

Repeated Aerosol-vapor JP-8 Jet Fuel Exposure Affects Neurobehavior and Neurotransmitter Levels in a Rat Model

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Four groups of Fischer Brown Norway hybrid rats were exposed for 5, 10, 15, or 20 d to aerosolized-vapor jet propulsion fuel 8 (JP-8) compared to freely moving (5 and 10-d exposures) or sham-confined controls (15 and 20-d exposures). Behavioral testing utilized the U.S. Environmental Protection Agency Functional Observational Battery. Exploratory ethological factor

analysis identified three salient factors (central nervous system [CNS] excitability, autonomic 1, and autonomic 2) for use in profiling JP-8 exposure in future studies. The factors were used as dependent variables in general linear modeling. Exposed animals were found to engage in more rearing and hyperaroused behavior compared to controls, replicating prior JP-8 exposure findings. Exposed animals also showed increasing but rapidly decelerating stool output (autonomic 1), and a significant increasing linear trend for urine output (autonomic 2). No significant trends were noted for either of the control groups for the autonomic factors. Rats from each of the groups for each of the time frames were randomly selected for tissue assay from seven brain regions for neurotransmitter levels. Hippocampal DOPAC was significantly elevated after 4-wk JP-8 exposure compared to both control groups, suggesting increased dopamine release and metabolism. Findings indicate that behavioral changes do not appear to manifest until wk 3 and 4 of exposure, suggesting the need for longitudinal studies to determine if these behaviors occur due to cumulative exposure, or due to behavioral sensitization related to repeated exposure to aerosolized-vapor JP-8.

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Jet propulsion fuel 8 (JP-8) is a kerosene-based middle distillate fuel that contains several performance additives, including benzene, naphthalene, *n*-hexane, toluene, and xylene, which are known neurotoxicants (Ritchie et al., 2001, for a comprehensive review of hydrocarbon fuels and neurotoxicant risk). JP-8 has a high flash point and low vapor pressure, which

reduce the potential for crash-related explosions and fires (Mattie et al., 1991). These characteristics have made JP-8 a preferred fuel source by the armed services since 1992 (Nordholm et al., 1999). Because of its widespread use in U.S. military and NATO operations, the opportunity for acute and chronic inhalation and dermal exposures to JP-8 poses even greater potential for toxicant risk to these personnel.

Inhalation toxicology studies have primarily examined the effects of JP-8 on pulmonary (Hays et al., 1995) and immune function (Harris et al., 1997) and on altered gene expression in lung tissue (Espinoza et al., 2005). Hays et al. (1995) showed consistent functional and adaptive changes in rat lungs in response to 28-d low- (500 mg/m³/h) and high-dose (813–1094 mg/m³/h) JP-8 exposures. Moderate (mg/m³) and high-dose (2500 mg/m³) short term (7 d) exposures to JP-8 culminated in prolonged immunosuppression and chronic immunotoxicity in a murine model (Harris et al., 1997). Short-term (7 d) exposure rates to JP-8 similar to occupational exposures experienced by military personnel showed marked alterations in gene expression, particularly genes involved in the protection of lung function against oxidative and toxicant-induced stress in a rodent model (Espinoza et al., 2005).

Several “natural” occupational studies reported neurobehavioral and neuropsychiatric consequences of jet fuel exposure (Knaue et al., 1976, 1978; Struwe et al., 1983). Knaue et al. (1976) found that workers with “heavy” compared to “less heavy” chronic exposures to A-1 and JP-1 jet fuels were more likely to report headache, dizziness, nausea, psychasthenia and neurasthenic symptoms, and polyneuropathy, as well as mild neurological signs with peripheral nerve dysfunction. Studies of workers with averaged jet fuel exposure levels of 300 mg/m³ and median employment durations of 19 yr showed more neurasthenic symptoms, performance irregularity on tests of complex reaction time, and attenuated performance in perceptual speed over time on a simple task of reaction time compared to workers who were not exposed to jet fuel (Knaue et al., 1978; Struwe et al., 1983). Bell et al. (2005) reported faster mean peripheral reaction times and faster post- versus pre-session mean central reaction times of unhealthy Gulf War 1 veterans exposed to subthreshold levels of JP-8 compared to unhealthy cohorts exposed to sham clean air.

Animal experiments of chronic exposure to various jet fuels have also documented neurobehavioral and neurochemical changes (Baldwin et al., 2001; Lin et al., 2004; Nordholm et al., 1999; Rossi et al., 2001). Nordholm et al. (1999) found that rats exposed to JP-4 vapor for 6 h/d for 14 consecutive days showed time-dependent postexposure differences in neurobehavioral capacity, as well as significantly elevated brain and blood serum 5-hydroxytryptamine (5-HT, serotonin) and serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels compared to sham controls. Rossi et al. (2001) exposed rats to JP-5 or JP-8 vapor for 6 h/d, 5 d/wk for 6 wk, and compared their neurobehavioral capacity to air controls following a 65-d rest period. The JP-5-exposed animals exhibited greater

forelimb grip strength, while the JP-8-exposed animals showed a significantly increased appetitive reinforcer approach sensitization compared to controls. Modulations in dopamine, the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were noted in JP-5-exposed animals, while 5-HIAA was differentially modulated in both JP-5- and JP-8-exposed rats compared to controls 85 d postexposure (Rossi et al., 2001). Gene expression alterations in whole brain tissue of rats exposed to low, moderate, and high levels of JP-8 vapor 6 h/d for 91 consecutive days showed gene expression changes in neurotransmitter signaling pathways and stress response functions (Lin et al., 2004).

Baldwin et al. (2001) documented greater hyperlocomotive behavior, increased arousal levels, and faster swim speed in rats exposed for 1 h/d, 5 d/wk, over 5 wk to moderate levels of aerosol-vapor JP-8 compared to sham-confined controls; however, learning, memory, and visual discrimination skills were not influenced by these exposures, based on Morris swim task findings. The progressive locomotor and arousal changes were observed between wk 4 and 5 of repeated/cumulative exposure (Baldwin et al., 2001), findings that were consistent with behavioral sensitization (progressively greater locomotor activity with daily cocaine administration) that is mediated by the mesolimbic dopaminergic system (Kalivas, 1992; Kalivas & Duffy, 1988; Kalivas & Stewart, 1991).

