III. POTENTIAL HAZARDS TO HEALTH AND SAFETY IN COAL LIQUEFACTION PLANTS

Characterization of workplace hazards associated with coal liquefaction plants in the United States must rely on pilot plant data because currently there are no commercial plants. These pilot plants are experimental units that process up to 600 tons (545 Mg) of coal per day. Because pilot plant operations are experimental, operating parameters and equipment configurations are frequently changed; consequently, exposures may be more severe than might occur in a commercial production facility. On the other hand, because pilot plants have operated for a relatively short time (less than 10 years), exposure effects over a working lifetime cannot be documented.

Available data are sufficient to qualitatively define the hazards that may occur in future commercial coal liquefaction plants, but not to quantify the degree of risk associated with long-term, low-level exposures. Industrial hygiene studies conducted at several pilot plants provide some information about worker exposure [36-39]. In addition, the toxicity of some of the coal-derived materials produced in these plants has been assayed in animals, bacteria, and cell cultures [5-7,9,14,15,40-52]. Only one epidemiologic study [53] of coal liquefaction workers has been conducted in the United States, and the cohort of 50 workers examined was small.

The opportunity for epidemiologic studies has been restricted. In the United States, the longest exposure period for a worker for whom health effects have been reported is approximately 10 years [54]. One foreign plant has operated for more than 23 years [55], but epidemiologic studies of the work force have not been published.

Laboratory analysis of the toxic hazards inherent in coal liquefaction processes is complicated by at least four major factors. First, process streams contain a mixture of many different substances, and isolation of any one potential toxicant can be difficult. Second, the various toxicants can produce diverse effects, ranging from skin irritation to cancer. Third, depending on the physical state of an individual toxicant, different biologic systems can be affected. For example, as an aerosol, a substance may more readily produce respiratory or systemic effects; as a liquid or solid, dermal effects may be more likely. Finally, dose levels are difficult to establish because the composition of process streams can vary, partitioning of process stream components after aerosolization may alter the distribution of components, and weathering of fugitive liquid emissions may alter the toxicity of process materials.

Although occupational safety and health research specifically related to coal liquefaction is limited, studies have been conducted in other industries where exposure to some of the same materials may occur. For example, polycyclic aromatic hydrocarbons (PAH's), which are present in coal tar products,

coke oven emissions, asphalt, and carbon black, are also present in coal liquefaction products [10,17,18,38,56,57]. Because some of these materials have reportedly caused severe long-term effects such as skin and lung cancer in workers in various industries [17,18], increased risk of cancer in coal liquefaction workers is possible. Other potential adverse health effects associated with constituent chemicals in coal liquefaction products include fatal poisoning from inhalation exposure [58,59], severe respiratory irritation [60], and chemical burns [61]. Fire and explosion are also significant hazards, because most systems in coal liquefaction operate at high temperatures and pressures and contain flammable materials.

Extent of Exposure

Coal liquefaction pilot plants currently operating in the United States (see Appendix II) employ approximately 100-330 workers and have production capacities of up to 600 tons (545 Mg) of coal per day [1].

In June 1980, the President called for a synthetic fuel production capacity equivalent of at least 2.0 million barrels of crude oil per day by 1992 [62,63]. Production of this amount of synthetic fuel by coal liquefaction processes would require approximately 12 plants, each of which would yield 50,000 barrels of fuel a day. Assuming that a commercial plant would employ at least 3 times as many workers as a large pilot plant, the projected 1995 work force would be approximately 12,000 workers [62,63].

Workers in pilot plants may be exposed to process liquids, solids, gases, aerosols, vapors, dusts, noise, and heat. Some of these potential hazards are summarized in Table III-1. Although coal liquefaction equipment is designed to operate as a closed system, it must still be opened for maintenance and repair operations, thereby exposing workers to potential hazards.

Processing of abrasive slurries, particularly at high operating temperatures and pressures, accelerates the erosion/corrosion effects on equipment such as piping, pressure vessels, seals, and valves in coal liquefaction plants. These effects increase the potential for worker exposure to process materials because leaks and fugitive emissions are more likely to occur [1]. Other sources of worker exposure to process materials include normal handling or inadvertent release of raw materials, products, and waste materials.

Hazards of Coal Liquefaction

According to a 1978 report [64], of an estimated 10,000 chemical compounds that may occur in coal, coal tar, and coal hydrogenation process and product streams, approximately 1,000 have been identified. For some of these chemicals, information is available on their potential hazard to workers. Appendix V summarizes the NIOSH-recommended limits and the current Federal

TABLE III-1

POTENTIAL OCCUPATIONAL HAZARDS IN COAL LIQUEFACTION PLANTS

System, Unit Operation, or Unit Process	Potential Hazards
Coal handling and preparation system	Coal dust, noise, fire, explosion, asphyxia (nitrogen and carbon monoxide gases), burns
Liquefaction system	Phenols, ammonia, tars, thiocyanates, PAH's, carbon monoxide, hydrogen sulfide, hydrocarbons, fires, explosions, burns, high pressures, noise, ash, slag, mineral residue, spent catalyst
Separation system	Oils, phenols, hydrogen cyanide, ammonia, hydrogen sulfide, burns, fires
Upgrading and gas purification	Light hydrocarbons, phenols, ammonia, hydrogen sulfide, carbon dioxide, carbon monoxide, burns, fire, explosion, high pressures
Shift conversion*	Tar, naphtha, hydrogen cyanide, fire, catalyst dust, burns, hot gases (carbon monoxide, hydrogen)
Methanation*	Carbon monoxide, methane, nickel carbonyl, spent catalyst dust, fire, burns
Waste treatment facilities	Hydrogen cyanide, phenols, ammonia, particulates, hydrocarbon vapors, sludges, spent catalyst, sulfur, thiocyanates

*Indirect liquefaction

Occupational Safety and Health Administration (OSHA) standards for various chemicals that have been identified in the process streams of coal liquefaction pilot plants.

Although exposure limits have been established for individual chemicals, in most cases the substances present in coal liquefaction plants will be complex mixtures of these and other compounds. Many of the chemicals listed in

Appendix V may be minor constituents in such mixtures. Other chemicals may be present that have no assessments of health effects. Some chemical constituents of coal liquids are presented in Appendix VI, grouped according to chemical structure.

Compounds that could present an acute hazard have been identified in pilot plant process and product streams [38]. These compounds include carbon monoxide and hydrogen cyanide, which are chemical asphyxiants, as well as hydrogen sulfide, which causes respiratory paralysis [58,65,66]. Workplace concentrations below NIOSH-recommended limits or OSHA standards for these compounds have been measured during normal pilot plant operations [38]. Plant malfunctions or catastrophic accidents could release lethal concentrations of these gases.

