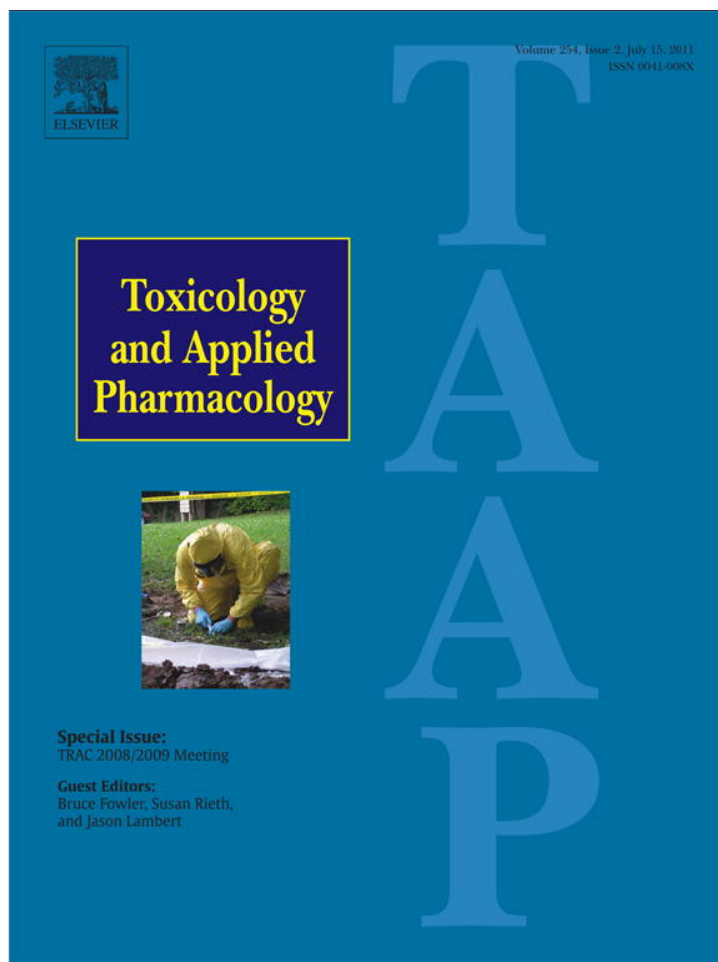


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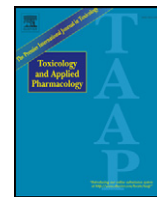
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journal homepage: www.elsevier.com/locate/yaapPast, present and emerging toxicity issues for jet fuel[☆]David R. Mattie^{a,*}, Teresa R. Sterner^b^a Applied Biotechnology Branch, Air Force Research Laboratory, AFRL/RHPB Bldg. 837, 2729 R Street, Wright-Patterson Air Force Base, OH 45433-5707, USA^b HJF, AFRL/RHPB Bldg 837, 2729 R Street, Wright-Patterson Air Force Base, OH 45433-5707, USA

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ABSTRACT

The US Air Force wrote the specification for the first official hydrocarbon-based jet fuel, JP-4, in 1951. This paper will briefly review the toxicity of the current fuel, JP-8, as compared to JP-4. JP-8 has been found to have low acute toxicity with the adverse effects being slight dermal irritation and weak dermal sensitization in animals. JP-4 also has low acute toxicity with slight dermal irritation as the adverse effect. Respiratory tract sensory irritation was greater in JP-8 than in JP-4. Recent data suggest exposure to jet fuel may contribute to hearing loss. Subchronic studies for 90 days with JP-8 and JP-4 showed little toxicity with the primary effect being male rat specific hydrocarbon nephropathy. A 1-year study was conducted for JP-4. The only tumors seen were associated with the male rat specific hydrocarbon nephropathy. A number of immunosuppressive effects have been seen after exposure to JP-8. Limited neurobehavioral effects have been associated with JP-8. JP-8 is not a developmental toxicant and has little reproductive toxicity. JP-4 has not been tested for immune, neurobehavioral or reproductive endpoints. JP-8 and JP-4 were negative in mutagenicity tests but JP-4 showed an increase in unscheduled DNA synthesis. Currently, JP-8 is being used as the standard for comparison of future fuels, including alternative fuels. Emerging issues of concern with jet fuels include naphthalene content, immunotoxicity and inhalation exposure characterization and modeling of complex mixtures such as jet fuels.

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Past

A wide cut fuel, labeled JP-4, was standardized under MIL-F-5624A and was issued for widespread use in May 1951. During the distillation of crude oil to make JP-4, a wide cut is taken of the distillate so as to include both the naphtha (gasoline) and kerosene fractions. JP-4 is typically composed of about 50–60% gasoline, and the remainder is kerosene. Combat experience, observed during the Vietnam War, revealed that the U.S. Air Force aircraft, using highly volatile JP-4, had higher combat losses than U.S. Navy aircraft, using lower volatility JP-5. Also, crash data indicated that the probability of a post-crash fire is nearly 100% when using JP-4, much higher than with a kerosene-based fuel such as JP-5 or commercial Jet A. The superior safety of kerosene fuels, as compared to wide cut fuels, such as JP-4, was also evident in the number and severity of ground handling accidents. JP-8 was developed to provide a safer, jet fuel (similar to commercial Jet A-1) for the Air Force. It was formulated to have adequate availability and an acceptable freeze point. The initial specification for JP-8 MIL-T-83133) was issued in 1976. Conversion from JP-4 to JP-8 was begun in 1979 and was completed in 1994.

