A Literature Review - Problem Definition Studies on Selected Toxic Chemicals

Volume 1 of 8

OCCUPATIONAL HEALTH AND SAFETY ASPECTS OF DIESEL FUEL AND WHITE SMOKE GENERATED FROM IT

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by

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**Title:** A Literature Review—Problem Definition Studies on Selected Toxic Chemicals. Volume I

**Abstract:**
Literature is reviewed (75 references) covering analysis, physical and chemical properties, human and animal toxicology, mammalian pharmacokinetics, industrial standards and occupational hazards of diesel fuel and white smoke (an aerosol mixture of diesel fuel, additives, diesel engine exhaust and pyrolysis products). Diesel fuel is an aliphatic and aromatic hydrocarbon mixture obtained from the straight-run distillation of petroleum and often blended with cracked fuels. Composition is controlled only by physical properties.
(boiling range, flash point, viscosity, cetane number); additives improve combustibility, reduce corrosiveness and reduce gum formation. The smoke is generated by feeding diesel fuel into the exhaust manifold of a diesel engine, creating a vapor which condenses into an opaque mass of microdroplets which may be useful in screening military equipment and personnel. The health hazards of exposure to white smoke have not been studied, although pure diesel fuel aerosols do not appear to be irritating to the respiratory tract or skin of humans during acute exposures to relatively low concentrations. Dermatitis following direct contact with diesel fuel is reportedly due to a combination of poor occupational hygiene and constitutional factors. Ingestion of diesel fuel results in gastritis and patchy destruction of the gastric mucosa. Systemic changes in mammals have been noted in studies of blood chemistry and hematology. Aspiration pneumonitis is a greater hazard to the health than ingestion. There are no long-term studies on diesel fuel in humans or laboratory animals. Likewise, there are no studies on teratogenicity, mutagenicity or metabolism of diesel fuel. Selected data on the toxicity and pharmacokinetics of somewhat similar hydrocarbon mixtures (kerosene, mineral seal oil) and concentrates of aromatic and aliphatic compounds occurring in diesel fuel (alkylbenzenes, hexadecane) are presented in an effort to identify possible risks, although the interactions of various hydrocarbons of the diesel fuel mixture in the organism are unknown. Prevention of exposure of personnel with heart, kidney, lung or liver disease, hydrocarbon allergy or chronic skin disease, protection against inhalation of white smoke aerosols and against skin exposure, limiting duration of exposure, medical surveillance for early detection of adverse effects, and documentation of all adverse effects of exposure are suggested. Recommendations for further research include chemical analysis as well as toxicological investigations of white smoke inhalation, and skin contact, feeding and metabolism of diesel fuel with additives.
EXECUTIVE SUMMARY

This report is a literature review (75 references) which covers physical and chemical properties, analytical methods, experimental animal studies and occupational health and safety of diesel fuel and the white smoke generated from it.

Diesel fuel is a refined, distilled petroleum product made from crude oil. The chemical composition of diesel fuel varies from batch to batch, but the fuel is required to have certain other properties which have been specified by the U.S. Armed Forces. Certain additives may be added to improve engine performance. The smoke which is produced from diesel fuel consists of tiny oil droplets suspended in air in a concentration great enough to produce a dense white cloud.

Inhalation of diesel oil smoke may cause lung damage, although further studies are needed. Irritation of the skin occurs in some individuals who handle diesel fuel in industry. The skin irritation consists of dryness, redness and pimples, sometimes severe enough to leave scars. Whether it is due to an allergy or to carelessness and uncleanliness is uncertain. Swallowing diesel fuel may severely damage the stomach lining. If diesel fuel is taken into the lungs, severe irritation, coughing, and inability to breathe may result. The lung condition may clear up or death may occur, especially in accidents when children drink and choke on diesel fuel. The possible harmful effects to humans during long-term exposure to diesel fuel white smoke have not been studied.

In studies on experimental animals, diesel fuel white smoke produced reduced ability to fight infection in rats, as well as loss of coordination, tiredness, and dry flaking skin. Rats fed diesel fuel developed abnormal blood, liver damage, and some died. When rat skin was painted with diesel fuel, pimples, hair loss and peeling of skin occurred. Some diesel fuel batches caused skin cancer after painting the fuel repeatedly on mouse skin. No studies are reported for effects of diesel fuel on reproduction in animals.

When taken into the body, some of the aromatic compounds of diesel fuel are metabolized in the liver. Arene oxides, formed as intermediates in the metabolism, may be cancer producing or cause mutations. Diesel fuel may be excreted from the body in the urine, or exhaled from the lungs.

Persons who may be exposed to diesel fuel white smoke should be protected from breathing the smoke and getting it in eyes, on skin and hair. Medical examinations should be provided to check for lung problems, skin problems or other disturbances in persons exposed to the smoke.
ABSTRACT

Literature is reviewed (75 references) covering analysis, physical and chemical properties, human and animal toxicology, mammalian pharmacokinetics, industrial standards and occupational hazards of diesel fuel and white smoke (an aerosol mixture of diesel fuel, additives, diesel engine exhaust and pyrolysis products). Diesel fuel is an aliphatic and aromatic hydrocarbon mixture obtained from the straight-run distillation of petroleum and often blended with cracked fuels. Composition is controlled only by physical properties (boiling range, flash point, viscosity, cetane number); additives improve combustibility, reduce corrosiveness and reduce gum formation. The smoke is generated by feeding diesel fuel into the exhaust manifold of a diesel engine, creating a vapor which condenses into an opaque mass of microdroplets which may be useful in screening military equipment and personnel. The health hazards of exposure to white smoke have not been studied, although pure diesel fuel aerosols do not appear to be irritating to the respiratory tract or skin of humans during acute exposures to relatively low concentrations. Dermatitis following direct contact with diesel fuel is reportedly due to a combination of poor occupational hygiene and constitutional factors. Ingestion of diesel fuel results in gastritis and patchy destruction of the gastric mucosa. Systemic changes in mammals have been noted in studies of blood chemistry and hematology. Aspiration pneumonitis is a greater hazard to the health than ingestion. There are no long-term studies on diesel fuel in humans or laboratory animals. Likewise, there are no studies on teratogenicity, mutagenicity or metabolism of diesel fuel. Selected data on the toxicity and pharmacokinetics of somewhat similar hydrocarbon mixtures (kerosene, mineral seal oil) and concentrates of aromatic and aliphatic compounds occurring in diesel fuel (alkylbenzenes, hexadecane) are presented in an effort to identify possible risks, although the interactions of various hydrocarbons of the diesel fuel mixture in the organism are unknown. Prevention of exposure of personnel with heart, kidney, lung or liver disease, hydrocarbon allergy or chronic skin disease, protection against inhalation of white smoke aerosols and against skin exposure, limiting duration of exposure, medical surveillance for early detection of adverse effects, and documentation of all adverse effects of exposure are suggested. Recommendations for further research include chemical analysis as well as toxicological investigations of white smoke inhalation, and skin contact, feeding and metabolism of diesel fuel with additives.
The U.S. Army Medical Research and Development Command has received the task of assessing occupational health and safety aspects of various chemicals to which army personnel may be exposed. In response to that assignment, this Problem Definition Study (PDS) has been prepared under contract number DAMD-17-77-C-7020 in order to provide the published information relating to occupational health and safety aspects of diesel fuel. Diesel fuel, which is a mixture of hydrocarbons obtained from petroleum distillates, is intended to be used to produce screening smoke, technically a fog, consisting of a suspension in air of minute droplets of the fuel. The subjects covered in this report include physical and chemical properties, methods of analysis, toxicological studies on humans and animals, metabolism, industrial hygiene and safety practices. An appendix lists the sources examined to locate relevant information in the literature. Also included in the appendix is a list of various organizations contacted to obtain relevant information concerning diesel fuel.

This Problem Definition Study is the first in a series of eight reports prepared under this contract.
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I. INTRODUCTION AND STATEMENT OF PROBLEM

White smoke is intentionally generated for purposes of screening personnel. Where personnel may be exposed the least toxic, noxious, and otherwise objectionable materials should be used. The "screening" use exposes personnel in the open, those partially protected in vehicles, operators of free-standing smoke generators, and operator-passengers of vehicles whose exhaust manifolds have been modified to generate smoke. Routes of exposure would be inhalation primarily, percutaneous secondarily, and ingestion.

Since it is anticipated that diesel fuel with its attendant additives may be used as a replacement for the somewhat different hydrocarbon mixture, fog oil, actually specified for white smoke generators, the toxic nature of the replacement must be ascertained to provide adequate safeguards for the most heavily exposed personnel.
II. TECHNICAL SUMMARY

A. Description of Diesel Fuel (and Additives) and Diesel Fuel Smoke

As purchased by the U.S. Armed Forces diesel fuel is a mixture of hydrocarbons (aliphatic and aromatic) obtained from the atmospheric distillation of petroleum, or a blend of the same with hydrocarbons obtained by breaking down yet higher boiling compounds. The composition is controlled only by the interaction of specifications covering boiling point, cetane number, viscosity, and flash point, which vary according to environmental conditions. Additives may also be present in very small quantities to: improve combustibility - alkyl nitrates; reduce corrosion of storage vessels - surfactants; reduce gum formation (improve compatibility of mixed source fuels) - mixed surfactant; antioxidant (gum retardation) - aromatic amines or phenols. The smoke, technically a fog, consists of a suspension in air of 0.5-1.0 micron droplets of the oil, individually translucent but opaque en masse.

B. Effects on Humans

Diesel fuel studies in humans have been limited in scope and number. Aerosols of diesel oil appear to be relatively harmless when exposures are short (0.33 mg/l, 10 min.). Skin and mucous membrane irritation are unusual. Inhalation of aerosol droplets into the lungs has not been studied, although it has been assumed that no pulmonary damage occurs by this route. Longer exposures to diesel fuel aerosols are not reported in the literature.

One skin effect of diesel fuel is dermatitis, which may occur in susceptible individuals. The skin reaction is characterized by erythema, burning, and pruritis in early stages, progressing to papular eruptions, folliculitis, scaling and fissures, and possible abscess formation. In the industrial setting, it is unclear what proportion of dermatitis is due to allergy or constitutional susceptibility, and what proportion is due to carelessness or poor personal hygiene.

Ingestion of diesel fuel may cause acute gastritis and temporary damage to the gastric mucosa. Aspiration (direct entry into lungs) results in respiratory distress and acute chemical pneumonitis which usually resolves, but large quantities may cause death if pulmonary damage is severe. In cases of poisoning in children, toxicity and mortality were due to aspiration of diesel fuel, not ingestion. No follow-up was undertaken. No epidemiologic analysis has been performed on any aspect of diesel fuel effects in humans.

Any effects of white smoke aerosols (containing diesel fuel, exhaust fumes, pyrolysis products and additives) have not been documented.
C. Animal Toxicity

Toxicological studies on diesel fuel per se are limited. Table 6 (pp. 31-33) summarizes the studies and results. Toxic effects of similar hydrocarbon compositions have been presented. Although the toxicity of aromatic hydrocarbons present in diesel fuel has been investigated, their presence in the diesel fuel does not seem to enhance its toxicity.

Rats exposed to aerosol concentrations of 10 mg/l of either diesel fuel or kerosene (time of exposure not given) showed no pulmonary surfactant reduction. In rabbits exposed to smoke from a mixture of diesel fuel and motor oil (16.6:1), effects on immunological response included reduced antibody production to typhoid-paratyphoid vaccine and absence of secondary response.

Exposure of rats to a saturated atmosphere (up to 0.1 mg/l) of kerosene vapors (similar hydrocarbon composition to diesel fuel; see section IV) for 8 hours had no significant adverse effects. However, when rats were exposed to aerosol concentrations of 6.9-9.6 mg/l of kerosene for 6 hours on 4 successive days, loss of coordination, sluggishness, and dryness and flaking of skin of extremities were observed. No effects were observed in cats upon a single 6-hour exposure to kerosene aerosol at 6.4 mg/l. Mice exposed to similar aerosol at 6.9 mg/l (duration not given) showed slight depression of respiratory rate.

Rats exposed to C₉-C₁₀ aromatic fractions distilling at the low end of diesel fuel boiling range at vapor concentrations of 5.2 mg/l for 18 hours had congested lungs and liver, hemorrhagic kidneys and enlarged spleens; similar exposure at 3.2 mg/l (18 hours/day) for 101 days caused reduced weight gain, decreased total white blood cell count, congestion and hemorrhage in the lungs, liver, kidneys, spleen and omentum; levels of 1 mg/l for 8 hours daily, 5 days a week (88 exposures) were ineffective. Monkeys exposed to vapor concentrations of 1 mg/l for 7 hours/day, 5 days/week (90 exposures) remained sedated during the exposure and showed significantly decreased total of white blood cell count, with an increase in numbers of neutrophils and a decrease in lymphocytes, hair loss, dry skin, and slight depression of myelocytic and erythrocytic activity of the bone marrow. No other gross or microscopic changes were noted. The inhalation LC₅₀ of C₉-C₁₀ aromatics in rats was 14.4 mg/l (7 hours) and 3.4 mg/l (3 3/4 hours), respectively. The oral LD₅₀ of diesel fuel in rats was 16 mg/kg body weight. Rats fed 20-25 mg/kg body weight daily showed marked changes in the peripheral blood picture after 7 days. Elevated serum enzyme levels of malate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were indicative of liver damage.

Skin application of diesel fuel in rats produced epidermal splitting and exfoliation, hair loss, and a papular rash. Diesel fuel did not produce skin sensitization in guinea pigs. The heavier grade diesel fuels have been found to be carcinogenic in mice after skin application; for the lighter grades
of diesel fuel conforming to military specifications, the carcinogenicity has not been established to date.

The following organs were adversely affected by administration of diesel fuel or related hydrocarbons to laboratory animals: blood, lungs, skin, central nervous system, bone marrow, spleen, liver, kidney, eye (cataract), gastrointestinal tract and bone.

There are no studies available on mutagenicity, teratogenicity or fertility for diesel fuel.