Liquefaction products generally include light and heavy oils, gases, tars, and char. Materials that may be used in the process in addition to coal vary according to the type of equipment used. These materials include hydrotreating catalysts, Claus catalysts, chemicals for wastewater treatment, heat exchange oils, such as phenylether-biphenyl mixtures, alkali carbonates from carbon dioxide removal, and filter-aid materials. Tetralin, anthracene oil, or other chemical mixtures may be used as recycle and/or startup solvents.

Numerous compounds are formed during various stages of liquefaction, upgrading, distillation, and waste treatment. Liquid streams consist of coal slurries and oils, which may be distilled into fractions having different boiling ranges. The liquids with higher boiling points are recycled in some processes. Solids are present in liquid and gas streams, filter residues, sludge from vacuum distillation units, spent catalysts, mineral residue from carbonizers, and sludge from wastewater treatment. Gas streams include hydrogen, nitrogen or inert gas, fuel gas, product gas, and stack gases. Occupational exposure to these materials is possible during maintenance and repair operations, or as the result of leaks, spills, or fugitive emissions.

Some of the compounds that have been identified in coal liquefaction process materials, eg, PAH's and aromatic amines, are known or suspect carcinogens. Kubota et al [10] analyzed PAH's in coal liquefaction-derived products and intermediates, including benzo(a)pyrene (40 µg/g of liquid) and benz(a)anthracene (20 µg/g of liquid). Industrial hygiene surveys at three direct liquefaction pilot plants [3,37,38] confirmed the presence of these and other PAH's in the workplace environment. Ketcham and Norton [37] measured benzo(a)pyrene levels at various locations in the coal liquefaction pilot plant at Institute, West Virginia, for durations varying from approximately 10 minutes to 2 days. Benzo(a)pyrene concentrations ranged from <0.01 to approximately 19 $\mu g/m^3$. Measurements of benzo(a)pyrene taken by personnel at the Fort Lewis, Washington, SRC pilot plant [38] ranged from 0.04 to 1.2 $\mu g/m^3$, and total PAH's ranged from <0.04 to 26 $\mu g/m^3$. Concentration ranges reported for both of these plants are based on high volume area sampling rather than personal breathing zone sampling.

A more recent industrial hygiene survey [13] reported potential worker inhalation exposure levels for PAH's, aromatic amines, and other compounds as 8-hour time-weighted averages (TWA's) in two coal liquefaction pilot plants: the SRC plant in Fort Lewis, Washington, and the CSF plant at Cresap, West Virginia. Workers at the Fort Lewis pilot plant were exposed to PAH concentrations (reported as the sum of 29 PAH's) ranging from 1 to 260 $\mu g/m^3$, with an average of 68 $\mu g/m^3$; exposures to PAH's in the CSF plant ranged from 0.02 to 0.5 $\mu g/m^3$, with an average of 0.2 $\mu g/m^3$. The higher exposure levels at the Fort Lewis pilot plant may be a result of it having processed more coal over a longer period of time than the CSF plant. This suggests that a greater deposition of process stream material may have occurred in the workplace through leaks, spills, and maintenance activities. Volatilization of these materials may have contributed to increased worker exposure.

Seven aromatic amines including aniline, o-toluidine, and o- and p-anisidine were also measured in the survey [13]. Exposure to these aromatic amines was of the same order of magnitude at both pilot plants. Concentrations measured were less than 0.1 ppm. The degree of risk of such exposures cannot be determined because toxicologic data for evaluation of effects at low exposure levels are unavailable.

Fluorescence is a property of benzo(a)pyrene and numerous other aromatic chemicals. Fluorescence has been used to observe droplets of material on the skin of workers under ultraviolet (UV) light [1,37]. This indicates that skin contact with airborne coal liquefaction materials or with contaminated equipment surfaces is also a potential route of exposure. There is, however, concern about the risk of skin sensitization and promotion of carcinogenic effects from excessive use of UV light. UV examination for skin contamination should only be conducted under medical supervision for demonstration purposes, preferably by a hand-held lamp.

Koralek and Patel [31] reviewed process designs at 14 plants and predicted the most likely sources of potential process emissions. According to their report, coal dust may escape from vents and exhausts used for coal sizing, drying, pulverizing, and slurrying operations. Hydrocarbon emissions from evaporation and gas liberation may occur in the liquefaction, physical separation, hydrotreatment, acid gas removal, product storage, wastewater treatment, and solid-waste treatment operations. Other potential air emissions included carbon monoxide, nitrogen oxides, hydrogen sulfide, sulfur dioxide, ammonia, and ash particulates. Potential solid waste materials included reaction wastes (particulate coal, ash, slag, and mineral residue), spent catalysts, spent acid-gas removal absorbents, water treatment sludges, spent water-treatment regenerants, tank bottoms for product storage tanks, and sulfur from the Claus unit. Wastewater could contain phenols, tars, ammonia, thiocyanates, sulfides, chlorides, and oils. Wastewater sources identified were quench water, process condensate, cooling water, gas scrubbers, and water from washdown of spills [31]. The potential for worker exposure to toxic materials is also significant for activities such as equipment repairs requiring vessel entry or line breaking, removal of waste materials, collection of process samples, and analysis of samples in a quality control laboratory.

Because some equipment and operations are similar, the nature and circumstances of injuries experienced in petroleum refining may approximate safety hazards that may exist in coal liquefaction plants. In 1977, the occupational injury and illness incidence rate, as reported to OSHA, for petroleum refining industries was 6.71 for the total number of cases and 1.38 for fatalities and lost workday cases [67]. For the entire petroleum industry, which includes areas such as exploration, drilling, refining, marketing, research and development, and engineering services, these figures were 4.52 and 1.56, respectively. The 1976 figures for all private sector industry were 9.2 and 3.5, respectively [68]. Incidence rates were calculated as:

$$\text{Incidence Rate} = \frac{\text{Number of Injuries and/or Illnesses} \times 200{,}000}{\text{Total Hours Worked During the Year}}$$

These results indicate that the total injury and illness incidence rate is greater for petroleum refining than for the entire petroleum industry. The fatality incidence rate in petroleum refining, however, is less than that of the petroleum industry as a whole. From May 1974 to April 1978, 58 deaths in the petroleum refining industry were reported to OSHA [69]. Contributing environmental factors in approximately half of these fatalities were gas, vapor, mist, fume, smoke, dust, or flammable liquid exposure [69]. About 33% of these deaths resulted from thermal burns or scalding injuries, and 16% from chemical burns. The primary source of injury was contact with or exposure to petroleum products, which accounted for approximately 28% of the total number of deaths. Fire and smoke accounted for approximately 17% of total deaths [69].

