) were identified in the urine of one or more fuel-exposed groups that were not present in the urine of controls.

Recently published research (Witzmann *et al.*, 2000a) indicates that repeated exposure of male Sprague-Dawley rats to 1000 mg/m<sup>3</sup> JP-8 vapor (6 hours/d, 5 d/wk for 6 w) resulted in a significant modulation of expression (from -36% to +315% of control) of several renal proteins, as measured 82 days post-exposure. These proteins were generally involved in kidney ultrastructure or in the detoxification of systemic xenobiotics. Exposure of male mice to aerosolized JP-8 (1000 mg/m<sup>3</sup> for 1 hour/d, for 5 d) resulted in a significant modulation of expression (from -22% to +178% of control) of several renal proteins related to ultrastructural abnormalities, altered protein processing, metabolic effects, and paradoxical stress protein/detoxification system responses (Witzmann *et al.*, 2000b).

## **17. Hepatic System Effects**

There are no published studies on hepatic function in humans exposed to JP-8, although Dossing *et al.* (1985) reported persisting increased liver metabolism (*i.e.*, antipyrine clearance) in 31 jet fueling personnel exposed repeatedly to European jet fuels.

Liver studies in rats and mice (MacEwen and Vernet, 1983, 1984, 1985) indicated alterations in serum biomarkers without significant hepatic histopathology following repeated exposure to JP-8 vapor/aerosol. Mattie *et al.* (1995) dosed male rats by oral gavage with JP-8 (0, 750, 1500, 3,000 mg/kg) daily for 90 days. Although there were no histopathological or weight changes in the livers of exposed rats, there was an increase in the liver enzyme aspartate aminotransferase (AST) and alanine aminotransferase (ALT). There was a significant liver/body weight increase in JP-8 exposed rats, in a dose-related manner, as well as an increase in total bilirubin and a decrease in triglycerides in exposed groups. Dudley *et al.* (2001) reported that oral gavage exposure of mice to 1000 or 2000 mg/kg/d JP-8 for 7 d resulted in significant increases in liver weight and liver-to-body weight ratio, compared to control.

Using electrophoretic techniques (proteomic assay), Witzmann *et al.* (2000) determined that exposure of male rats to 1000 mg/m<sup>3</sup> JP-8 vapor for 6 hours/d, 5 d/wk, for 6 weeks resulted in a persisting numerical, but not significantly different, increase in total abundance of lamin A (NCBI Accession No. 1346413) in the liver. Lamin A is hypothesized to be important in nuclear membrane integrity.

Grant *et al.* (2000), exposing H4IIE liver cells to JP-8, demonstrated a mean inhibitory concentration (IC<sub>50</sub>) of 12.6 +/- 0.4 ug/ml. Comparison of JP-8 toxicity for exposure of hepatic (H4IIE) cells with similar exposure of several central nervous system cell lines, indicated significantly less sensitivity in liver cells

## **18. Endocrine System Effects**

Mattie *et al.* (1995) reported no histopathological changes in the adrenal glands or pancreas of male rats administered 750, 1500, or 3,000 mg/kg JP-8 by gavage once daily for 90 days. There are no published studies of JP-8 induced deficits on the endocrine systems of humans.

## **19. Metabolic Effects**

There are no published studies of metabolic function in humans following JP-8 exposure. In rodents, there are a large number of studies indicating reduced weight, or rate of weight gain, as compared to controls during inhalation, dermal, or oral exposure to JP-8. In many cases, however, no differences in mean body weight between fuel-exposed and control animals can be measured 7-21 days post-exposure (Ritchie *et al.*, 2001a).

## **20. Genotoxic Effects**

In an extensive study, JP-8 was evaluated for genotoxicity using the Ames assay, the mouse lymphoma assay, the unscheduled DNA synthesis assay, and the dominant lethal assay (Air Force, 1978). JP-8 was not mutagenic in the Ames assay and did not induce mutation in mouse lymphoma cells. In the unscheduled DNA synthesis tests, it was shown that JP-8 exposure induced significant incorporation of radiolabeled thymidine, indicating moderate unscheduled DNA synthesis. In the dominant lethal assay, JP-8 exposure did not induce genetic damage in germ cells. In all assays, JP-8 was cytotoxic at concentrations of  $\leq 5$  microliters ( $\mu\text{L}$ )/ml.

Most recently, Grant *et al.* (2001) investigated the genotoxicity of JP-8 on H4IIE rat hepatoma cells *in vitro*. DNA damage was evaluated using the comet (single cell gel electrophoresis) assay. Cells were exposed for 4 hours to JP-8 [solubilized in ethanol at 0.1% (v/v)] to concentrations ranging from 1 to 20 microgram ( $\mu\text{g}$ )/ml. Exposure to JP-8 resulted in an overall increase in mean comet tail moments. Addition of DNA repair inhibitors hydroxyurea (HU) and cytosine arabinoside (Ara-C) to cell culture with JP-8 resulted in accumulation of DNA damage strand breaks and an increase in comet tail length. JP-8, in the concentrations used in this study, did not result in cytotoxicity or significant apoptosis, as measured using the terminal deoxynucleotidyl transferase (TDT)-mediated dUTP-X nick end labeling (TUNEL) assay. These results demonstrated that dose-relevant exposures to JP-8 result in DNA damage to H4IIE cells, and suggested that DNA repair is involved in mitigating these effects.

## **21. Blood System Effects**

There are no published studies identifying effects of JP-8 exposure on the blood in humans. Mattie *et al.* (1995) exposed male Sprague-Dawley rats by oral gavage for 90 days to 750, 1500 or 3,000 mg/kg/d JP-8. Immediately following the exposure, rats were sacrificed and a blood sample analyzed for red blood cell count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell distribution width, mean corpuscular hemoglobin concentration, hematocrit, platelet count, and differential leucocyte count. Although results were not necessarily dose-related, the following significant differences were found, compared to controls, in at least one of the three-exposed group:

- % Neutrophils (increased)
- % Eosinophils (reduced)
- % Basophils (decreased)
- % Lymphocytes (decreased)
- Number of Platelets (increased)

## **22. Acute Lymphocytic Leukemia (ALL) or Acute Myelogenous Leukemia (AML) as Possibly Related to JP-8 Exposure**

There are no published data relating JP-8 exposure to development of ALL or AML. However, JP-8 contains up to 0.8% (volume/volume) benzene (MSDS, Phillips Chemical Co., 1995). There are over 100 published studies indicating a possible relationship between repeated exposure to benzene or compounds containing benzene

(i.e., gasoline, solvent products, and tobacco products) and the development of AML or other leukemias (Rushton and Romaniuk, 1997). While there is little or no evidence that benzene exposure results in development of ALL (Lewis *et al.*, 1997; Rushton and Romaniuk, 1997), it must be remembered that exposure to JP-8, in at least some rodent strains, can result in severe immunosuppression (see Section 14). Severe immune suppression may reduce protection against specific viral infections, a possible causative factor in development of ALL, AML or other cancers (zur Hausen, 1991, Dorak, 1996). Co-exposure to JP-8, gasoline, diesel fuels, some hydrocarbon solvents, and/or tobacco smoke may provide a body burden of benzene that is significantly greater than would be expected from occupational or environmental exposure to JP-8 alone. A review of benzene-induced health effects is presented in Section 23B.

Additionally, *in vitro* human T cell (HPB-ALL and Jurcat line) models have shown that exposure to various PAHs found in JP-8 (e.g., benzo [a] pyrene, anthracene, benz [a] anthracene) can, through cell binding, result in modulation of  $Ca^{+2}$  mobilization and significant suppression of lymphocytic immune cell function (Krieger *et al.*, 1994). These authors additionally reference a number of previously published studies indicating similar outcomes for *in vivo* exposures to PAHs (for example, Blanton *et al.*, 1986). A more complete discussion of PAH-induced health effects is presented in Section 23A.

Smith (1996) developed a hypothesis for the mechanism underlying development of other non-lymphoblastic leukemias from benzene exposure. This theory contains the following key components: 1) inhalation, oral ingestion, or dermal penetration of benzene; 2) activation of blood-transported benzene in the liver to phenolic metabolites (phenol, hydroquinone, catechol, and 1,2,4-benzenetriol); 3) blood transport of these metabolites to the bone marrow; 4) metabolic conversion of these metabolites in the bone marrow (via peroxidase enzymes) to semiquinone radicals and quinones; 5) generation of active oxygen species via redox cycling; 6) damage to tubulin histone proteins, topoisomerase II, and other DNA associated proteins; 7) consequent genetic damage, including DNA strand breakage, mitotic recombination, chromosome relocations, and aneuploidy, resulting in possible development of a leukemic clone. Smith (1996) has further hypothesized that maternal exposure to benzene and other environmental toxicants may provide the most likely mechanism for induction of non-lymphoblastic leukemias. However, it is not clear what the dose-response properties of this mechanism are; it may require prolonged exposure to substantial concentrations of benzene to trigger this potential pathway.

## **23. Human, Animal and *In vitro* Effects of Acute or Long-Term Exposure to Selected Chemical Constituents of JP-8**

**23A. Polycyclic Aromatic Hydrocarbons (PAHs):** JP-8 raw fuel (0.29-3% v/v) and particularly exhaust (20-4,000 ng/m<sup>3</sup>) from JP-8 partial combustion contain polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs, predominated by naphthalenes, and including at least benzo [a] pyrene (BaP), fluoranthene, pyrene, phenanthrene, anthracene, and chrysene. The addition of performance additives to hydrocarbon fuels can increase PAH levels in emissions (Mi *et al.*, 1998). PAHs are distributed in the air in vapor phase or in the particulate phase through adsorption or condensation on the surface of respirable particles (Childers *et al.*, 2000), or from spills

in fuel-contaminated soil and groundwater. Aislabie *et al.* (1999), for example, measured samples ranged from 41 to 8105 nanograms/g (dried soil) of naphthalene and other PAHs in samples collected at a spill site in Antarctica.