D. Pharmacokinetics

It is probable that the organism absorbs, metabolizes, stores or excretes each of diesel fuel's hydrocarbons as individual chemicals; the influences of one component upon the others have not been investigated. The absorption rate of diesel fuel through the skin of rats has been found to be too slow for large accumulations to occur in the body.

Normal aliphatic hydrocarbons such as hexadecane and octadecane are emulsified and partly absorbed from the mammalian gastrointestinal tract; some oxidation to fatty acids of the same carbon number occurs in the intestinal mucosa prior to absorption, while a portion of the alkanes is absorbed unaltered. Absorption into lymph is greater than directly into portal blood. After application of hexadecane to the skin of guinea pigs, about 20% is absorbed. Alkane mixtures with more than 20 carbon atoms reduced penetration; repeated applications of hexadecane enhanced absorption.

Aromatic hydrocarbons are absorbed slowly through the skin, gastrointestinal tract and respiratory epithelium.

Normal alkanes and fatty acid metabolites are cleared from the bloodstream by the liver, and some deposits in fatty tissue occur. The C_{16} fatty acid is incorporated into liver lecithin, oleic, palmitic and stearic acids. Microsomal oxidation of hexadecane and octadecane occurs in the liver and lungs of mice.

Aromatic hydrocarbons such as alkylbenzenes are bound to red blood cells; smaller amounts are adsorbed on lipoproteins or dissolved in chylomicrons in the bloodstream. Because of their lipid solubility, aromatic hydrocarbons easily cross membranes and distribution and accumulation occur in fat-containing tissues.

Kerosene and naphthalene increase the urinary glucuronic acid and organic sulfate levels in rabbits, indicating that biotransformation is one metabolic pathway for these hydrocarbons. Polycyclic aromatic compounds are metabolized in the mammalian liver to phenols, trans-dihydriodiols, glutathione conjugates and mercapturic acids via intermediary arene oxides (epoxides). The possible cytotoxicity, mutagenicity and carcinogenicity of arene oxides, which bind to intracellular nucleic acids and other macromolecules, are presently being studied.
Other than urinary excretion of biotransformation products of some hydrocarbons, unmetabolized aromatic compounds may be excreted unchanged in the urine or exhaled from the lungs. There is no evidence that excretion occurs through the skin.
III. RECOMMENDATIONS AND HAZARD ANALYSIS

A. Recommendations for Further Research

At the present time, almost no research on white diesel fuel smoke has been reported. Specific areas where work is needed include: (1) determining smoke density or concentration; (2) qualitative and quantitative analyses of white smoke; (3) consideration of the method of generation of the aerosol, with respect to the temperatures reached in the exhaust manifold where the diesel fuel is introduced; and (4) the proportion of engine exhaust mixed with the white smoke. Diesel fuel should be chemically analyzed to formulate a "standard" for testing purposes.

The biologic effects of white smoke exposure have not been studied. Observations (if existent) of the effects on military personnel employed in fogging operations have not been made available. Until the smoke mixture is analyzed, experimental conditions for conducting animal studies need to be established. Refer to Table 1 for a more detailed description of recommended toxicological studies.

B. Hazard Analysis

Due to the limited studies undertaken so far, it is impossible to assess hazards. Possible dangers exist in inhalation (respiratory tract irritation, absorption and systemic effects). Effects on skin, mucous membranes and conjunctivae also need to be studied at the same smoke concentrations and over the same duration to which army personnel are exposed.

Since laboratory animals have shown changes in lungs, skin, central nervous system, blood, bone marrow, spleen, liver, kidney, eye, gastrointestinal tract and bone, the effects on humans need evaluation in terms of organ toxicity.

C. Recommendations for Protective Equipment

Until it can be stated whether or not diesel fuel white smoke is toxic, protection should be provided to exposed personnel. Face masks capable of filtering diesel fuel aerosol particles around 0.5 microns in diameter, as well as exhaust fumes, should be worn. Protective clothing is recommended where skin exposure is expected. Personnel with dry, eczematous skin may be further protected by using skin softeners and protective ointments on exposed areas. Cleansing after exposure, and removing wet clothing promptly to avoid prolonged skin contact are recommended.

In addition, exposure time should be limited as much as practical, and careful medical surveillance should be provided for early detection of symptoms or signs of intoxication, dermatitis or other adverse effects.
**TABLE 1. GAPS IN TOXICOLOGICAL KNOWLEDGE**

<table>
<thead>
<tr>
<th>Compounds:</th>
<th>White Smoke&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diesel Fuel with Additives</th>
<th>Diesel Fuel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHASE I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute oral LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Acute dermal LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Acute inhalation LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Eye and skin irritation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Metabolism in various animals&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Mutagenesis in microbes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>PHASE II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14-Day feeding</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>90-Day feeding</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Sub-acute inhalation studies</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><strong>PHASE III</strong></td>
<td></td>
<td></td>
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<tr>
<td>2-Year feeding&lt;sup&gt;d&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>180-Day feeding</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chronic inhalation studies</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Fertility, reproduction</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Teratology</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Metabolism in various animals&lt;sup&gt;e&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Notes:**
* X-marks indicate that study is recommended.
  
  a. Aerosol containing mixture of diesel fuel, additives, diesel engine exhaust and pyrolysis products.
  
  b. Including absorption, distribution, excretion and biotransformation, using radio-labeled material.
  
  c. Ames test, including activation.
  
  d. Including carcinogenicity evaluation.
  
  e. Including identification and possible isolation of any metabolites.
IV. PHYSICAL AND CHEMICAL PROPERTIES

Before examining the effects of diesel fuel on the organism, data are presented on its physical and chemical properties. This section will include Federal Specifications, grades of fuel, hydrocarbon composition, distillation, additives, and the generation of white smoke. Similar hydrocarbon mixtures will be discussed in order to acquaint the reader with the full scope of compounds presented in the toxicity and pharmacodynamics sections.

A. Specifications for Diesel Fuel

Under consideration in this report are fuels designed for vehicles with high-speed diesel engines (tanks, trucks, etc.). For this use three grades have been designated in Federal Specification VV-F-800B (1), and their property requirements are presented in Table 2. Grade DF-A is for arctic use, DF-1 for general winter use and DF-2 for warmer climatic conditions. The three grades differ in viscosity and boiling range; further, the grades designed for cold weather use probably have a higher proportion of volatile components to make engine starting easier (2).

This Specification was designed to ensure proper performance characteristics of the fuel, but does not stipulate the chemical composition necessary to pass the required tests; diesel fuels of widely varying composition pass the Specifications. Fuels intended for use in marine, railroad, or slow and medium speed stationary engines have other Specifications, which are available, but are not considered here.

B. Origin of Diesel Fuel

Diesel fuel is a very complex mixture of hydrocarbons containing small quantities of additives. Some diesel fuel is obtained directly from the fractional distillation of crude petroleum oil; the fuels under investigation correspond to that fraction boiling approximately in the range of 160-371°C. Fuels produced in this manner are termed "straight run" distillates by the petroleum industry. The "straight run" distillate usually contains a high proportion of normal alkanes relative to branched chain alkanes and aromatic compounds; it is this high n-alkane content which makes it valuable as a diesel fuel.

An accepted measure of the quality of a diesel fuel is the "cetane number". The cetane number (cetane is a synonym for hexadecane) corresponds to the octane number for gasoline engines. However, in gasoline engines the fuel is ignited by a spark, whereas in diesel engines the fuel is ignited by the heat of compression of the piston. Because of the different method of fuel ignition, fuels with a high normal alkane content yield a valuable diesel fuel with a high cetane number, whereas fuels with a high content of aromatic hydrocarbons and branched chain paraffins yield a high octane number, but give low cetane number diesel fuels.
Table 2. Property Requirements of Diesel Fuels for High Speed Engines in Federal Specification VV-F-800B

<table>
<thead>
<tr>
<th>Properties</th>
<th>Grade DF-A</th>
<th>Grade DF-1</th>
<th>Grade DF-2: CONUS(^1)</th>
<th>OCONUS(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity, °API</td>
<td>Report</td>
<td>Report</td>
<td>Report</td>
<td>32.9 to 41.0</td>
</tr>
<tr>
<td>Flash point, °F(°C) min.</td>
<td>100(37.8)</td>
<td>100(37.8)</td>
<td>125(51.7)</td>
<td>133(56)</td>
</tr>
<tr>
<td>Cloud point, °F(°C) max.(^2)</td>
<td>-60(-51)</td>
<td>-4(^3)</td>
<td>4(^4)</td>
<td>5(^5)</td>
</tr>
<tr>
<td>Pour point, °F(°C) max.(^3)</td>
<td>Report</td>
<td>Report</td>
<td>Report</td>
<td>6(^6)</td>
</tr>
<tr>
<td>Kinematic viscosity @ 100°F (37.8°C), cSt</td>
<td>1.2 to 2.5</td>
<td>1.4 to 3.0</td>
<td>2.0 to 4.3</td>
<td>1.8 to 9.5</td>
</tr>
<tr>
<td>Flash point, °F(°C) min.</td>
<td>Report</td>
<td>Report</td>
<td>Report</td>
<td>32.9 to 41.0</td>
</tr>
<tr>
<td>Cloud point, °F(°C) max.(^2)</td>
<td>-60(-51)</td>
<td>-4(^3)</td>
<td>4(^4)</td>
<td>5(^5)</td>
</tr>
<tr>
<td>Pour point, °F(°C) max.(^3)</td>
<td>Report</td>
<td>Report</td>
<td>Report</td>
<td>6(^6)</td>
</tr>
<tr>
<td>Kinematic viscosity @ 100°F (37.8°C), cSt</td>
<td>1.2 to 2.5</td>
<td>1.4 to 3.0</td>
<td>2.0 to 4.3</td>
<td>1.8 to 9.5</td>
</tr>
<tr>
<td>Distillation, °F(°C):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% evaporated</td>
<td>Report</td>
<td>Report</td>
<td>Report</td>
<td></td>
</tr>
<tr>
<td>90% evaporated, max.</td>
<td>550(288)</td>
<td>550(288)</td>
<td>640(338)</td>
<td>675(337)</td>
</tr>
<tr>
<td>End point, max.</td>
<td>572(300)</td>
<td>626(330)</td>
<td>700(371)</td>
<td>700(371)</td>
</tr>
<tr>
<td>Carbon residue on 10% bottoms, % wt., max.(^7)</td>
<td>0.10</td>
<td>0.15</td>
<td>0.35</td>
<td>0.20</td>
</tr>
<tr>
<td>Sulfur, % wt., max.</td>
<td>0.25</td>
<td>0.50</td>
<td>0.50</td>
<td>0.70</td>
</tr>
<tr>
<td>Copper strip corrosion, 3 hrs. @ 122°F(50°C)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>max. rating</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ash, % wt., max.</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Water &amp; Sediment, % max.</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Accelerated stability, total insolubes</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>mg/100 ml. max.(^8)</td>
<td>0.05</td>
<td>--</td>
<td>--</td>
<td>0.10</td>
</tr>
<tr>
<td>Neutralization number, TAN, max.</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Particulate contamination, mg/liter, max.</td>
<td>40</td>
<td>45</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Cetane number, min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1\(^{\text{1)}} - CONUS stands for continental U.S.; OCONUS for outside continental U.S.
2\(^{\text{2)}} - cloud point—temperature at which solid substances begin to separate from the fuel under conditions of ASTM Method D97.
3\(^{\text{3)}} - pour point—lowest temperature at which fuel will pour or flow under conditions of ASTM Method D97.
4\(^{\text{4)}} - to be specified by purchaser.
5\(^{\text{5)}} - DF-2 destined for Europe and S. Korea shall have a maximum limit of 9°F (-13°C). For other OCONUS areas, the maximum limit must be specified by the procuring activity.
6\(^{\text{6)}} - DF-2 destined for Europe and S. Korea shall have a maximum limit of 0°F (-18°C). For other OCONUS areas, the maximum limit must be specified by the procuring activity.
7\(^{\text{7)}} - The maximum limits do not apply for samples containing cetane improvers. In those instances, the test must be performed on the base fuel blend.
8\(^{\text{8)}} - This requirement is applicable only for military bulk deliveries intended for tactical, OCONUS, or long term storage (greater than six months) applications (i.e., Army depots, etc.).
The demand for high octane gasoline has created the need for raw material with a greater proportion of aromatic hydrocarbons and branched chain paraffins than is ordinarily present in crude oil. To provide this material, portions of crude oil distillate and nonvolatile residue are processed further in a catalytic cracker (see below). This "cracked" product is then fractionally distilled to produce gasoline, other petroleum fractions, more residue, and a component boiling in the diesel fuel range which is available for blending with "straight run" distillate. Since the high content of branched paraffins and aromatic hydrocarbons (which are desirable for high octane gasoline) yields a diesel fuel of lower quality (low cetane number), high quality diesel fuels are usually made by blending "straight run" distillate with "cracked" products, in proportions of 0-100% of each. A cetane improver, as discussed below, is then added (2).

In the refinery the crude oil is heated continuously and transferred to a fractionation column. Distillate is collected in various fractions on the column; the more volatile a fraction is, the higher in the column it will condense. Proceeding up the column, heavy gas oil (for furnaces) is encountered first, then light gas oil (No. 2 fuel oil and diesel fuel) and kerosene, gasoline, naphtha, and finally, gases are at the top. A nonvolatile residue remains at the bottom. The light gas oil is also called middle distillate and generally covers the boiling range 160-343°C. Kerosene is in this range, but is likely to contain more lower boiling material. The light gas oil may go directly to a hydrogenation unit where sulfur and nitrogen are removed; olefins and partially saturated aromatics containing two or three fused rings are converted to saturates and partially hydrogenated compounds. After blending of any required additives, the material is stored. Alternatively, the light gas oil may be combined with the heavy gas oil and residue and transferred to a catalytic cracker. Here, in contact with the catalyst, assisted by heat and pressure, larger molecules are broken down into smaller ones, and rearrangements occur yielding after fractionation: high octane gasoline, cracked light gas oil, cracked heavy gas oil, and residue. As with ordinarily derived light gas oil (called straight run distillate) the cracked version goes to the hydrogenation unit (3). Cracked and straight run distillate may then be blended.