Carcinogenic, Mutagenic, and Other Effects

(a) Epidemiologic Evidence in Coal Liquefaction Plants

Epidemiologic data on coal liquefaction employees are scarce, primarily because of the early stage of development of this technology. Data that are available come from medical surveillance programs conducted for employees of pilot plants. These programs were instituted because of toxic effects known for some chemicals in the coal liquefaction processes.

Between 1952 and 1959 a coal liquefaction hydrogenation pilot plant operated at Institute, West Virginia. Many changes and repairs of equipment were necessary in the early phases of the operation. According to a report by Sexton [4] about the medical surveillance program, "These early and intermittent start-ups resulted in excessive exposure to some employees, the extent of which is not known and much of which was not recorded in the medical files." Operating pressures in this plant were much higher (5000-10,000 psi or 350-690 MPa) [70] than expected for other processes and may have increased potential for release of air contaminants and escape of some oil, which would

contaminate equipment surfaces. Extensive protective measures were not implemented until 1955.

During the plant's 7 years of operation, the 359 male employees regularly assigned to the coal liquefaction operation were given annual physical examinations and, after 1955, quarterly skin examinations. The author [4] reported that this medical surveillance revealed 63 skin abnormalities in 52 men (later corrected by Palmer to be 50 men [53]). Diagnostic criteria were not specifically defined; nevertheless, diagnoses of cutaneous cancer were reported for 10 men and precancerous lesions for 42 men. The expected number of cases was not reported. Duration of exposure to coal tar, pitch, high boiling aromatic polycyclic compounds, and other compounds for the 359 men, including prior exposures, ranged from several months to 23 years. All cancerous and precancerous lesions were in men with less than 10 years of exposure, however. One worker was found to have two skin cancers, one occurring after only 9 months and the other after 11 months of exposure.

The author [4] acknowledged some doubt that 9 months of exposure to the process could have resulted in a carcinoma of the skin; other risk factors and sources of exposure must be analyzed before a causal relationship can be suggested for this case. According to the author, the age-adjusted skin cancer incidence rate for this population of workers was 16 times greater than the incidence rate for US white males as reported by Dorn and Cutler [71] and 22 times greater than the "normal" incidence as reported by Eckardt [72]. Sexton [4] concluded that an increased incidence of skin cancer was found in workers exposed 9 months or more to coal hydrogenation chemicals.

It was stated [4] that these workers were also exposed to UV radiation to demonstrate that their skin was not always clean after normal showering. Although skin cancer has been reported in workers exposed to the UV radiation of sunlight [73], it is questionable that a brief exposure to UV radiation in this pilot plant could have substantially contributed to the excess risk observed. The excess risk may have been overestimated, however; the incidence in a carefully surveyed cohort was compared with that in the general population (where underreporting of skin cancer was believed to be common) [74]. Taking this underreporting into account would reduce the observed to expected incidence ratio but not eliminate the excess risk. Several other features of the medical surveillance study also hindered accurate quantification of excess risk [4]. Prior occupational exposures of these workers were inadequately assessed in this paper and could have contributed somewhat to observed risk. In addition, specific exposure data were not ascertained. Because specific diagnostic criteria were not established, diagnoses conflicted among the consulting physicians in the study. In spite of these flaws, an excess risk to skin cancer is suggested, and better control of the worker exposure to the chemicals identified in the coal liquefaction process is therefore warranted.

A followup mortality study on the 52 employees with skin lesions was undertaken to determine whether or not these men were at an increased risk for

systemic cancer mortality [53]. A review of the records revealed previous double counting; the number of affected employees with cancerous skin lesions was 10 and with precancerous skin lesions was 40. All but 1 of the 50 cases were followed up, and, after an 18- to 20-year latency period, 5 deaths had occurred. The five deaths were reported as cardiac-related, two with pulmonary involvement. No autopsies were performed on them. The expected number of deaths in this population was not reported. The author [53] stated that the results did not indicate an increased risk for systemic cancer mortality for the group. Since most occupationally associated cancers occur 20 or more years after initial exposure [73], the followup period in this study would not be expected to reveal an increased risk to systemic cancer mortality in this small and select cohort (the workers who developed skin lesions). A better risk estimate would have been derived if (1) the disposition of all the 359 men who had worked in the pilot plant was ascertained and (2) if the followup period had been longer than 20 years. The fact that all five deaths were cardiac-related may deserve special attention. It may be an early indication of adverse heart changes similar to the decreased functional capabilities of the cardiovascular and respiratory systems observed among carbon black workers [56].

Findings from the medical surveillance program at the SRC plant in Fort Lewis, Washington, were reported by Moxley and Schmalzer [75]. No discernible changes were revealed by comparing the exposed employees' medical records prior to and following the initiation of coal liquefaction production. The only known occupational health problems encountered at the SRC pilot plant were eye irritation and mild transient dermatitides from skin contact with coal-derived materials; eye irritation was the most common medical problem. Neither the number of exposed workers nor the length of time the medical surveillance program was operative was stated. However, the pilot plant had been operating for only 5 years at the time of the report. In this short time the surveillance program could not possibly have detected chronic effects having long latency periods.

The medical surveillance program for approximately 150 full-time workers at another pilot plant revealed 25-30 cases of contact dermatitis per year and 150-200 cases of thermal burns per year [1].

Preceding reports demonstrate that the available epidemiological data on exposed coal liquefaction workers concentrate on the acute hazards of exposure (dermatitis, eye irritation, and thermal burns). Preliminary evidence suggests the presence of a potential carcinogenic hazard, as illustrated by an apparent excess incidence of skin cancer [4]. However, no conclusive statement on the full potential of cancer or other diseases of long latency from occupational exposure to the coal liquefaction process can be made on the basis of current epidemiologic data. Nevertheless, the known carcinogenic and noncarcinogenic properties of the many chemicals in the liquefaction process mandate every possible precautionary measure be taken to protect workers.

Although this assessment is primarily concerned with the direct coal liquefaction process, the possibility of obtaining epidemiologic data from plants utilizing the indirect process should not be overlooked. The medical facilities of SASOL I commonly see cases of burns (steam, tar, and thermal) and eye irritations [18,21]. No epidemiologic study has been published, however, on the SASOL facility.