Acute inhalation exposure to bicyclic aromatic hydrocarbon naphthalenes has been shown in mice or rats to result in damage to pulmonary epithelial cells, while repeated exposure may be associated with renal damage. Naphthalene is thought to be metabolized under the influence of cytochrome P450 to toxic, electrophilic intermediates (*i.e.*, 1,2-naphthalene oxide, 1,4-naphthoquinone) that mediate damage to nonciliated bronchiolar epithelial cells (Clara cells) and proximal renal tubules in at least mice (Kawabata and White, 1990). Whether this metabolism occurs strictly in liver hepatocytes, and the metabolite is transported to other target organs or whether metabolism also occurs within target organs containing P450 (*i.e.*, lung Clara cells, splenocytes, *etc.*) remains conjectural. Kawabata and White (1990) reported mild immune suppression in splenocyte cultures exposed to high doses (> 200 micromolar) of naphthalene metabolites.

Knuckles *et al.* (2001) exposed male and female F-344 rats orally to BaP at doses of 0, 100, 600 or 1000 mg/kg/d for 14 days (acute group) or 0, 50 or 100 mg/kg/d for 90 (subchronic group) days. In the acute study, white blood cells were significantly decreased and mean cell-hemoglobin concentration was significantly increased in BaP exposed males. Additionally, the liver to body ratio was increased up to 30% in both males and females. In the subchronic study, mean body weight was significantly decreased in exposed males and the liver to body weight ratio was significantly increased. In both male and female BaP exposed groups, red blood cells, hematocrit, and hemoglobin were all significantly decreased. The histopathological examination of selected tissues indicated significant abnormalities (*i.e.*, tubular casts) in the kidneys of some BaP exposed males.

Exposure to respirable polycyclic PAHs is thought to represent a significant human cancer risk (Holland *et al.*, 1981; Eighth Report on Carcinogens, 1998), particularly for the oral areas, lung, skin, and possibly kidneys. BaP and fluoranthene, for example, have been ranked by the Agency for Toxic Substances and Disease Registry (ATSDR, 1997) and the Environmental Protection Agency (EPA) as among the most hazardous substances in the environment.

Again, the carcinogenicity of PAHs is based on their bioactivation to yield carcinogenic intermediates that can be uptaken into cells. The glutathione S-transferase genes *GSTM1* and *GSTT1*, cytochrome P450 (particularly *CYP1A*, *CYP1A1*, and *CYP1B1*), and microsomal epoxide hydrolase have been identified in catalyzing the dihydrodial epoxide (+)-(7R, 8S)-dihydroxy- (9S, 10R)-epoxy-7, 8,9,10-tetrahydrobenzo [a] pyrene, the ultimate carcinogenic form of BaP (Vakharia *et al.*, 2001). Related biomarkers for possible carcinogenicity include 7,8-dihydroxy-9, 10-epoxy-7, 8,9,10-tetrahydrobenzo [a] pyrene-DNA adducts or BaP metabolites in the urine (Hecht, 2001).

Eaton and Chapman (1992, 1995) and others have hypothesized pathways by which highly specific bacterial strains can initiate the metabolism of PAHs to compounds with toxicity potential. For example, *Pseudomonas putida* can, through naphthalene 1,2 deoxygenase, initiate a metabolic cascade with possible end products of salicylate, then catechol. *Pseudomonas* and a number of other hydrocarbon-degrading strains commonly exist in contaminated soils and groundwater. Ferrari *et al.* (1998) collected



350 (fuel/water interface) samples from jet fuel storage systems (*i.e.*, tanks, trucks, and pipelines). The aerobic microorganisms in fuel samples were mainly fungi; 85% of samples containing  $\leq 100$  colony forming units (cfu)/l [range 0 ( $< 1$  cfu/l) to 2000 cfu/l]. The predominant fungi were *Cladosporium* and *Aspergillus*. The aerobic heterotrophic microorganisms found in water samples were mostly bacteria, counts varying from 100 to  $8.8 \times 10(7)$  cfu/ml, with 85% of samples containing  $10(4)$ - $10(7)$  cfu/ml. There was a preponderance of *Pseudomonas spp.* Bacterial contaminants belonging to the genus *Flavobacterium* and *Aeromonas* were also identified. Sulfate reducing bacteria were detected in 80% of water samples.

**23B. Benzene:** Although some relatively clear health effects of exposure to benzene have been identified, no definitive dose response pattern has been determined. The evidence suggests the major health problems resulting from benzene exposure, aplastic anemia and leukemia, require more than a single exposure, however, it is still unknown what levels and lengths of exposure result in increased risk. For instance, it has yet to be resolved whether or not there are risk differences associated with multiple lower-dose exposures over a long period of time, versus fewer high dose exposures over a shorter period of time. Even so, recognition of the health effects of benzene exposure has a relatively long history, with reports as early as 1897 (Santesson) and 1916 (Selling) of illness and death in chronically exposed workers. A link with leukemia was first reported in 1928 (Delore and Borgomano). Today it is widely accepted that the primary health effect of benzene exposure is a depression of bone marrow that often culminates in aplastic anemia or leukemia. A third health problem associated with benzene exposure is myelofibrosis where bone marrow is replaced with fibrous tissue (Zoloth, *et al.*, 1986). Much recent research into the health effects of benzene exposure has concentrated on identifying the molecular mechanisms that underlie the decline in bone marrow function that is evidenced with benzene exposure.

Pancytopenia, a reduction in red and white blood cells and platelets as a result of depressed bone marrow characterizes aplastic anemia. Epidemiological research suggests a connection between the development of the disease and benzene exposure (Aksoy *et al.*, 1971, Yin *et al.*, 1987, 1989). Similarly, epidemiological studies provide evidence for the link between benzene exposure and leukemia (Aksoy and Erdem, 1978; Infante *et al.*, 1977b; Rinsky *et al.*, 1981, 1987; Ott *et al.*, 1978; Bond *et al.*, 1986; Yin *et al.*, 1987, 1989). The onset of acute myelocytic leukemia (AML) related to benzene exposure is often preceded by myelodysplastic syndrome (MDS) (Forni and Moreo, 1967, 1969, van den Berghe *et al.*, 1979). It is proposed that benzene-related MDS is a precursor to the later developing AML (Le Beau *et al.*, 1986; Irons and Stillman, 1996; Irons, 2000). All these conditions (aplastic anemia, MDS, AML) involve a compromised hematopoietic system that may be caused by benzene toxicity. It is posited that one major cause of the compromised bone marrow is damage to the stromal cells responsible for normal bone marrow function (Garnett *et al.*, 1983; Snyder, 2000). Benzene exposure detrimentally affects these stromal cells (Gaido and Wierda, 1984, 1985, Chertkov *et al.*, 1992) that are critical for establishing the hematopoietic environment necessary for the normal maturation of stem cells into blood cells. In addition to indirectly inhibiting the maturation of stem cells, there is evidence that benzene directly damages proliferating stem cells (Uyeki *et al.*, 1977; Boyd *et al.*, 1982).

Although benzene may directly cause some of the health effects that have been observed, more recent data suggest the majority of the problems are caused by benzene metabolites. Compounds that promote benzene metabolism have been demonstrated to increase the toxic effects (Gad-El-Karim *et al.*, 1985, 1986), while an inhibition of benzene metabolism will reduce the amount of cytotoxic damage (Morimoto *et al.*, 1983; Tice *et al.*, 1982). Indeed, it has been reported that toluene inhibits benzene metabolism and reduces benzene toxicity (Andrews *et al.*, 1977), a significant factor in exposure to compounds such as JP-8 that contain both benzene and toluene. Hydroquinone is one metabolite that directly affects bone marrow and the immune system. It interferes with stromal cell activity (Gaido and Weirida, 1984, 1985, 1987; Renz and Kalf, 1991) that is necessary for proper hematopoietic functioning. Hydroquinone also suppresses nuclear factor kappa B, a transcription factor that regulates genes critical for normal T lymphocyte activation (Pyatt *et al.*, 1998). Likewise, it also suppresses interleukin 2 (IL-2), a cytokine required for the proliferation of T-cells (Pyatt *et al.*, 1998). Although the data support the theory that benzene metabolites underlie the toxic effects of exposure, there is not sufficient evidence to clearly implicate a single metabolite, or combination of metabolites, that are responsible for benzene cytotoxicity (Ross, 2000). Indeed, it is likely that combinations of the different metabolites have synergistic toxic effects (Eastmond *et al.*, 1987; Guy *et al.*, 1991; Snyder *et al.*, 1989).

To further support the evidence that benzene metabolism is a critical component of the observed health effects, animal research shows that genetic factors controlling the metabolism of benzene correlate with benzene cytotoxicity. For instance, CYP2E1 is an enzyme important for benzene metabolism. In transgenic mice negative for the enzyme, the cytotoxic and genotoxic effects of benzene exposure were not observed, although they were found in mice of the control groups (Valentine *et al.*, 1996). This finding was not supported in a study of benzene exposed workers where CYP2E1 expression was not correlated with incidence of benzene-related disease (Rothman, 1997). A second enzyme, NAD (P) H: quinone oxidoreductase (NQO1) is also important for benzene metabolism. It is a quinone reductase that acts to detoxify some benzene metabolites, and appears to reduce the resulting cytotoxic insult (Cadenas *et al.*, 1992; Smith, 1999; Wiemels *et al.*, 1999). Reports from epidemiological studies suggest an increased susceptibility to benzene toxicity in people lacking NQO1 (Larson *et al.*, 1999, Rothman *et al.*, 1997, 1998).

In summary, the data suggest a link between benzene exposure and development of hematopoietic disorders such as aplastic anemia and non-lymphoblastic leukemia. The goal of current research is to determine the molecular mechanisms of benzene and its metabolites that contribute to such problems.

**23C. Toluene:** Although there is often concomitant exposure to benzene and toluene, the data suggest toluene is not a cause of the same blood problems as benzene. It has been proposed that a difference in chemical structure, an alkyl group attached to the benzene ring, is the reason toluene does not have the myelotoxic effects observed with benzene (Gerarde, 1956.) In cases where toluene exposure is correlated with anemia and leukemia, it is likely that there was a combination exposure including benzene (Tahti *et al.*, 1981; Banfer, 1961; NIOSH, 1973; Moszczynski and Lisiewicz,

1984, 1985; Yin *et al.*, 1987). In a study where the exposure was toluene without benzene or xylene, rotogravure printers and assistants were occupationally exposed for about 3 years while their blood constituents and bone marrow were consistently monitored (Banfer, 1961). No evidence of hematopoietic problems was observed. In contrast to the hematopoietic effects of benzene, toluene has more neurological effects. The acute effects of a toluene exposure include a narcotic effect, as well as impaired cognitive and neuromuscular function. Similar effects, albeit often to a lesser degree, are found to persist following exposure.