Initial studies of jet fuel toxicity noted that many observed effects are attributable to the components of the fuel such as benzene, toluene, xylene and *n*-hexane. Studies by [Struwe et al. \(1983\)](#) and [Knave et al. \(1976, 1978\)](#) have documented the occurrence of symptoms of neurasthenia, psychasthenia and polyneuropathy in civilian and military aircraft workers exposed to JP-4. They also reported cases of sexual dysfunction which were possibly neurologic in origin. Government reviews of the literature summarizing the health effects of jet fuel exposure have been published ([Air Force, 1989](#); [ATSDR, 1993](#)), primarily focusing on JP-4. The major findings have been skin irritation and defatting, neurotoxicity, nephrotoxicity and, in male rats, renal carcinogenicity.

Present

The awareness of JP-4's health effects resulted in comprehensive toxicity studies of the newer JP-8 fuel. Consequently, a number of toxicity reviews for JP-8 and jet fuel in general are available. These include military reviews ([Air Force, 1989](#); [Ritchie et al., 2003](#)) as well as those performed by other government agencies ([ATSDR, 1998](#); [COT, 1996, 2003](#)).

Since the USAF is using JP-8 as its standard fuel, all data for toxicity of new fuels will be compared to JP-8. This fuel has the largest database of all currently used jet fuels and additional JP-8 toxicity studies are still being released. Periodically, further investigations into the toxicological endpoints of interest for JP-8 are necessary in order

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to update the baseline and compare to new alternative fuels. The following is a brief summary of JP-8 effects for studies that are most appropriate for the risk assessment of JP-8. The toxicity of JP-8 will be compared to JP-4.

Acute toxicity

To reduce fuel fouling in current systems and to provide additional heat sink and thermal stability for future systems, the U.S. Air Force developed an improved JP-8 fuel (JP-8 + 100) that offers a 100°F improvement in thermal stability and a 50% increase in fuel heat sink capability. The approach for determining the acute toxicity of JP-8 + 100 was to perform a battery of tests, including oral, dermal and inhalation (vapor and aerosol) acute lethality tests, dermal irritation assays and dermal sensitization tests. These assays had been conducted for JP-8, either previously or simultaneously.

Lethality: Lethality tests indicate that JP-8 is considered slightly toxic at concentrations tested. Male and female F344 rats were gavaged with neat (pure) jet fuel and monitored for 14 days. The oral dose in rats that resulted in lethality of 50% of the tested population (LD_{50}) was greater than 5.0 g/kg (highest dose tested) for both JP-8 and JP-8 + 100. For the dermal route of exposure, patches on the back of male and female New Zealand white rabbits were clipped and neat jet fuel was applied evenly and occluded for 24 h; the rabbits were monitored for 14 days. The dermal LD_{50} in rats was >2.0 g/kg (highest dose tested) for both fuels. Similarly, in the acute inhalation tests, male and female F344 rats were exposed (whole-body) to vapor only or vapor plus aerosol fuel for 4 h and monitored for 14 days. The inhaled vapor concentration LC_{50} was >3.43 mg/l, while the vapor and aerosol combined LC_{50} was >4.39 mg/l, the highest concentrations tested in both fuels (Wolfe et al., 1996).

For JP-4, male Sprague–Dawley rats were dosed by gavage with up to 8 g/kg without any lethality, indicating the LD_{50} is greater than 8.0 g/kg. Male Sprague–Dawley rats were exposed to essentially a saturated vapor of JP-4 (38.3 mg/l) for 6 h. Although coordination was affected as early as 10 min into exposure, followed by sporadic convulsions, no rats died during the exposure or during the 14 days post-exposure observation period (Kinkead et al., 1993).

Irritation: JP-8 was evaluated for primary eye irritation and skin irritation using standard Draize protocols. JP-8 was found to be non-irritating in the rabbit primary eye irritation test. New Zealand white rabbits were given a topical anesthetic in both eyes; one eye was exposed to 0.1 ml JP-8 and the other used for control response comparison (Smith et al., 1981, Kinkead et al., 1992a). Results in the corresponding rabbit skin irritation test ranged from non-irritating to slightly irritating and back to non-irritating (Smith et al., 1981, Kinkead et al., 1992a, Wolfe et al., 1996). In each test, 0.5 ml JP-8 was applied to clipped skin on the backs of New Zealand white rabbits, occluded and evaluated at multiple time points. JP-4 was considered to be non-irritating in the rabbit primary eye irritation test and slightly irritating in the rabbit skin irritation test (Kinkead et al., 1992b).

The rabbit skin irritation test, conducted three times for JP-8 with variable results and once for JP-4, ultimately was not predictive for human exposure. JP-4 was not found to be irritating to workers even though the rabbit test indicated that it was slightly irritating to the skin. The two negative results for dermal irritation in rabbit studies for JP-8 gave the impression that JP-8 would be the same as JP-4 or at least less irritating to humans. In actuality, JP-8 has proven to be more irritating to humans than JP-4. This indicates the importance of testing new fuels for dermal irritation potential while comparing the new fuel to JP-8 as part of the same test to better understand the real potential for dermal irritation in humans. Additional *in vitro* tests with human skin may be necessary as well.

Dermal Sensitization: In the guinea pig skin sensitization test, results ranged from non-sensitizing to a weak dermal sensitizer (10%

sensitization response) and back to non-sensitizing (Smith et al., 1981, Kinkead et al., 1992a, Wolfe et al., 1996, respectively). JP-8 + 100 packages were all negative in the skin irritation and sensitization tests (Wolfe et al., 1996). In this test, Hartley albino guinea pigs were dermally exposed to 0.1 ml JP-8 on 4 separate occasions within 10 days; after a 2-week induction period, a dermal challenge of 0.1 ml JP-8 was applied to a different skin location to test for a sensitized response. Minor Variations in how the studies were conducted may have resulted in slightly different outcomes. However, murine local lymph node assays also indicate that JP-8, but not JP-8 + 100, is a weak dermal sensitizer. In this assay, 25 μ l JP-8 was applied to the ear flaps of female CBA/Ca mice for three consecutive days; allergic response was measured by increased uptake of [3H]-methyl thymidine into auricular lymph node cells compared to concurrent controls (Kanikkannan et al., 2000).