(b) Other Related Industries

PAH's in coal liquefaction products have also been identified in coke oven emissions, coal tar products, carbon black, asphalt fumes, and coal gasification tars. NIOSH has previously reviewed the health effects data for a variety of these materials in different industrial environments [16-18,56,76].

In the coal tar products criteria document [17], NIOSH concluded that coal tar, coal tar pitch, and creosote could increase the risk of lung and skin cancer in exposed workers. This conclusion was based on considerable evidence, including the identification of product components that by themselves are carcinogenic (benzo(a)pyrene, benz(a)anthracene, chrysene, and phenanthrene), the results of animal experiments, and the incidence of cancer in the worker populations studied.

In the carbon black criteria document [56], NIOSH concluded that carbon black may cause adverse pulmonary and heart changes. Investigations of the adsorption of PAH's on carbon black, retention of these materials in the lung, and the elution of PAH's from carbon black by human blood plasma were reviewed. The reports suggest a potential risk of cancer from PAH's adsorbed on carbon black, which workers should be protected from. Other health effects associated with carbon black exposure were lung diseases (pneumoconiosis and pulmonary fibrosis), dermatoses, and myocardial dystrophy. Although carbon black workers are exposed primarily to dusts and coal liquefaction workers to process liquids and vapors, similarities in substances such as PAH's could result in the same adverse health effects, including cancer.

In the criteria document on asphalt fumes [76], NIOSH concluded that available evidence did not clearly demonstrate that a direct carcinogenic hazard is associated with asphalt fumes. Three studies were cited that quantified PAH's in asphalts and coal tar pitches. Benzo(a)pyrene and benz(a)anthracene concentrations in eight asphalts ranged from "not detected" to 35 ppm; benzo(a)pyrene and benz(a)anthracene concentrations in two coal tar pitches were in the range of 0.84-1.25% by weight. NIOSH is concerned that future investigations may suggest a greater occupational hazard from asphalt fumes than is currently documented [76].

(c) Animal Studies

During the 1950's, Hueper [5,6,8] tested the carcinogenic potential of oils produced in the experimental and large-scale production plants using the Bergius and Fischer-Tropsch processes. Tests were performed on mice, rabbits, and rats by cutaneous and intramuscular (im) administration.

(1) Cutaneous Administration

Hueper [8] examined the carcinogenic effects of various fractions of Bergius oil or Fischer-Tropsch oil when applied to the skin of mice. Three Bergius oils (heavy, light, and centrifuge residue) obtained from the experimental operation at Bruceton, Pennsylvania, were tested in two different strains of mice. Three groups of 100 strain A mice were exposed to a 50% solution of each oil fraction once a week for 15 months. Two groups of 25 strain C57 black mice were exposed to the heavy oil or the centrifuge residue in concentrations of 100, 25, and 10% once a week for 14 months. Ethyl ether was used as a diluent in all cases, but only the study involving C57 black mice had a control group.

Post-mortem examinations were performed on all mice that died or were killed, with the exception of those that were cannibalized. Histopathologic examinations of the skin, the thigh tissues, and the organs of the chest and abdomen were made [8].

Skin papillomas and carcinomas were observed in both strains of mice with all fractions of oil. In strain A mice, three adenomas occurred (one animal from each treatment group), and four mice had leukemia. The author's observations, as shown in Table III-2, indicate that the carcinogenic potency of the centrifuge residue extract and the heavy oil fraction was greater than that of the light oil. The number of lesions observed in this study decreased with the progressive dilution of the oils [8].

In the same study, Hueper [8] tested light and heavy oils and reaction water, ie, the "liquor" containing the water-soluble products, of Fischer-Tropsch oils in each of three strains of mice: strains A, C, and C57 black. Each experimental group consisted of 125 mice. Fractions were applied with a micropipette to the skin of the mice once a week for a maximum of 18 months. The heavy oil was diluted with ethyl ether at a ratio of 1:2 by weight; the light oil was undiluted; the reaction water was diluted with water at a ratio of 1:4 to reduce its toxicity. No diluent or untreated controls were used, and the source of the diluent water was not mentioned.

Repeated applications of Fischer-Tropsch heavy oil, light oil, and reaction water to mice resulted in neoplastic reactions. Five lesions occurred in male strain C mice treated with light oil: one intestinal cancer, one breast cancer, two lung adenomas, and one incidence of leukemia. The only lesion that occurred in female strain C mice treated with heavy oil was one breast

TABLE III-2

INCIDENCE OF SKIN CARCINOMA IN MICE AFTER REPEATED CUTANEOUS APPLICATION OF BERGIUS OILS

Concentration	Strain	Incidence in Mice Examined (%)		
		Centrifuge Extract	Heavy Oil	Light Oil
50%	A	4 of 78 (5)	4 of 64 (6)	1 of 82 (1)
100%	C57	5 of 22 (23)	6 of 17 (35)	-
25%	C57	2 of 12 (17)	3 of 22 (14)	-
10%	C57	3 of 21 (14)	-	-

Adapted from reference 8

cancer. In strain A mice four lesions were observed following treatment in reaction water; three were lung adenomas and one was a breast cancer [8]. In strain A mice treated with heavy oil, five males had lesions; four were hepatomas, and the fifth a breast cancer. One male strain C57 mouse had a skin papilloma following treatment with reaction water.

The author [8] dismissed the neoplasms of the breasts, lungs, and hematopoietic tissues as spontaneous tumors, although no control animal data were presented. In addition, he dismissed the single skin papilloma and the intestinal adenocarcinoma because they were the only ones that occurred. However, the author attributed the hepatomas to the application of heavy oil because most of the livers observed had extensive necrotic changes. Cirrhotic lesions associated with local bile duct proliferations were also seen in one case. Because no diluent or untreated control groups were used and the same number, strain, and sex of mice were not tested with each fraction, the validity of this study is reduced.

Hueper [6] carried out a followup study on product samples obtained from the US Bureau of Mines (BOM) Synthetic Fuels Demonstration Plant in Louisiana, Missouri, which used the Fischer-Tropsch process. He applied five fractions of these oils, each with a different boiling point, by dropper to the nape of the neck of 25 male and 25 female 6-week-old strain C57 black mice twice a week for life. The use of control animals or a diluent control group was not

mentioned. The five fractions used were (1) thin synthesis condensate, corresponding to a one- to four-part mixture of diesel oil with gasoline, (2) cracking stock, (3) diesel oil, (4) raw gasoline, and (5) used coolant oil diluted with xylene. The only skin lesions observed were one small papilloma in each of two mice painted with Fraction 4. At necropsy, one mouse (sex unspecified) had a liver sarcoma. The specific times when these lesions appeared and when the animals died were not mentioned. According to the author [6], the effects revealed at necropsy of mice that died in the latter part of the study were not uncommon in untreated mice of the same strain. These effects included nephritis and amyloid (starchlike) lesions of the spleen, liver, kidneys, and adrenal glands.