Since the residue from the ordinary distillation contains many polycyclic aromatic molecules which break apart in the cracker and are later hydrogenated, the cracked distillate contains more cycloparaffins (naphthenes) than does straight run distillate. The naphthenes generally contain one or two 5- or 6-membered rings with side chains.

Several other petroleum products which are also contained in the same "middle distillate" or "light gas oil" fraction as diesel fuels, and are therefore likely to have a similar hydrocarbon composition, are presented below:

kerosene - also known as range oil, coal oil, Grade 1
distillate heating oil, Grade 2 heating oil
- a broad term for straight run material boiling
at 160-288°C
fuel oil No. 1 - characterized by a 10% boiling point of 214°C and a 90% value of 288°C

fuel oil No. 2 - the 90% boiling point must fall at 282-350°C
- both fuel oils are for home heating.

C. Composition

Diesel fuel mainly consists of three hydrocarbon types: aliphatic, olefinic and aromatic. The hydrocarbon composition varies considerably in different samples, for many reasons. Small quantities of additives are also present, the nature and function of which will be discussed in this section.

1. Hydrocarbons

Because diesel fuel is obtained from crude oils of widely varying hydrocarbon composition, and also because different fuels contain different proportions of "cracked" material, the hydrocarbon composition will vary from supplier to supplier and even batch to batch. Further, the variation of viscosity and boiling ranges of the three grades given in Table 2 implies that the distribution of molecular weights and/or hydrocarbon types differs among them. For these three reasons it is possible to indicate only the normal ranges of hydrocarbon types in describing the composition of diesel fuel.

The carbon atom number range for diesel fuels is not rigidly defined, and may readily encompass C₉-C₂₄ (4). Regardless of refinery process there is a saturated component (normal paraffins, branched paraffins and cycloparaffins) and an aromatic fraction (5); olefins are currently considered of negligible occurrence (6). The aromatics generally include: substituted benzenes - one ring; indenes and naphthalenes - two fused rings; biphenyls - two non-fused rings; acenaphthylenes, anthracenes, fluorenes, phenanthrenes - three rings; and polycyclic aromatic hydrocarbons. Partially unsaturated aromatics occur: indanes, dihydronaphthalenes, tetrahydronaphthalenes (tetralins), and acenaphthenes. The cyclic compounds, almost as a rule, have one or more n-paraffinic side chains each ranging from C₁ to about C₁₄. This is one reason why even a narrow boiling range sample may contain representatives of all of these hydrocarbon types (7, 8).

Diesel fuel compositions as available in the 1959-1976 literature are summarized in Tables 3 and 4. Analyses of 30 straight run samples failed to detect any olefins by a method which could detect as little as 1% (9). An effort to determine 3,4-benzpyrene located 2.6 µg/100 ml, compared with 1-2 µg/100 g of dried leaves (10).

In the compositional analysis of diesel fuel, certain problems arise. The aliphatic side chains on the aromatics tend to cause tailing in column chromatographic schemes for separating saturates from aromatics; these side chains also tend to confound spectrometric methods for indicating saturated vs aromatic nature as the instruments do not readily differentiate between free paraffins and side chains. Methods used by the publishers of the data in Tables 3 and 4 include:
### TABLE 3. Composition Ranges for Hydrocarbon Types in Straight Run Diesel Fuels

<table>
<thead>
<tr>
<th>Hydrocarbon type</th>
<th>Range (in %)</th>
<th>Reported values (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60-80(a) 39-80(b) 74(c) 59(d) 74(e) 77(f) 65-70(g) 68-70(g) 72.6(h)</td>
</tr>
<tr>
<td>Paraffinic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal alkanes</td>
<td>39-80</td>
<td>24 12 39 52 1-44 37-43</td>
</tr>
<tr>
<td>branched alkanes</td>
<td>12-24</td>
<td></td>
</tr>
<tr>
<td>cyclic alkanes</td>
<td>25-42</td>
<td>21 24 18-25 33 26-32</td>
</tr>
<tr>
<td>monocyclic</td>
<td>21-25</td>
<td></td>
</tr>
<tr>
<td>dicyclic</td>
<td>6-32</td>
<td>12 9 6-12</td>
</tr>
<tr>
<td>polycyclic</td>
<td>1-5</td>
<td>2 1-5</td>
</tr>
<tr>
<td>Olefinic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alkylbenzenes</td>
<td>20-61</td>
<td>20-40 20-61 26 41 26 23 30-35 30-32 27</td>
</tr>
<tr>
<td>dicyclic</td>
<td>4-25</td>
<td>25 7 6-11 4</td>
</tr>
<tr>
<td>tricyclic</td>
<td>9-22</td>
<td>9 15 10-22 15</td>
</tr>
<tr>
<td>polycyclic</td>
<td>0-10</td>
<td>5 3.5 0-9 10</td>
</tr>
</tbody>
</table>

### TABLE 4. Composition Ranges for Hydrocarbon Types in Cracked* Diesel Fuels

<table>
<thead>
<tr>
<th>Hydrocarbon type</th>
<th>Range (in %)</th>
<th>Reported values (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>55(d) 50(f) 34.6(i) 39(j) 37(j)</td>
</tr>
<tr>
<td>Paraffinic</td>
<td>34.5-55</td>
<td></td>
</tr>
<tr>
<td>normal alkanes</td>
<td>34.5-55</td>
<td>19 15 15</td>
</tr>
<tr>
<td>branched alkanes</td>
<td></td>
<td>19 15 15</td>
</tr>
<tr>
<td>cyclic alkanes</td>
<td>5-7.5</td>
<td>7.5 6.6 5</td>
</tr>
<tr>
<td>monocyclic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dicyclic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>polycyclic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olefinic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alkylbenzenes</td>
<td>5-7.5</td>
<td>45 50 58 54 58</td>
</tr>
<tr>
<td>dicyclic</td>
<td>45-58</td>
<td>10 20</td>
</tr>
<tr>
<td>tricyclic</td>
<td>10-20</td>
<td>10 25</td>
</tr>
<tr>
<td>polycyclic</td>
<td>10-18</td>
<td>18 10</td>
</tr>
<tr>
<td></td>
<td>0.5-3</td>
<td>3 0.5</td>
</tr>
</tbody>
</table>

*Possibly blended with straight run fuel. All values listed in one column come from the indicated reference: a=(17-Teasley); b=(6-Shoolery and Rudde); c=(11-Herron); d=(12-Fitzgerald et al.); e=(18-Ehrler et al.); f=(19-Fitzgerald et al.); g=(20-Gerarde); h=(41-Keen); i=(7-Snyder); j=(5-Kearns et al.). In fuel from reference (a), naphthenes were included in aromatics. The second samples of references (g) and (j) are No. 2 fuel oil. In fuel from reference (i), 2.5% nitrogenous heterocycles were present in aromatics.

In Table 4, samples d,f, and i are cracked fuels; both j samples are blends.
do not readily differentiate between free paraffins and side chains. Methods used by the publishers of the data in Tables 3 and 4 include:

1) natural abundance C-13 nuclear magnetic resonance spectrometry—overestimates saturates (6)

2) silica gel column chromatography with a differential refractometer and an ultraviolet spectrometer to monitor the cuts—gel particle size and elution solvent important to avoid tailing (9, 11)

3) high resolution, low voltage mass spectrometry (as opposed to high voltage methods)—presence of sulfur compounds obscures detection of carcinogenic types of aromatics (11)

4) ultraviolet spectrometry—useful for determining presence of particular types of aromatics as they tend to have "signature" type absorption frequencies (12)

5) molecular sieve column chromatography—for separating n- from iso- and cycloparaffins (12)

6) infrared spectrometry—useful in identification of individual isomers of aromatics (13)

7) gas liquid chromatography—useful for separation but not type characterization (14).

2. Additives

Small quantities of additives may be formulated into diesel fuels to bring them up to specifications. The Federal Specification for Diesel Fuel accepts cetane improvers, corrosion inhibitors, and antioxidants. Cetane improvers, which help to control burning velocity (increase cetane number) in the engine, are effective in very small amounts. In grade DF-A up to 0.25% by weight is allowed, and in grades DF-1 and DF-2 as much as 0.5% may be added. The actual agents may be isomeric mixtures of primary hexyl or amyl nitrates.

The corrosion inhibitors function to protect storage containers; they are generally surfactants which coat the container surface. A review of patent literature (15) provides the best indication of the potential chemical nature of these agents. The MIL Spec. for corrosion inhibitors (16) does not require specific chemicals. Six classes seemed especially significant:

1. Bis(1,3-alkylamino)-2-propanol and reaction products with phosphoric anhydride

2. Reaction products of a mixture of sulfur, alkylen polyamine, and nitro-nitrito alkane

3. Fatty acid amides—possibly mixtures of R(CO₂H)₂ with various RNH₂(NHR')₂, and fatty amines
4. 2-Hydroxy-5-cetylbenzene-1,3-dicarboxylic acid

5. Alkyl sulfoxides, R-S(0)-R'; one of the R groups must have an acid, ester, or alcohol function

6. R-substituted dimercaptotriazoles, where R is aliphatic or aromatic.

Class 6 serves to prevent corrosion of copper fittings from sulfur compounds. The Qualified Products List (QPL) of various manufacturers' products under MIL-I-25017C (16) specifies maximum amounts of 0.002-0.006% by weight. Permissible antioxidants as listed in Federal Spec. VV-F-800B (1) are:

N,N'-diisopropyl-para-phenylenediamine
N,N'-discecondary butyl-para-phenylenediamine
2,6-diteriary butyl-4-methylphenol
2,4-dimethyl-6-tertiary butylphenol
2,6-diteriary butylphenol
75 percent min. 2,6-diteriary butylphenol and 25 percent max. tertiary and triteriary butylphenols
60 percent min. 2,4-diteriary butylphenol and 40 percent max. mixed tertiary butylphenols.

They serve to deter the formation of gums, which sometimes occur when fuels are blended. Maximum concentration is 0.003% of active ingredient.

D. Generation of Smoke

Historically "smoke" screening of this type for troops, equipment, and facilities has been created using a stationary generator and a higher boiling oil than diesel fuel. Experience indicated that only the operator of the generator might be exposed to unhealthful amounts of hydrocarbons, and that a standard face mask would provide adequate protection. Now that diesel fuel is under consideration as a substitute oil under conditions whereby the smoke might be generated by a vehicular diesel engine exhaust system, a reexamination of the physical and chemical nature of the smoke is required.

A private communication from Teledyne Corporation indicated that the diesel fuel was being fed at about 4 l/min into the exhaust manifold of the engine, at a point where the temperature was 540-620°C. An older publication had given diesel exhaust temperatures of 260-427°C (21). In theory the fuel is instantly vaporized by the exhaust gases. Emerging into the open air the vaporized fuel rapidly condenses into particles 0.5-1.0 μ in diameter which, although individually transparent, appear opaque en masse. The particles are dispersed by the wind to provide wide area screening (22).

Direct information on concentration in air of these particles is lacking. A private communication from FMC Corporation indicated a possible vapor concentration in the exhaust pipe of about 46 mg/l. Visibility in the smoke is reduced to a meter or less according to the Naval Systems Sea Command and the U.S. Army-private communications.
Analysis of condensed vapor from a mineral oil smoke showed that very little cracking occurred; the mid-boiling point of this oil, 416°C, indicates it is likely to be more heat-stable than a typical diesel fuel. Pyrolysis was postulated to be possible when the fuel contacted the manifold wall prior to vaporization; presence of air in the system described in this patent (23) did not lead to significant combustion. A test hydrocarbon, n-hexadecane, heated for six hours at 371 or 399°C in a closed system containing various metal surfaces showed about 4% decomposition at the lower temperature, somewhat more at the higher one. New compounds formed ranged from C₁-C₁₆ and higher, plus hydrogen. In an addendum it was disclosed that naphthenes pyrolyze faster than n-hexadecane at 371°C, the weakest bond proving to be the ring-side chain juncture (24). Thus it seems likely that pyrolysis can occur, but the brief residence time in the highest temperature zone minimizes it. Highly reactive compounds including aldehydes, olefins and others produced in the combustion of fuel in the engine will mix and may possibly react with the added diesel fuel.

E. Other Hydrocarbons as Models for Diesel Fuel

In the human and animal toxicology and pharmacokinetics sections which follow, studies are presented in which substances other than diesel fuel were tested. The similarities of these hydrocarbons to diesel fuel or its fractions justified their inclusion, in view of the insufficient literature on diesel fuel.

Deodorized kerosene is a mixture of straight chain, branched and cyclic aliphatic hydrocarbons, with a very low aromatic content. Kerosene has a higher aromatic content. Both substances have boiling ranges similar to, although lower than diesel fuel, and a somewhat similar composition.

Alkynaphthalenes and alkylbenzenes are found in the aromatic fraction of diesel fuel. The C₉-C₁₂ alkyl aromatics boil at low temperatures within the diesel fuel distillation range; therefore, the vapors of diesel fuel would contain relatively greater quantities of C₉-C₁₂ aromatic hydrocarbons than the liquid or aerosolized forms.

Octadecane and hexadecane, C₁₈ and C₁₆ straight chain saturated hydrocarbons, respectively, are found in the paraffinic fraction of diesel fuel.

Other mixtures, such as heating oil and Mentor 28, are fairly similar in composition, boiling range and viscosity to diesel fuel.
V. HUMAN TOXICITY

Diesel fuel has not been well studied. Many of the reports presented in this discussion can only be considered indicative of possible effects of the agent, until further investigation is undertaken. Because of this paucity of data, information on several of its components and on petroleum products, such as kerosene, of somewhat similar composition has also been included in this discussion (see section IV.E).

A. Conditions and Extent of Exposure

Diesel fuel mists and aerosols, in particles ranging from 0.5 to 1.0 micron in diameter can be inhaled directly into the lung. Operators of smoke-generating equipment and personnel maneuvering under the fog cover are at risk. In most cases, the exposure is limited to microdroplets of the liquid; however, when the aerosolization occurs at sufficient temperatures for pyrolysis, the resulting transformed hydrocarbon products may be mixed with the fog. Since the fog is to be produced in the exhaust manifold of a running diesel engine exposure to exhaust fumes will occur concomitantly.