This study investigated the behavioral consequences of moderate levels of aerosolized-vapor JP-8 for 1 h/d using 4 groups of rats for 5-, 10-, 15-, or 20-d exposures. Animal behavior was assessed with the U.S. Environmental Protection Agency (EPA)-endorsed Functional Observational Battery (FOB). The aims of the study included replication of increased locomotor and arousal behaviors recorded in the original study (Baldwin et al., 2001) and to extend the literature by (1) identifying salient factors derived from the FOB that can be used as a framework for future JP-8 exposure studies in a rat model and (2) determining changes in brain neurotransmitter levels associated with changes in observed behaviors.

MATERIALS AND METHODS

Subjects

This study utilized four different sets of rats over four different exposure time frames. As in the original study (Baldwin et al., 2001), 6-mo-old male Fischer-344 Brown Norway hybrid rats weighing 375–400 g, obtained from the National Institute on Aging colony at Harlan Sprague-Dawley (total $n = 72$), were used in this experiment. All animals were housed in the rat facility of the Department of Animal Resources at the University of Arizona Health Sciences Center. All rats were housed in the same room, but according to types of exposure to reduce the chance for control exposure to JP-8 via breath exhalation. They were placed into groups of 2 or 3 rats, and each group was placed in a 45.7 cm × 38.5 cm × 38.5 cm (18 in × 12

in $\times 12$ in) Plexiglas tub and maintained on full food and water. Rats were tested and exposed during the light phase of their 12-h light:dark cycles at approximately the same time each day. University of Arizona IACUC approval was obtained prior to implementation of this study.

Jet Fuel Exposure Protocol

The four groups of rats were exposed over four independent time periods in order to examine repeated/cumulative effects of JP-8 exposure. All rats were randomly assigned to groups. Nonexposed groups included freely moving or “sham-confined” animals to examine confinement stress versus jet fuel exposure effects on behavior using statistical modeling. Groups were studied over time in the following manner:

1. One week of JP-8 exposure ($n = 18$; 12 exposed/6 “freely moving” controls).
2. Two weeks of JP-8 exposure ($n = 18$; 12 exposed/6 “freely moving” controls).
3. Three weeks of JP-8 exposure ($n = 18$; 12 exposed/6 “sham-confined” controls).
4. Four weeks of JP-8 exposure ($n = 18$; 12 exposed/6 “sham-confined” controls).

Each group of JP-8 exposed animals was exposed for 1 h/d, 5 d/wk (no weekend exposures to simulate 5-d work weeks), for a total of 5, 10, 15, or 20 d. Ten to 15 ml of JP-8 was aerosolized with a DeVilbiss Ultra Neb nebulizer (model 99 [Somerset, PA]). Rats were exposed to the aerosol-vapor mixture via nose-only presentation while confined in individual loading tubes. Each tube was nose-cone-fitted to receiving adapters that originated from a common anodized aluminum exposure chamber (volume = 0.5 m^3 [IN-TOX, Albuquerque, NM]). The IN-TOX chamber is operated by a vacuum drawn system set at a flow rate of 0.143 L/s. Rat nares were positioned in the exposure port to reduce the chance of systemic dermal absorption or absorption through self-grooming following exposure.

Animals exposed to JP-8 were rotated daily through the 12 adapter positions of the exposure chamber to minimize the proximity of the exposed animals to the jet fuel source as a variable in exposure concentration or composition. A T-arm from the ventilator circuit was positioned to the portal of the IN-TOX (Albuquerque, NM) exposure chamber. Aerosol production rates were manipulated to achieve the desired JP-8 concentration delivery to the exposure chamber. The vacuum was applied to the exposure chamber on the opposite side of the fuel source. Chambers were thoroughly cleaned prior to exposures. A seven-stage cascade impactor attached to the jet-fuel delivery system measured the total fuel concentration and determined the size range of the fuel particulates. The relative laboratory temperature was $21.33 \pm 1^\circ\text{C}$ standard error of the mean (SEM), the relative humidity was $42.6 \pm 1\%$ SEM, and the average barometric pressure was $697.6 \pm 0.6 \text{ mm Hg}$. The average particulate size was $1.7 \pm 2.3 \mu\text{m}$ (standard geometric deviation).

Sham-exposed controls were confined in a manner identical to those animals exposed to jet fuel, with the exception that only room air was introduced into the exposure chamber. Tubes used in the sham-exposed condition were never used in the exposure chamber to further reduce any inadvertent JP-8 contamination. The rats were sham exposed at the same time and for the same length of time as the jet fuel exposed animals; however, sham-exposed animals were kept in a separate enclosed room from the exposure chamber to prevent any chance of ambient JP-8 inhalation. Freely moving animals were maintained in their Plexiglas tubs. Investigators who tested the animals on the FOB and the rotarod activity were blinded to the groups throughout the experiments.

Source of JP-8 and Averaged Exposure Rates

The original source of JP-8 used throughout this experiment was the Air Force Laboratory at Wright-Patterson Air Force Base (lot 3509). Exposure levels for each of the groups (\pm standard deviation) averaged over their respective exposure time frames were as follows: 5 d = $1318.5 (\pm 1318.5) \text{ mg/m}^3$; 10 d = $1755.6 (\pm 1856.1) \text{ mg/m}^3$; 15 d = $1568.8 (\pm 910.1) \text{ mg/m}^3$; and 20 d = $1774.1 (\pm 1133.7) \text{ mg/m}^3$. It is difficult to ascertain reasons for the large standard deviations. Laboratory environment remained constant. Placement or accidental movement of the nebulizer tubing during exposure may, in part or whole, account for the differences.

Functional Observational Battery

The FOB is a neurobehavioral assessment tool endorsed by the U.S. EPA for use in animal testing of insecticides/pesticides (Moser et al., 1997, 1998; Tilson & Moser, 1992). The FOB has proven to be reliable, sensitive, and robust in detecting neurotoxic potentials of chemicals across a range of laboratory conditions (Moser et al., 1997). It was suggested that the FOB assessment of animals parallels the clinical neurological evaluations in humans in terms of rating the presence and severity of neurobehavioral signs (Tilson & Moser, 1992). The FOB contains both home-cage and open-field observations that test physiologic (weight, temperature), autonomic (urination, defecation, pupillary response, lacrimation, salivation), muscle tone (gait, righting reflex, foot splay), sensory/motor affective (approach, startle, touch responses, tail pinch), and spontaneous/CNS excitability functions (arousal, rearing, home cage posture, ease of removal and handling, grooming, appearance, palpebral closure). Monitoring of motor coordination was included, but grip strength was not conducted in this study. Each rat was initially tested to establish a baseline value 1 d prior to the first day of JP-8 exposure, and behaviorally tested the day after each of the consecutive 5 d of their respective 5, 10, 15, or 20 d of exposure. The baseline (preexposure measures) and all subsequent weekly assessments for the control and exposure groups were used in the ethological factor analysis. The factors that emerged as relevant to JP-8

exposure were used in the general linear model (GLM) analysis. Rats were not exposed or sham confined on weekends. Rats were behaviorally tested on the same day of the week at approximately the same time of day to reduce the chance for diurnal variation.