A large review of the research for toluene has been evaluated in a report for the U.S. Department of Health and Human Services (1994). The data for human health effects overall suggest the effects of toluene are primarily neurobehavioral effects. For instance, a 6-hr exposure to 100 ppm toluene caused deficits in visual perception, the ability to discriminate colors, and the ability to do multiplication calculations (Baelum *et al.*, 1985). Similar exposures have resulted in deficits in some, but not all, tests of short-term memory (Echeverria *et al.*, 1991). In this study, no differences were reported for tasks of sensory motor skills such as reaction time, hand-eye coordination, finger tapping, or critical tracking. Also, there were no exposure effects on mood or vigilance. As documented in a NIOSH report on occupational exposure to toluene (NIOSH, 1973), the most carefully controlled exposure to toluene was conducted with 3 volunteers that were exposed to different concentrations of toluene for 8 hours, two times each week for 3 months (von Oettingen *et al.*, 1942a, b). No myelotoxic effects were observed in these volunteers, however, neurobehavioral effects were apparent. In general, the effects during exposure consisted of fatigue, headaches, incoordination and muscle weakening, with a worsening of symptoms as the concentrations increased from 50-800 ppm. At the highest doses, neurobehavioral effects such as mental confusion, exhilaration, and lack of self-control were reported. There were no lasting effects at concentrations up to 100 ppm. However, lasting problems with fatigue, general confusion, headaches, insomnia and skin paresthesia were evident following exposures at higher doses.

Less controlled studies of toluene effects have been conducted in cases of "huffing" where toluene is used as a drug of abuse, and health effects are monitored in the abusers. It should be noted that in studies with abusers, hypoxia is a major confounding variable and should be considered when interpreting results. For instance, there are reports of atrophy in several locations throughout the brain (Rosenberg *et al.*, 1988; Damasceno and de Capitani, 1994; Fornazzari *et al.*, 1983; Lazar *et al.*, 1983), oculo-motor deficits (Mass *et al.*, 1991), and the emergence of personality disorders (Byrne and Zibin, 1991) in toluene abusers. Scores on neuropsychological evaluation tests show few deficits in simple tasks, such as reaction time or finger tapping, but there are deficits on more involved tests of short term memory and spatial skills (Foo *et al.*, 1990), or even more cognitively challenging tasks (Hanninen *et al.*, 1976). A reduction in IQ scores from before versus after exposure has also been reported (Byrne *et al.*, 1991). Evidence from animal models of neurobehavior following toluene exposure also suggest deficits with several different tests of cognitive processing (Evans *et al.*, 1985; Wada *et al.*, 1988; Ikeda and Miyake, 1978; Miyake *et al.*, 1983; Taylor and Evans, 1985). No differences were reported in measures of open field activity or wheel activity tests (Ikeda and Miyake, 1978). Although these results suggest limited problems with

the more basic brain functions, there is evidence to suggest a slowing in the rate of auditory information traveling to the brain for further processing (Abbate *et al.*, 1993).

Toluene abuse also has reproductive and teratological effects. Specifically, an absence of effects on menstruation variables was reported (Ng *et al.*, 1992a), but an increase in spontaneous abortions has been reported (Ng *et al.*, 1992b). In males, hormonal changes have been reported, although there was not a systematic analysis of the effects on fertility (Svensson *et al.*, 1992a, 1992b). The effects were reductions in luteinizing hormone, follicular stimulating hormone, and testosterone. Infants of mothers who abuse toluene often have craniofacial features that resemble those of children with fetal alcohol syndrome, even if the mother did not consume alcohol during pregnancy (Hersh *et al.*, 1985; Toutant and Lippman, 1979; Pearson *et al.*, 1994). Other effects on the embryos and infants include digital hypoplasia, urinary tract anomalies, intrauterine growth retardation, prenatal microcephaly, and developmental delays (Arnold *et al.*, 1994; Goodwin, 1988; Hersch, 1989; Pearson *et al.*, 1994; Wilkins-Haug *et al.*, 1991).

In summary, toluene exposure does not seem to cause hematopoietic problems; however, it does appear to have neurotoxic properties. Its acute effects are narcotic-like, and the lasting effects include fatigue, as well as cognitive confusion and impaired motor coordination. The major detrimental effects related to permanent brain damage have been evidenced in toluene abusers who are exposed via "huffing."

**23D. Trimethylbenzenes:** Trimethylbenzene (TMB) has three common isomers: 1,2,3-; 1,2,4-; and 1,3,5-. The three show quantitatively different, and in a few cases qualitatively different, effects. Rats exposed to 1,2,4-TMB at 123 to 1230 mg/m<sup>3</sup> showed low system toxicity, with no changes in body weight gain or organ weight when compared to controls. However, at the highest concentration a decrease in red blood cells and increase in white blood cells was noted (Korsak *et al.*, 2000a). When rats were exposed to the same regimen of 1,2,3-TMB, a liver weight increase was noted in male rats at the highest exposure, in addition to the blood effects noted for 1,2,4-TMB (Korsak *et al.*, 2000b). In a similar finding, exposure to 1,2,4-TMB for 90 days increased macrophages, polymorphonuclear leukocytes, and lymphocytes at all tested concentrations (Korsak *et al.*, 1997).

Like many hydrocarbons, excessive exposure to TMB produces neurological and behavioral effects. Exposure of rat neural synaptosomes to 1,2,4-TMB produced a dose-dependent increase in reactive oxygen and nitrogen species (Myhre and Fonnum, 2001). Rats exposed 6 hours/d, 5 d/wk to each of the common isomers of TMB at 100 ppm showed significant effects in learning and spontaneous behavior 2 weeks after exposure; however, rats exposed to 1,2,3-TMB had fewer effects (Gralewicz and Wiaderna, 2001). Acute (single-dose) oral exposure of rats increased spontaneous locomotor activity (Tomas *et al.*, 1999) and inhibited brain activity (Tomas *et al.*, 2000).

It is worth noting that exposure to TMB as a part of a hydrocarbon mixture does not affect uptake into the system at large, or into specific organs (Eide and Zahlsten, 1996); however, it does interfere with clearing the compound from the human system (Jamberg *et al.*, 1998). It is important, therefore, to consider TMB exposure in context.

All three common isomers were tested for *in vitro* endpoints (Jamik-Spiechowicz *et al.*, 1998). Only the 1,2,3 isomer was positive in the Ames test, and that without S9 liver extract (tests with enzymatic transformation did not show increased mutation

rates). All three isomers caused increased sister chromatid exchange in mouse bone marrow cells, with 1,2,3- stimulating exchange at the lowest concentration. These tests provide incomplete evidence that all trimethylbenzenes are mutagens, with a higher likelihood for the 1,2,3- isomer.

**23E. Xylenes:** Human occupational exposure monitored by personal diffusive sampling with a mean of 21 ppm xylene showed no toxicity to hematopoietic organs, liver or kidney (Uchida *et al.*, 1993); it did show an increased number of subjective symptoms, as well as eye, nose, and throat irritation. In a series of twelve chronic and subchronic animal exposures (reviewed in Low *et al.*, 1989), no substantial or consistent effects were seen in blood, liver, kidneys, or lungs. Non-lethal effects of acute oral exposure of rats and mice to doses up to 4,000 mg/kg reversed by 2 weeks post-exposure (National Toxicology Program, 1986). Exposure of pregnant dam rats to xylene vapors 6 hours/d, 5 d/wk throughout the gestational period disrupted prenatal and postnatal development (Mirkova *et al.*, 1983)

Epidemiological studies in man suggest that even significant occupational exposure is not carcinogenic. A twenty-year study of several thousand Finnish workers with occupational exposure showed no evidence of increased cancer risk (Anttila *et al.*, 1998). A Montreal study of 3,370 cancer patients of 15 types (non-leukemia) found no evidence of excess risk associated with exposure to xylene for most cancer sites; there was limited evidence which suggested a possible link with colorectal cancer (Gerin *et al.*, 1998). Studies by the National Toxicology Program (1986) showed no evidence of carcinogenicity in mice.

**23F. n-Hexane:** Hexane is perhaps the most toxic of the alkanes by oral exposure; ingestion causes nausea, vertigo, and bronchial and intestinal irritation. It is believed that 50 grams is a fatal dose in humans (Bingham *et al.*, 2000). Current standards set maximum vapor exposure at 100 ppm for 8 hours/d (O'Donoghue, 1985); however, there is some evidence that humans occupationally exposed to less than 100 ppm can have small, cumulative effects in the peripheral nervous system.

Hexane metabolites are cytotoxic to Schwann cells (Kamijima *et al.*, 1996), reducing DNA synthesis in a dose-dependant manner. Mice exposed to 2000-ppm hexane 24 hours/d, 6 d/wk for one year exhibited hind leg muscle degeneration. Rats exposed 13 weeks to 10,000 ppm hexane for 6 hours/d, 5 d/wk showed decreased locomotor activity, along with decreased weight gain and nasal irritation (Dunnick *et al.*, 1989). Hexane can cause significant lung damage. Rabbits exposed to 3,000 ppm 8 hours/d for 8 d developed emphysema and scattered microhemorrhages (Lungarella *et al.*, 1980); the same exposure 8 h/d, 5 d/wk for 24 weeks led to pulmonary fibrosis and papillary tumors (Lungarella *et al.*, 1984). When pregnant female rats were exposed to 1000 ppm 6 hours/d for 9 d, the pups showed reduced postnatal growth (Bus *et al.*, 1979). Rats given single oral doses of several hexane metabolites displayed thymic atrophy after 7 d; however, thymuses from rats given the metabolites for 7 d did not atrophy (Upreti *et al.*, 1986).

A large cancer study using 800 rats and mice was recently reported for hexane exposure (Daughtrey *et al.*, 1999). Animals were exposed for 6 hours/d, 5 d/wk for 2 years to hexane concentrations up to 9,000 ppm. There were no significant differences

in mortality among rats or mice. Rats had no differences in tumor incidence for either sex at any concentration. Female mice showed a decreased incidence of severe cystic endometrial hyperplasia, and an increase in hepatocellular adenomas and carcinomas. No reports link leukemia or lymphoma to hexane exposure.