Exposure to diesel fuel occurs in individuals handling or transporting it, as well as in work areas where diesel engines are maintained or repaired, such as in the railroad industry. In addition to direct skin contact with the liquid, contamination of clothing worn next to the skin, breathing of vapors and splashing into the eye must be considered.

Incidentally, children and persons with suicidal intent have been known to drink diesel fuel. Although it is a chemical irritant to the gastrointestinal tract, the greater danger is aspiration directly into the lungs, which can be fatal.

B. Exposure to Aerosols and Vapors

Inhalation is the major type of exposure to be encountered in screening smoke operations. The effects of diesel fuel and similar hydrocarbon mixtures are included.

1. Diesel fuel

Diesel oil aerosol of unspecified content was used as a control atmosphere in the study of the effect of atmospheric pollutants (25). Normal subjects exposed to dispersions of 0.33 mg/l and 0.17 mg/l reported practically no eye, nose or throat irritation after 10 minutes of exposure. Pulmonary effects were not considered.

There is no other literature on diesel fuel aerosol inhalation in humans.

Diesel fuel analysis reveals that it is composed of paraffins, cycloparaffins (naphthenes) and aromatic hydrocarbons in varying concentrations, depending upon the crude oil source, whether it is a straight
run distillate or cracked, and other factors. (See section IV). An examination of the effects of individual hydrocarbon classes on an organism will permit some conclusions about toxicity, although the possibility of additive or antagonistic behavior of the various aromatic and aliphatic hydrocarbons must ultimately be evaluated.

2. Kerosene

The paraffin component of diesel fuel is represented in kerosene, a hydrocarbon mixture which contains around 80% of C_9-C_{16} saturated hydrocarbons. According to Gerarde (20), "toxicologically and pharmacologically the diesel fuels are similar to kerosene".

Subjects inhaling deodorized kerosene (treated to remove most aromatics) mean vapor-air concentrations of 0.14 mg/l (20 ppm) for 15 minutes reported no eye, nose or throat discomfort or irritation during or following the exposure (26). This concentration was the highest obtainable, representing saturation at 25°C. Other subjects inhaled a series of vapor-air concentrations of deodorized kerosene over a period of 2 days, with 10-second exposures to each sample. The odor threshold was determined to be approximately 0.0006 mg/l (0.09 ppm) for 83% of the subjects. With this practically aromatic-free hydrocarbon mixture (3.9% aromatics), there was essentially no irritation or toxicity due to inhalation of vapors.

The effects of the cycloparaffinic components in diesel fuel aerosols have not been extensively investigated (27). Kerosene contains more cycloparaffins (over 40%) than diesel fuel (approximately 30%). As indicated in the above study, deodorized kerosene did not irritate the eye, nose or throat in saturated air-vapor concentrations (26), so it is reasonable to suggest that the cycloparaffins in diesel fuel are equally inoffensive.

3. Aromatic hydrocarbons

The aromatic hydrocarbons present in diesel fuel are more toxic than the saturated components, as indicated by the following studies. Alkynaphthalenes, which may constitute 9-20% of diesel fuel, have been studied with particular reference to 1- and 2-methylnaphthalene (20). Prolonged exposure to aerosols or mists of these substances has resulted in irritation to the eyes, mucous membranes, skin and respiratory passages of laboratory animals, and the possibility of the same effects in humans must be considered. According to Gerarde, the low vapor pressure of monomethylated naphthalenes makes it improbable that acute intoxication might result from inhalation of vapors (20).

The alkylbenzenes (found in diesel fuel) are highly irritating when concentrated. Tetramethylbenzenes such as durene, in mists and vapors, can be expected to affect the respiratory membranes (20).

Nau et al. (28) studied the effects of aromatic hydrocarbons boiling in the low end of the diesel fuel range, characterized by carbon numbers C_9-C_{12}. Vapor concentrations as low as 137.5 mg/l (25 ppm) of C_9-C_{10} aromatics produced lacrimation and/or irritation of the eyes, skin and
mucous membranes of experimental subjects. For the C\textsubscript{11}-C\textsubscript{12} fraction, the minimum vapor level producing these effects was 104.5 mg/l (19 ppm). Odor detection thresholds were 11 mg/l (2 ppm) and 2.5 mg/l (0.5 ppm), respectively, for the C\textsubscript{9}-C\textsubscript{10} and the C\textsubscript{11}-C\textsubscript{12} fractions.

There is insufficient information to state that mists or aerosols of diesel fuel are not harmful to humans. Of the hydrocarbon components, it is probable that the aromatic fraction is responsible for whatever upper respiratory tract irritation occurs, whereas the paraffinic and cycloparaffinic constituents appear to be innocuous. Because of the presence of additives, exhaust components, and possible pyrolysis products in any fog, further investigation is necessary.

C. Skin Contact

Dermatitis and other skin conditions have been reported after industrial exposure to diesel fuel and related substances. Skin sensitivity factors have been evaluated.

1. Diesel fuel dermatitis

Diesel fuel produces acute dermatitis in some persons, while others who are exposed for months or years may experience no adverse effects. Il'in et al. (29) reported acute dermatitis in six workers. The skin reaction occurred about 18 hours following exposure, even though the area was washed thoroughly. Feelings of coolness were followed by burning and pruritis. The dermatitis lasted for a few minutes to several hours.

Repeated contact with diesel fuel leads to constant skin erythema in sensitive individuals. Tense, painful, dry and cracked skin is characteristic and especially marked in skin folds. Eruptions usually contain a purulent exudate (29). In 15 workers, prolonged skin contact caused a slight but gradually progressive dermatitis. Chronic dermatitis lead to pyoderma in another 18 cases. Three of the 18 suffered acute dermatitis, characterized by transient hyperemia, edema, vesiculation and sweating of exposed skin, and continued contact caused abscess formation. The pyoderma was usually localized to the face, forearms and back of the hands. Abscess development was favored by hypothermia in cold weather, fever and excessive perspiration. Inadequate personal hygiene was also a contributing factor (29).

A very high percentage of workers in an asbestos-cement factory developed skin lesions, which were attributed to the use of diesel oil (composition unspecified) as a molding medium. The dermatitis was characterized by folliculitis and furuncles. It would worsen in warm weather, and poor personal hygiene was also a factor in the severity of the condition. The frequency of dermatitis decreased markedly when a paraffin-water emulsion was substituted for diesel oil (32).

Diesel engine vapors and exhaust were implicated in a case of a 32-year-old diesel truck driver who developed an intermittent follicular dermatitis (as well as a nervous condition). The papular eruption was distributed symmetrically over the inner skin of the arms and legs, skin
folds of the axilla and trunk. Histologically, subacute infiltrating inflammation, dermal sclerosis, intracellular vacuolization and epidermal hyperplasia were described. The heavy vapors and exhaust of volatile hydrocarbons and gas oil contaminated the cabin when ventilation was insufficient, and the skin condition would worsen for example, in cold weather, when the windows of the unheated cabin were closed (34).

2. Diesel fuel injury

Yaxley (35) presented a case of a diesel injection injury. In the diesel engine, fuel is forced from small jets at a high pressure (6,000 lb/in$^2$) in a stream of finely divided particles suitable for combustion. An injection tester accidently had diesel fuel forced into the pulp of his index finger. The traumatized finger was numb at first, becoming swollen and painful, with lymphangitis of the dorsal hand. Although the finger was incised for drainage, by the eighth day after the injury, the wound had extended through the terminal phalanx, which became dry and gangrenous by the 22nd day, necessitating amputation. The occurrence of necrosis and gangrene following the subcutaneous tissue trauma and contact with diesel fuel is supported by the fact that small amounts of the substance produced sterile abscesses when injected subcutaneously into rabbit ears, according to the author.

3. Kerosene burn

Prolonged skin contact with kerosene-contaminated clothing was responsible for a case of epidermal necrolysis, which occurred on the second day of a camping trip in a 12-year-old backpacker who was carrying a fuel container which leaked onto his clothing. The skin eruption involved extensive erythema and detachment of epidermal tissue overlying a purulent exudate (31). This acute chemical burn situation may be equivalent to the industrial setting in which poor personal hygiene practices, such as wearing contaminated work clothes, occur.

4. Aromatic hydrocarbons

The aromatic hydrocarbons produce skin changes including vasodilation, redness and irritation. Shorter side chains and increased branching increase the potency. Prolonged skin exposure to liquid aromatics leads to defatting, drying, scaling and fissuring in some cases. However, skin cancer has not been reported after prolonged contact with diesel fuel. Petroleum products containing material boiling above 370°C, obtained from severe cracking processes, and containing polycyclic aromatic components are carcinogenic in laboratory animals. In products similar to diesel fuel, the high-boiling components are inactive carcinogens, unless the conditions of synthesis or processing have favored the production of highly condensed aromatic hydrocarbons, according to Gerarde (20).

Aromatic hydrocarbon fractions containing C$_9$-C$_{12}$ constituents may produce skin lesions severe enough to cause scarring (28). Pure monomethylated naphthalenes, which are found in diesel fuel, produce only negative or slight skin reactions in persons undergoing 24-hour patch tests. Gerarde believes that repeated overexposure to monomethylated naphthalenes may result in local irritation of the eyes, mucous membranes and skin. Prolonged skin contact with durene, a tetramethylbenzene, causes dehydration and defatting of the skin, leading to dermatitis.
5. Constitutional factors in skin sensitivity

Rao (33) noted that industrial dermatitis occurred in some individuals after a few months, while in others, resistance continued for years. He investigated the relationship between blood group (ABO system) and occurrence of dermatitis. Twenty-five workers affected with industrial dermatitis were compared with 25 workers who were unaffected after at least 3 years of exposure. The A blood group was evenly distributed between the 2 groups of workers. Two out of 3 persons with B blood developed dermatitis, as did 1 out of 3 with blood group 0. There were too few individuals with AB blood to make a similar comparison. Rao concluded that "blood grouping provides a rough screening test for the prospective employees to be engaged in work connected with diesel oil during pre-placement medical examination".

Hydrocarbon mixtures boiling in the 177°-316°C range were used in 24-hour skin patch tests. Kerosene caused skin irritation, of varying degrees, in some subjects. A naphthene-rich kerosene fraction in the same boiling range gave more positive skin reactions. The strongest skin irritation response followed patch testing with a highly aromatic hydrocarbon mixture also in the same boiling range. The lower boiling fractions were found to be more irritating to normal skin than the higher boiling, oily fractions. The low boiling fractions, characteristic of diesel fuel, produced an eczematous reaction, while higher boiling distillates produced keratosis, acne, melanosis, photosensitivity and epitheliomas (30).

D. Ingestion and Aspiration

These exposure routes are secondary in comparison with inhalation. When swallowed, gastritis is reported. The substance may cause coughing, by which fuel may reach the trachea and lungs. Respiratory distress and acute pneumonitis may follow if a sufficient quantity is aspirated.

1. Gastritis

It has been pointed out that diesel fuel may be an irritant, and that the aromatic constituents are probably responsible for this effect. Uncomplicated ingestion (without aspiration) of diesel fuel will irritate the mucous membranes of the mouth, throat and upper gastrointestinal tract. Lejeune et al. (36) reported a case of a worker who swallowed a quantity of fuel oil (of unspecified composition) during a siphoning operation. The result was an acute gastritis, with epigastric pain and slight hematemesis, which became subacute but persisted for 3 weeks. Early x-rays revealed gastric mucosal lesions and gastric hypermotility. The lesions were completely resolved by 1 1/2 months after the episode, i.e. there were no radiologic sequellae. No systemic involvement was noted, but it appears that if any of the fuel was absorbed, it was without clinical effect.

It is probable that this is not the only worker who has swallowed fuel accidentally, but this is the only reported instance of simple ingestion.

2. Aspiration pneumonitis

The danger of ingesting diesel fuel is that aspiration and the resulting pneumonitis almost always follow. Accidental ingestion of gasoline,
Kerosene and furniture polish were studied by Olsen (37). When swallowed, these substances cause intense irritation of the upper gastrointestinal tract, and vomiting usually occurs immediately. Cough, dyspnea and a blood-tinged frothy discharge from the mouth or nose indicate that material has entered the lungs.

There are two mechanisms proposed for the development of pneumonitis (37). The more probable is direct aspiration. The second mechanism is based on the observation that some hydrocarbons which are absorbed from the digestive tract into the bloodstream are excreted through the pulmonary capillaries and exhaled, resulting in the pulmonary irritation.

Szamosi et al. (38) reported 30 cases of diesel fuel ingestion by children under 6 years old. Signs and symptoms included: cough in 13; dyspnea in 3; pneumonia (x-ray evidence) in 25; clinical pneumonia in 10; tachycardia in 19; somnolence in 13; cardiac dilatation in 10; vomiting in 20; fever (38°-39°C) in 15; increased erythrocyte sedimentation rate in 13; and breath and vomitus of characteristic odor in 18 of the 30 cases. Aspiration was chiefly responsible for toxicity and mortality. Pneumonia and fever were frequent in diesel fuel intoxication, in contrast with gasoline, which caused pulmonary edema and death.

Aspiration of diesel fuel (unspecified composition) in a 44-year-old man caused initial coughing and emesis, followed by marked dyspnea, sinus tachycardia and low-grade fever within a few hours. Progressive infiltration of the right lung lead to cardiopulmonary insufficiency and death after 17 days. Autopsy revealed extensive bilateral pulmonary necrosis and gangrene. Small deposits of Sudan positive (fatty) material were found near the necrotic areas. The severity of the condition may have been due to the quantity of fuel ingested and/or to hypersensitivity from repeated occupational exposure (39).

Liquid aromatic hydrocarbon mixtures cause chemical pneumonitis with pulmonary edema, hemorrhage and necrosis after direct contact with lung tissue in animals (see section VI.B.). Aspiration of a small quantity causes extensive injury because the low surface tension of the substance enables it to spread over a large area. Liquid alkylbenzenes, alkylnaphthalenes, indanes, indenes and commercial hydrocarbon mixtures containing these substances will cause similar damage, according to Gerardo (20). It appears that the aromatic components in diesel fuel are responsible for the severity of the pulmonary injury after aspiration; the paraffinic constituents tend to produce granulomas, as these substances are walled off by the lung (37).