All testing activity was carried out in an isolated dedicated laboratory room in the University of Arizona College of Medicine. Except for infrared (IR) activity monitoring and grip strength, testing and scoring protocols were followed as outlined by Moser et al. (1997). Specifically, while animals remained in their home cages, the observers first documented each rat's eyelid closure, and presence or absence of writhing, circling, biting, or vocalizations. Each rat was then removed and rated for ease of removal and handling, then observed for signs autonomic or behavioral changes, such as exophthalmos, piloerection, puffiness around the eyes, tail or paw bite marks, fur appearance, tearing, or salivation, all of which were rated and recorded. Each rat was then placed in a large, clear 38.5 cm × 38.5 cm × 45.7 cm (12 in × 12 in × 18 in) photosensor-surrounded Plexiglas container, the floor of which was lined with paper towels. Rats were observed for a 3-min period in this open-field cage. A timer measured latency to the first step, during which a counter was used to record number of rears (supported and nonsupported were not distinguished), as well as grooming episodes. Gait characteristics and arousal level were also recorded at this time.

Arousal, a subjective assessment, was determined with the scale proposed by Moser et al. (1988), such that 1 = very low (stupor, coma, little or no responsiveness); 2 = low (some stupor, "dulled," some head or body movement); 3 = somewhat low (slight stupor, some exploratory movements with periods of immobility); 4 = high (slight excitement, tense, sudden darting or freezing) and 5 = very high (hyperalert, excited, sudden bouts of running or body movements). Following a 3-min period, the number and type (i.e., soft, formed, hard) of fecal boluses and the number and size (cm) of urine pools were noted.

Reflex testing was then implemented and included documentation of each rat's response to finger snap, tail pinch, approach of a pencil, touch to the hindquarter, and pupillary changes to a penlight. This assessment was followed with hind-limb rotation, hind-limb thrust, righting reflex, and catalepsy. Hind paws were then pressed onto a nontoxic stamp pad to measure landing foot splay. Finally, the rat was weighed and returned to its home cage. If any unusual behaviors or changes were noted, separate from activities listed on the FOB, these observations were also recorded. For example, prior FOB testing of JP-8-exposed animals suggested excessive shedding and internal rotation of the left hind limb (possible lateralization effect) (Baldwin et al., 2001); therefore, shedding and limb rotation were included in this assessment. Rectal temperatures were obtained on the day of FOB testing, immediately following the turning on of lights of the light/dark cycle in the animal housing area.

FOB Activity Monitoring

Animal activity was monitored by means of an "Opto-Micro" computerized animal activity measuring system (Columbus Instruments, Columbus, OH) in order to more accurately capture and record vertical and horizontal movement in lieu of visual observation and counting. Activity measurement consisted of eight sensory sets that consist of eight infrared (IR) emitters and eight IR receivers placed around the Plexiglas cage used for field testing. Sensors were spaced 1 inch apart. When positioned opposite another similar sensor, such pairs produce 16 IR light beams intersecting the animal cage. Sensor pairs were positioned in horizontal and vertical directions to provide information about both horizontal and vertical activity. The status of IR beam interruptions by the presence of animals was transferred from all sensors to a personal computer via RS-232 link 10 times per second. From received information about beam interruptions, the computer documented the number of ambulatory and nonambulatory animal movements, as well as animal position along each sensor. Photosensor data were downloaded for statistical analysis after each testing session.

Rotarod Testing

Rotarod treadmills have been used to assess the effect of drugs, motor coordination, and fatigue resistance on rats. An Economex accelerating rotarod (Columbus Instruments, Columbus, OH) was used for testing in this experiment. Each animal was placed on a textured spindle to avoid slipping. Clear Plexiglas front panels were used for viewing during testing. When the animal dropped onto the individual platform below the rotarod, the automated treadmill acceleration speed and duration of activity were hand-recorded by two research assistants. Combined speed and duration were data entered immediately following FOB testing.

Neurochemical Assay Substudy

The neurochemical substudy was designed to determine effects of JP-8 exposure on neurotransmitter levels and their metabolites in rodent brain. Rats (6 per group) were randomly selected and decapitated within 24–48 h of completion of FOB and rotarod testing in order to determine neurotransmitter levels. Brains were removed in the University of Arizona Lung Injury Laboratory and dissected into seven regions (cerebral cortex, cerebellum, striatum, hippocampus, hypothalamus, midbrain, and brainstem). These brain samples were rapidly frozen and shipped on dry ice to JP-8 collaborators at the Waisman Center, University of Wisconsin–Madison, Molecular and Genetic Sciences Unit.

Frozen brain samples were homogenized in 0.1 N perchloric acid containing 0.1% cysteine and stored at –80°C. Neurotransmitter levels were determined by high performance liquid chromatography/electrochemical detection (HPLC/EC). Two

assays were performed and analyzed for each sample. In the first, chromatography, levels of the biogenic amines and their metabolites were determined (norepinephrine and dopamine and its metabolites DOPAC and HVA, 5HT, and 5-HIAA) and in the second, levels of amino acids were determined following derivation with ophthalaldehyde.

Statistical Analyses

Several analytical steps were used for the neurobehavioral assessment. Exploratory (ethological) factor analysis was performed for the purpose of extracting the patterns of intraindividual variability (Figueredo et al., 1992). Ethological factor analysis was applied to determine multiple convergent indicators of species typical patterns (i.e., study of states, not traits). This approach was used in an attempt to identify patterns not idiosyncratic to individual rats, but all rats; assessing indicators of what is occurring in the rats can imply internal events or malfunctions. A pattern of behaviors that co-occur is a better indicator of what is going on internally than a single index. Computation used factor score estimations by means of unit-weighted factor scaling (Gorsuch, 1983). Criterion scaling was done to determine the effect of time on the control group relative to experimental group in an exposure-response fashion; the expected effect of time was imputed, and statistically controlled for using general linear modeling (Cohen & Cohen, 1983). Polynomial regression was used to identify quadratic terms (Cohen & Cohen, 1983).