JP-4 was found to not cause dermal sensitization in albino guinea pigs. This test was conducted using male Hartley albino guinea pigs in the same manner as for JP-8, above (Kinkead et al., 1992b). Neither JP-4 or JP-8 has been implicated as a sensitizer among fuel handlers.

Respiratory tract sensory irritation was examined for JP-4, JP-8 and JP-8 + 100 in male Swiss-Webster mice. Groups of mice were exposed head-only to JP-4 (685, 956, 1888 or 11430 mg/m³), JP-8 (681, 708, 1090, 1837 or 3565 mg/m³) or JP-8 + 100 (777, 1519 or 2356 mg/m³) for 30 min. The calculated concentration at which the respiratory rate decreased 50% (RD_{50}) was 4842, 2876 and 1629 mg/m³ for each fuel, respectively (Whitman and Hinz, 2001).

Dermal toxicity

Few dermal systemic toxicity tests, aside from the acute irritation tests above, have been performed with JP-8. A review of the dermal toxicity of petroleum distillates closely related to JP-8 can be found in McDougal and Rogers (2004). Much work has been performed to examine the absorption and local effects of JP-8, including several *in vitro* studies. Again, it is clear from the body of knowledge combined with reports from military personnel working with JP-8 that the fuel does cause considerable skin irritation over continued use and exposure.

One subchronic dermal study of JP-8 was conducted by Baker and coauthors in 1999. Dermal histological changes were investigated in male F344 rats. A daily un-occluded dermal exposure to 0.156 ml JP-8, JP + 100 or JP-4 for 4 weeks was followed by a 3-week recovery period. Proliferative, degenerative and inflammatory changes were significantly greater in the fuel exposed skin versus non-exposed control skin sites on the same animal immediately post-exposure, but fuel treatment results did not differ from each other. Following the recovery period, the dermal histology of all the exposed skin sites had returned to control scores.

Hearing loss

JP-8 exposure has been shown to increase hearing damage from noise. Male Long-Evans rats were exposed, nose-only, to 1000 ppm JP-8 for 4 h. A subset was then exposed to noise (97 or 102 dB) for 1 or 4 h. Exposures were performed either singularly or repeated for five consecutive days. JP-8 alone did not affect hearing but JP-8 followed by noise resulted in small but consistent disruption of outer hair cell function and hair cell loss greater than for noise alone. The effect was only partially reversible with time (4 weeks) (Fechter et al., 2007). These results are corroborated by an occupational study among jet fuel and noise exposed military workers. JP-8 estimated exposures below 350 mg/m³ (the permissible exposure limit (PEL) at the time the study was performed) for 3 or 12 years, combined with noisy working environments, were associated with increased odds of hearing loss. Incidence of hearing loss also increased with time on

the job (Kaufman et al., 2005). Possible hearing damage specifically associated with jet fuel exposure was not assessed for JP-4.

Subchronic

Subchronic 90-day studies were conducted through inhalation and oral pathways. Male and female F344 rats and C57BL/6 mice were exposed (whole-body) to JP-8 vapor (0, 500 or 1000 mg/m³) continuously over 90 days. Decreased bodyweight occurred in male rats, along with renal alpha 2-microglobulin hydrocarbon nephropathy, a male rat specific response to hydrocarbons that is not related to human health (Alden, 1986; Flamm and Lehman-McKeehan, 1991). No additional treatment-related adverse effects were found (Mattie et al., 1991). In a subsequent study, male Sprague–Dawley rats were dosed with 0, 750, 1500 or 3000 mg/kg neat JP-8 by gavage daily for 90 days. Decreased bodyweight and hydrocarbon nephropathy occurred in all JP-8 dosed rat groups. Additional effects included gastritis, perianal dermatitis and increased liver enzymes (AST and ALT). JP-8 was concluded to have minimal toxic effects outside the male rat specific nephropathy (Mattie et al., 1995).

For JP-4, male and female F344 rats, female C57BL/6 mice and male and female beagle dogs were exposed (whole-body) to JP-4 jet fuel vapor at concentrations of 0, 500 or 1000 mg/m³ continuously over 90 days (Kinkead et al., 1995). At the end of the 90 days of exposure, all dogs and one third of the rodents were euthanized and the remaining animals held for either a 19- or 21-month post-exposure observation for tumorigenesis. There was no treatment-related respiratory toxicity seen in any species. Pathologic findings were limited to male rats in which the alpha 2-microglobulin nephropathy was the only treatment-related kidney toxicity. There were no target organ toxicity or carcinogenesis caused by inhalation exposure to JP-4 for 90 days.

No chronic study was conducted for JP-8. For JP-4, F344 rats and C57BL/6 mice of both sexes were exposed (whole-body) to JP-4 jet fuel vapor at concentrations of 0, 500 or 1000 mg/m³ for 6 h per day, 5 days per week for 12 months (Bruner et al., 1993). At the end of exposure, 10% of the rodents were euthanized and the remaining animals held for a 12-month post-exposure observation for tumorigenesis. Again pathologic findings were limited to male rats in which the alpha 2-microglobulin nephropathy was the treatment-related renal toxicity. Renal neoplasms were consistent with the male rat nephropathy as well. No other neoplasms were seen as the result of JP-4 exposure. There was no treatment-related respiratory or any other target organ toxicity seen after inhalation exposure to JP-4 for 1 year.