#### **24. Summary**

1. There is little or no evidence that acute or long-term JP-8 exposures result directly in cancer, serious organic disease, or death in humans.
2. Health effects of JP-8 exposure may be subtle, but persisting, and may occur over prolonged periods of low-dose exposure.
3. Some JP-8 induced health effects may require complex neurobehavioral, proteomic, genomic and metabolomic tests for early identification.
4. There appears to be major differences in JP-8 induced health effects as a function of the duration (acute versus long-term), route of administration (dermal versus respiratory versus oral), and exposure phase (vapor versus aerosol versus raw fuel).
5. From animal studies, it appears that brief exposure to JP-8, in at least aerosol or raw fuel phase, can result in severe and persisting immunosuppression.
6. Animal and *in vitro* studies indicate that exposure to JP-8 can result in modulation of dermal, pulmonary, hepatic, ocular, and renal systems involved in the metabolism, detoxification, and/or elimination of constituent chemicals of JP-8, as well as other xenobiotics.
7. Results of both human and animal studies would appear to indicate that prolonged "occupational-level" exposure to JP-8 could result in persisting changes in brainstem/cerebellar systems, as well as in neurobehavioral performance capacity.
8. Animal and *in vitro* studies indicate that acute or long-term exposure to JP-8, at least in aerosol phase, can result in persisting damage to the pulmonary system.
9. Human, animal and *in vitro* studies indicate that acute or long-term dermal exposure to JP-8 can result in damage to the skin (possible necrosis). There is limited evidence from animal studies that repeated dermal exposure to JP-8 might result in skin cancer.
10. There is limited evidence from animal studies that exposure of females to JP-8 can result in developmental deficits in offspring.
11. There is no direct evidence that JP-8 exposure can result in acute lymphocytic leukemia (ALL). There is minimal evidence that repeated exposure to benzene, at JP-8 occupational levels, can result in development of acute myelogenous leukemia (AML). It is generally unknown if possible immunosuppressive effects of JP-8 exposure, as well

as JP-8 induced changes in detoxification systems (*i.e.*, skin, liver, *etc.*) are correlated with the development of leukemia or other cancers.

## **25. References**

1. Abbate, C, Giorgianni, C, Munao, F, Brecciaroli, R. Neurotoxicity induced by exposure to toluene. An electrophysiologic study. *Int Arch Occup Environ Health* 1993 64:805-811.
2. Air Force. Mutagen and oncogen study of JP-8. 1978 Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, OH. NTIS publication no. AD-A064-948/3.
3. Aislabie J, Balks M, Astori N, Stevenson G, and Symons R. Polycyclic aromatic hydrocarbons in fuel-oil contaminated soils, Antarctica. *Chemosphere* 1999 Dec;39(13):2201-7.
4. Aksoy, M, Dincol, K, Akgun, T, Erdem, S, Dincol, G. Hematological effects of chronic benzene poisoning in 217 workers. *Br J Ind Med* 1971 28:296-302.
5. Aksoy, M, Erdem, S. Follow-up study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic benzene exposure. *Blood* 1978 52(2):285-292.
6. Alden, CL. A review of the unique male rat hydrocarbon nephropathy. *Toxicol Pathol.* 14:109-11.
7. Allen DG, Riviere JE, and Monteiro-Riviere NA. Identification of early biomarkers of inflammation produced by keratinocytes exposed to jet fuels jet A, JP-8, and JP-8(100). *J Biochem Mol Toxicol.* 2000;14(5):231-7.
8. Allen DG, Riviere JE, and Monteiro-Riviere NA. Cytokine induction as a measure of cutaneous toxicity in primary and immortalized porcine keratinocytes exposed to jet fuels, and their relationship to normal human epidermal keratinocytes. *Toxicol Lett* 2001 Mar 8;119(3):209-17.
9. Andersson H, Lindqvist E, Westerholm R, Gragg K, Almen J, and Olson L. Neurotoxic effects of fractionated diesel exhausts following microinjections in rat hippocampus and striatum *Environ Res* 1998 Jan;76(1):41-51. Anttila A, Pukkala E, Riala R, Sallmen M, Hemminki K. 1998. Cancer incidence among Finnish workers exposed to aromatic hydrocarbons. *Int Arch Occup Environ Health* 71(3):187-93.

10. Andrews, LS, Lee, EW, Witmer, CM, Kocsis, JJ, Snyder, R. Effects of toluene on metabolism, disposition, and hematopoietic toxicity of (<sup>3</sup>H) benzene. *Biochem Pharmacol* 1977 26:293-300.
11. Armbrust Aviation Group. 1998 World Jet Fuel Almanac. 1998 The Armbrust Group: Palm Beach Gardens, FL.
12. Arnold, G, Kirby, RS, Langendoerfer, S, Wilkinson-Haug, L. Toluene embryopathy: Clinical delineation and developmental follow-up. *Pediatrics* 1994 93:216-220.
13. Baelum, J, Andersen, I, Lundqvist, GR, Molhave, L, Pedersen, OF, Vaeth, M, Wyon, DP. Response of solvent-exposed printers and unexposed controls to six-hour toluene exposure. *Scand J Work Environ Health* 1985 11:271-280.
14. Baker, W, Dodd, D, McDougal, J, and Miller, T. Repeated Dose Skin Irritation Study on Jet Fuels - Definitive Study. Air Force Research Laboratory Technical Report, AFRL-HE-WP-TR-1999-0022.
15. Baldwin, CM, Houston, FP, Podgornik, MN, Young, RS, Barnes, CA, and Witten, ML. Effects of aerosol-vapor JP-8 jet fuel on the Functional Observational Battery, and learning and memory in the rat. 2001 *Arch Environ Health*, in press.
16. Banfer, W. [Studies on the effect of pure toluene on the blood picture of photogravure printers and helper workers. (Ger)] *Zentralbl Arbeitsmed* 1961 11:35-40.
17. Bingham, E, Cohrssen, B, and Powel, CH. 2000. *Patty's Toxicology* 5th Ed. 4:32-39. John Wiley Sons: New York.
18. Blanton RH, Lyte M, Myers MJ, Bick PH. Immunomodulation by polyaromatic hydrocarbons in mice and murine cells. *Cancer Res.* 1986 Jun;46(6):2735-9.
19. Bond, GG, McLaren, EA, Baldwin, C, Cook, RR. An update of mortality among chemical workers exposed to benzene. *Br J Ind Med* 1986 43:685-691.
20. Boyd, R, Griffiths, J, Kindt, V, Snyder, R, Caro, J, Erslev, A. Relative toxicity of five benzene metabolites on CFU-GM cultures. *Toxicol* 1982 2:121.
21. Briggs, GB, Price, WA, Walsh, AF, and Still, KR. Evaluation of Military Fuel Potential to Produce Male Reproductive Toxicity. Abstract. The Society for Risk Assessment, 1999 Annual Meeting.
22. Briggs, GB, Price, WA, Murray, J., and Still, KR. Evaluation of Military Fuel JP-8 Potential to Produce Male Reproductive Toxicity. Abstract. 2001 *The Toxicologist* 60(1):1194.



23. Broddle, WD, Dennis, MW, Kitchen, DN, and Vernot, EH. Chronic dermal studies of petroleum streams in mice. 1996 *Fund Appl Toxicol* 30: 47-54.
24. Brown, EL, Rivas, JM, Ullrich, SE, Young, CR, Norris SJ, and Kripke, ML. Modulation of immunity to *Borrelia burgdorferi* by ultraviolet irradiation: Differential effect on Th1 and Th2 type immune responses. *Eur J Immunol*.
25. Bruner, RH. Pathologic findings in laboratory animals exposed to hydrocarbon fuels of military interest. In *Renal Effects of Petroleum Hydrocarbons* (MA Mehlamn, GP Hemstreet, JJ Thorpe, and NK Weaver, eds.). *Advances in Modern Environmental Toxicology*, Vol. 7. Princeton Scientific Publishers, Inc., Princeton, NJ. pp. 133-140.
26. Bus JS, White EL, Tyl RW, Barrow CS. 1979. Perinatal toxicity and metabolism of n-hexane in Fischer-344 rats after inhalation exposure during gestation. *Toxicol Appl Pharmacol* 51(2):295-302.
27. Byrne, A., Zibin, T. Toluene-related psychosis. *Br J Psych* 1991 158:578.
28. Byrne, A, Kirby, B, Zibin, T, Ensminger, S. Psychiatric and neurological effects of chronic solvent abuse. *Can J Psychiatry*, 1991 36:735-738.
29. Cadenas, E, Hochstein, P, Ernster, L. Pro- and antioxidant functions of quinones and quinone reductases in mammalian cells. *Adv Enzymol Relat Areas Mol Biol* 1992 65:97-146.
30. Chen H, Witten, ML, and Pfaff JK. JP-8 jet fuel exposure increases alveolar epithelial permeability in rats. 1992 *FASEB J* 6(4):A1064.
31. Chertkov, JL, Lutton, JD, Jiang, S, Da Silva, JL, Abraham, NG. Hematopoietic effects of benzene inhalation assessed by murine long term bone marrow culture. *J Lab Clin Med* 1992 119:412-419.
32. Childers, JW, Witherspoon, CL, Smith, LB, and Pleil, JB. Real-time and integrated measurement of potential human exposure to particle-bound polycyclic aromatic hydrocarbons (PAHs) from aircraft exhaust. *Environ Health Perspect* Sept. 2000 108(9): 853-63.
33. Clark, CR, Ferguson, PW, Katchen, MA, Dennis, MW, and Craig, DK. Comparative acute toxicity of shale and petroleum derived distillates. *Toxicol Ind Health* 1989 Dec;5(6):1005-16.
34. Committee on Toxicology (COT). Permissible exposure levels for selected military fuel vapors. 1996 National Academy Press: Washington, DC.