All neurotransmitter data were expressed as nanograms neurotransmitter per milligram protein. Results were expressed as mean \pm standard deviation (SD). All groups were tested for normality and equal variance. If those tests passed, one-way analysis of variance (ANOVA) was used to determine significance, followed by Dunnett's test when appropriate. If the normality test failed, Kruskal-Wallis one-way ANOVA on ranks was used to determine significance followed by Dunnett's test when appropriate. The criterion for significance was set at $p < .05$.

RESULTS

Functional Observational Battery

Given the focus on factor analysis and general linear modeling to replicate and extend our prior study (Baldwin et al., 2001), the data set was constructed for these types of analyses; however, in order to provide a "snapshot" of changes, means and standard deviations for controls ($n = 6$) compared to exposed ($n = 12$) for the final day of testing for each of the exposure time frames (i.e., d 5, 10, 15 or 20) are provided in Table 1 to display leading variables that would result in the three salient factors described below.

CNS excitability/spontaneous activity. As in the prior study (Baldwin et al., 2001), significant differences were noted primarily in this parameter of the FOB. Exposed animals were significantly more likely to exhibit rearing behavior and

increased ambulation, particularly in the 20-d exposure group. Rats in the 5-d exposure group showed greater freezing behavior; however, freezing appears to attenuate across the other exposure groups. Although not statistically significant within each of the exposure groups, numeric values suggest heightened arousal. Other items in this parameter that showed differences in the direction of greater CNS excitability, although not significantly so, were ease of handling and removal. There were no significant differences between groups for grooming, vocalizations, writhing, circling, biting, tail or paw bite marks, exophthalmos, piloerection, puffiness around the eyes, and tonic/clonic convulsions.

Autonomic parameter. Exposed animals showed significantly more fecal boli during the 10-d exposure, which tapered off during the 15- and 20-d exposures. Fecal odor, type, and color also differed significantly between control and exposed animals at 20 d of exposure. The 5-d-exposed animals were also more likely to produce more urine compared to the controls; however, there were no significant difference between groups for the remaining exposure categories. There were no differences between groups for other autonomic variables, including papillary changes, lacrimation, and salivation.

Physiologic parameter. The exposed animals were significantly more likely to exhibit weight loss during 5 and 10 d of exposure, as reported in our prior study (Baldwin et al., 2001). Weight between groups was equivalent in the 15-d exposure groups and greater in the 20-d exposure group, but not significantly so. There were no differences between groups for temperature.

Muscle tone parameter. There were no significant differences between groups across exposure days for gait, righting reflex, hind limb thrust, rotation, or splay.

Sensory/motor affective parameter. Neither group exhibited significant differences for approach/avoidance responses, startle, touch or tail-pinch response, or catalepsy.

Motor coordination. As shown in Table 1, there were no significant differences between groups on rotarod performance for duration in seconds. The control groups for the 10- and 20-d exposure comparisons showed higher duration times, but were not significant.

Other observations that were first noted in the original study (Baldwin et al., 2001), but not included in the FOB, were claw contracture and lateralization of the contracture. As in the previous study, claw contracture was noted in 2 exposed animals, one during the 15-d exposure and the second during the 20 d exposure. Lateralization of the claw contracture was noted in the exposed animal during the 20-d exposure group.

Ethological Factor Analysis

Data from all four groups of rats, including days of testing (learning effects over time) and cumulative exposure levels of JP-8 using plate weights from the cascade impactor, were entered into the analysis. Behaviors that were believed to

TABLE 1
Patterns of FOB Variables Correlated With JP-8 Exposure Over Time

FOB variables	Days exposed			
	5	10	15	20
CNS excitability				
Rearing				
• Control	2.3 ± 2.3	6.5 ± 2.9	4.7 ± 4.3	4.3 ± 2.7*
• Exposed	6.7 ± 4.0	6.8 ± 4.6	7.5 ± 6.3	8.6 ± 5.2
Ambulation				
• Control	40 ± 29*	83 ± 39	96 ± 134	117 ± 80*
• Exposed	99 ± 63	105 ± 57	147 ± 105	166 ± 115
Arousal				
• Control	2.5 ± 0.5	3.8 ± 0.4	3.8 ± 0.8	3.8 ± 0.8
• Exposed	3.5 ± 0.7	4.0 ± 0.4	4.0 ± 1.0	4.2 ± 0.7
Freezing				
• Control	0.67 ± 08*	0.54 ± 0.8	0.21 ± 0.4	0.33 ± 0.7
• Exposed	1.17 ± 0.9	0.50 ± 0.7	0.08 ± 0.3	0.00
Autonomic 1				
Defecation (bolus number)				
• Control	1.0 ± 1.2	1.5 ± 1.5*	3.0 ± 3.5	3.2 ± 1.6
• Exposed	0.5 ± 1.3	2.1 ± 2.0	2.3 ± 2.6	2.1 ± 2.9
Motor coordination				
Rotarod Activity				
• Control	27 ± 12	19 ± 4	16 ± 8	17 ± 8
• Exposed	29 ± 14	15 ± 12	17 ± 10	11 ± 4
Autonomic 2				
Urination				
• Control	0*	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4
• Exposed	0.5 ± 1.2	0.1 ± 0.3	0.3 ± 0.8	0.1 ± 0.3
Physiological				
Weight (g)				
• Control	395 ± 17*	407 ± 32*	397 ± 18	390 ± 23
• Exposed	370 ± 30	380 ± 30	397 ± 25	408 ± 23
Other observations				
Lateralization (number observed)				
• Control	0	0	0	1
• Exposed	0	0	0	1
Claw contracture (number observed)				
• Control	0	0	0	0
• Exposed	0	0	1	1

Note. Data presented as mean ± SD. Asterisk indicates significance at $p < .05$.

factor load together based on the FOB item findings from our prior study (Baldwin et al., 2001) were suggested for analysis. Unit-weighted factor scores were computed on unresidualized data. This was done by standardizing indicators (FOB behaviors) using z scores. Negative items were reverse scored by multiplying by -1 . Averaged z scores of salient items were

given on each factor, which provided unit-weighted factor scores.

Cronbach's alpha suggested good internal consistency on three factors: CNS excitability (Cronbach's $\alpha = .69$) that correlated with ambulation, arousal, no freezing behavior, ease of handling, rearing, and ease of removal; autonomic 1

(Cronbach's $\alpha = .77$) that correlated with stool color, type, odor, number, rotarod activity, and no fur loss; and autonomic 2 (Cronbach's $\alpha = .77$) that correlated with urine color, odor, amount, number, body weight, no observed lateralization or claw contracture (Table 2). We tested for significance the part-whole correlations for each item on each factor in the current data. The magnitude of the correlations was also very high (.70 or greater), suggesting that these items belong in these factors in these data. Control groups were analyzed separately in order to assess the effects of time and confinement. Exposed animals were excluded because exposure was confounded with time. In the exposure group, it was assumed that true effects of time would be identical to the controls. The dependent variables CNS excitability, autonomic 1, and autonomic 2 were analyzed using confinement condition to control for time.