Immune

Immunological parameters, host resistance and thyroid hormones were evaluated by Keil et al. (2003) in F1 mice exposed in utero to JP-8. C57BL/6 pregnant dams (mated with C3H/HeJ males) were gavaged daily on gestation days 6–15 with JP-8 in a vehicle of olive oil at 0, 1000 or 2000 mg/kg. At weaning (3 weeks of age), no significant differences were observed in body, liver, spleen or thymus weight, splenic and thymic cellularity, splenic CD4/CD8 lymphocyte subpopulations, or T-cell proliferation. Yet, lymphocytic proliferative responses to B-cell mitogens were suppressed in the 2000 mg/kg treatment group. In addition, thymic CD4⁺/CD8⁺ cells were significantly increased. By adulthood (8 weeks of age), lymphocyte proliferative responses and the alteration in thymic CD4⁺/CD8⁺ cells had returned to normal. However, splenic weight and thymic cellularity were altered, and the IgM plaque forming cell response was suppressed by 46% and 81% in the 1000 and 2000 mg/kg treatment groups, respectively. Furthermore, a 38% decrease was detected in the total T₄ serum hormone level at 2000 mg/kg. In F1 adults, no significant alterations were observed in natural killer cell

activity, T-cell lymphocyte proliferation, bone marrow cellularity and proliferative responses, complete blood counts, peritoneal and splenic cellularity, liver, kidney or thymus weight, macrophage phagocytosis or nitric oxide production, splenic CD4/CD8 lymphocyte subpopulations, or total T₃ serum hormone levels. Host resistance models in treated F1 adults demonstrated that immunological responses were normal after challenge with *Listeria monocytogenes*, but heightened susceptibility to B16F10 tumor challenge was seen at both treatment levels. This study demonstrated that prenatal exposure to JP-8 can target the developing murine fetus and result in impaired immune function and altered T₄ levels in adulthood.

A study by Keil et al. (2004) examined the effects of JP-8 on humoral and cell-mediated and hematological parameters. A suite of immunotoxicological endpoints was evaluated in adult female B6C3F1 mice gavaged with JP-8 (in an olive oil vehicle) ranging from 250–2500 mg/kg/day for 14 days. One day following the last exposure, significant increases in liver mass were detected beginning at exposure levels of 1000 mg/kg/day, while thymic mass was decreased at exposure levels of 1500 mg/kg/day and above. Decreases in thymic cellularity, however, were only observed at exposure levels of 2000 mg/kg/day and above. Mean corpuscular volume was increased (1500–2500 mg/kg/day), while the hematocrit, hemoglobin concentration and red blood cell count were decreased only at the 2500 mg/kg/day exposure level. Natural killer cell (NK) activity and T- and B-cell proliferation were not altered. Decreases in the plaque forming cell (PFC) response were dose responsive at levels of 500 mg/kg/day and greater, while unexpectedly, serum levels of anti-SRBC immunoglobulin M (IgM) were not altered. Alterations were detected in thymic and splenic CD4/8 subpopulations, and proliferative responses of bone marrow progenitor cells were enhanced in mice exposed to 2000 mg/kg/day of JP-8. This study showed that humoral immune function is impaired with lower exposure levels of JP-8 than are required to affect primary and secondary immune organ weights and cellularities, CD4/8 subpopulations and hematological endpoints. Immune studies are not available for JP-4.

Neurobehavioral

Neurobehavioral effects have been assessed in adult rats following JP-8 vapor inhalation. Changes in behavioral response were observed in two studies where rats were exposed to 0, 500 or 1000 mg/m³ for 6 h/day, 5 days a week for 6 weeks. JP-8 inhalation affected performance on very specific tasks and did not cause a generalized deficit. When animals were subjected to different operant tasks with varying levels of complexity, the low and high exposure groups scored the same as control animals on all tests except for the most complex tasks. In two operant tests, group differences emerged in which low dose animals demonstrated better performance than high dose animals while neither group performed differently from controls (Ritchie et al., 2001). In a second study using the same exposure methods, animals were tested in a large battery of neurobehavioral tasks. No exposure group differences were found in acoustic startle responses, forelimb grip strength, nociception, social interaction, the forced swim test, spontaneous locomotor activity, passive avoidance or Morris watermaze performance. However, differences were found in a test for behavioral sensitization. The appetitive stimulus approach sensitization (ARAS) measures the time an animal spends proximal to an appetitive stimulus versus a neutral stimulus. Animals exposed to JP-8 spent more time than control animals investigating the appetitive stimulus, suggesting behavioral sensitization and altered neural pathways related to the dopaminergic system (Rossi et al., 2001). Overall, the data suggest very specific, versus generalized, neurobehavioral effects of JP-8 vapor exposure in adult rats.