35. Committee on Toxicology (COT). Evaluating Chemical Exposures for Reproductive and Developmental Toxicity. 2001 National Academy Press: Washington, DC.
36. Cooper, JR, and Mattie, DR. Developmental toxicity of JP-8 jet fuel in the rat. *J Appl Toxicol* 1996 May-Jun;16(3):197-200.
37. Damasceno, BP, de Capitani, EM. Cerebellar atrophy related to chronic exposure to toluene. *Arz Neuropsychiatr* 1994 52(1):90-91.
38. Daughtrey W, Newton P, Rhoden R, Kirwin C, Haddock L, Duffy J, Keenan T, Richter W, Nicolich M. 1999. Chronic inhalation carcinogenicity study of commercial hexane solvent in F-344 rats and B6C3F1 mice. *Toxicol Sci* 48(1):21-9.
39. Delore, P, Borgomano. Leucemie aigue au cours de l'intoxication benzenique. Sur l'origine toxique de certaines leucemies aigues et leurs relations avec les anemies graves. *J Med Lyon* 1928 9:227-233.
40. Dorak, MT. The implications for childhood leukemia of infection with adenovirus. *Trends Microbiol* 1996 Feb;4(2):60-3
41. Doull, J., C.D. Klaassen, and M. D. Amdur (eds.).1980. Casarett and Doull's Toxicology. 2nd ed. New York: Macmillan Publishing Co. P. 469.
42. Dossing, M, Loft, S, and Schroeder, E. Jet fuel and liver function. *J Work Environ Health* 1985 Dec;11(6):433-7.
43. Dunnick JK, Graham DG, Yang RS, Haber SB, Brown HR. 1989. Thirteen-week toxicity study of n-hexane in B6C3F1 mice after inhalation exposure. *Toxicology* 57(2):163-72.
44. Dudley, AC, Peden-Adams, MM, EuDaly, J, Pollenz, RS, and Keil, DE. An aryl hydrocarbon receptor independent mechanism of JP-8 jet fuel immunotoxicity in Ah-responsive and Ah-nonresponsive mice. *Toxicol Sci* 2001 Feb;59(2):251-9.
45. Eastmond, DA, Smith, MT, Irons, RD. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. *Toxicol Appl Pharmacol* 1987 91:85-95.
46. Eaton, RW, and Chapman, PJ. Bacterial metabolism of naphthalene: construction and use of recombinant bacteria to study ring cleavage of 1,2-dihydroxynaphthalene and subsequent reactions. *J Bacteriol* 1992 Dec;174(23):7542-54.

47. Eaton, RW, and Chapman, PJ. Formation of indigo and related compounds from indolecarboxylic acids by aromatic acid-degrading bacteria: chromogenic reactions for cloning genes encoding dioxygenases that act on aromatic acids. *J Bacteriol* 1995 Dec;177(23):6983-8.
48. Echeverria, D, Fine, L, Langolf, G, Schork, T, Sampaio, C. Acute behavioral comparisons to toluene and ethanol in human subjects. *Br J Ind Med* 1991 48:750-761.
49. Eighth Report on Carcinogens. Washington, D.C.: US Dept. of Health and Human Services. 1998.
50. Evans, HL, Bushnell, PJ, Pontecorvo, MJ, Taylor, JD. Animal models of environmentally induced memory impairment. *Ann NY Acad Sci* 1985 444:513-514.
51. Ferrari, MD, Neirotti, E, and Albornoz, C. Occurrence of heterotrophic bacteria and fungi in an aviation fuel handling system and its relationship with fuel fouling. *Rev Argent Microbiol* 1998 Jul-Sep;30(3):105-14.
52. Flamm, WG, and Lehman-McKeeman, LD. The human relevance of the renal tumor-inducing potential of d-limonene in male rats: implications for risk assessment. *1991 Reg Toxicol Pharmacol* 5: 785-86.
53. Foo, SC, Jeyaratnam, J, Koh, D. Chronic neurobehavioural effects of toluene. *Br J Ind Med* 1990 47:480-484.
54. Fornazzari, L, Wilkinson, DA, Kapur, BM, Carlen, PL. Cerebellar, cortical and functional impairment in toluene abusers. *Acta Neurol Scand* 1983 67(6):319-29.
55. Forni, A, Moreo, L. Cytogenetic studies in a case of benzene leukaemia. *Eur J Cancer* 1967 3(4):251-255.
56. Forni, A, Moreo, L. Chromosome studies in a case of benzene-induced erythroleukaemia. *Eur J Cancer* 1969 5(5):459-463.
57. Freeman, JJ, Federici, TM, McKee, RH. Evaluation of the contribution of chronic skin irritation and selected compositional parameters to the tumorigenicity of petroleum middle distillates in mouse skin. *1993 Toxicology* Jul 28;81(2):103-12.
58. Gad-EI-Karim, MM, Ramanujam, VM, Ahmed, AE, Legator, MS. Benzene myeloclastogenicity: A function of its metabolism. *Am J Ind Med* 1985 7:475-484.
59. Gad-EI-Karim, MM, Sadagopa Ramanujam, VM, and Legator, MS. Correlation between the induction of micronuclei in bone marrow by benzene exposure and

- the excretion of metabolites in urine of CD-1 mice. *Toxicol Appl Pharmacol* 1986 85:464-477.
60. Gaido, KW, Wierda, D. Modulation of stromal cell function in DBA/2J and B6C3F1 mice exposed to benzene or phenol. *Toxicol Appl Pharmacol* 1985 81(3):469-75.
  61. Gaido, KW, Wierda, D. *In vitro* effects of benzene metabolites on mouse bone marrow stromal cells. *Toxicol Appl Pharmacol* 1984 76(1):45-55.
  62. Gaido, KW, Wierda, D. Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin. *Toxicol Appl Pharmacol* 1987 89(3):378-90.
  63. Garnett, HM, Cronkite, EP, Drew, RT. Effect of *in vivo* exposure to benzene on the characteristics of bone marrow adherent cells. *Leuk Res* 1983 7(6):803-10.
  64. Gerarde, HW. Toxicological studies on hydrocarbons II. A comparative study of the effects of benzene and certain mono-n-alkylbenzenes on hemopoiesis and bone marrow metabolism in rats. *AMA Arch Ind Health* 1956 13:468-474.
  65. Gerin M, Siemiatycki J, Desy M, Krewski D. 1998. Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: results of a case-control study in Montreal. *Am J Ind Med* 34(2):144-56
  66. Goodwin, TM. Toluene abuse and renal tubular acidosis. *Obstet Gynecol* 1988 71:715-718.
  67. Gralewicz S, Wiaderna D. 2001. Behavioral effects following subacute inhalation exposure to m-xylene or trimethylbenzene in the rat: a comparative study. *Neurotoxicology* 22(1):79-89
  68. Grant, GM, Shaffer, KM, Kao, WY, Stenger, DA, and Pancrazio, JJ. Investigation of *in vitro* toxicity of jet fuels JP-8 and Jet A. *Drug Chem Toxicol* 2000 Feb;23(1):279-91.
  69. Grant, GM, Jackman, SM, Kolanko CJ, and Stenger DA. JP-8 jet fuel-induced DNA damage in H4IIE rat hepatoma cells. *Mutat Res* 2001 Jan 25;490(1):67-75.
  70. Guy, RL, Hu, P, Witz, G, Goldsetin, BD. Depression of iron uptake into erythrocytes in mice by treatment with the combined benzene metabolites p-benzoquinone, muconaldehyde, and hydroquinone. *J Appl Toxicol* 1991 11:443-446.

71. Hanninen, H, Eskelinen, L, Husman, K, Nurimen, M. Behavioral effects of long-term exposure to a mixture of organic solvents. *Scand J Work Environ Health* 1976 4:240-255.
72. Hersh, JH. Toluene embryopathy. *J. Med. Genet.* 1989 26:333-337.
73. Hersh, JH, Podruch, PE, Rogers, G, Weisskopf, B. Toluene embryopathy. *J Pediatr* 1985 106:922-927.
74. Harris, DT, Sakiestewa, D, Robledo RF, and Witten M. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol Ind Health* 1997a Jan-Feb;13(1):43-55.
75. Harris, DT, Sakiestewa, D, Robledo, RF, and Witten, M. Short-term exposure to JP-8 jet fuel results in long-term immunotoxicity. *Toxicol Ind Health* 1997b Sep-Oct;13(5):559-70.
76. Harris, DT, Sakiestewa, D, Robledo, RF, and Witten M. Protection from JP-8 jet fuel induced immunotoxicity by administration of aerosolized substance P. *Toxicol Ind Health* 1997c Sep-Oct;13(5):571-88.
77. Harris, DT, Sakiestewa, D, Robledo, RF, Young, RS, and Witten, M. Effects of short-term JP-8 jet fuel exposure on cell-mediated immunity. *Toxicol Ind Health* 2000 Feb;16(2):78-84.
78. Hays, AM, Parlaman, G, Pfaff, JK, Lantz, RC, Tinajero, J, Tollinger, B, Hall, JN, and Witten, ML. Changes in lung permeability correlate with lung histology in a chronic exposure model. *Toxicol Ind Health* 1995 May-Jun;11(3):325-36.
79. Hecht, SS. Carcinogen biomarkers for lung or oral cancer chemoprevention trials. *IARC Sci Publ* 2001;154:245-55
80. Henz, K. Survey of Jet Fuels Procured by the Defense Energy Support Center, 1990-1996. 1998 Defense Logistics Agency, Ft. Belvoir, VA.
81. Holland, JM, Wolf, DA, and Clark, BR. Relative potency estimation for synthetic petroleum skin carcinogens. *Environ Health Perspect* 1981 Apr;38:149-55.
82. Ikeda, T, Miyake, H. Decreased learning in rats following repeated exposure to toluene: Preliminary report. *Toxicol Lett* 1978 1:235-239.
83. Infante, PF, Rinsky, RA, Wagoner, JK, Young, RJ. Benzene and leukemia. *Lancet* 1977a 2:868-869.
84. Infante, PF, Rinsky, RA, Wagoner, JK, Young, RJ. Leukemia in benzene workers. *Lancet* 1977b 2:76-78.