Certain factors that would affect the author's conclusions were not addressed. These include the use of both untreated and diluent-treated control animals, the maintenance of the fraction at the site of application, the prevention of absorption due to animals licking the site, and the amount of hair remaining at the site following scissoring rather than shaving or clipping, which would interfere with absorption of the material. In addition, no criteria for the necropsies or microscopic evaluations were presented, nor was it mentioned whether the xylene used as a diluent was assayed for benzene contamination.

In the same report [6], Hueper discussed the effects of applying these same five fractions of Fischer-Tropsch oils twice a week to the skin of five 3-month-old Dutch rabbits. Neither the sex of the rabbits nor the concentrations of the fractions were reported. The skin sites included the dorsal surfaces of the ears and three areas on the back. Applications were continued for up to 25 months and followed a rotation scheme that allowed each fraction to be tested on all areas. Several fractions, however, were applied to the same rabbit at different sites. As with the mice, the hair at each site was first cut with scissors. None of the rabbits developed any neoplastic lesions.

Interpretation of this lack of neoplastic lesions is hindered by three considerations: (1) the number of animals used was small, (2) the adherence of the fractions to the site of application was not verified, and (3) the amount of the fraction absorbed was indeterminate. No evidence of cutaneous absorption was given. Painting the same rabbit with different fractions invalidates the results because if tumors were found away from the site of application there would be no way to identify which fraction was responsible. In addition, no control groups were used, and necropsy and microscopic examination criteria were lacking.

In one of two studies with Bergius oil, Hueper [5] tested nine different fractions of Bergius coal hydrogenation products obtained in a large-scale production process operated by the US BOM at its Synthetic Fuels Demonstration Plant at Louisiana, Missouri. These fractions ranged from pitch to finished gasoline and had different boiling points and physiochemical properties. Each

fraction was applied by dropper twice a week to the nape of the neck of 25 male and 25 female 6-week-old strain C57 black mice. Applications continued throughout life, except that supplies of Fraction 9 ran out after about 6 months. Post-mortem examinations were performed on all animals used. Histologic examinations of the various tissues and organs were made whenever any significant pathologic changes were found at necropsy.

Papillomas were found at the primary contact site in mice treated with Fractions 1, 2, and 3. Ten squamatous carcinomas occurred with all fractions except Fractions 1, 2, and 8. In addition to these, leukemic or lymphomatous conditions were noted in one mouse treated with Fraction 1, in two mice treated with Fraction 3, and three mice treated with Fraction 7. The author [5] was unsure about the relation of these reactions to the cutaneously applied oils. However, he attributed the fact that none of the mice survived more than 16 months to the high toxicity of the Bergius products. He also concluded that with the exception of finished gasoline, Bergius products possess carcinogenic properties for mice.

Hueper [5] also reported on the application of the same nine Bergius fractions to the skin of ten 3-month-old Dutch rabbits twice a week for 22 months. However, four or five of the fractions were applied to each rabbit at different sites, ie, the dorsal surfaces of ears and back, so that an additional 10 rabbits were used for the study. The skin preparation and mode of application were the same as for the mice. Applications continued for up to 22 months, except that Fraction 9 was discontinued after 6 months because supplies ran out. Hueper [5] performed necropsies on all of the rabbits and histologic examinations on 19. He found 10 carcinomas and 18 papillomas at the primary contact site. In addition, he observed extensive mononuclear leukemic infiltrations in the liver, abdominal lymph nodes, and pancreas in one rabbit treated with Fractions 5 to 9.

Table III-3 shows the distribution by the different oil fractions of benign and malignant tumors at the site of primary contact in mice and rabbits. The author [5] suggested that the greater number of skin tumors in rabbits may have been caused by a greater susceptibility in rabbits than in mice. He did not report the use of untreated or diluent control mice or rabbits, the doses applied to the skin, the steps taken to ensure that the substance remained on the skin, or observations of any tumors at the application sites.

In a series of three separate experiments, Holland et al [9] tested the carcinogenicity of synthetic and natural petroleums when applied to the skin. In these studies SPF male and C3H/fBd female mice were exposed to test materials at various concentrations. The number of animals varied from 20 to 50 per dose group. The effects of coal liquid A, produced by the Synthoil catalytic hydrogenation process, and coal liquid B, produced by the pyrolytic COED process using Western Kentucky coal, were compared with the carcinogenicity of a pure reference carcinogen, benzo(a)pyrene, tested in in vitro tissue

TABLE III-3

INCIDENCE OF CARCINOMAS AND PAPILLOMAS IN MICE AND RABBITS TREATED WITH BERGIUS OIL FRACTIONS

Fraction No.	Product	Carcinomas		Papillomas	
		Mice	Rabbits	Mice	Rabbits
1	Centrifuge residue	0	2	3	4
2	Heavy oil let-down	0	3	1	5
3	Light oil bottoms	1	2	1	2
4	Middle oil	2	3	1	2
5	Cold catchpot liquid	1	0	0	3
6	Cold catchpot vapor	1	0	0	0
7	Raw gasoline	1	0	0	2
8	Finished gasoline	0	0	0	0
9	Pitch flash distillation residue	4	0	0	0

Adapted from reference 5

culture studies. In the same series of studies, three other fossil liquids were also tested: crude shale oil, single-source natural petroleum, and a blend of six natural petroleums. All fractions were analyzed for PAH content by acid-base solvent partition. Three regimens were followed, and in each, the animals were given pasturized feed and hyperchlorinated-acidified water. The test materials were dissolved by sonication or dispersed in a solvent of 30% acetone and 70% cyclohexane, by volume [9]. Fifty microliters of each test material were applied to the dorsal skin of the mice.

In the first of the three treatment regimens, four groups of 15 male and 15 female mice were treated with 25 mg of four of five test materials, which were applied three times a week for 22 weeks [9]. (Single-source petroleum was not tested.) A 22-week observation period followed. With coal liquid A treatment, 20 animals had died by the end of the study, and the final percentage of carcinomas (squamous epidermal tumors) was 63%; for coal liquid B,

these figures were 3 and 37%, respectively; and for shale oil, 37 and 47%. No carcinomas or deaths occurred in the animals treated with blended petroleum. The average latency period in animals treated with coal liquid A was 149 days (standard error: 8); in animals treated with coal liquid B, 191 days (14); and in animals treated with crude shale oil, 154 days (9).