As a continuation of the reproductive study by Mattie et al. (2000), the pups from the females were assessed for potential developmental neurobehavioral deficiencies after exposure in utero and during

lactation to JP-8 (Mattie et al., 2001). Litters were standardized to four male and four female pups at PND 4; all eight pups in a litter were tested for surface righting and negative geotaxis. JP-8 did not affect age of onset for surface righting reflex in pups tested on PND 4. Negative geotaxis abilities, tested on PND 5 through 8, developed at the same age for pups in all JP-8 groups; however, all females met the criterion sooner than males. Development of motor coordination related to swimming was tested in one male and one female pup from each litter every other day from PNDs 6 through 20. A dose-related difference in composite scores for swimming abilities was observed on PNDs 8 and 14, indicating a delay in development of coordinated motor movements related to the swimming task. On PND 8, pup scores from all doses were $\geq 20\%$ lower than control scores. On PND 14, composite swimming scores were 8% lower in the 750 and 1500 mg/kg/day dose groups versus controls. Pups were tested in an M swimming maze on PNDs 70 and 77. JP-8 did not affect the number of trials to criterion on either test date; on PND 77, male pups met the criterion of five errorless trials in fewer attempts than females. The effects of JP-8 exposure found in results from developmental neurobehavioral testing indicated developmental delays in pups caused by exposure of dams to JP-8 in utero and during lactation may be associated with key developmental milestones in the developing cerebellum of the pups. Although recovery occurred, a delay in motor development has potential adverse impacts. The lowest dose of 325 mg/kg/day would be the lowest observed adverse effect level (LOAEL) for developmental neurobehavioral effects.

A study by Smith et al. (1997) used the postural stability technique to investigate the neurological effects of cumulative low-level exposure to JP-8 jet fuel vapors in aircraft maintenance personnel. All subjects performed two sets of four 30-s postural sway tests. The results of mean cumulative exposure levels (in parts per million \pm standard error of the mean) were as follows: naphthas, 1308 ± 292 ; benzene, 21.2 ± 5.7 ; toluene, 23.8 ± 6.1 ; and m-, o-, p-xylenes, 22.7 ± 5.4 . Covariate adjusted regression analysis of the exposed group data showed a statistically significant association ($p < 0.05$) between the solvents (benzene, toluene and xylenes) and increased postural sway response. For all solvent exposures, the “eyes closed, on foam” test provided the strongest association between sway length and JP-8 benzene (r^2 range, 0.45–0.52), implying subtle influence on vestibular/proprioception functionalities. Neurobehavioral studies are not available for JP-4.

Developmental/reproductive

A developmental toxicity study indicated that JP-8 is not a teratogen in the rat (Cooper and Mattie, 1996). Female rats were dosed with 0, 500, 1000, 1500 or 2000 mg/kg neat JP-8 daily by gavage on days 6 through 15 of gestation. Maternal body weights significantly decreased in the 1000, 1500 and 2000 mg/kg/day dose groups while fetal weights decreased in the 1500 and 2000 mg/kg/day groups. Fetal malformations and variations did not differ significantly between control and treatment groups.

Two reproductive studies were performed as part of an aforementioned multi-step investigation (Mattie et al., 1995). JP-8 was shown not to be a reproductive toxicant in rats. In the first study, male rats were given 0, 750, 1500 or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naïve females to assess fertility and sperm parameters. After 70 days of dosing, body weights in the 3000 mg/kg group were over 30% lower than control weights. There were no significant changes for pregnancy rate, gestation length or sperm parameters as compared to control values (Mattie et al., 2000).

In the second reproductive study, general toxicity, fertility and reproductive endpoints were assessed in female rats dosed with neat JP-8 (0, 325, 750 or 1500 mg/kg) daily by gavage for a total of 21 weeks (90 days plus mating with naïve males, gestation and

lactation). Results of general toxicity revealed a significant dose-dependent decrease in body weights of the female rats. Significant organ weight ratio increases were seen for the liver/body, liver/brain and kidney/brain weights. Corresponding histopathologic changes and increases in liver enzymes (ALT, AST) were not observed although there was an increase in liver weight. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis. There were no statistically significant changes from control values for gestation length, pregnancy rate and numbers of pups per litter. There was a trend for decreased pup weight with increasing dose from postnatal days 4 through 21 with the 1500 mg/kg pups statistically and biologically significantly lower on these days. Recovery occurred by 90 days. Based on the results of both reproductive studies, the no observed adverse effect level (NOAEL) for JP-8 reproductive and development effects is 750 mg/kg with 1500 mg/kg as a LOAEL based on decreased pup weights (Mattie et al., 2000). Data from developmental or reproductive studies are not available for JP-4.

Mutagenicity/oncogenicity

Brusick and Matheson (1978) tested JP-8 for mutagenicity in a number of test systems. JP-8 was not mutagenic for Salmonella in the Ames Bacterial Reverse Mutation Test. The chemical was toxic to most of the bacteria strains at concentrations above 1 μL per plate. In the Mouse Lymphoma Assay, JP-8 did not induce gene mutation in mouse cells. The material was moderately toxic in this assay at 0.16 $\mu\text{L}/\text{mL}$. JP-8 induced significant levels of 3H-thymidine incorporation in the Unscheduled DNA Synthesis Assay. The increase in activity of the WI-38 cells was moderate; the effect plateaued and was not dose related. The dose of 5.0 $\mu\text{L}/\text{mL}$ was beginning to show clear evidence of cytotoxicity. These data suggest that JP-8 could interact with DNA producing nonspecific lesions. The dominant lethal assays (DLA) showed that JP-8 was only moderately toxic for mice and rats. The dose levels used for mice were 0.13, 0.4 and 1.3 ml/kg per day for 5 days. The dose levels employed for rats were 0.1, 0.3 and 1.0 ml/kg per day for 5 days. Mouse and rat DLA test results for JP-8 were negative. None of the parameters measured in either study showed compound-induced effects. The positive control values for this study were clearly elevated but were not as high as usual. No evidence for mutagenicity was evident in the test battery and the indications for mutagenic and carcinogenic potential for JP-8 are minimal at best. There is no suggestion of significant genetic risk associated with this material according to Brusick and Matheson (1978).