E. Long-term Effects and Epidemiology

There is almost no literature on long-term effects of diesel fuel in humans other than what has been presented in the skin exposure section. Chronic industrial dermatitis has not received much attention. In 1950, around 1.5% of compensated cases in New York, Illinois and Minnesota were industrial dermatitis. According to Plunkett (40) dermatitis was the "most disabling industrial disease, accounting for 60% of time lost from work".

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There are no reports of effects on humans from exposure to war or peacetime fogging operations where diesel fuel may have been used.

Laignel-Lavastine et al. (34) reported the development of neurological manifestations in a diesel truck driver. Symptoms included vertigo, staggering, nausea, vomiting, tingling paresthesias, dizziness and headache, persisting for several minutes to a few hours. The symptoms would appear most frequently when the cabin of the truck was poorly ventilated, and engine exhaust, containing gas oil, volatile hydrocarbons and carbon monoxide would accumulate. There would be periods of complete freedom from symptoms. Neurological examination revealed no abnormalities. There was a concurrent dermatitis in this case. Evidence is insufficient to implicate diesel fuel as the neurotoxic agent, since the exhaust vapors contained other substances as well.
VI. ANIMAL TOXICITY

The section presents results of aerosol inhalation, skin application, ingestion and intratracheal administration of diesel fuel and related hydrocarbons. Each route of exposure or administration is subdivided according to species. Table 6 (pp. 31-33) summarizes the effects of diesel fuel after various routes of administration.

A. Exposure to Aerosols and Vapors

Diesel fuel studies are limited in scope and number. However, deodorized kerosene and aromatic fractions have been more thoroughly investigated in laboratory animals.

1. Rats

The effect of diesel fuel on the surfactant layer of the lung was investigated in Wistar rats weighing 175 to 300 g. The animals were placed in a 12-l chamber into which diesel fuel, vaporized on a hot plate at 400°C and then water cooled, was introduced in aerosol concentrations up to 10 mg/l. Droplet size averaged less than 2 microns in diameter. The duration of exposure and temperature of the chamber were varied in an unspecified manner. Inhalation did not damage the pulmonary surfactant layer, as demonstrated by pressure-volume lung deflation curves (hysteresis) at low transpulmonary pressures. The diesel fuel contained 72.6% saturates, 0.4% olefins and 27.0% aromatics, with a boiling range of 193°C-335°C (41). When kerosene was substituted for diesel fuel, under the same conditions, the pulmonary effects were equally negative.

Deodorized kerosene (boiling range 208°C-272°C; 55% paraffinic, 41% naphthenic, 3.9% aromatic) vapor inhalation was investigated by Carpenter et al. (26). Six male albino rats weighing 90-120 g, were placed in an atmospheric chamber saturated with kerosene vapors for 8 hours. They were then observed for 14 days and sacrificed. The rats remained normal in appearance and weight gain and there were no remarkable autopsy findings. Tests were performed on 6 male albino rats inhaling deodorized kerosene aerosols with droplet size averaging under 1 micron in diameter. The rats inhaled kerosene aerosol for 6 hours per day for 4 consecutive days. On day 1, the concentration was 9.6 mg/l; day 2 - 6.9 mg/l; day 3 - 7.0 mg/l; and day 4 - 7.4 mg/l. On the first day of exposure, loss of coordination and sluggishness occurred, and following the second exposure day, there was redness of the extremities. After the 4th day of exposure and an additional day of rest, the skin of the extremities was dry and flaking, and remained in this condition for 4 days following exposure. One of the 6 had slight hair loss. Body weight remained normal during and up to 14 days following the exposure.

Carpenter et al. also investigated the subacute inhalation toxicity of deodorized kerosene, monitoring body weight change and blood and urine analyses in male rats (26). A group of 25 rats was exposed to an aerosol concentration of 0.10 mg/l for 6 hr/day, 5 days/wk, for 13 weeks. There were also 25 unexposed rats. One rat died of pneumonia after 30 days. No weight loss had been noted. Mean urine pH was significantly higher (P < 0.05) and specific gravity lower than
controls after 8 weeks of exposure but both had returned to normal values by 13 weeks. One rat which was killed after 8 weeks of exposure had a significantly elevated serum alkaline phosphatase activity, related to the pleural adhesions and abscess bronchopneumonia noted on autopsy. The other rats developed no abnormalities considered to be related to exposure. There were no other statistically significant deviations from the control group in any of the monitored criteria. Of 25 rats exposed to 0.05 mg/l of deodorized kerosene aerosol in the same schedule as above, one rat lost 40 g of weight in 7 days and died after 16 days of exposure, due to bronchopneumonia. In the other rats, the mean erythrocyte count was slightly depressed at 8 weeks, although still within normal limits, returning to control levels by 13 weeks. Histopathological reports of lesions did not differ either in type or frequency for exposed compared to control rats. Sporadic findings of slight tubular regeneration of the kidneys were not considered to be treatment-related. Carpenter et al. concluded that these subacute exposures did not produce progressive dosage-related effects which could be considered adverse effects caused by the treatment.

In contrast to studies with diesel fuel and deodorized kerosene (which contained <3.9% aromatics), where minimal effects were produced from aerosol or vapor inhalation, animals inhaling aromatic hydrocarbon distillates in the lower end of the diesel fuel boiling range suffered severe damage. Nau et al. (28) studied the C₉-C₁₂ fractions, containing alkylbenzenes, naphthalenes, indanes, cyclopentanes and paraffins (see Table 5) in rats, using the following criteria to detect changes from normal: appearance, behavior, weight gain, organ weights, hematological findings, bone marrow changes and gross and histopathologic changes. Laboratory rats exposed to 5.2 mg/l C₉-C₁₀ vapors for 18 hours every day developed lung and liver congestion, enlarged spleens and hemorrhagic kidneys at the end of one day of exposure. By the 8th day, the total white blood cell count was significantly lowered, with increased neutrophil and decreased lymphocyte counts. Expected weight gains did not occur, and there was increased transparency and fragility of the femurs. When rats were exposed to 3.2 mg/l C₉-C₁₀ vapors, under the same conditions for as long as 2,424 hours there was a significantly lower weight gain and a significant (p<0.5) fall in the total white blood cell count. Congestion and hemorrhages were present in the lungs, liver, kidneys, spleen and omentum after a few days of exposure. After 54 hours of exposure, there were focal inflammatory changes in the lungs and fatty livers. After 400 hours, there were hemorrhages around the nose and mouth. Bilateral cataracts developed in 70% of rats which were set aside for 2 months with no additional exposure, where none of the controls developed cataracts. Eye changes included focal accumulations of enlarged epithelial cells with vacuolated cytoplasm, areas of fibroblastic proliferation but no inflammatory cells. There were no significant changes in organ weight. Numbers of myelocytic precursors in the bone marrow were increased.

Rats exposed to 3.2 mg/l of vapors of C₉-C₁₀ alkyl aromatics for 23.5 hr/day for 7 days developed hemorrhages of the kidneys, omentum and subcutaneous tissue. Rats exposed continuously to 3.2 mg/l for 24, 36, 54 or 72 hours showed transient blood changes only, whereas no changes occurred in rats exposed to 3.2 mg/l for 18 hours per day, every other day (total of 3 exposures) and then set aside for observation for 9 months. Lower concentrations (1.0 mg/l, and 0.26 mg/l) produced no changes in rats after more than 700 hours of exposure for 8 hours a day, 5 days a week.
Table 5. Alkyl Aromatic Composition of Fractions used Experimentally by Nau et al. (28)

<table>
<thead>
<tr>
<th>Components</th>
<th>C&lt;sub&gt;9&lt;/sub&gt;-C&lt;sub&gt;10&lt;/sub&gt; Fraction</th>
<th>BP:155-200°C</th>
<th>C&lt;sub&gt;11&lt;/sub&gt;-C&lt;sub&gt;12&lt;/sub&gt; Fraction</th>
<th>BP:200-249°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffins</td>
<td>20 mol %</td>
<td>6 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycloparaffins</td>
<td>6 mol %</td>
<td>0 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylbenzenes</td>
<td>74 mol %</td>
<td>44 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indans</td>
<td>0 mol %</td>
<td>27 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthalenes</td>
<td>0 mol %</td>
<td>23 mol %*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricyclics</td>
<td>0 mol %</td>
<td>0 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylbenzenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;9&lt;/sub&gt;</td>
<td>42 mol %</td>
<td>7 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;10&lt;/sub&gt;</td>
<td>29 mol %</td>
<td>10 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;11&lt;/sub&gt;</td>
<td>3 mol %</td>
<td>24 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;</td>
<td>0 mol %</td>
<td>5 mol %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;13&lt;/sub&gt;</td>
<td>0 mol %</td>
<td>1 mol %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*13.5 mol % naphthalene; 9.5 mol % 2-methyl naphthalene

Rats inhaling 3.2 mg/l of C<sub>11</sub>-C<sub>12</sub> alkyl aromatic vapors, for 18 hours every day did poorly; 50% of 37 animals died after one 18-hour period. There was severe weight loss, engorgement of all organs with blood, small spleen, and liquid and gaseous material filling the intestines. Exposures for 8 hours every day caused rapid deterioration and weight loss after 15 days. Autopsy findings included small spleen, hemorrhagic lungs and liver and bloody and gaseous intestinal contents. Exposures for 5 hours a day caused hair coarsening, emaciation and vesicles around the eyes, nose and feet, marked decrease in the total white blood cell count, with increased neutrophil and decreased lymphocyte counts, possible CNS depression and low rates of weight gain. Femoral softening and "watery" bone marrow were observed when these rats were exposed for up to 1,583 hours. There were no cataracts. Good recovery occurred over 4 months following the end of the experiment.

Rats exposed to 1.28 mg/l of C<sub>11</sub>-C<sub>12</sub> vapors for 8 hours a day, 5 days a week (90 exposures) showed a fall in white blood cell count and a slower weight gain than expected. One of the 17 rats developed a cataract. Bone marrow revealed an increase in erythrocyte precursors and a delayed decrease in the proportion of myelocyte precursors. Rats exposed to 0.32 mg/l (same schedule) showed slight slowing of expected weight gain, and the same bone marrow changes as rats exposed to 1.28 mg/l.
Acute LC₅₀ values for the alkyl aromatics (28) in 150 g rats were 14.4 mg/l of C₉–C₁₀ for 7 hours, and 4.6 mg/l for C₁₁–C₁₂ for 7 hours. In the latter case, 4 out of 10 rats died within 96 hours following exposure, whereas with the lower-boiling fraction, 4 out of 10 died after 7 hours of exposure.

In rats receiving 27 daily one-hour exposures to methylated naphthalene solvent mist (an aromatic hydrocarbon), at a concentration of 2.83 mg/l, median droplet size of 3.15 microns, there were no deaths, no weight losses, and no abnormal hematological findings (20).

2. Rabbits

In groups of rabbits intermittently exposed to diesel fumes, Samal et al. (42) found alterations in immunologic response. Animals were exposed for 2 hours each day for 80 days to smoke from a mixture of diesel and motor oil (100:6). Subcutaneous injections of typhoid-paratyphoid vaccine were given on day 0 (0.5 ml) and on day 50 (1.0 ml), and antibody titers were estimated by the Widal test (flocculation and precipitation), before exposure, and then every 10 days throughout the experiment. The exposed rabbits generated less antibody to the vaccine than controls. In the controls, immune titers showed an initial rise, levelled off at day 40 and fell after day 70, while in the experimental animals there was a steady increase in immune titer for 70 days, indicating absence of the secondary response. Other possible effects of inhalation of the fumes were not discussed.

Rabbits inhaling methylated naphthalene solvent mist (an aromatic hydrocarbon) at a concentration of 2.83 mg/l, median droplet size of 3.15 microns, for one hour each day for 27 days developed redness of the ears after the 4th hour exposure. There were no hematological abnormalities and no deaths. Moderate liver fat deposition and slight epithelial hyperkeratosis of the ears were the only pathological findings (20).

3. Mice

Mouse respiratory rates were not affected by high kerosene vapor concentrations (26). Six mice exposed to 6.9 mg/l of deodorized kerosene aerosol developed a slightly depressed breathing rate. There was no respiratory tract irritation from exposure to saturated vapors at room temperature or from 6.9 mg/l aerosol (duration not given).

Mice exposed to 2.83 mg/l of methylated naphthalene solvent mist for one hour daily for 27 days developed dyspnea, restlessness, prostration, irritation of eyes and redness of ears after the second hour of exposure, and slight weight loss occurred. Hematological studies were normal. Eight out of 20 died; the majority of these deaths occurred during the first three days of the experiment. Autopsy findings included bronchopneumonia, parabronchial alveolar septal thickening, edema and emphysema (20).
Nau et al. (28) determined acute inhalation LC₅₀ values for C₉-C₁₂ alkyl aromatic hydrocarbons in 27-gram CFW mice. The vapor concentrations were 9.7 and 3.4 mg/l for 3 3/4 hours for C₃-C₁₀ and C₁₁-C₁₂, respectively.

4. Rhesus monkeys

Nau et al. (28) studied the effect of vapors of C₉-C₁₀ alkyl aromatic hydrocarbons on Rhesus monkeys. Three monkeys, weighing 1.8 kg, inhaled 1.0 mg/l for 7 hours a day, 5 days a week for 90 exposures. They appeared groggy or sedated during exposures. Tremor developed during the first week, and then diminished throughout the experiment. Total white blood cell counts decreased, with increased neutrophil, and decreased lymphocyte counts. There was hair loss and dry leathery skin, but no gross or microscopic changes other than a slight decrease in erythrocytic and myelocytic precursors in the bone marrow. Monkeys inhaling 0.26 mg/l under the same exposure schedule for 90 exposures showed no changes except an elevated hematocrit and peripheral white blood cell changes similar to those in the above-mentioned monkeys.