In the second regimen, groups of 20 mice (10 male and 10 female) each were tested with one of four materials: coal liquid A, coal liquid B, shale oil, and single-source petroleum, at one of four dose levels: 25, 12, 6, and 3 mg. The applications were administered twice a week for 30 weeks, followed by a 20-week observation period. No skin lesions and no deaths were seen in animals treated with single-source petroleum at any dose level, with crude shale oil and coal liquid B at the 6- or 3-mg levels, or with coal liquid A at the 3-mg level. Other results of this study are presented in Table III-4.

As indicated in the table, all of the syncrudes tested were capable of causing malignant squamous epidermal tumors. Dose-response was observed for the syncrudes. Coal liquid A also appeared to be a tumorigenic agent at the reduced dose level as compared with coal liquid B and shale oil.

In the third regimen, the doses were considerably reduced per application although the frequency of applications was increased from two to three times a week for 24 months [9]. The length of time that the applications were allowed to remain on the skin was also increased. The doses per application for each material were 1.0, 0.3, 0.2, and 0.04 mg for coal liquid A; 0.8, 0.3, 0.17, and 0.03 mg for coal liquid B; 2.5, 0.5, 0.3, and 0.1 mg for shale oil; and 2.0, 0.4, 0.3, and 0.08 mg for composite petroleum. The number of mice used in this regimen was also increased to 50 (25 of each sex) per dose level.

In general, the results of this regimen were similar to those of the second regimen with the higher dose and shorter application time. However, this longer exposure at lower doses allowed time for carcinoma induction and expression in the blended petroleum group. This result was not seen in the previous regimen. The design of the study, ie, using several dose levels, produced evidence that a sufficient amount of fraction was being applied to produce effects. In each case, no effects or a weak effect (0-4% carcinoma) was produced at the lowest doses and a much stronger effect was produced at the highest doses (up to 92%). The results of this regimen are shown in Table III-5.

The authors [9] compared the data from the third regimen with results obtained by applying benzo(a)pyrene in the same solvent three times weekly to the same strain of mice. Fifty mice (25 of each sex per dose level) were tested with 0.05, 0.01, and 0.002 mg of benzo(a)pyrene. At the two highest doses, the percentage of skin carcinomas observed was 100%, with an average latency of 139 days at the 0.05-mg level and 206 days at the 0.01-mg level.

TABLE III-4

CARCINOGENICITY OF SYNCRUDES APPLIED TO MICE TWICE A WEEK FOR 30 WEEKS

Material	Dose/Application (mg)	Final Percentage of Carcinomas
Coal liquid A	25	80
	12	35
	6	10
Coal liquid B	25	10
	12	5
Crude shale oil	25	35
	12	5

Adapted from reference 9

TABLE III-5

CARCINOGENICITY OF SYNCRUDES APPLIED TO MICE THREE TIMES A WEEK FOR 24 MONTHS

Material	Dose/Application (mg)	Final Percentage of Carcinomas
Coal liquid A	1.0	92
	0.3	26
	0.2	8
	0.04	4
Coal liquid B	0.8	8
	0.3	4
	0.17	2
	0.03	2
Shale oil	2.5	90
	0.5	2
	0.5	2
	0.3	0
Composite petroleum	2.0	8
	0.4	0
	0.3	0
	0.08	0
Vehicle (30% acetone + 70% cyclohelane)	--	0

Adapted from reference 9

At the lowest dose (0.002 mg) the percentage of skin carcinomas observed was 90%, with an average latency of 533 days. At 24 months, 58% mortality was observed. The dose that most closely approximated the carcinogenicity of the syncrudes was the 0.05-mg dose. The authors indicated that this amount was one five-hundredth of the amount of coal liquid A that would be required to elicit a comparable skin tumor incidence.

In addition to the study discussed above, the same authors [9] analyzed the percentages of PAH's and benzo(a)pyrene in each sample. The PAH content did not correlate with the carcinogenicity of the materials, but the benzo(a)pyrene concentration did agree with the potency of each mixture. The percentages of PAH's by weight for coal liquid A, coal liquid B, shale oil, and blended petroleum were 5.1, 6.0, 2.0, and 2.6, respectively. Single-source petroleum was not analyzed. The micrograms of benzo(a)pyrene per gram for coal liquid A, coal liquid B, shale oil, blended petroleum, and single-source petroleum were 79, 12, 30, approximately 1, and 1, respectively.

In a study of 15 coal hydrogenation materials, Weil and Condra [7] applied samples from streams and residues to the skin of 15 groups of 30 male mice, three times a week for 51 weeks. Two species of mice were tested, Rockland All-Purpose (20% of animals) and C3H (80%). The authors compared the results with positive (0.2% methyl cholanthrene in benzene) and negative (benzene and water) control agents, and concluded that the light and heavy oil products were "mildly" tumorigenic, ie, predominantly produced papillomas. A high incidence of carcinogenicity was seen for middle oil stream, light oil stream residue, pasting oil, and pitch product (Table III-6). The specific types and numbers of papillomas and carcinomas were not reported. In general, the incidence of carcinogenicity increased as the boiling points of the fractions rose.

Renne et al [51] recently published results of studies of skin carcinogenesis in mice. The materials tested were heavy and light distillates from solvent-refined coal, shale oil, and crude petroleum. Also tested were reference carcinogens benzo(a)pyrene and 2-aminoanthracene. A mixture of 50 ml of the test materials in acetone was applied 3 times per week to the dorsal surface of C3Hf/HeBd mice of both sexes.

After 465 days of exposure, the mice showed high incidences of skin tumors with heavy distillate, shale oil, and benzo(a)pyrene. Petroleum crude (Wilmington) and light distillate showed less tumorigenic activity. The two groups of mice treated with the highest doses of heavy distillate (22.8 and 2.3 mg per application), shale oil (21.2 and 2.1 mg per application) and benzo(a)pyrene (0.05 and 0.005 mg per application) showed almost 100% tumor incidence. In contrast, only one mouse in each of the high (20 mg per application) and medium (2.0 mg per application) dose groups exposed to light distillate developed skin tumors. The latency period for tumors was shortest (56 days at the highest concentration) for mice exposed to heavy distillate. All tumors were malignant squamous cell carcinomas, regardless of the treatment group. Untreated and vehicle-treated (acetone) control mice did not

TABLE III-6

PERCENTAGE OF TUMORS AND CANCERS PRODUCED BY COAL PROCESS MATERIALS IN MICE*

Coal Process Material	Boiling Point °C	Percentage of Tumors	Percentage of Cancers
Heavy oil product	--	30	0
Light oil product	--	27	4
Light oil stream	260	0	0
Middle oil stream	260-320	46	15
Light oil stream residue**	260-380	66 87	38 39
Pasting oil**	320-450	78 100	56 100
Pitch product**	>450	54 77	12 63
Stabilizer overhead	35-100	0	0
Crude nitrogen bases	195-260	0	0
Neutral light oil	115-260	0	0
Crude naphthalene	200-230	0	0
High boiling hydrocarbons	230-260	0	0
Low boiling phenols	180-230	0	0
High boiling phenols	230-260	0	0
Phenolic pitch	260+	0	0

*Eighty percent of the experimental mice were of the C3H strain. The remaining mice were identified as Rockland All-Purpose from Rockland Farms. No tumors or cancers were observed in control mice.
**Two different samples were tested.