Mice were treated dermally with either a single or multiple applications of JP-8 and Jet A fuels in the Mammalian Micronucleus Test. Peripheral blood and bone marrow smears were prepared to examine the incidence of micronuclei (MN) in polychromatic erythrocytes (PCEs). In all experiments, using several different exposure regimens, no statistically significant increase in the incidence of MN was observed in the bone marrow and/or peripheral blood of mice treated with JP-8 or Jet A when compared with those of untreated control animals (Vijayalaxmi et al., 2006).

In dominant lethal experiments, male mice and rats were exposed to JP-4 and allowed to mate with unexposed females in dominant lethal experiments. Statistically significant dominant lethal effects were not observed for either mice or rats (Air Force, 1978). However, because of the small sample size of pregnant females, the results were not considered conclusive. In vitro studies were negative for chromosome aberrations in Chinese hamster ovary cells (EPA, 1982a,b; Galloway, 1982a,b), gene mutations in mouse L5178Y lymphoma cells (Air Force, 1978; Cifone, 1982a,b; EPA, 1982a,b), and gene mutations in *Saccharomyces cerevisiae* (Air Force, 1978) and *Salmonella typhimurium* (Air Force, 1978; EPA, 1982a, 1982b; Jagannath, 1982; Rabenold, 1982). The only positive effect that

could potentially be significant was seen in human diploid WI-38 cells that are derived from embryonic lung. These cells were treated with JP-4 and examined for unscheduled deoxyribonucleic acid (DNA) synthesis (Air Force, 1978). Unscheduled DNA synthesis (UDS) is an indirect measurement of DNA damage. Cells treated with 0.1, 0.5, 1.0 and 5.0 $\mu\text{L}/\text{mL}$ (in water), in both activated and nonactivated systems, showed a dose-dependent increase in UDS activity. This indicated that JP-4 can produce repairable damage in the DNA of these human WI-38 cells. In addition, cells cultured in the presence of metabolic activators exhibited greater UDS activity, suggesting the involvement of toxic metabolites.

Emerging issues

Immunotoxicity and suppression

Immunotoxic properties of chemicals are a growing concern for the toxicology and regulatory communities and have also become an issue for jet fuel. Studies using aerosolized JP-8 jet fuel have shown immunosuppression in rodents (Harris et al., 1997a,b). However, there have been no indications of immune suppression in industry or Air Force studies. The review by the National Research Council, Committee on Toxicology (COT, 2003) questioned past exposures and stated that definitive inhalation bioassays were needed.

Two pilot studies were conducted by the Naval Health Research Center, Environmental Health Effects Laboratory (NHRC/EHEL) with analytical support by AFRL/RHPB. Exposures to Jet A were carefully generated and the chamber concentrations were characterized for both vapor and aerosol concentrations. Target doses were 500, 1000 and 2000 mg/m^3 for 4 h per day for 14 days in both studies. The first study used female Sprague–Dawley rats while the second study compared Female Fischer 344 and Sprague–Dawley rats. No significant toxicity or immune effects were seen from the exposure to Jet A fuel.

As a result, the petroleum industry sponsored a definitive immunotoxicity bioassay involving exposing rats for 28 days by inhalation and examining the full battery of recommended immune endpoints. A second study using mice was also conducted by industry. The studies have been completed and a publication will be forthcoming.

Naphthalene and naphthalene content in jet fuel

Another concern involving jet fuel is naphthalene. It is a natural constituent of petroleum-based fuels such as JP-8 with concentrations in the 0.1–0.2% range. There are many sources of naphthalene including engine exhaust. There are also many uses for naphthalene such as moth balls. Because of the extent of Air Force fuel operations, potential exposure is wide spread. The toxicity of high naphthalene exposures results in respiratory, CNS, kidney and liver effects, anemia and even coma. However, the U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) Program has targeted naphthalene due to positive carcinogenic results in rodent inhalation bioassays. As a result the classification for naphthalene could change to "... reasonably anticipated to be a human carcinogen ...". The initial cancer risk assessment indicated 2 ppt as a potential new level for compliance. This would have a significant impact on the U.S. Air Force because its fuels would be branded as carcinogens. According to 29CFR 1910.1000, if a component in the mixture is $\geq 0.1\%$, the mixture is considered a carcinogen. There would be many environmental and occupational safety implications for the use of jet fuel and potential exposure to fuels containing naphthalene. Currently the EPA is waiting for new data from industry to address the carcinogenic issue in rodents that is driving the lowering of the standard for humans.

Mixtures modeling and inhalation exposure characterization

The individual components of JP-8 may interact with each other in the body, both on the pharmacokinetic and pharmacodynamic levels. In order to develop pharmacokinetic and toxicokinetic models for such complex interacting mixtures and to characterize target site dosimetry for examination of dose–response effects, a stepwise approach was proposed by the University of Georgia (UGA) and our laboratory. UGA will focus primarily on the kinetics of vapor and aerosol characterization and absorption, while our laboratory will focus on the development and validation of the complex mixture kinetic model for the fuel as a whole. The first step in the model development process was to build PBPK models for a number of representative components of JP-8. Suitable representative components have been identified as nonane and decane (representative of shorter-chain length, volatile aliphatic hydrocarbons), benzene and naphthalene (aromatics) and tetradecane (longer chain aliphatics). A model for tetradecane in planned. Initial models for nonane and decane were developed by our laboratory and UGA (Perleberg et al., 2004), respectively. Because of the similarity of these chemicals and some significant differences between the respective PBPK models, the models for nonane and decane were harmonized and are being published this year. Models and parameter values for benzene, toluene and naphthalene have been published in the literature (Haddad et al., 2001, Quick and Shuler, 1999, Willems et al., 2001). To date benzene, toluene, nonane, decane and naphthalene have been harmonized and incorporated into an integrated interaction model. Model refinements for naphthalene and validation studies *in vivo* are planned for this year by our laboratory. The goal is to develop a mixtures model that is predictive for JP-8 jet fuel. The model, after validation, can then be adapted to predict the behavior of the new alternative jet fuels of the future or a blend of JP-8 and an alternative fuel.