General Linear Modeling

The three factors were entered into the JP-8 cumulative exposure rat model. General linear modeling (GLM) (Cohen & Cohen, 1983) was used to determine any linear, quadratic, and/or cubic trends for each of the three factors. Time was controlled for by taking the mean values from the CNS excitability, autonomic 1, and autonomic 2 of the control animals and subtracting these from the mean values of the CNS excitability, autonomic 1, and autonomic 2 of the exposed animals in order to examine specifically the effects of CNS excitability, autonomic 1, and autonomic 2 of cumulative exposure to JP-8.

A significant positive linear trend (curve estimate = 0.13) was noted for the CNS excitability factor of exposed rats and a negative linear trend for both control groups (curve estimate = -0.33). A significant positive linear (curve estimate = 0.15) and negative quadratic (curve estimate = -0.43) trend for exposed animals and a nonsignificant negative linear trend

(curve estimate = -0.44) for control animals were observed for the autonomic 1 (defecation) factor. A negative marked linear trend (curve estimate = -0.16) was found for exposed rats, while both sets of control animals showed a nonsignificant negative linear trend (curve estimate = -0.16) for the autonomic 2 (urine) factor.

Hence, the experimental groups were significantly different from the controls. Because significant effects were found on the differences, this logically implies that the differences were significant. Had the scores of the experimental and control animals not been significantly different, there would have been no systematic effects found of JP-8 exposure on the rats. Subtracting the effects of time (estimated from the control group) from the scores of the experimental animals would have left nothing but random error in the residuals, which could not have correlated significantly with JP-8 exposure.

No quadratic trends were observed for the three factors. No differences were noted between freely moving, sham confined, or exposed rats in the 5-d exposure protocol. Freely moving and sham-confined control animals differed on the CNS excitability factor; that is, sham-confined animals became less active from baseline, whereas freely moving animals changed very little from baseline.

Neurotransmitter Results

Dihydroxyphenylacetic acid (DOPAC) in the hippocampal region of the rats in the 20-d cumulative exposure group was significantly elevated compared to the freely moving and sham-confined animals (Table 3). The DOPAC levels increased incrementally from exposure groups 1 through 4, but not significantly. The freely moving and sham-confined groups did not differ significantly from each other. There were no other significant findings between control and exposure groups for any other biogenic amine or amino acid levels for any of the brain regions.

TABLE 2
Part-Whole Correlations of Unit Weighted Factors With Cronbach's Alpha for FOB and Other JP-8 Exposure Indicators

Hyper behavior ($\alpha = .69$)*	<i>r</i>	Stool ($\alpha = .77$)*	<i>r</i>	Urine ($\alpha = .77$)*	<i>r</i>
Ease of handling	.70*	Number of boluses	.67*	Number of pools	.68*
Nonfreeze behavior	.70*	Odor	.65*	Amount	.67*
Ease of removal	.69*	Type (e.g., soft)	.64*	Odor	.67*
Arousal	.61*	Color	.64*	Color	.67*
Ambulation	.60*	Rotarod activity	.85*	Body weight	.83*
Rearing	.59*	No fur loss	.84*	No lateralization	.80*
				No claw contracture	.80*

Note. Asterisk indicates significance at $p < .05$.

TABLE 3
DOPAC Levels in Hippocampus of JP-8 Exposed Rats

Group	DOPAC levels \pm SD (ng/mg)
No treatment	0.102 \pm 0.026
Sham exposed	0.104 \pm 0.035
1 wk exposed (5 exposures)	0.083 \pm 0.023
2 wk exposed (10 exposures)	0.110 \pm 0.026
3 wk exposed (15 exposures)	0.118 \pm 0.027
4 wk exposed (20 exposures)	0.148 \pm 0.043*

Note. Asterisk indicates significant difference, $p < .05$, from no treatment and sham-confined groups.

DISCUSSION

Results of this study showed that the exposed animals engaged in more rearing behavior and became more hyperaroused, while the control animals habituated to their surroundings, replicating our prior FOB findings with JP-8 exposure (Baldwin et al., 2001). Our study also provided three salient factors, using exploratory factor analysis, derived from FOB testing that are relevant to assessing JP-8 exposure in an animal model. These factors may be used as profiles in future studies specific to models of JP-8 exposure.

In addition to the CNS excitability factor, the autonomic 1 and autonomic 2 factors assist in providing a more holistic representation of what may be occurring with repeated JP-8 aerosol-vapor exposure. Exposed animals showed significant positive linear and negative quadratic trends for the autonomic 1 factor, suggesting increasing but rapidly decelerating stool output. Future JP-8 studies will need to replicate this autonomic finding and determine its relationship to rotarod performance, which correlated with this factor. Exposed animals also showed a significant negative linear trend for the autonomic 2 (urine) factor. Changes in body weight correlated with this factor. Our prior FOB study showed that animals experienced significant weight loss compared to controls during the first 15 d of aerosolized exposure to JP-8 (Baldwin et al., 2001). Additional studies are needed to replicate these findings and to determine the potential influence of JP-8 exposure on increased urinary output and weight loss. No significant trends were noted for freely moving or sham-confined controls for either the autonomic 1 or autonomic 2 factor, suggesting that these functions are influenced by repeated and/or cumulative exposure to JP-8.

Although several of the item loadings seem counterintuitive, there is a literature that provides potential explanations for the interrelationship between these seemingly unrelated functional systems (Pradhan & Arunasmitha, 1990; Sanberg & Norman, 1989; Witzmann et al., 2000). Defecation is a commonly used indicator of emotionality in rats when placed in unfamiliar or arousing situations (Sanberg & Norman, 1989). Pradhan and Arunasmitha (1990) showed a parallel relationship between hyperlocomotion and elevated striatal dopamine

and its metabolites, and a simultaneous negative correlation between dopamine activity and motility with defecation in rats. These results are consistent with our findings for hyperlocomotion (open-field ambulation) and a reduction in the number of fecal boluses produced, particularly in the 20-d exposure group, which occurred in tandem with significantly elevated DOPAC, suggesting increased release of dopamine secondary to increased dopaminergic activity in the hippocampus.