85. Irons, RD. Molecular models of benzene leukemogenesis. J Toxicol Env Health, Part A 2000 61:391-397.
86. Irons, RD, Stillman, WS. The effects of benzene and other leukaemogenic agents on haematopoietic stem and progenitor cell differentiation. Eur J Haematol Suppl 1996 60:119-24.
87. Janik-Spiechowicz E, Wyszynska K, Dziubaltowska E. 1998. Genotoxicity evaluation of trimethylbenzenes. Mutat Res 412(3):299-305.
88. Jarnberg J, Johanson G, Lof A, Stahlbom B. 1998. Toxicokinetics of 1,2,4-trimethylbenzene in humans exposed to vapours of white spirit: comparison with exposure to 1,2,4-trimethylbenzene alone. Arch Toxicol 72(8):483-91
89. Kamijima M, Sobue G, Ichihara G, Shibata E, Ono Y, Kondo H, Villanueva MB, Itoh T, Mitsuma T, Takeuchi Y. Toxic effects of hexane derivatives on cultured rat Schwann cells. Toxicology 108(1-2):25-31.
90. Kanikkannan, N, Jackson, T, Sudhan Shaik, M, and Singh, M. Evaluation of skin sensitization potential of jet fuels by murine local lymph node assay. Toxicol Lett 2000 Jul 27;116(1-2):165-70.
91. Kanikkannan, N, Burton, S, Patel, R, Jackson, T, Shaik, MS, and Singh, M. Percutaneous permeation and skin irritation of JP-8(100) jet fuel in a porcine model. Toxicol Lett 2001a Feb 28;119(2):133-42.
92. Kanikkannan, N, Patel, R, Jackson, T, Shaik, MS, and Singh, M. Percutaneous absorption and skin irritation of JP-8 (jet fuel). Toxicology. 2001b Mar 21;161(1-2):1-11.
93. Kawabata, TT, and White, KL. Effects of naphthalene and naphthalene metabolites on the *in vitro* humoral immune response. 1990 J Toxicol Environ Health 30:53-67.
94. Kinkead, ER, Salins, SA, and Wolfe, RE. Acute irritation and sensitization potential of JP-8 jet fuel. 1992 J Am Coll Toxicol 11: 700.
95. Knuckles, ME, Inyang, F, and Ramesh, A. Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. Toxicol Sci 2001 61: 382-388.
96. Kobayashi, A, and Kikukawa, A. Increased formaldehyde in jet engine exhaust with changes to JP-8, lower temperature, and lower humidity irritates eyes and respiratory tract. Aviat Space Environ Med 2000 Apr;71(4):396-9.
97. Korsak Z, Rydzynski K, Jajte J. Respiratory irritative effects of trimethylbenzenes: an experimental animal study. Int J Occup Med Environ Health 10(3):303-11.

98. Korsak Z, Stetkiewicz J, Majcherek W, Stetkiewicz I, Jajte J, Rydzynski K. 2000a. Sub-chronic inhalation toxicity of 1,2,4-trimethylbenzene (pseudocumene) in rats. *Int J Occup Med Environ Health* 13(2):155-64.
99. Korsak Z, Stetkiewicz J, Majcherek W, Stetkiewicz I, Jajte J, Rydzynski K. 2000b. Subchronic inhalation toxicity of 1,2,3-trimethylbenzene (hemimellitene) in rats. *Int J Occup Med Environ Health* 13(3):223-32.
100. Krieger JA, Born JL, Burchiel SW. Persistence of calcium elevation in the HPB-ALL human T cell line correlates with immunosuppressive properties of polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol*. 1994 Aug;127(2):268-74.
101. Lanier, LL. Natural killer cells fertile with receptors for HLA-G? *Proc Natl Acad Sci USA* 96:5343-45.
102. Larson, RA, Wang, Y, Banerjee, M, Wiemels, J, Hartford, C, Le Beau, MM, Smith, MT. Prevalence of the inactivating 609CT polymorphism in the NAD(P)H:quinone oxidoreductase (NQO1) gene in patients with primary and therapy-related myeloid leukemia. *Blood* 1999 94:P803-P807.
103. Lazar, RB, Ho, SU, Melen, O, Daghestani, AN. Multifocal central nervous system damage caused by toluene abuse. *Neurology* 1983 33(10):1337-40.
104. Le Beau, MM, Albain, KS, Larson, RA, Vardiman, JW, Davis, EM, Blough, RR, Golomb, HM, Rowley, JD. Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromosomes no. 5 and 7. *J Clin Oncol* 1986 4(3):325-45.
105. Lemasters, GK, Olsen, DM, Yin, JH, Lockey, JE, Shukla, R, Selevan, SG, Schrader, SM, Toth, GP, Evenson, DP, and Huszar, GB. Male reproductive effects of solvent and fuel exposure during aircraft maintenance. *Reprod Toxicol* 1999 May-Jun;13(3):155-66.
106. Lewis, SJ, Bell, GM, Cordingley, N, Pearlman, ED, and Rushton, L. Retrospective estimation of exposure to benzene in a leukaemia case-control study of petroleum marketing and distribution workers in the United Kingdom. 1997. *Occup Environ Med* 54:152-166.
107. Lungarella G, Fonzi L, Centini F. 1980. Respiratory tract lesions induced in rabbits by short-term exposure to N-hexane. *Res Commun Chem Pathol Pharmacol* 29(1):129-39.
108. Lungarella G, Barni-Comparini I, Fonzi L. 1984. Pulmonary changes induced in rabbits by long-term exposure to n-hexane. *Arch Toxicol* 55(4):224-8.

109. MacEwen, JD, and Vernet, EH. Toxic Hazards Research Unit Annual Technical Report. AMRL-TR-83-64. 1983 1984 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Dayton, OH.
110. MacEwen, JD, and Vernet, EH. Toxic Hazards Research Unit Annual Technical Report. AMRL-TR-84-001. 1984 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Dayton, OH.
111. MacEwen, JD, and Vernet, EH. Toxic Hazards Research Unit Annual Technical Report. AMRL-TR-85-058. 1985 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Dayton, OH.
112. Mass, EF, Ashe, J, Spiegel, P, Zee, DS, Leigh, RJ. Acquired pendular nystagmus in toluene addiction. *Neurol* 1991 41:282-285.
113. Material Data Safety Sheet (MSDS). 1995 Jet Fuel JP-8. Phillips Chemical Co.
114. Mattie, DR, Alden, CL, Newell, TK, Gaworski, CL, and Flemming, CD. A 90-day continuous vapor inhalation toxicity study of JP-8 jet fuel followed by 20 or 21 months of recovery in Fischer 344 rats and C57BL/6 mice. *Toxicol Pathol* 1991 19(2):77-87.
115. Mattie, DR, Marit, GB, Flemming, CD, and Cooper, JR. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. *Toxicol Ind Health* 1995 Jul-Aug;11(4):423-35.
116. Mattie, DR, Sterner, TR, Bekkedal, MY, Schimmel, BD, Bausman, TA, Young, SM, and Cooper, JR. Developmental neurobehavioral effects of JP-8 jet fuel on pups from female Sprague-Dawley rats exposed by oral gavage. Abstract. 2001 *The Toxicologist* 60(1):1035.
117. McDougal, JN, Pollard, DL, Garrett, CM, Davis, RM, and Miller, TE. Dermal Absorption of JP-8 Jet Fuel and its Components. 1999 Operational Toxicology Branch, Human Effectiveness Directorate, Wright-Patterson Air Force Base, OH. Technical Report ARFL-HE-WP-TR-1999-0021.
118. McDougal, JN, Pollard, DL, Weisman, W, Garrett, CM, and Miller, TE. Assessment of skin absorption and penetration of JP-8 jet fuel and its components. *Toxicol Sci* 2000 Jun;55(2):247-55.
119. McGuire, S, Bostad, E, Smith, L, Witten, M, Siegel, FL, and Kornguth, S. Increased immunoreactivity of glutathione-S-transferase in the retina of Swiss Webster mice following inhalation of JP-8(100) aerosol. *Arch Toxicol* 2000 Jul;74(4-5):276-80.



120. McInturf, SM, Bekkedal, MY, Ritchie, GD, Nordholm, AF and Rossi III, J. Effects of repeated JP-8 jet fuel exposure on eyeblink conditioning in humans. Abstract. 2001 Abstract. 2001 *The Toxicologist* 60(1):555.
121. McMichael AJ. 1988. Carcinogenicity of benzene, toluene and xylene: epidemiological and experimental evidence. *IARC Sci Publ* 85:3-18
122. Mi, HH, Lee, WJ, Chen, SJ, Lin, TC, Wu, TL, and Hu, JC. Effect of the gasoline additives on PAH emission. *Chemosphere* 1998 Apr;36(9):2031-41.
123. Mirkova E, Zaikov C, Antov G, Mikhailova A, Khinkova L, Benchev I. 1983. Prenatal toxicity of xylene. *J Hyg Epidemiol Microbiol Immunol* 27(3):337-43.
124. Miyake, H, Ikeda, T, Maehara, N, Harabuchi, I, Kishi, R, Yokota, H. Slow learning in rats due to long-term inhalation of toluene. *Neurobehav Toxicol Teratol* 1983 5:541-548.
125. Morimoto, K, Wolff, S, Koizumi, A. Induction of sister chromatid exchanges in human lymphocytes by microsomal activation of benzene metabolites. *Mutat Res* 1983 119:355-360.
126. Moszczynski, P, Lisiewicz, J. Occupational exposure to benzene, toluene, and xylene and the lymphocyte lysosomal N-acetyl-beta-D-glucoaminidase. *Ind Health* 1984 23:47-51.
127. Moszczynski, P, Lisiewicz, J. Occupational exposure to benzene, toluene and xylene and the lymphocyte lysosomal N-acetyl-beta-D-glucoaminidase. *Ind Health* 1985 23:47-51.
128. Myhre O, Fonnum F. 2001. The effect of aliphatic, naphthenic, and aromatic hydrocarbons on production of reactive oxygen species and reactive nitrogen species in rat brain synaptosome fraction: the involvement of calcium, nitric oxide synthase, mitochondria, and phospholipase A. *Biochem Pharmacol* 62(1):119-28.
129. National Institute for Occupational Safety and Health. Occupational Exposure to Toluene. U.S. Department of Health, Education, and Welfare. 1973
130. National Toxicology Program. 1986. Toxicology and Carcinogenesis Studies of Xylenes (Mixed) (60% m-xylene, 14% p-xylene, 9% o-xylene, and 17% ethylbenzene) (CAS No. 1330-20-7) in F344/N Rats and B6C3F<sub>1</sub> Mice (Gavage Studies). NTP document TR-327.
131. Ng, TP, Foo, SC, Yoong, T. Menstrual function in workers exposed to toluene. *Br J Ind Med* 1992a 49:799-803.