Adapted from reference 7

develop any tumors. Tumor incidence in the 2-aminoanthracene positive control group was 25/32 and 0/49 at dose levels of 0.05 and 0.005 mg, respectively [51].

(2) Intramuscular Injections

In addition to the dermal studies using mice and rabbits, Hueper [5] also conducted injection studies using rats. Each of the nine fractions of Bergius oils previously described were injected im into the right thighs of groups of ten 3-month-old Wistar rats once a week for 3 successive weeks. This regimen was repeated with rats surviving after 6 months. Each fraction was diluted at a ratio of 1.1 g oil to 16.5 cc tricaprylin. An individual 0.3-cc dose of this mixture contained 0.02 g of oil. Fraction 3 was administered to 20 additional rats because of high mortality early in the experiment (weeks 5-6). The time when these extra animals were added to the study was not mentioned.

The controls consisted of two groups of 30 rats each. One control group was injected in the marrow cavity of the right femurs with 0.1 cc of a 2% gelatin solution in physiologic saline. The same amount of solution was injected into the nasal sinuses of the other control group through a hole drilled in the frontal bone. The purpose of this second control was not reported. After a 2-year observation period, all surviving animals were killed and histologic examinations of selected tissues and organs were made in cases where pathologic changes were observed. No diluent (tricaprylin) control animals were studied.

Rats died throughout the study period, with the highest incidences per group (5-9 deaths) between weeks 7 and 10 for all fractions except Fraction 3. For Fraction 3, 28 deaths were reported, 23 of which occurred during weeks 5 and 6. A total of 13 tumors away from the site of injection were observed in the 100 treated animals. Of these, 11 tumors were malignant [5].

Rats treated with Fractions 3, 4, 6, 7, 8 (three cases), and 9 showed large round-cell sarcomas. Ovarian fibromas were also observed in one rat in each of the groups injected with Fractions 1 and 9; an ovarian adenocarcinoma was observed in one rat given Fraction 3. A retroperitoneal fibrosarcoma was found in a rat treated with Fraction 7, and a small squamous-cell lung carcinoma was found in one rat treated with Fraction 9. In addition, a fibrosarcoma was found at the injection site in each of two rats. Nine malignant and two benign tumors were observed in the 60 rats used as controls; these tumors included four round-cell abdominal lymph node sarcomas, five spindle-cell liver sarcomas, one squamous-cell papilloma of the forestomach, and one breast adenofibroma. The author [5] concluded that one of the spindle-cell liver sarcomas was due to the presence of a parasitic infection and originated from the wall of a cyst. Tumors were not observed at the site of gelatin injection in either treated or control animals.

Several factors detract from the conclusiveness of Hueper's findings [5]. For example, neither the time of appearance of these lesions nor the time of necropsy were reported. In addition, the incidence of lesions occurring away from the injection site was reported to be 13 lesions in 100 rats, but this total does not agree with the total number of deaths and necropsies (110) reported for the treated animals. This discrepancy may be a result of the author not including the original 10 rats tested with Fraction 3 in his reports. Additional discrepancies were: 11 deaths and necropsies were reported for rats treated with Fraction 7 although only 10 animals were studied; 9 deaths were reported for rats treated with Fraction 9 but no mention was made of the 10th animal in that group; and 9 malignant tumors were observed in the 60 control animals, but the author did not indicate in which of the two control groups these lesions were seen.

Hueper [6] also conducted one study consisting of three series of im injections of the five Fischer-Tropsch oil fractions using Wistar rats. There was a 2-month interval between the first and second series and a 4-month interval between the second and third series. Two injections were given in each series, and there was an interval of 1 week between the injections. Each of the five treatment groups consisted of 15 Wistar rats of both sexes. At the end of a 2-year observation period, all surviving rats were killed for histopathologic examination. Pathologic studies were done on 41 rats.

This study revealed that Fischer-Tropsch products showed definite carcinogenic properties for rats when administered by im injections. Fischer-Tropsch products are species and tissue specific. The carcinogenic effects of these oils may not be restricted to the tissues in which these materials are deposited. The histopathologic results showed that lesions occurring at the site of injection varied. For Fraction 1, necrosis and multicystic fat tissue were observed; for Fractions 2 and 5, granulomas were seen; and for Fraction 3, fibrosis occurred. Fraction 4 produced no lesions. A total of 19 tumors in 75 rats was observed. Two tumors, a breast adenofibroma and an adrenal hemangioma, were benign, and the 17 others were malignant. These were spindle-cell sarcomas or fibrosarcomas of the right thigh, spindle-cell abdominal lymph node sarcomas, round-cell abdominal sarcomas, kidney adenocarcinomas, and squamous-cell lung carcinomas. The spindle-cell and round-cell sarcomas produced had metastasized to other organs such as the spleen, liver, kidney, and lung. The tumors that resulted from each of the five oil fractions injected im into rats are listed in Table III-7.

According to Hueper [6], the proximity of the spindle-cell thigh sarcomas to the injection site implicated the injected materials (Fractions 2, 4, and 5). The types of cancer observed indicated that Fraction 5 seemed to be the most carcinogenic and Fractions 2 and 4 followed in degree of carcinogenicity, while the carcinogenic potency for Fractions 1 and 3 was uncertain. Hueper did not explain the relationship of the other cancers to the materials tested except to say that the materials may have been transported through the lymph nodes to the remote sites. This study did not include any untreated or diluent control animals.