It is difficult to ascertain potential reasons for rotarod performance to correlate with the autonomic 1 (defecation) factor. Pradhan and Arunasmitha (1991) indicated that emotional defecation might serve as a heuristic approach for understanding drug effects, akinesia, or central dopamine functions in animal models. The hyperlocomotion demonstrated by the exposed rats, if viewed as a variant of akinesia, may provide one possible explanation for rotarod performance correlating with the autonomic 1 factor. Future studies of JP-8 exposure will need to replicate these interrelationships for this factor.

Although defecation and urination are autonomic functions, each of these functions emerged as independent factors in this study. Reasons for this are not clear. While dopamine is involved in both functions, dopamine receptor binding (Elwan & Soliman, 1995) or other differences in neural substrates may contribute to changes in defecation and urination in exposure studies. Changes in kidney tissue per se might have resulted from exposure to JP-8. For example, Witzmann et al. (2000) showed that mice exposed to 1000 mg/m³ of JP-8 for 1 h/d for 5 d resulted in significant changes in soluble kidney proteins, indicating evidence for JP-8 nephrotoxicity. In the present study, changes in rat body weight correlated with the autonomic 2 (urine) factor. Means scores indicated significant weight loss for the 5- and 10-d exposure groups compared to their respective control groups. Weight was equivalent or greater for the 15- and 20-d exposure groups. These weight changes as well as the increase in urination for the 5-d exposure group mirror our previous findings (Baldwin et al., 2001). One might speculate that weight loss is associated with the polyuria that occurred in the early stages of exposure. It is suggested that future JP-8 studies examine kidney tissue as outlined by Witzmann et al. (2001) so as to include specific data points over at least 20 d of exposure and correlate these findings with weight loss and urinary output.

Behavioral Sensitization, Mesolimbic Dopaminergic Substrates, Gene Expression Changes

JP-8 is a complex mixture of aliphatic and aromatic hydrocarbons, including various solvents. Repeated intermittent exposure to toxicants such as JP-8, formaldehyde, or toluene initiates progressive amplification or sensitization and, eventually, bidirectional oscillation of dopamine-dependent behavioral activation responses over time in animals (Antelman, 1994; Caggiola et al., 1998). This process of progressive amplification of host responses to repeated intermittent exposures to a foreign substance or stressor, known as time-dependent

sensitization (Antelman, 1994), involves dopaminergic mesolimbic pathways in the brain in reward and appetitive behaviors (Ferber & Kuchinsky, 1996; Rossi et al., 2001; Sorg, 1992; Steketee et al., 1992). These environmental agents can cross-sensitize with known dopaminergic agents such as cocaine and apomorphine (Caggiula et al., 1996, 1998; Ferger & Kuchinsky, 1996; Research Advisory Committee on Gulf War Veterans' Illnesses, 2004). Dopaminergic pathways in the interconnected mesolimbic system and prefrontal cortex play an important role in modulation or expression of sensitization processes (Ferber & Kuchinsky, 1996; Sorg et al., 2001; Vanderschuren & Kalivas, 2000) and the ability to attend to stimuli (Coull, 1998; Vanderschuren & Kalivas, 2000).

The hyperactive behavior of rats exposed to JP-8 compared to the habituation seen in both freely moving and sham-confined rats suggests that changes in arousal levels may be a manifestation of behavioral sensitization in this rodent model. This observation is underscored by the concomitant neurotransmitter finding of a greater amount of DOPAC, a metabolite of dopamine, in the hippocampal region of the rats randomly selected for assay from the 20-d exposure group compared to each of the control groups.

Hyperlocomotion in response to repeated aerosolized JP-8 exposure was observed in human and animal studies following solvent exposure (Colotla et al., 1980; Morrow, 1994; Morrow et al., 1992). Colotla et al. (1980) noted rate-dependent paint-thinner exposure effects that were similar to effects seen following sensitization to amphetamine exposure in rats (Antelman et al., 1992, 1997; Austin & Kalivas, 1991; Kalivas, 1992). Stimulant-like arousal effects of the aerosolized JP-8 are a possible factor in the current findings for the CNS excitability factor.

Lin et al. (2004) identified gene expression changes in the rat central nervous system with repeated exposure to JP-8 vapor levels that simulated possible occupational exposures over 91 d for 6 h/d. Two categories of expression were delineated according to their function: neurotransmitter signaling pathways and stress response. Relevant to this research, rats exposed to JP-8 vapor at high levels demonstrated a greater range of gene expression changes, including the dopamine D3 receptor signal response, which is associated with increased locomotor activity.

Sensitization and Unexplained Illnesses

There are converging animal and human studies to suggest that repeated intermittent exposures to subthreshold levels of chemicals, such as the neurotoxicant additives found in JP-8, might initiate various unexplained illnesses that frequently overlap, such as fibromyalgia, chronic fatigue syndrome, sick building syndrome, chemical odor intolerance (CI, the focal symptom of the controversial multiple chemical sensitivity [MCS]), and Gulf War Syndrome (Baldwin et al., 2000; Bell et al., 1997, 1998a, 1998b, 2001, 2005). These syndromes present as multiple symptoms in multiple organ systems, including

neuromuscular, musculoskeletal, gastrointestinal, cardiac, affective, airway, and cognitive complaints. Attention deficits, with concomitant effects on processing load, were described in workers with histories of organic solvent exposure (Morrow, 1994; Morrow et al., 1992). Bell et al. (2005) examined divided attention test performance of 1991 Gulf War Veterans with and without CI. Using nasal-delivered subthreshold levels of JP-8 derived from the same source as that used in the Baldwin et al. (2001) study, the veterans who received the aerosolized JP-8 demonstrated faster peripheral reaction times over sessions compared to unexposed veterans, which was consistent with time-dependent sensitization to low-level exposures (Bell et al., 2005).

Limitations

Although the findings in this study are consistent with behavioral sensitization to a variety of toxicants reported in other animal studies, they differ from a study of JP-8 vapor exposure in one rodent study (Rossi et al., 2001). Rossi et al. (2001) exposed rats to JP-8 jet fuel vapor for 6 h/d, 5 d/wk for 6 wk, and compared their neurobehavioral capacity to air controls following a 65-d rest period. The JP-8-exposed animals did not exhibit hyperlocomotion compared to controls, nor was dopamine turnover reported; however, 5-HIAA was differentially modulated in the JP-8 exposed rats compared to controls at 85 d postexposure. The differences in findings between studies may be related to any of several factors, including (1) vapor versus aerosolized-vapor exposure, (2) differences in test batteries, and (3) different strains of rats, as well as (4) length of time that elapsed between postexposure testing and assay prior to the assessment of neurotransmitter levels. These protocol differences underscore the need for future studies to replicate findings of JP-8 studies using similar protocols and time frames.