Four Rhesus monkeys exposed to 1.28 mg/l of vapors of C₁₁-C₁₂ alkyl aromatics for 7 hours a day, 5 days a week (90 exposures) developed eye and facial irritation, diarrhea after 2 days, neutrophilia and lymphocytopenia of peripheral blood, and increased erythrocytic and decreased myelocytic precursors in the bone marrow. At vapor concentrations of 0.3 mg/l (same exposure schedule) monkeys developed diarrhea by the third day and the same changes as the monkeys exposed to 1.28 mg/l.

5. Dogs

The subacute inhalation toxicity of deodorized kerosene was investigated in male beagles (26). Body weight, blood and urine parameters were monitored. No adverse effects were noted with the 13-week inhalation of 0.10 mg/l for 6 hours a day, 5 days a week. There was a slight elevation of polymorphonuclear leukocytes. Beagles exposed to 0.02 mg/l of deodorized kerosene aerosol showed a borderline but significant (p<0.5) mean weight increase after 13 weeks. There were no treatment-related histopathological findings.

Dogs exposed to 2.83 mg/l methylated naphthalene mist for one hour a day developed marked salivation after the 4th hour of exposure. There were no abnormal hematological findings and no deaths (20).

6. Cats

Deodorized kerosene aerosol concentrations of 6.4 mg/l, produced no signs of neurotoxicity during the 6-hour exposure, and no alterations in weight gain for 14 days following the exposure, in 4 male cats of mixed breed (26). No treatment-related abnormalities were revealed at the autopsies performed 14 days after exposure.

7. Guinea pigs

Six male guinea pigs were exposed to methylated naphthalene solvent
mist, in a concentration of 2.83 mg/l, median droplet size 3.15 microns, for one hour a day for 27 days. Dyspnea occurred following each exposure. There were no weight losses or hematological abnormalities. One animal died around the 10th day of exposure, with pulmonary edema evident at autopsy (20).

It would seem that diesel fuel inhalation toxicity is low. There are no LC50 values or other experimental data in available literature. The aromatic content of diesel fuel did not affect its toxicity, as shown by study comparing it with deodorized kerosene, a mixture with 3.9% aromatic compounds. When aromatic hydrocarbons found in diesel fuel were inhaled by animals, they did produce abnormalities in skin, blood, bone marrow and internal organs. These aromatics are lower boiling and would be found in high relative concentration in vapor, but probably not in high concentration in aerosols of diesel fuel. Study of diesel fuel aerosol toxicity in animals is warranted.

B. Ingestion and Aspiration

Diesel fuel ingestion by farm or laboratory animals leads to changes in blood chemistry, hematologic picture, digestive system involvement, and pulmonary damage if the material is aspirated.

1. Rats

Diesel fuel was found to have low toxicity in rats, in a study performed by Starok et al. (43). In 136 Wistar rats, weighing 180-340 g, given diesel oil (unspecified content) by stomach tube, an oral LD50 of 16.0 ml/kg of body weight (range of 6.7-38.4 ml/kg) was established. The peripheral blood picture in rats fed 20 and 25 ml/kg/day revealed a significant drop in hemoglobin after 14 days, significant reticulocytosis after 7 and 14 days, elevations of neutrophilic granulocytes after 7 days and reduced lymphocytes, thrombocytes and platelets. Erythrocyte counts were normal. Elevated serum enzyme levels of malate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were found, but alkaline phosphatase levels remained normal. The subacute lethal dose of diesel fuel in rats was calculated to be 43.2% of the acute LD50, i.e. 6.9 ml/kg of body weight per day, based on observations in 5 rats orally administered increasing daily dosages from 0.06 - 10.8 ml/kg of body weight over a 32-day period. Three died when the dose reached 3.2 ml/kg on day 18; the other 2 died when the dose reached 10.8 ml/kg on day 32.

Wistar rats weighing 175-300 g were given 2.0 ml to 3.0 ml of kerosene by direct instillation into the duodenum. In the 9 experiments, there were no changes in lung stability 18 to 24 hours later as measured by pressure-volume determinations of deflation under low transpulmonary pressure (hysteresis) (41). No other parameters were studied. This experiment showed that ingestion of distillates somewhat similar to diesel fuel does not damage the lungs if the material is not aspirated.

As discussed in section V.D., entry of diesel fuel into the lungs causes pulmonary damage and signs of respiratory distress. Wistar rats weighing 175-300 g were subjected to instillation of 0.01-0.10 ml of the substance into the trachea via a cannula. The animals which were killed
15 minutes later showed significant, dose dependent differences in pulmonary stability (hysteresis), indicating damage to the surfactant layer. An intratracheal dose of 0.05 ml of diesel fuel produced 50% mortality. In animals remaining alive, lung stability increased and returned to normal levels after 48 hours. In another group of rats given 0.1 ml/kg body weight, after 14 days the lungs were macroscopically normal, although microscopically there were small patches of resolving pneumonia. When kerosene or mineral seal oil (boiling range 196-292°C; 76.6% paraffinic, 1.4% olefinic, 22% aromatic) were instilled into the trachea in the same experimental setup, the pulmonary effects were indistinguishable from those of diesel fuel. The ability of diesel fuel and similar oils to damage the lung lining was proportional to the quantity which was introduced into the trachea, but was not influenced by the relative proportions of aromatic and paraffinic hydrocarbons in the oils tested (41).

Gerarde (47) studied diesel oil aspiration in 2 male albino Wistar rats weighing 300-400 g. The animals were given 0.02 ml (a mouthful) while aspnic, and the material was aspirated when the animals regained their ability to breathe. This dose was lethal in 24 hours for 2/2 rats. Lung findings were characteristic of acute chemical pneumonitis: severe pulmonary edema, hemorrhage, and liver-like appearance. Clinically, the animals developed tachypnea, dyspnea, cyanosis, and a blood-tinged frothy nasal discharge. In a discussion of related hydrocarbon mixtures, Gerarde (20) reported that rats aspirating 0.2 ml of kerosene also developed acute chemical pneumonitis. Liquid alkylbenzenes, alkylnaphtalenes, indanes, indenes and commercial hydrocarbon mixtures containing these substances also damaged the lungs when aspirated.

2. Rabbits

Ten rabbits, weighing around 2.5 kg were fed 1 ml/kg body weight of a fuel oil having a boiling range between 150° and 300°C. Blood sugar levels were determined after 30 minutes, 2, 5, 7, 9 and 12 hours. There was a gradual drop in blood sugar, reaching minimum values (as low as 23% below normal) between 5 and 7 hours and returning to control levels by 12 hours. Petroleum derivatives boiling in slightly lower ranges caused similar changes (46).

After observing a patient with fatal pulmonary gangrene following diesel fuel aspiration, Haraszt (39) was able to reproduce the pathologic effects in the laboratory. In rabbits, 6-8 days following intratracheal administration of 1 ml of diesel fuel, pulmonary changes of progressive interstitial infiltration (hyperemia, capillary endothelial damage, edema, alveolar hemorrhage or exudation and bronchiolar necrosis) were noted.

3. Farm animals

Two veterinary reports of poisoning, in a cow and an ewe, are presented. Although the amount of fuel which was ingested could only be approximated, clinical findings in the blood and gastrointestinal tract, among others, are indicative of systemic toxicity.
The ingestion of up to 7 liters of diesel fuel by a cow lead to slight fever (39.5°C), reduced heart rate, palpitations, reduction in appetite and slowing of peristalsis in the first stomach. This was followed by mild diarrhea, then constipation, a considerable decrease in milk production and a stiff and uncertain gait suggestive of muscular weakness. Painful swelling of the hind fetlocks occurred 5 days after the poisoning. The animal slowly recuperated with treatment after the 8th day (44).

Ranger (45) reported a case of poisoning in an ewe after the consumption of diesel fuel-soaked grass. The animal was depressed, weak, had lost weight, and had a strong odor of diesel fuel emanating from the breath, urine and feces. Slight dyspnea, respiratory signs and increased rumenal peristalsis were also reported. After 10 days without improvement, the contents of the rumen were removed and replaced. The rumenal wall contained an area with raised nodular lesions up to 3 cm in diameter, with ulcerating caseous centers. Following the rumenotomy the animal recovered, although complete loss of fleece occurred. Hematological changes included neutrophilic leukocytosis and moderate normochromic anemia, but there were no changes in serum concentrations of liver enzymes.

C. Skin Contact

In this section, experiments are reported in which diesel fuel and related hydrocarbon mixtures were topically applied. Mechanism of action and protective effects of topically applied hydrocarbons and the findings in the literature concerning carcinogenicity are cited.

1. No sensitization

Sensitization studies with diesel oil, using the Landsteiner and Jacobs method, produced only negative results on 24 white male 400-g guinea pigs (48).

2. Mild primary irritant

Diesel oil was classified as a mild primary irritant on the skin and conjunctiva of the eye, utilizing the Draize et al. method in 10 white Belgian rabbits, weighing about 3500 g (48).

3. Topical application - rats

In male Wistar rats weighing around 250 g, undiluted diesel oil (of unspecified composition) was applied to the skin of the tail for 6 hours each day for 10 days. Epidermal splitting and exfoliation, hair loss, and a papular rash occurred, and generally receded 2-3 weeks after termination of exposure (48). There was no mortality or significant weight loss. Blood studies did reveal decreased hemoglobin concentrations (11% below normals on 17th day of the experiment). The red blood cell count was decreased by 13% on day 17, 24 and 31. The elevated reticulocyte count, mean 58% above normal, was significant (p<0.05) on day 17. The leukocyte count was up by 61% on the 11th and 17th day.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Route of Exposure</th>
<th>Dose;Duration</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar rats</td>
<td>Inhalation of aerosol (2μ diam.)</td>
<td>10 mg/l 2%</td>
<td>no damage to pulmonary surfactant</td>
<td>(41-Keen)</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Exposure to &quot;smoke&quot; diesel fuel: motor oil (16:6:1)</td>
<td>2 hr/day 80 days</td>
<td>less antibody response to typhoid-paratyphoid vaccine; absence of secondary response</td>
<td>(42-Samal et al.)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>intragastric</td>
<td>16.0 ml/kg b.w.</td>
<td>decreased Hb after 14 days reticulocytosis after 7 &amp; 14 days neutrophilic granulocytosis after 7 days decreased lymphocytes, thrombocytes and platelets RBC - no change elevated MDH, Asp-AT, Ala-AT</td>
<td>(43-Starek et al.)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>p.o.</td>
<td>20-25 mg/kg b.w./day</td>
<td>subacute toxicity (\text{LD}_{50})</td>
<td>(43-Starek et al.)</td>
</tr>
<tr>
<td>Cow</td>
<td>p.o.</td>
<td>about 7 liters</td>
<td>low-grade fever, reduced appetite, weight loss, weakness, decreased milk production; recovery in 8 days</td>
<td>(44-Messerli)</td>
</tr>
<tr>
<td>Ewe</td>
<td>ingestion of fuel-soaked grass</td>
<td></td>
<td>weakness; weight loss; fuel odor in breath, urine, feces; dyspnea; loss of fleece; neutrophilic leukocytosis; normochromic anemia; nodular caseating ulcers in rumen</td>
<td>(45-Ranger)</td>
</tr>
<tr>
<td>Animal</td>
<td>Route of Exposure</td>
<td>Dose:Duration</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------</td>
<td>-----------------</td>
<td>--------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Rabbits</td>
<td>p.o.</td>
<td>1 ml/kg b.w.</td>
<td>23% drop in blood sugar in 5-7 hr; recovery in 12 hr</td>
<td>(46-Tani)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>intratracheal</td>
<td>0.01-0.10 ml</td>
<td>respiratory distress; reduction in pulmonary surfactant</td>
<td>(41-Keen)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>intratracheal</td>
<td>0.05 ml</td>
<td>50% mortality</td>
<td>(41-Keen)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>intratracheal</td>
<td>0.01 ml</td>
<td>respiratory distress; pneumonitis; resolution in 2 wk</td>
<td>(41-Keen)</td>
</tr>
<tr>
<td>Albino Wistar rats</td>
<td>aspiration</td>
<td>0.02 ml</td>
<td>2/2 died in 24 hr; acute pneumonitis; respiratory distress; liver-like lungs</td>
<td>(47-Gerarde)</td>
</tr>
<tr>
<td>Rabbits</td>
<td>intratracheal</td>
<td>1 ml</td>
<td>progressive interstitial infiltration of lungs after 6-8 days</td>
<td>(39-Baraszti and Sovari)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>topical application to tail skin</td>
<td>? 6 hr/day for 10 days</td>
<td>epidermal splitting, exfoliation, depilation, papular rash</td>
<td>(48-Stage et al.)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>topical</td>
<td>? 6 hr/day for 10 days</td>
<td>no mortality; no weight change; 11% decreased 4th-day 17; 13% decreased RBC count-day 17, 24, 31; 58% increased reticulocytes-day 17; 61% increased leukocytes-day 11, 17; 175% increased neutrophils-day 11, 17, 24; increased Asp-T; increased LDH (2,3,4); decreased LDH (1,5); damage to myocardial cells, RBC and hepatocytes</td>
<td>(48-Stage et al.)</td>
</tr>
<tr>
<td>Animal</td>
<td>Route of Exposure</td>
<td>Dose; Duration</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
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<td>----------------------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Rats</td>
<td>dorsal skin implant</td>
<td>? 10 days</td>
<td>dose-dependent stimulation of granulation tissue</td>
<td>(49-Bien and Buntrock)</td>
</tr>
<tr>
<td>Mice</td>
<td>topical</td>
<td>? daily</td>
<td>18/100-tumors; 3/18 malignant (heavy-grade dirty fuel)</td>
<td>(51-Twort and Twort)</td>
</tr>
<tr>
<td>Mice</td>
<td>topical</td>
<td>? 2 times/wk</td>
<td>1/100 tumors; 0 malignant (heavy-grade dirty fuel)</td>
<td>(51-Twort and Twort)</td>
</tr>
<tr>
<td>Mice</td>
<td>topical</td>
<td>? daily</td>
<td>dermatitis (light-grade fuel)</td>
<td>(51-Twort and Twort)</td>
</tr>
<tr>
<td>White Belgian</td>
<td>topical</td>
<td>Dana et al. method</td>
<td>mild primary irritant of skin and eye conjunctiva</td>
<td>(48-Starek et al.)</td>
</tr>
<tr>
<td>rabbits</td>
<td></td>
<td></td>
<td>desquamation, depilation, ulceration, crusting</td>
<td></td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>topical</td>
<td>?</td>
<td>no effect (protective)</td>
<td>(49-Bien and Buntrock)</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>topical</td>
<td>1-10% mineral oil in diesel fuel</td>
<td>no sensitization</td>
<td>(48-Starek et al.)</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>topical</td>
<td>Landsteiner &amp; Jacobs method</td>
<td>day 5-erythema; day 12- ulceration and crusting; day 19-worsening of skin, restlessness; day 29-recovery; day 34-hair regrowth</td>
<td></td>
</tr>
<tr>
<td>Albino guinea</td>
<td>topical to dorsal skin</td>
<td>5 times/wk</td>
<td>total 19 days</td>
<td></td>
</tr>
<tr>
<td>pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations used**

-? - Dose or duration not specified in original source

p.o. - oral administration

b.w. - body weight

RBC - red blood cells

Hb - hemoglobin

MDH - malate dehydrogenase

LDH - lactate dehydrogenase

Asp-AT - Aspartate aminotransferase

Ala-AT - Alanine aminotransferase
Neutrophilic granulocytes increased by 175%, which was statistically significant (p<.05) on day 11, 17 and 24. There were no changes in thrombocyte or lymphocyte counts. Blood serum chemistry revealed increases in aspartate aminotransferase and the intermediate lactate dehydrogenase (LDH) isoenzymes, and decreased activity of heart and liver (muscle) fractions of lactic dehydrogenase. The authors concluded that absorption of diesel fuel through the skin caused a direct effect on blood-forming organs, leading to decreased production of red blood cells and stimulation of reticulocyte formation, neutrophilia and lymphocytopenia. LDH isoenzyme changes may have been secondary to cell damage of myocardium, erythrocytes and hepatocytes.