References

- Air Force, 1978. Mutagen and oncogen study on JP-4. Aerospace Medical Research Laboratory, Aerospace Division, Air Force Systems Command, Wright-Patterson Air Force Base, OH. AMRL-TR-78-24, AD-A064952.
- Air Force, 1989. The Installation Restoration Program Toxicology Guide, Vol. 4. DOE interagency agreement no. 1891-A076-A1. AAMRL, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, OH.
- Alden, C.L., 1986. A review of unique male rat hydrocarbon nephropathy. *Toxicol. Pathol.* 14, 109–111.
- ATSDR, 1993. Draft Toxicological Profile for Jet Fuels JP-4 and JP-7. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
- ATSDR, 1998. Toxicological Profile for JP-5 and JP-8. Agency for Toxic Substances and Disease Registry, Atlanta, GA. <http://www.atsdr.cdc.gov/toxpro2.html>.
- Baker, W., Miller, T., Dodd, D., McDougal, J., 1999. Repeated dose skin irritation study on jet fuels - a histopathology study. Operational Toxicology Branch, Wright-Patterson AFB, OH. AFRL-HE-WP-TR-1999-0022.
- Bruner, R.H., Kinkead, E.R., O'Neill, T.P., Flemming, C.D., Mattie, D.R., Russell, C.A., Wall, H.G., 1993. The toxicologic and oncogenic potential of JP-4 jet fuel vapors in rats and mice: 12-month intermittent inhalation exposures. *Fundam. Appl. Toxicol.* 20, 97–110.
- Brusick, D. J. and Matheson, D. W., 1978. Mutagen and oncogen study on JP-4. Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH. AMRL-TR-78-24.
- Cifone, M. A., 1982a. Mutagenicity evaluation of L13B in the mouse lymphoma forward mutation assay. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. Document no. 88-8100388.
- Cifone, M. A., 1982b. Mutagenicity evaluation of L14-B in the mouse lymphoma forward mutation assay. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. Document no. 88-8100388.
- Cooper, J.R., Mattie, D.R., 1996. Developmental toxicity of JP-8 jet fuel in the rat. *J. Appl. Toxicol.* 16, 197–200.
- COT, 1996. Permissible Exposure Levels for Selected Military Fuel Vapors. National Research Council, Committee on Toxicology. National Academy Press, Washington, DC.
- COT, 2003. Toxicologic Assessment of Jet-Propulsion Fuel 8. National Research Council, Committee on Toxicology. National Academy Press, Washington, DC.
- EPA, 1982a. Overview summary compound L13-B. U.S. Environmental Protection Agency, Office of Toxic Substances. Washington, D.C. Document no. 88-8100388.
- EPA, 1982b. Overview summary compound L14-B. U.S. Environmental Protection Agency, Office of Toxic Substances. Washington, D.C. Document no. 88-8100388.