132. Ng, TP, Foo, SC, Yoong, T. Risk of spontaneous abortion in workers exposed to toluene. *Br J Ind Med* 1992b 49:804-808.
133. Nordholm, AF. Comparative neurobehavioral toxicity assessment of three hydrocarbon fuels. 1998 U.S. Army Medical Research and Materiel Command, Report No. ADA368457.
134. O'Donoghue, J.L. (ed.). 1985. Neurotoxicity of Industrial and Commercial Chemicals. Volume II. Boca Raton, FL: CRC Press, Inc. P. 63.
135. Pearson, MA, Hoyme, HE, Seaver, LH, Rimsza, ME. Toluene embryopathy: Delineation of the phenotype and comparison with fetal alcohol syndrome. *Pediatrics* 1994 93:211-215.
136. Ott, MG, Townsend, JC, Fishbeck, WA, Langner, RA. Mortality among individuals occupationally exposed to benzene. *Arch Environ Health* 1978 33:3-10.
137. Pfaff, J, Parton, K, Lantz, RC, Chen, H, Hays, AM, and Witten, ML. Inhalation exposure to JP-8 jet fuel alters pulmonary function and substance P levels in Fischer 344 rats. *J Appl Toxicol* 1995 Jul-Aug;15(4):249-56.
138. Pfaff, JK, Tollinger, BJ, Lantz, RC, Chen, H, Hays, AM, and Witten, ML. Neutral endopeptidase (NEP) and its role in pathological pulmonary change with inhalation exposure to JP-8 jet fuel. *Toxicol Ind Health* 1996 Jan-Feb;12(1):93-103.
139. Pfeiffer, KD. A numerical model to predict the fate of jettisoned aviation fuel. 1994 Wright-Patterson AFB, OH, Air Force Institute of Technology.
140. Pleil, JD, Smith, LB, and Zelnick, SD. Personal exposure to JP-8 jet fuel vapors and exhaust at air force bases. *Environ Health Perspect* 2000 Mar;108(3):183-92.
141. Porter, HO. Aviators intoxicated by inhalation of JP-5 fuel vapor. *Aviat Space Environ Med* 1990 61:654-56.
142. Pyatt, DW, Stillman, WS, Irons, RD. Hydroquinone, a reactive metabolite of benzene, inhibits NF-kappa B in primary CD4+ lymphocytes. *Toxicol Appl Pharmacol* 1998 149:178-184.
143. Renz, JF, Kalf, GF. Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: inhibition of conversion of pre-IL-1 alpha to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1 alpha. *Blood* 1991 78(4):938-44.

144. Rinsky, RA, Smith, AB, Hornung, R, Filloon, TG, Young, RJ, Okun, AH, Landrigan, PJ. Benzene and leukemia: An epidemiologic risk assessment. *N Engl J Med* 1987 316:1044-1050.
145. Rinsky, RA, Young, RJ, Smith, AB. Leukemia in benzene workers. *Am J Ind Med* 1981 2:217-245.
146. Ritchie, GD, Still, KR, Alexander, WK, Nordholm, AF, Rossi, J III, and Mattie, DR. A review of the neurotoxicity risk of selected hydrocarbon fuels. 2001a *J Toxicol Environ Health B* 4:101-91.
147. Ritchie, GD, Rossi III, J, Nordholm, AF, Still, KR, Carpenter, RL, Wenger, GR, and Wright, DR. Effects of repeated exposure to JP-8 jet fuel on learning of simple and difficult operant tasks by rats. 2001b *J Toxicol Environ Health, Part A*, In press.
148. Robledo, RF, and Witten ML. Acute pulmonary response to inhaled JP-8 jet fuel in mice. *Inhal Toxicol* 1998 10: 531-553.
149. Robledo, RF, Barber, DS, and Witten, ML. Modulation of bronchial epithelial cell barrier function by *in vitro* jet propulsion fuel 8 exposure. *Toxicol Sci*. 1999a Sep;51(1):119-25.
150. Robledo RF, and Witten ML. NK1-receptor activation prevents hydrocarbon-induced lung injury in mice. *Am J Physiol* 1999b Feb;276(2 Pt 1):L229-38.
151. Robledo RF, Young RS, Lantz RC, and Witten ML. Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. *Toxicol Pathol* 2000 Sep-Oct;28(5):656-63.
152. Riviere, JE, Brooks, JD, Monteiro-Riviere, NA, Budsaba, K, Smith, CE. Dermal absorption and distribution of topically dosed jet fuels jet-A, JP-8, and JP-8(100). *Toxicol Appl Pharmacol* 1999 Oct 1;160(1):60-75.
153. Rosenthal, DS, Simbulan-Rosenthal, CM, Liu, WF, Stoica, BA, and Smulson ME. Mechanisms of JP-8 jet fuel cell toxicity. II. Induction of necrosis in skin fibroblasts and keratinocytes and modulation of levels of Bcl-2 family members. *Toxicol Appl Pharmacol* 2001 Mar 1;171(2):107-16.
154. Rosenberg, NL, Spitz, MC, Filley, CM, Davis, KA, Schaumburg, HH. Central nervous system effects of chronic toluene abuse – clinical, brainstem evoked response and magnetic resonance imaging studies. *Neurotoxicol Teratol* 1988 10:489-495.
155. Ross, D. The role of metabolism and specific metabolites in benzene-induced toxicity: Evidence and issues. *J Toxicol Env Health, Part A* 2000 61:357-372.

156. Rossi III, J, Nordholm, AF, Carpenter, RL, Ritchie, GD, and Malcomb, W. Effects of repeated exposure of rats to JP-5 or JP-8 jet fuel vapor on neurobehavioral capacity and neurotransmitter levels. 2001 *J Toxicol Environ Health A* 63:101-32.
157. Rothman, N, Bechtold, WE, Yin, SN, Dosemeci, M, Li, GL, Wang, YZ, Griffith, WC, Smith, MT, Hayes, RB. Urinary excretion of phenol, catechol, hydroquinone and muconic acid by workers occupationally exposed to benzene. *Occup Environ Med* 1998 55:705-711.
158. Rothman, N, Traver, RD, Smith, MT, Hayes, RB, Li, GL, Campleman, S, Dosemeci, M, Zhang, L, Linet, J, Wacholder, S, Yin, SN, Ross, D. Benzene poisoning, a risk factor for hematological malignancy, is associated with NQ01 C 609C to T mutation and fractional excretion of chlorzoxazone. *Cancer Res* 1997 57:2839-2842.
159. Rushton L, Romaniuk H. A case-control study to investigate the risk of leukaemia associated with exposure to benzene in petroleum marketing and distribution workers in the United Kingdom. *Occup Environ Med*. 1997 Mar;54(3):152-66.
160. Sanagi S, Seki Y, Sugimoto K, Hirata M. 1980. Peripheral nervous system functions of workers exposed to n-hexane at a low level. *Int Arch Occup Environ Health* 47(1):69-79.
161. Santesson, CG. Uber chronische Vergiftung mit Steinkohlenteerbenzin: Vier Todesfalle. *Arch Hyg Berl* 1897 31:336-376.
162. Selden, A, and Ahlberg, G, Jr. Causes of death and cancer morbidity at exposure to aviation fuels in the Swedish armed forces. An Update (Swed.), Orebro, Department of Occupational Medicine. In: IARC (1989) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Occupational Exposures in Petroleum Refining; Crude Oil and Major Petroleum Fuels, 1987, Lyon, France, Vol. 45, pp. 203-18.
163. Selden, A, and Ahlberg, G. Mortality and cancer morbidity after exposure to military aircraft fuel. 1991 *Aviat Space Environ Med* 62:789-94.
164. Selling, L. Benzol as a leukotoxin. Studies on degeneration regeneration of blood and hematopoietic organs. *Johns Hopkins Hosp Rep* 1916 17:83-142.
165. Smith, LB, Bhattacharya, A, Lemasters, G, Succop, P, Puhala, E 2nd, Medvedovic, M, and Joyce, J. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel. *J Occup Environ Med* 1997 Jul;39(7):623-32.

166. Smith, MT, The mechanism of benzene-induced leukemia: A hypothesis and speculations on the causes of leukemia. *Environ Health Perspect* 1996 104:1219-25.
167. Smith, MT. Benzene, NQO1 and genetic susceptibility to cancer. *Proc Natl Acad Sci USA* 1999 96:7624-7626.
168. Snyder, R. Overview of the toxicology of benzene. *J Toxicol Env Health, Part A* 2000 61:339-346.
169. Snyder, R, Dimitriadis, E, Guy, R, Hu, P, Cooper, KR, Bauer, H, Witz, G, Goldstein, BD. Studies on the mechanism of benzene toxicity. *Environ Health Perspect* 1989 82:31-35.
170. Stoica, BA, Boulares, AH, Rosenthal, DS, Hamilton, ID, and Smulson, ME. Mechanisms of JP-8 jet fuel toxicity. I. Induction of apoptosis in rat lung epithelial cells. *Toxicol Appl Pharmacol* 2001 Mar 1;171(2):94-106.
171. Svensson, BG, Nise, G, Erfurth, EM, Nilsson, A, Skerfving, S. Hormone status in occupational toluene exposure. *Am J Ind Med* 1992a 22:99-107.
172. Svensson, BG, Nise, G, Erfurth, EM, Olsson, H. Neuroendocrine effects in printing workers exposed to toluene. *Br J Ind Med* 1992b 49:402-408
173. Tahti, H, Karkkainen, S, Pyykko, K, Rintala, E, Kataja, M, Vapaatalo, H. Chronic occupational exposure to toluene. *Int Arch Occup Environ Health* 1981 48:61-69.
174. Taylor, JD, Evans, HL. Effects of toluene inhalation on behavior and expired carbon dioxide in macaque monkeys. *Toxicol Appl Pharmacol* 1985 80:487-495.
175. Tice, RR, Vogt, TF, Costa, DL. Cytogenetic effects of inhaled benzene on murine bone marrow. In Tice, R.R., Costa, D.L., Schaich, K.M. (eds.). *Genetoxic Effects of Airborne Agents*. Plenum Press, New York. 1982 pp.257-275.
176. Tomas T, Lutz P, Wiaderna D. 2000. Changes in electrocortical arousal following acute trimethylbenzene administration in rats. *Int J Occup Med Environ Health* 13(1):67-78.
177. Tomas T, Swiercz R, Wiaderna D, Gralewicz S. 1999. Effects of acute exposure to aromatic hydrocarbons C 9 on locomotor activity in rats. Trimethylbenzene isomers. *Int J Occup Med Environ Health* 12(4):331-43.
178. Toutant, C, Lippman, S. Fetal solvents syndrome. *Lancet* 1979 i:1356.