Starek and Cembala (50) investigated the skin effects of Mentor 28, a substance used in the electroerosive industry. This hydrocarbon mixture distills in the range of 278°-320°C; its composition is 77.0% paraffinic and naphthenic, 19.9% aromatic and 3.1% olefinic. When applied to the tail skin of Wistar rats for 6 hours a day for 10 consecutive days, splitting, exfoliation of epidermis, hair loss and nodular exanthema resulted. Cosmetic naphtha (99% paraffinic) and turbine oil (unspecified content) produced less severe skin changes, leading the authors to conclude that the higher aromaticity of Mentor 28 was responsible for its greater activity. When Starek et al. (48) compared the skin effects of Mentor 28 with those of diesel fuel, using identical tail painting schedules, the rats' skin reactions were similar in both experiments, consisting of epidermal splitting and exfoliation, hair loss, and nodular exanthema.

4. Topical application - guinea pigs

Skin compatibility to diesel fuel (boiling range 180-340°C) was tested in 5 albino guinea pigs weighing 270-550 g. The substance was applied to the shaved skin of the back 5 times a week until a skin reaction appeared. By the 5th day, there was slight erythema, followed by desquamation, induration, hair loss and rhagades while treatment continued. The experiment was stopped on day 19 due to increasing restlessness of the animals as well as to advancing skin ulceration and intensified crustation which had first been noted on the 12th day. Recovery was rapid, with the skin healing in 10 days and new hair growth in 15 days (49).

Hoekstra and Phillips (9) studied the effects of various petroleum fractions on the skin of male albino guinea pigs weighing 300-500 g. Applications were made every other day for a total of 4 exposures, and the animals were observed for 20 days following the first application. The oils were characterized as aromatic-rich, paraffin-rich and naphthenel-rich fractions. All fractions produced hyperplasia, hyperkeratosis and hair loss, but the aromatic and isoparaffinic fractions were more potent than the paraffinic and naphthenic fractions. In addition, the higher-boiling fractions were less noxious than lower-boiling ones. The transition from non-damaging to noxious was abrupt, occurring in the range of 350 ± 15°C. Aliphatic fractions boiling above 350°C (21-23 carbon atoms) were protective when mixed with the noxious fractions, i.e., the mixture caused less skin reaction than the low-boiling fraction alone. Fractions
with 14-19 carbon atoms caused the most severe reactions. The aromatic hydrocarbons were skin damaging even at the highest boiling point studied (402°C), unlike the aliphatics.

5. Topical application - mice

C9-C12 alkyl aromatic hydrocarbons boiling in the diesel fuel range were applied to the skin of 20 male C3H mice, in doses of 0.10-0.15 g, 3 times a week for 150 applications. Skin became thick, dry and scaly, and there was evidence of hyperkeratosis in 31%, epidermal atrophy in 24%, inflammatory reaction in 41%, perikeratosis in 23% and ulceration in 25% of the group of 79 mice painted with the C9-C10 fraction. The C11-C12 fraction, applied to 85 mice, also resulted in thick, dry and scaly skin, and hyperkeratosis in 30%, perikeratosis in 16%, epidermal atrophy in 29%, inflammation in 23% and ulceration in 6% (28).

6. Skin carcinogenicity - mice

In a report by Twort and Twort (51) in which various catalytically cracked oils were topically applied to mice in a search for carcinogens, they also observed that "lighter grade diesel fuel oils are non-carcinogenic for the skins of mice, but a heavier grade tested was definitely carcinogenic". When lighter grade oils having very low viscosity and the appearance of colored kerosene were applied daily to the interscapular region of 100 mice, only dermatitis was observed. The heavier grade diesel fuel oil, an "older-type dirty-looking oily mixture with a kinematic viscosity of 13.7", induced 18 tumors in 100 mice after daily application; three of the 18 tumors became malignant. With two applications per week in another 100 mice, only one tumor was observed.

According to Fisher et al. (52) and Gerarde (20), only very high-boiling petroleum products display appreciable carcinogenicity in mice. The carcinogenic constituents occur in distillates boiling over 370°C, and are associated only with the aromatic fraction, and especially with the polynuclear aromatics. Heating oils and related products which are lower-boiling are not carcinogenic, even when they are partly aromatic. Many high-boiling products are also inactive, unless the creation of highly condensed aromatic hydrocarbons occurs in their synthesis or processing.

Exhaust manifold temperatures can reach 540°-607°C during diesel fuel fog generation; polynuclear aromatic compounds may be formed. However, no qualitative or quantitative investigations have been reported to date.

7. Topical application - rabbits

The aromatic hydrocarbons generally produced vasodilation, redness and irritation of skin. The shorter and fewer the alkyl groups, or the more branched side chains there were, the more irritating the hydrocarbons became. In rabbits exposed to repeated application of mono-methylated
naphthalene solvent to intact skin, severe irritation and sloughing occurred with 1.0 ml/kg for 21 days. At doses of 2-4 ml/kg, deaths occurred after 6 to 8 applications (20).

8. Protective effect of higher-boiling fractions

The mechanism by which diesel oil and related hydrocarbons damage the skin is discussed by Hoekstra and Phillips (8). They feel that changes such as hyperplasia, hyperkeratosis and depilation are a general response to lipid-soluble solvents, and are unrelated to the specific reactive groups or type of structure. The length of the carbon chains is important. The skin barrier is selectively permeable to molecules of a given size; penetration of the hydrocarbon is necessary for the initiation of the tissue damage. They also stated that the dermatotoxicity of a given petroleum product is unrelated to the crude source, viscosity or degree of refinement.

The protective effect of higher-boiling fractions was demonstrated in guinea pigs (49). Diesel oil (which by itself caused desquamation, hair loss, ulceration and crusting) was mixed with mineral oil in concentrations of 1-10%. The skin effects were eliminated.

9. Dermal implants - rats

The effect of diesel oil on formation of granulation tissue in Wistar rats, weighing 100-170 g was investigated by Bien and Buntrock (49). The diesel oil fraction distilled in the boiling range of 180°-370°C. Plastic rings were implanted in the dorsal skin of the rats, the liquid was added and left in contact for 10 days, and then the granulation tissue formed was removed, examined histologically, and analyzed for hydroxyproline content and ratio of moist to dry weight. The diesel fuel promoted formation of granulation tissue in a dose dependent manner, giving higher dry tissue weight than control substances, but there was no significant difference in amount of hydroxyproline between diesel fuel-treated and control tissues.
VII. PHARMACOKINETICS IN HUMANS AND ANIMALS

Although the absorption, distribution, storage, biotransformation, and excretion of the diesel fuel hydrocarbon mixture have not been reported in the literature to date, it is likely that the pharmacokinetics of the different hydrocarbon types, e.g. aromatics, paraffins, and olefins would proceed through different mechanisms. Further studies are needed to evaluate this possibility. Reported data include the skin absorption rate of diesel fuel, absorption, metabolism and distribution of normal paraffins from the digestive tract, distribution and metabolism of selected alkyl aromatic compounds, and urinary biotransformation products of aromatic hydrocarbons.

A. Absorption

Although literature is scanty on the pharmacokinetics of diesel fuel in humans, it is likely that the body handles the hydrocarbon mixture according to its components. The general lipid solubility of the fuel allows it to be absorbed through the respiratory epithelium, mucous membranes, gastrointestinal tract and epidermis, although actual rates of absorption have not been investigated, with the exception of one study, performed by Starek et al. (43). The authors measured the absorption of diesel oil through the intact tail skin of Wistar rats; it was 2.57 ± 0.52 mg/cm²/hr. The significance of this is that no rats died; it was concluded that the rate of absorption was too slow to allow accumulation of the substance in the body.

The normal aliphatic portion of diesel fuel can be represented by octadecane and hexadecane for purposes of studying absorption. It was originally believed that these paraffins were completely unabsorbed and metabolically inert. El Mahdi and Channon (53) found that rats fed n-hexadecane daily for 3 weeks (doses from 0.035 - 0.12 ml) were able to absorb about 7% of the total dose. Because there was no increase in unsaponifiable material in the livers of these rats, they concluded that the substance was either metabolized or stored in an organ other than the liver. Hexadecane was isolated from tissue lipids after prolonged feeding of the substance to rats (54). Stetten, Jr. (54) found that growing rats absorbed about 80 mg/day of deuterated hexadecane, of which 15% of the isotope was recovered in fatty acids. This was evidence that oxidation took place. The isotope content of the liver was higher than that of the feces, indicating the site of oxidation was not exclusively the gastrointestinal tract. There was no accumulation of unoxidized hydrocarbon in the liver.

The oxidation of normal aliphatics to fatty acids, occurring in the intestinal wall of rats fed deuterated octadecane, hexadecane, tetradecane, dodecane and decane, was studied by Bernhard et al. (55). The reaction proceeds in 2 steps: an intestinal dehydrase removes hydrogen from the 1,2-position; oxidation follows.

The products of the reaction were recovered in lymph. McCarthy (56) found that oxidation occurred in perfused goat rumen prior to absorption into the blood. Hexadecane and octadecane underwent ω-oxidation to fatty
acids of the same carbon number. A portion of these hydrocarbons was also absorbed unaltered from the gut and found in liver lipids after 6 hours, as determined from experimental feeding of radioactive octadecane and hexadecane to rats.

Oxidation of radioactive hexadecane occurs in subcellular fractions of guinea pig intestinal mucosa (57). The highest activity was found in the microsomal fraction, although the high-speed supernatant was also active. Microbial enzymes from the flora of the small intestine did not contribute to oxidation. Maximal conversion of hexadecane to hexadecanol and palmitic acid required $\text{NAD}^+$, $\text{NADP}^+$ and glucose-6-phosphate, and the reaction was inhibited by carbon monoxide, indicating involvement of cytochrome $P_{450}$ in oxidation.

The major site of absorption of normal alkanes was found to be the small intestine in rats. There were no differences in uptake among duodenum, jejunum and ileum. Duodenal sections of intestine released radioactive hexadecane and its metabolic products more rapidly than sections of ileum. The primary route of absorption was into the lymph, as determined by cannulation of the intestinal lymph duct. Paraffins with more than 29 carbon atoms were not significantly absorbed, and there were no significant differences between retention of branched, cyclic and unsaturated hydrocarbons and saturated aliphatics with the same carbon number. (Retention was calculated as 100% minus the percentage excreted in feces). In rats injected intraduodenally with radioactive hexadecane in an emulsion of synthetic rat bile, the lymph and blood levels of $^{14}$C increased almost linearly over 7 hours. Portal blood was slightly more radioactive than heart blood, suggesting the possibility of direct absorption of the hydrocarbon or metabolites into the portal circulation. The authors postulated that absorption of aliphatic hydrocarbons involves emulsification in the gastrointestinal tract, some absorption of intact molecules into the lymph, and some oxidation to fatty acids of the same carbon number at the time of absorption (58).

The absorption of radioactive hexadecane after topical application to the skin of male albino guinea pigs for 48 hours was about 20% of the total applied dose. Mineral oil, docosane and heptane reduced the penetration of the labelled alkane, while repeated applications of hexadecane increased its uptake (59). It is proposed that heavy mineral oil and alkanes over 20 carbons long reduce the dermatotoxic effect of hexadecane by interfering with its penetration to the site of action, which appears to be deep in the epidermis. The use of protective pastes on the skin to reduce dermatitis after exposure to industrial solvents and fuels is based on this observation. The mechanism of the protective effect may be either one of simple dilution or a more specific interference of penetration.

$^1\text{NAD}^+$ - nicotinamide-adenine dinucleotide
$^2\text{NADP}^+$ - nicotinamide-adenine dinucleotide phosphate
Aromatic hydrocarbons are absorbed slowly through the skin. Gerarde (20) feels that systemic intoxication with these compounds following topical exposure is improbable for this reason. Absorption of alkylbenzenes, for example, into the bloodstream may cause local irritation of the endothelium and permeability changes in capillaries, leading to edema, petechiae or gross hemorrhage. Alkylbenzenes are absorbed in decreasing levels from the gastrointestinal tract with increased chain length and branching.