Future longitudinal studies are also needed to confirm the factors identified in this ethological analysis, and to determine if these behaviors occur due to cumulative exposure, or behavioral sensitization. The neurotransmitter findings also warrant future longitudinal studies with animals from the exposed and control groups randomly selected on a weekly basis over a minimum of a 4-wk time period for neurotransmitter assay, pharmacologic challenge with amphetamine antagonists (e.g., haloperidol), or perfusion and pathology analysis of relevant brain structures (e.g., limbic system and frontal lobes). Data suggest that freely moving and sham-confined animals differed on the CNS excitability factor (i.e., sham-confined became less active from baseline, whereas freely moving animals changed very little from baseline). In our prior study (Baldwin et al., 2001), as in behavioral sensitization studies (Antelman et al., 1997; Caggiula et al., 1998; Ferger & Kuchinsky, 1996; Kalivas & Stewart, 1991; Sorg et al., 2001) behavioral changes did not appear until 3 to 4 wk of exposure. Hence, future behavioral studies need to extend exposure studies for at least 4 wk, and use sham-confined animals to control for all factors other than exposure.

Summary

Analyses identified three factors relevant to assessing JP-8 aerosol-vapor exposure in an animal model. Findings from the CNS excitability factor support our earlier finding of hyperarousal (increased rearing, locomotion and arousal) (Baldwin et al., 2001). The hyperarousal with repeated exposure to JP-8 compared to habituation seen in both sets of control animals, in combination with the increase in DOPAC in the hippocampal regions noted in the 4-wk exposure group, supports the occurrence of time-dependent sensitization noted in prior drug and toxicant exposure studies in rodents. The autonomic 1 (predominantly changes in defecation) and autonomic 2 (predominantly changes in urination) factors also assist in providing a more comprehensive picture of what may be occurring with repeated exposure to JP-8. Taken together, the profile of aerosol-vapor JP-8 exposure serves as a heuristic approach for identifying and understanding the subtle behavioral and autonomic outcomes using a rodent model. These findings may be extrapolated to military and civilian studies of JP-8 exposure.

REFERENCES

- Antelman, S. M. 1994. Time-dependent sensitization in animals: A possible model of multiple chemical sensitivity in humans. *Toxicol. Ind. Health* 10:335–342.
- Antelman, S. M., Kocan, D., Knopf, S., Edwards, D. J., and Caggiula, A. R. 1992. One brief exposure to a psychological stressor induces long-lasting, time-dependent sensitization of both the cataleptic and neurochemical responses to haloperidol. *Life Sci.* 51:261–266.
- Antelman, S. M., Soares, J. C., and Gershon, S. 1997. Time-dependent sensitization—Possible implications for clinical psychopharmacology. *Behav. Pharmacol.* 8:505–514.
- Austin, M. C., and Kalivas, P. W. 1991. Dopaminergic involvement in locomotion elicited from the ventral pallidum/substantia innominata. *Brain Res.* 542:123–131.
- Baldwin, C. M., Bell, I. R., Fernandez, M., and Schwartz, G. E. 2000. Multiple chemical sensitivity. In *Women and health*, eds. M. B. Goldman and M. C. Hatch, pp. 1129–1139. San Diego, CA: Academic Press.
- Baldwin, C. M., Houston, F. P., Podgornik, M. N., Young, R. S., Barnes, C. A., and Witten, M. L. 2001. Effects of aerosol-vapor JP-8 jet fuel on the Functional Observational Battery, and learning and memory in the rat. *Arch. Environ. Health* 56:216–226.
- Bell, I. R., Baldwin, C. M., and Schwartz, G. E. 1998a. Illness from low levels of environmental chemicals: Relevance to chronic fatigue syndrome and fibromyalgia. *Am. J. Med.* 105:74S–82S.
- Bell, I. R., Baldwin, C. M., and Schwartz, G. E. 2001. Sensitization studies in chemically intolerant individuals: Implications for individual difference research. *Ann. NY Acad. Sci.* 933:38–47.
- Bell, I. R., Brooks, A. J., Baldwin, C. M., Fernandez, M., Figueredo, A. J., and Witten, M. L. 2005. JP-8 jet fuel exposure and divided attention test performance in 1991 Gulf War Veterans. *Aviat. Space Environ. Med.* 76:1136–1144.
- Bell, I. R., Rossi J. III, Gilbert, M. E., Kopal, G., Morrow, L. A., Newlin, D. B., Sorg, B. A., and Wood, R. W. 1997. Testing the neural sensitization and kindling hypothesis for illness from low levels of environmental chemicals. *Environ. Health Perspect.* 105(suppl. 2):539–547.
- Bell, I. R., Warg-Damiani, L., Baldwin, C. M., Walsh, M. E., and Schwartz, G. E. 1998b. Self-reported chemical sensitivity and wartime chemical exposures in Gulf War veterans with and without decreased global health ratings. *Mil. Med.* 163:725–732.
- Caggiula, A. R., Antelman, S. M., Kucinski, B. J., Fowler, H., Edwards, D. J., Austin, M. C., Gershon, S., and Stiller, R. 1998. Oscillatory-sensitization model of repeated drug exposure: Cocaine's effects on shock-induced hypoalgesia. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* 22:511–521.
- Caggiula, A. R., Antelman, S. M., Palmer, A. M., Kiss, S., Edwards, D. J., and Kocan, D. 1996. The effects of ethanol on striatal dopamine and frontal cortical D-[3H]aspartate efflux oscillate with repeated treatment. Relevance to individual differences in drug responsiveness. *Neuropsychopharmacology* 15:125–132.
- Cohen, J., and Cohen, P. 1983. *Applied multiple regression/correlation analysis for the behavioral sciences*, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates.
- Colotla, V. A., Lorenzana-Jiminez, M., and Rodriguez, R. 1980. Toward a behavioral toxicology of paint thinner. *Neurobehav. Toxicol.* 2:31–36.
- Coull, J. T. 1998. Neural correlates of attention and arousal: Insights from electrophysiology, functional neuroimaging and psychopharmacology. *Prog. Neurol. Biol.* 55:343–361.
- Elwan, M. A., and Soliman, M. R. 1995. Alteration of D1 and D2 dopaminergic receptor kinetics in specific rat brain regions following repeated administration of opiates. *Pharmacology* 51:73–83.
- Espinoza, L. A., Valikhani, M., Cossio, M. J., Carr, T., Jung, M., Hyde, J., Witten, M. L., and Smulson, M. E. 2005. Altered expression of gamma-synuclein and detoxification-related genes in lungs of rats exposed to JP-8. *Am. J. Respir. Cell Mol. Biol.* 3:192–200.
- Ferger, B., and Kuschinsky, K. 1996. Effects of cocaine on the EEG power spectrum of rats are significantly altered after its repeated administration: Do they reflect sensitization phenomena? *Naunyn-Schmeideberg's Arch. Pharmacol.* 353:545–551.
- Figueredo, A. J., Petronovich, L., and Ross, D. M. 1992. The quantitative ethology of the Zebra finch: A study in comparative psychometrics. *Multivariate Behav. Res.* 27:413–436.
- Gorsuch, R. L. (1983). *Factor analysis*. Hillsdale, NJ: Lawrence Erlbaum.
- Harris, D. T., Sakiestewa, D., Robledo, R. F., and Witten, M. L. 1997. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol. Ind. Health* 13:43–55.
- Hays, A. M., Parlaman, G., Pfaff, J. K., Lantz, R. C., Tinajero, J., Tollinger, B., Hall, J. N., and Witten, M. L. 1995. Changes in lung permeability correlate with lung histology in a chronic exposure model. *Toxicol. Ind. Health* 11:325–336.
- Kalivas, P. W. 1992. Neural substrate of sensitization to psychostimulants. *Clin. Neuropharmacol.* 15:648A–649A.
- Kalivas, P. W., and Duffy, P. 1988. Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. *J. Neurochem.* 50:1498–1504.
- Kalivas, P. W., and Stewart, J. 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res. Rev.* 16:223–244.
- Knave, B., Persson, H. E., Goldberg, J. M., and Westerholm, P. 1976. Long-term exposure to jet fuel: An investigation on occupationally exposed workers with special reference to the nervous system. *Scand. J. Work Environ. Health* 2:152–164.
- Knave, B., Olson, B. A., Elofsson, S., Gamberale, F., Isaksson, A., Mindus, P., Persson, H. E., Struwe, G., Wennberg, A., and Westerholm, P. 1978. Long-term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. *Scand. J. Work Environ. Health* 4:19–45.
- Lin, B., Ritchie, G. D., Rossi J. III, and Pancrazio, J. J. 2004. Gene expression profiles in the rat central nervous system induced by JP-8 jet fuel vapor exposure. *Neurosci. Lett.* 363:233–238.
- Mattie, D. R., Alden, C. L., Newell, T. K., Gaworski, C. L., and Flemming, C. D. 1991. 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.* 19:77–87.
- Morrow, L. A. 1994. Cueing attention: Disruptions following organic solvent exposure. *Neuropsychology* 8:471–476.
- Morrow, L. A., Robin, N., Hodgson, M. J., and Kamis, H. 1992. Assessment of attention and memory efficiency in persons with solvent neurotoxicity. *Neuropsychologia* 30:911–922.
- Moser, V. C., Becking, G. C., Mac Phail, R. C., and Kulig, B. M. 1997. The IPCS collaborative study on neurobehavioral screening methods. *Fundam. Appl. Toxicol.* 35:143–151.