- Fechter, L.D., Gearhart, C., Fulton, S., Campbell, J., Fisher, J., Na, K., Cocker, D., Nelson-Miller, A., Moon, P., Pouyatos, B., 2007. JP-8 jet fuel can promote auditory impairment resulting from subsequent noise exposure in rats. *Toxicol. Sci.* 98, 510–525.
- Flamm, W.G., Lehman-McKeeman, L.D., 1991. The human relevance of the renal tumor-inducing potential of d-limonene in male rats: implications for risk assessment. *Regul. Toxicol. Pharmacol.* 13, 70–86.
- Galloway, S. M., 1982a. Mutagenicity evaluation of L13-B in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. Document no. 88–8100388.
- Galloway, S. M., 1982b. Mutagenicity evaluation of L14-B in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. Document no. 88–8100388.
- Haddad, S., Beliveau, M., Tardif, R., Krishnan, K., 2001. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. *Toxicol. Sci.* 63, 125–131.
- Harris, D.T., Sakiestewa, D., Robledo, R.F., Witten, M., 1997a. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol. Ind. Health* 13, 43–55.
- Harris, D.T., Sakiestewa, D., Robledo, R.F., Witten, M., 1997b. Short-term exposure to JP-8 jet fuel results in long-term immunotoxicity. *Toxicol. Ind. Health* 13, 559–570.
- Jagannath, D. R., 1982. Mutagenicity evaluation of L13-B in the Ames salmonella/microsome plate test. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. Document no. 88–8100388.
- Kanikkannan, N., Jackson, T., Sudhan, S.M., Singh, M., 2000. Evaluation of skin sensitization potential of jet fuels by murine local lymph node assay. *Toxicol. Lett.* 116, 165–170.
- Kaufman, L.R., Lemasters, G.K., Olsen, D.M., Succop, P., 2005. Effects of concurrent noise and jet fuel exposure on hearing loss. *J. Occup. Environ. Med.* 47, 212–218.
- Keil, D.E., Warren, D.A., Jenny, M.J., EuDaly, J.G., Smythe, J., Peden-Adams, M.M., 2003. Immunological function in mice exposed to JP-8 jet fuel in utero. *Toxicol. Sci.* 76, 347–356.
- Keil, D.E., Dudley, A.C., EuDaly, J.G., Dempsey, J., Butterworth, L., Gioffre, F., Peden-Adams, M.M., 2004. Immunological and hematological effects observed in B6C3F1 mice exposed to JP-8 jet fuel for 14 days. *J. Toxicol. Environ. Health A* 67, 1109–1129.
- Kinthead, E.R., Wolfe, R.E., Flemming, C.D., Solomon, R.A., Mattie, D.R., Grabau, J.H., Marit, G.B., 1995. Toxicologic and oncogenic potential of JP-4 vapor: 90-day continuous inhalation exposure. *Inhal. Toxicol.* 7, 239–253.
- Kinthead, E.R., Salins, S.A., Wolfe, R.E., 1992a. Acute irritation and sensitization potential of JP-8 jet fuel. *Acute Toxic. Data* 11, 700.
- Kinthead, E.R., Salins, S.A., Wolfe, R.E., 1992b. Acute irritation and sensitization potential of petroleum-derived JP-4 jet fuel. *Acute Toxic. Data* 11, 699.
- Kinthead, E.R., Wolfe, R.E., Salins, S.A., 1993. Acute oral and inhalation toxicity of petroleum-derived JP-4 jet fuel. *Acute Toxic. Data* 12, 635.
- Knave, B., Persson, H.E., Goldberg, J.M., 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., 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.
- Mattie, D.R., Alden, C.L., Newell, T.K., Gaworski, C.L., 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.
- Mattie, D.R., Marit, G.B., Flemming, C.D., Cooper, J.R., 1995. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. *Toxicol. Ind. Health* 11, 423–435.
- Mattie, D. R., Marit, G. B., Cooper, J. R., Sterner, T. R., Flemming, C. D., 2000. Reproductive effects of JP-8 jet fuel on male and female Sprague-Dawley rats after exposure by oral gavage. Air Force Research Laboratory, Human Effectiveness Directorate, Wright-Patterson AFB, OH. AFRL-HE-WP-TR-2000-0067, ADA453146.
- Mattie, D. R., Cooper, J. R., Sterner, T. R., Schimmel, B. D., Bekkedal, M. Y. V., Bausman, T. A., Young, S. M., 2001. Developmental neurobehavioral effects of JP-8 jet fuel on pups from female Sprague-Dawley rats exposed by oral gavage. Air Force Research Laboratory, Applied Toxicology Branch, Wright-Patterson AFB, OH. AFRL-HE-WP-TR-2001-0186, ADA428272.
- McDougal, J.N., Rogers, J.V., 2004. Local and systemic toxicity of JP-8 from cutaneous exposures. *Toxicol. Lett.* 149, 301–308.
- Perleberg, U.R., Keys, D.A., Fisher, J.W., 2004. Development of a physiologically based pharmacokinetic model for decane, a constituent of Jet Propellant-8. *Inhal. Toxicol.* 16, 771–783.
- Quick, D.J., Shuler, M.L., 1999. Use of *in vitro* data for construction of a physiologically based pharmacokinetic model for naphthalene in rats and mice to probe species differences. *Biotechnol. Prog.* 15, 540–555.
- Rabenold, C., 1982. Mutagenicity evaluation of L14-B in the Ames salmonella/microsome plate test. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. Document no. 88–8100388.
- Ritchie, G.D., Rossi III, J., Nordholm, A.F., Still, K.R., Carpenter, R.L., Wenger, G.R., Wright, D.W., 2001. Effects of repeated exposure to JP-8 jet fuel vapor on learning of simple and difficult operant tasks by rats. *J. Toxicol. Environ. Health A* 64, 385–415.
- Ritchie, G.D., Still, K.R., Rossi, J., Bekkedal, M.Y.V., Bobb, A.J., Arfsten, D.P., 2003. Biological and health effects of exposure to kerosene-based jet fuels and performance additives. *J. Toxicol. Environ. Health B* 6, 357–451.
- Rossi III, J., Nordholm, A.F., Carpenter, R.L., Ritchie, G.D., 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.
- Smith, L.H., Haschek, W.M., Witschi, H., 1981. Acute toxicity of selected crude and refined shale oil- and petroleum-derived substances. Ch. 11. In: Griest, W.H., Guerin, M.R., Coffin, D.L. (Eds.), Health effects investigation of oil shale development. Ann Arbor Science Publishers, Inc., Ann Arbor, MI, pp. 141–160.
- Smith, L.B., Bhattacharya, A., Lemasters, G., Succop, P., Puhala, E., Medvedovic, M., Joyce, J., 1997. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel. *J. Occup. Environ. Med.* 39, 623–632.
- Struwe, G., Knave, B., 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.
- Vijayalaxmi, V., Kligerman, A.D., Prihoda, T.J., Ullrich, S.E., 2006. Micronucleus studies in the peripheral blood and bone marrow of mice treated with jet fuels, JP-8 and Jet-A. *Mutat. Res.* 608, 82–87.
- Whitman, F. T. and Hinz, J. P., 2001. Sensory irritation study in mice: JP-4, JP-8, JP-8 + 100. AF Institute for Environment, Safety and Occupational Health Risk Analysis, Brooks AFB, TX. IERA-RS-BR-SR-2001-0005.
- Willems, B.A.T., Melnick, R.L., Kohn, M.C., Portier, C.J., 2001. A physiologically based pharmacokinetic model for inhalation and intravenous administration of naphthalene in rats and mice. *Toxicol. Appl. Pharmacol.* 176, 81–91.
- Wolfe, R. E., Kinthead, E. R., Feldmann, M. L., Leahy, H. F., Jederberg, W. W., Mattie, D. R., Still, K. R., 1996. Acute toxicity evaluation of JP-8 jet fuel and JP-8 jet fuel containing additives. Armstrong Laboratory, Wright-Patterson AFB, OH. AL/OE-TR-1996-0136.