In one report of a 2-year-old boy who drank heating oil (composition unspecified) and was given mineral oil therapeutically, it was postulated that the mechanism of absorption involved formation of an emulsion of the oils in the small intestine. Microdroplets of the hydrocarbons then were able to pass into the lymph, and into the bloodstream. An alternate theory is that the oils may have been resorbed by direct passage through the intestinal wall without degradation, in a manner similar to persorption of starch granules. The mechanism remains unknown (60).

B. Distribution

Once in the bloodstream, aliphatic hydrocarbons in the form of free fatty acids are cleared by the liver. McCarthy (56) found that in goats, radioactive octadecane was rapidly cleared from the bloodstream after i.v. administration and there was increased radioactivity in liver phospholipid and fatty acid fractions, indicating that the liver took up the octadecane before conversion to fatty acids.

Bernhard (61) fed rats deuterated octadecane and found the isotope in oleic, palmitic and stearic acids. In another experiment, rats fed labelled octadecane and hexadecane developed deposits of these paraffins in the liver and fatty tissues.

The distribution of octadecane was studied by Popovic (62). After i.v. administration of a radioactive emulsion of n-octadecane to rats, the radioactivity was concentrated in the liver (33%), fat (18%) and spleen (8.3%), while after p.o. administration, the most radioactivity was in the liver (1.28%), fat (0.66%) and intestine (2.41%). This indicates that only a small portion of octadecane emulsion was absorbed from the gastrointestinal tract. Expired $^{15}$CO$_2$, determined every 30 minutes after administration, was also much lower when the emulsion was given orally than intravenously. The octadecane was incorporated into fatty acids in the liver, especially lecithin.

Goat and rat liver homogenates metabolized hexadecane more readily than carbon chains either longer or shorter than C$_{16}$, according to McCarthy (56). The microsomal fraction of mouse liver hydroxylates long-chain aliphatic hydrocarbons. Radioactive hexadecane was oxidized in the presence of NADPH$^1$ and molecular oxygen to palmitate and a smaller quantity of hexadecanal. Mouse lung microsomes showed weak hydroxylation activity, and the kidney microsomal fraction was inactive (63). P$_{450}$ cytochrome involvement was shown by inhibition of the reaction with carbon monoxit.

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$^1$NADPH = reduced form of NADP
After topical application of radioactive hexadecane in guinea pigs, the dermis contained about 0.1% of the applied dose, and the liver and kidneys a total of about 0.1%. Measurable amounts of $^{14}C$ were not found in the blood (59).

Alkylbenzenes and related aromatic hydrocarbons are found in the bloodstream adsorbed on lipoproteins or dissolved in chylomicrons, after gastrointestinal absorption, but the majority is bound to red blood cells, due to high lipid solubility of the hydrocarbons. They accumulate in body tissues in proportion to their fat content (20). They may be dissolved in neutral body fat, may enter the nervous system and cerebrospinal fluid and may cross the placenta into fetal blood, as do other lipid soluble substances.

C. Excretion

Excretion of diesel fuel in humans has not been studied, but it can be compared to excretion of heating oil, which is briefly discussed in the report of the 2-year-old boy who drank the substance. The oil could be detected spectroscopically in the urine of the child (60).

In albino rabbits given, orally, various organic compounds at half the single lethal dose, urinary inorganic sulfates and glucuronic acid were determined by quantitative spectrophotometry with a modified Tollens test (64). Kerosene and naphthalene caused a marked increase in glucuronic acid excretion and a slight or moderate increase in urinary inorganic sulfates, usually on the second day after dosing. This may be a valuable method for assessing the extent of absorption of organic compounds in industrially exposed persons.

In comparison with kerosene, diesel fuel is excreted slowly by rats fed large doses, and accumulation of the substance occurred. The toxicity is discussed in section VI.B. (43).

Excretion of aromatic hydrocarbons, such as alkylbenzenes, occurs either as unchanged hydrocarbons, or as water soluble urinary biotransformation products, conjugated with glucuronic acid, sulfuric acid or glycine. Unmetabolized aromatics may also be exhaled from the lungs (20). Figure 1 shows the curve of aromatic hydrocarbons in blood following a 1.25 ml intragastric dose of kerosene. After an initial rise, the blood concentration fell over the next 12 hours, and by 32 hours, less than 5 ppm could be detected.

Urinary biotransformation products of aromatic hydrocarbons have been studied and the metabolic pathways described. Hepatic monoxygenases convert naphthalene to naphthalene-1,2-oxide, which then either spontaneously isomerizes to 1-naphthol, is enzymatically hydrated to a trans-dihydrodiol, or conjugates both spontaneously and by enzymatic catalysis with glutathione. The conversion of polycyclic hydrocarbons to oxides of phenanthrene and benzanthracene also occurs. Glutathione conjugates are metabolized to premercapturic acids, by loss of glycine and glutamic acid residues, followed by N-acetylation of the substituted cysteine. The premercapturic acids are dehydrated to mercapturic acids (65).
Fig. 1. Absorption-elimination curve for aromatic hydrocarbons in kerosene in rats dosed by gastric intubation (1.25 ml per animal).

Ref: Gerarde (20).
VIII. CARCINOGENICITY, MUTAGENICITY, TERATOGENICITY

There is no direct information in the literature concerning the teratogenic or mutagenic effects of diesel fuel. It is obvious that some investigations are needed in these two areas. Carcinogenicity of diesel fuel after chronic topical application is touched upon in one reference. The metabolism of aromatic compounds via intermediate arene oxides is reviewed.

A. Skin Carcinogenicity

A discussion of available literature is presented in section VI.C. (p.30). Until further study is done, it may be tentatively concluded that diesel fuels meeting federal specifications are not carcinogenic after topical application.

However, certain paraffins, olefins and alkyl derivatives of cyclohexane, benzene and naphthalene are capable of accelerating the induction of skin cancer in C3H mice. There are probably no liquid hydrocarbons which retard the rate of induction of tumors by repeated application of solutions of methylcholanthrene or benzo(a)pyrene to C3H mouse skin. Dodecylbenzene and dodecane induced tumors at a much higher rate for a given concentration of carcinogen than other hydrocarbon solvent solutions such as sec-amylobenzene and octane, which are of lower molecular weight. By themselves, these two accelerators are not carcinogenic for mouse skin. The accelerating hydrocarbons precondition the skin of C3H mice, rendering it much more responsive to subsequent applications of oils containing carcinogens (76).

B. Intermediary Metabolism of Aromatic Hydrocarbons

Aromatic compounds are metabolized in mammals to phenols, trans-di-hydrodiols, glutathione conjugates and (pre)mercuric acids via intermediate arene oxides; aromatic compounds in which a formal double bond has undergone epoxidation. The rate of formation and metabolism of arene oxides as well as spontaneous isomerization to phenols or addition of glutathione or sulfhydryl groups, determine steady state concentrations in the liver. Covalent bonding of these intermediates to intracellular macromolecules provides the molecular basis for aromatic hydrocarbon cytotoxicity and carcinogenicity of polycyclic hydrocarbons (65, 66).

In vitro experiments indicate that various arene oxides and phenols of polycyclic hydrocarbons bind to nucleic acids. The results were obtained by monitoring changes in UV absorption and fluorescence spectra of poly-guanosine acid after reaction with various arene oxides and phenols (67). Arene oxides of benzpyrene were mutagenic in strains of Salmonella typhimurium and in Chinese hamster V79 cells. There was no relationship between cytotoxicity and mutagenicity. There were large differences in response of hamster and bacterial cells to arene oxides in mutagenicity and cytotoxicity. The value of mutation experiments in determining carcinogenic activity of the compounds was found to be uncertain (68).
IX. INDUSTRIAL HYGIENE PRACTICES AND STANDARDS

The untoward effects of exposure to diesel fuel white smoke are unknown. Furthermore, the composition of the smoke has not been sufficiently studied. In order to limit exposure as much as possible, therefore, certain precautions are suggested. As new experimental data becomes available, appropriate revisions should be considered.

A. Standards

No standards for diesel fuel fumes, smokes, fogs or liquid have been established.

B. Protective Equipment

In fogging operations, inhalation of aerosol particles consisting of diesel fuel, exhaust products, pyrolyzed substances and various additives, can be controlled by protective field masks, such as MIL-M-50079E(MU) (69), containing activated charcoal filters.

Control of skin contact with diesel fuel through the use of protective skin pastes which may prevent absorption of the fuel is recommended by some authors. One paste, described by Rao (33), contains a silicone oil ointment. It is effective in controlling dermatitis, and is soothing and without untoward systemic effects. Il'in et al. (29) recommended the application of protective skin pastes prior to exposure, and the use of cleaning pastes after exposure. Acceptable cleansers included: soap and lanolin; industrial or liquid soap; and lukewarm 0.25-0.5% ammonia solution. The possible skin protection offered by mineral oil, when mixed with diesel oil, was discussed in section VII.A.

Skin softeners may control the severity of dermatitis by helping to prevent abscess formation (29). These authors favored a solution of equal parts of ethyl alcohol, glycerine, ammonia and water, and 0.5% chloramine solution.

Other mechanical barriers, such as gloves and aprons, may be useful if they do not interfere with performance. Some maintain that the use of gloves contributes to sloppy personal hygiene, and that only scrupulous cleanliness and prophylactic measures can effectively control diesel fuel exposure (32, 33). Others highly recommend protective clothing where practical (28, 40).

C. Medical Surveillance

Constitutional factors play an important role in individual susceptibility to irritation of the skin or mucous membranes. Dry, senile skin is sensitive to agents which may cause dermatitis. The skin of Caucasians is more sensitive to chemical irritants than the skin of Blacks (30). Some individuals may be occupationally exposed for years before developing dermatitis to diesel fuel.
Screening of all individuals prior to assignment to tasks in which they will be exposed to diesel fuel or smoke is necessary in order to exclude persons with the following conditions: organic disease of the heart, lungs, kidneys or liver; history of allergy to hydrocarbons; chronic skin disease; hematologic abnormalities; and possibly a history of benzene intoxication. Gerarde (20) states that exposure to aromatic hydrocarbons or mixtures containing them must be avoided by these individuals.

Periodical medical examinations are essential for the early detection of dermatitis as well as possible inhalation effects of diesel fuel smokes. Documentation of exposure effects is necessary, in view of the limited information which now exists in this area.

D. Control Measures

In conditions where it is impossible to prevent exposure, personnel must be educated in the use of protective skin creams and clothing, the use of proper cleansers after exposure and the importance of cleanliness of work clothes. Early recognition of symptoms of respiratory, skin and other untoward reactions is essential, and requires periodical medical surveillance. Enforcement of safety and cleanliness regulations is equally essential.

Intravehicular exposures can be minimized by the use of activated charcoal filtering masks, or by filters covering ventilation intake ports. Precautions to exclude unfiltered air and smoke from the smoke-generating vehicle cabin, such as keeping windows closed, are necessary.
X. SAMPLING AND ANALYTIC TECHNIQUES

No reports were found dealing specifically with sampling in air, or analysis in biologic fluid or tissue, for diesel fuel. Therefore, methods for kerosene or oil mists which appeared to be applicable also to diesel fuel have been described.

A. Atmospheric Sampling

A Mine Safety Appliances 60-25 Electrostatic Sampler was used, operating at 18 l/min for 30 min; before and after the sampling period the precipitator tube was weighed to determine the weight of material collected.

A special grade of paper (S & S 2045 BM) was used to absorb the hydrocarbon droplets in the air drawn through it.

An acidic potassium dichromate solution was used to bathe the sample, but it was not clear if vapors only, or also aerosols, would be absorbed. Vapors would interfere with aerosol determination.

Fiberglass filter paper was used to adsorb the droplets from the sample; portable apparatus, for personal use, was as suitable as larger equipment intended for room averaging. At an expected value of 5 mg/m³, the air is drawn at 2 l/min for 10 min through a 25 or 37 mm filter for personal sampling - 190 min for 0.5 mg/m³.

B. Analysis

1. Air (after sampling)

A microscope-photometer arrangement was used to determine increase in translucence of chromatographic grade paper through which the sample had been drawn.

Ol'khovskaya measured the change in color of a 0.01% solution of potassium dichromate in concentrated sulfuric acid (through which the sample had been drawn); sensitivity was 20 µg/3 ml reagent for kerosene when a 1.5-l air sample was used; wavelength monitored was not given.

The Institute of Petroleum Occupational Hygiene Subcommittee reviewed accepted methods only commented on one sampling technique, but discussed three ways of determining amount collected. A gravimetric method involved solvent extraction of the filter, evaporation and weighing; its sensitivity was 0.1 mg. A drawback was possible loss of volatiles during the solvent removal step. Extraction of the filter and measurement of the UV absorbance at 200-300 nm was useful if a known was available to prepare a calibration curve; the sensitivity was 0.1 mg when the curve had unit slope. Alternatively, the IR absorbance of the
extract in the 3.4 μm region could be checked against a standard; sensitivity was 0.05 mg.

2. Biologic fluids

A technique for blood kerosene, which should be applicable to diesel fuel, was found but it is only suitable when an original sample is available to calibrate the method. Five ml of blood is hemolyzed in dilute hydrochloric acid, then extracted with carbon tetrachloride. The extract is mixed with a formaldehyde-sulfuric acid reagent - which reacts with aromatics - and the color produced measured at 490 nm in a colorimeter. The method was good to a lower limit of 10 ppm when 14% aromatics were present (20).

3. Biologic tissue

No methods applicable to diesel fuel were located.

C. Characterization of Unknown Sample as Diesel Fuel

A problem exists with complex mixtures of hydrocarbons as to how to identify one as a particular product of the petroleum industry. In this case, boiling range overlap occurs with kerosene, diesel fuel, fuel oils, and a product known as mineral seal oil.

A series of gas-liquid chromatograms (GLC) intended to represent standards for various gasolines, jet fuels, kerosenes, and diesel fuels was presented. It is readily seen that diesel fuels, except for arctic grade, are distinctively different from kerosene (74). Another GLC for diesel fuel was depicted (75) with the additional datum that there are diagnostic peaks adjoining the major peaks for hepta- and octadecane. The latest publication, also presenting GLC comparisons of jet fuels and diesel fuels, commented on the usefulness of these "pictures" for estimating possible deviation from certain specifications (4).
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