179. Uchida Y, Nakatsuka H, Ukai H, Watanabe T, Liu YT, Huang MY, Wang YL, Zhu FZ, Yin H, Ikeda M. 1993. Symptoms and signs in workers exposed predominantly to xylenes. *Int Arch Occup Environ Health* 64(8):597-605.
180. Ullrich, SE. Dermal application of JP-8 jet fuel induces immune suppression. *Toxicol Sci* 1999 Nov;52(1):61-7.
181. Ullrich, SE, and Lyons, HJ. Mechanisms involved in the immunotoxicity induced by dermal application of JP-8 jet fuel. *Toxicol Sci* 2000 Dec;58(2):290-8.
182. Upreti RK, Singh KP, Saxena AK, Shanker R. 1986. Effect of 2,5-hexanedione on lymphoid organs of rats: a preliminary report. *Environ Res* 39(1):188-98
183. US Department of Health and Human Services. Toxicological Profile for JP-5 and JP-8. August 1998.
184. U.S. Department of Health and Human Services. Toxicological profile for toluene. TP-93/14. 1994.
185. Uyeki, EM, Ashkar, AE, Shoeman, DW, Bisel, TU. Acute toxicity of benzene inhalation to hemopoietic precursor cells. *Toxicol Appl Pharmacol* 1977 40:49-57.
186. Vakharia, DD, Liu, N, Pause, R, Fasco, M, Bessette, E, Zhang, QY, and Kaminsky, LS. Effect of metals on polycyclic aromatic hydrocarbon induction of CYP1A1 and CYP1A2 in human hepatocyte cultures. *Toxicol Appl Pharmacol* 2001 Jan 15;170(2):93-103.
187. Valentine, JL, Lee, SST, Seaton, JJ, Asgharian, B, Farris, G, Corton, JC, Gonzalez, FJ, Medinsky, MA. Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. *Toxicol Appl Pharmacol* 1996 141:205-213.
188. van den Berghe, H, Louwagie, A, Broeckaert-van-Orshoven, A, David, G, Verwilghen, R. Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene. *Blood* 1979 53:558-566.
189. von Oettingen, WF, Neal, PA, Donahue, DD. The toxicity and potential dangers of toluene: Preliminary report. *JAMA* 1942a 118:579-584. Wada, H, Hosokawa, T, Saito, K. Repeated toluene exposure and changes of response latency in shock avoidance learning. *Neurotoxicol. Teratol.* 1988 10:387-391.
190. von Oettingen, WF, Neal, PA, Donahue, DD, Svirebely, JL, Baernstein, HD, Monaco, AR, Valaer, PJ, Mitchell, JL. The toxicity and potential dangers of toluene with special reference to its maximal permissible concentration, bulletin 279. Public Health Service, pp. 50, 1942b.

191. Washington Headquarters Service. Military Personnel Statistics: Active Duty Military Strength Report for May 2001, URL is at <<http://www.defenselink.mil/pubs/almanac/people/activeduty.html>>.
192. White, RD. Refining and blending of aviation turbine fuels. *Drug Chem Toxicol* 1999 Feb;22(1):143-53.
193. Wiemels, J, Wiencke, JK, Varykoni, A, Smith, MT. Modulation of the toxicity and macromolecular binding of benzene metabolites by NAD(P)H: Quinone oxidoreductase in transfected HL-60 cells. *Chem Res Toxicol* 1999 12:467-475.
194. Wilkins-Haug, L, Gabow, P. Toluene abuse during pregnancy: Obstetric complications and perinatal outcome. *Ob Gyn* 1991 77:504-509.
195. Witten, ML. Chronic effects of JP-8 jet fuel exposure on the lungs. 1992 Govt. Reports Announcements & Index (GRA&I), Issue 17.
196. Witten, ML. Chronic effects of JP-8 jet fuel exposure on the lungs. 1993 Govt. Reports Announcements & Index (GRA&I), 17.
197. Witzmann, FA, Bauer, MD, Fieno, AM, Grant, RA, Keough, TW, Kornguth, SE, Lacey, MP, Siegel, FL, Sun, Y, Wright, LS, Young, RS, and Witten, ML. Proteomic analysis of simulated occupational jet fuel exposure in the lung. *Electrophoresis* 1999 Dec;20(18):3659-69.
198. Witzmann, FA, Carpenter, RL, Ritchie, GD, Wilson, CL, Nordholm, AF, Rossi III, J. Toxicity of chemical mixtures: proteomic analysis of persisting liver and kidney protein alterations induced by repeated exposure of rats to JP-8 jet fuel vapor. *Electrophoresis* 2000a Jun;21(11):2138-47.
199. Witzmann, FA, Bauer, MD, Fieno, AM, Grant, RA, Keough, TW, Lacey, MP, Sun, Y, Witten, ML, and Young, RS. Proteomic analysis of the renal effects of simulated occupational jet fuel exposure. *Electrophoresis* 2000b Mar;21(5):976-84.
200. Wolfe, RE, Kinead, ER, Feldmann, ML, Leahy, HF, and Jederberg, WW. Acute toxicity evaluation of JP-8 jet fuel and JP-8 jet fuel containing additives. 1997 Govt. Reports Announcements & Index (GRA&I), Issue 09.
201. Zoloth SR, Michaels DM, Villalbi JR, Lacher M. Patterns of mortality among commercial pressmen. *J Natl Cancer Inst.* 1986 Jun;76(6):1047-51.
195. zur Hausen H. Viruses in human cancers. *Science* 1991 Nov 22;254(5035):1167-73.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports (070488), 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>				
1. REPORT DATE (DD-MM-YYYY) 17-09-01		2. REPORT TYPE Final		3. DATES COVERED (From - To) 2001
4. TITLE AND SUBTITLE BIOLOGICAL AND HEALTH EFFECTS OF JP-8 EXPOSURE			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Glenn D. Ritchie, Ph.D., Marni Y. V. Bekkedal, Ph.D., LT Andrew J. Bobb (Ph.D.), MSC, USNR, CAPT Kenneth R. Still (Ph.D.), MSC, USN			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Health Research Center Detachment Toxicology NHRC/TD 2612 Fifth Street, Building 433 Area B Wright-Patterson AFB, OH 45433-7903			8. PERFORMING ORGANIZATION REPORT NUMBER  TOXDET-01-01	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Naval Health Research Center Detachment Toxicology NHRC/TD 2612 Fifth Street, Building 433 Area B Wright-Patterson AFB, OH 45433-7903			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT  Approved for public release; distribution is unlimited.				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT (Maximum 200 words) This report summarizes available human, animal and <i>in vitro</i> studies investigating biological and health effects from acute or long-term exposure to JP-8, its combustion products, and each of six major chemical constituents of JP-8 with known human toxicity potential.				
15. SUBJECT TERMS JP-8, jet fuel, toxicity.				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  UL	18. NUMBER OF PAGES  34
a. REPORT UNCL.	b. ABSTRACT UNCL.	c. THIS PAGE UNCL.		
			19b. TELEPHONE NUMBER (Include area code) (937) 255-6058	

Standard Form 298 (Rev. 8/98)

Prescribed by ANSI Std. Z39.18



## INSTRUCTIONS FOR COMPLETING SF 298

**1. REPORT DATE.** Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year and be Year 2000 compliant, e.g. 30-06-1998; XX-06-1998; xx-xx-1998..

**2. REPORT TYPE.** State the type of report, such as final, technical, interim, memorandum, master's theses, progress, quarterly, research, special, group study, etc.

**3. DATES COVERED.** Indicate the time during which the work was performed and the report was written, e.g., Jun 1997 - Jun 1998; 1-10 June 1996; May - November 1998; Nov 1998.

**4. TITLE.** Enter titles and subtitle with volume number and part number, if applicable. On classified documents, enter the title classification in parentheses

**5a. CONTRACT NUMBER.** Enter all contract numbers as they appear in the report, e.g. F33615-86-C-5 169.

**5b. GRANT NUMBER.** Enter all grant numbers as they appear in the report, e.g. AFOSR-82-1234.

**5c. PROGRAM ELEMENT NUMBER.** Enter all project numbers as they appear in the report, e.g. 61101 A.

**5d. PROJECT NUMBER.** Enter all project numbers as they appear in the report, e.g. 05; RF0330201; T4112.

**5e. TASK NUMBER.** Enter all task numbers as they appear in the report, e.g. 001; AFAPL30480105.

**5f. WORK UNIT NUMBER.** Enter all work unit numbers as they appear in the report, e.g. 001; AFAPL30480105.

**6. AUTHOR(S).** Enter name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. The form of entry is the last name, first name, middle initial, and additional qualifiers separated by commas, e.g. Smith, Richard, J, Jr.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES).** Self-explanatory.

**8. PERFORMING ORGANIZATION REPORT NUMBER.** Enter all unique alphanumeric report numbers assigned by the performing organization, e.g. BRL-1234; AFWL-TR-85-4017-Vol-21 PT-2.

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES).** Enter the name and address of the organization(s) financially responsible for and monitoring the work.

**10. SPONSOR/Monitor's ACRONYM(S).** Enter, if available, e.g. BRL, ARDEC, NADC.

**11. SPONSOR/Monitor's REPORT NUMBER(S).** Enter report number as assigned by the sponsoring/monitoring agency, if available, e.g. BRL-TR-829; -215.

**12. DISTRIBUTION/AVAILABILITY STATEMENT.** Use agency-mandated availability statements to indicate the public availability or distribution limitations of the report. If additional limitations/restrictions or special markings are indicated, follow agency authorization procedures, e.g. RD/FRD, PROPIN, ITAR, etc. Include copyright information.

**13. SUPPLEMENTARY NOTES.** Enter information not included elsewhere such as: prepared in cooperation with; translation of; report supersedes: old edition number, etc.

**14. ABSTRACT.** A brief (approximately 200 words) factual *summary* of the most significant information.

**15. SUBJECT TERMS.** Key words or phrases identifying major concepts in the report.

**16. SECURITY CLASSIFICATION.** Enter security classification in accordance with security classification regulations, e.g. U, C, S, etc. If this form contains classified information, stamp classification level on the top and bottom.

**17. LIMITATION OF ABSTRACT.** This block must be completed to assign a distribution limitation to the abstract. Enter UU (Unclassified Unlimited) or SAR (Same as Report). An entry in this block is necessary if the abstract is to be limited.