- Moser, V. C., McCormick, J. P., Creason, J.P., and MacPhail, R. C. 1988. Comparison of chlordimeform and carbaryl using a functional observational battery. *Fundam. Appl. Toxicol.* 11:189–206.
- Nordholm, A. F., Rossi J. III, Ritchie, G. D., McInturf, S., Hulme, M. E., McCool, C., Narayanan, L., MacMahon, K. L., Eggers, J., Leahy, H. F., and Wolfe, R. E. 1999. Repeated exposure of rats to JP-4 vapor induces changes in neurobehavioral capacity and 5-HT/5-HIAA levels. *J. Toxicol. Environ. Health A* 56:471–499.
- Pradhan, N., and Arunasmitha, S. 1991. Correlations of motility, defecatory behavior and striatal dopaminergic activity in rats. *Physiol. Behav.* 50:135–138.
- Research Advisory Committee on Gulf War Veterans' Illnesses. 2004. *Scientific progress in understanding Gulf War veterans' illnesses: Report and recommendations*. Washington, DC: Department of Veterans Affairs.
- Ritchie, G. D., Still, K. R., Alexander, W. K., Nordholm, A. F., Wilson, C. L., Rossi, J. III, and Mattie, D. R. 2001. A review of the neurotoxicity risk of selected hydrocarbon fuels. *J. Toxicol. Environ. Health A* 64:223–312.
- Rossi, J. III, Nordholm, A. F., Carpenter, R. L., Ritchie, G. D., and Malcomb, W. 2001. Effects of repeated exposure of rats to JP-5 or JP-8 jet fuel vapor on neurobehavioral capacity and neurotransmitter levels. *J. Toxicol. Environ. Health A* 63:397–428.
- Sanberg, P. R., and Norman, A. B. 1989. Underrecognized and undersearched side effects of neuroleptics. *Am. J. Psychiat.* 146:411–412.
- Sorg, B. A. 1992. Mesocorticolimbic dopamine systems: Cross-sensitization between stress and cocaine. *Ann. NY Acad. Sci.* 654:136–144.
- Sorg, B., Tschirgi, M. Swindell, S., Chen, L., and Fang, A. 2001. Repeated formaldehyde effects in an animal model for multiple chemical sensitivity. *Ann. NY Acad. Sci.* 933:57–67.
- Steketee, J. D., Sorg, B. A., and Kalivas, P. W. 1992. The role of the nucleus accumbens in sensitization to drugs of abuse. *Prog. Neuropsychopharmacol. Biol. Psychiat.* 16:237–246.
- Struwe, G., Knave, B., and Mindus, P. 1983. Neuropsychiatric symptoms in workers occupationally exposed to jet fuel—A combined epidemiological and casuistic study. *Acta Psychiat. Scand. Suppl.* 303:55–67.
- Tilson, H. A., and Moser, V. C. 1992. Comparison of screening approaches. *Neurotoxicology* 13:1–13.
- U.S. Environmental Protection Agency. 1998. Neurotoxicity Screening Battery, Series 870 Health Effects Test Guidelines #870.6200.
- Vanderschuren, L. J., and Kalivas, P. W. 2000. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: A critical review of preclinical studies. *Psychopharmacology* 151:99–120.
- Witzmann, F. A., Bauer, M. D., Fieno, A. M., Grant, R. A., Keough, T.W., Lacey, M. P., Witten, M. L., and Young, R. S. 2000. Proteomic analysis of the renal effects of simulated occupational jet fuel exposure. *Electrophoresis* 21:976–984.