TABLE III-7

MALIGNANT TUMORS IN RATS INTRAMUSCULARLY INJECTED WITH FISCHER-TROPSCH OILS

Fraction No.	Fraction Name	No. of Tumors	Types of Tumors
1	Synthesis condensate	1	Spindle-cell abdominal sarcoma
2	Cracking stock	5	2 Spindle-cell abdominal sarcomas 2 Spindle-cell thigh sarcomas 1 Lung carcinoma
3	Diesel oil	4	1 Spindle-cell abdominal sarcoma 3 Round-cell abdominal sarcomas
4	Raw gasoline	3	1 Spindle-cell thigh sarcoma 1 Round-cell abdominal sarcoma 1 Kidney carcinoma
5	Coolant oil	4	4 Spindle-cell thigh sarcomas

Adapted from reference 6

These studies show that the fractionation products obtained through the hydrogenation of coal are, in general, carcinogenic in at least one animal species; that the incidence of carcinogenicity seems to decrease as the boiling points of the fractionation products decrease; and that carcinogenicity may not be restricted to the tissues into which the material was originally administered. Although treatment schedules were not the same as possible daily industrial exposures, and the numbers of animals tested were small (5-100) or not reported at all, the results of these studies indicate that certain coal liquefaction products contain carcinogenic chemicals.

(d) Mutagenicity Studies

Mutagenicity studies have been conducted using strains of the bacterium Salmonella typhimurium that require histidine for growth [40-45,50]. Two of these strains (TA 100 and 1535) are used to detect base-pair mutations, and others (TA 98, 1536, 1537, and 1538) are used to detect frameshift mutations. Rubin et al [42] tested 14 fractions of syncrude from the COED process using S typhimurium strains TA 1535, 1536, 1537, and 1538. An unspecified number of control plates was used for spontaneous reversion and sterility checks. The results showed an increase in the number of revertants (1,000 colonies over background) with four fractions when the system was metabolically activated. These fractions were benzene/ether (TA 1536, 1537, and 1538), hexane/benzene (TA 1537 and 1538), and hexane (TA 1537 and 1538), and one ether-soluble fraction (TA 1537 and 1538).

Using chemicals supplied by the manufacturers, Teranishi et al [41] reported results of mutagenicity tests on PAH's found in coal liquefaction processes by observing metabolic activation in S typhimurium strains TA 1535, 1536, 1537, and 1538. In at least one strain, the authors found at least a doubling of the number of revertants above those shown in the dimethylsulfoxide controls. Using this criterion, benzo(a)pyrene, benz(a)anthracene, 7,12-dimethyl-benz(a)anthracene, and dibenzo(a,i)pyrene were mutagenic. Anthracene, benzo(e)pyrene, dibenzo(a,c)pyrene, and dibenz(a,h)anthracene did not produce a doubling of the number of revertants above that of the controls.

Shults [40] reported on preliminary studies with 17 fractions of COED syncrude tested at Oak Ridge National Laboratory (ORNL) using four S typhimurium strains (TA 1535, 1536, 1537, and 1538). Mutagenicity was recorded for 8 of 17 fractions: hydrochloric acid insolubles, ether soluble bases and weak acids, first and second cyclohexane extracts, phenanthrene-benz(a)anthracene, benz(a)anthracene, and benzo(a)pyrene. Fractions that did not induce mutagenicity included ether-soluble strong acids, neutrals, polar compounds in water, dimethylsulfoxide residuals, phenanthrene, insoluble weak and strong acids, insoluble bases, and insoluble sodium hydroxide. No mention was made of whether or not metabolic activation was incorporated.

Epler and coworkers [44] tested fractions of Synfuels A and B for mutagenic activity in S typhimurium strains TA 98 and 100 using metabolic activation. The results indicated mutagenicity from both Synfuel A (1, 2, and 3) and Synfuel B, although the insoluble base fraction (a) showed a greater increase in the number of revertant colonies than did the neutral fraction, the insoluble sodium hydroxide fraction, or the diethyl ether-soluble fraction. Fractions producing little or no increase in revertant colonies over that of the control product, composite crude oil, included the insoluble weak and strong acids, the water-soluble strong acids, the water-soluble bases,and the insoluble base fraction (b).

In the same laboratory, Rao et al [45] tested four fractions of Synfuel A-3. He detected an increase in the number of revertant colonies after metabolic activation of the test materials in the tester strains (TA 100 and 1535) designated to detect mutagenicity by base-pair substitution. Strains TA 98, 1537, and 1538, designated to detect frameshift mutations, proved to be more sensitive to metabolically activated fractions, with strains TA 98 and 1538 exhibiting a 20- to 75-fold increase over the incidence of spontaneous reversion.

Using selected fractions of Synfuels A and B that provided a large number of revertant colonies in the S typhimurium assay, Epler and coworkers [43] compared their results by using other systems. Comparative systems included forward and reverse mutation assays in Escherichia coli and Saccharomyces cerevisiae, chromatid aberrations in human leukocytes, and gene mutation in Drosophila. The results of the E coli 343/113 (K-12, gal RS 18, arg 56, nad 113) assay supported the results obtained with S typhimurium. Results with S cerevisiae strain XA4-8Cρ⁻, his1-7, with forward mutants to canavanine resistance (CAN-can) and revertants to histidine prototrophy indicated antagonistic effects with metabolic activates. In this assay, 1.2×10^8 cells in an unspecified amount of buffer were used. Treatment of the human leukocytes with the fractions did not produce chromatid aberrations; however, metabolic activation was not attempted, and in the Drosophila sex-linked recessive lethal test, no fraction gave a significant increase over the spontaneous level. The genus and number of Drosophila used and the number of chromosomal preparations from human peripheral leukocytes were not specified.

Pelroy et al [50] recently published the results of studies that used the S typhimurium test system to evaluate the mutagenicities of light, middle, and heavy distillates from the SRC-II process, raw shale oil, crude petroleums, and some SRC-I process materials. Tests were performed in both the presence and absence of mammalian liver microsomal enzymes (S9) in several strains of S typhimurium. Significant mutagenic activity was seen with high boiling point materials from the SRC-II (heavy distillate) and the SRC-I (process solvent) processes. In strain TA 98, 90.0±23 and 12.3±1.9 revertants per mg of heavy distillate (SRC-II) and process solvent (SRC-I), respectively, were observed. The light and middle distillates showed no mutagenic activity. Raw shale oil (Paraho-16, Paraho-504, and Livermore L01) had very low mutagenic activity. Crude petroleum (Prudhoe Bay and Wilmington) showed less than 0.1 revertants per mg of material. When the mutagenic activities of the coal liquefaction materials were compared with those of the pure reference carcinogens benzo(a)pyrene and 2-aminoanthracene in strain TA 98, benzo(a)pyrene was 3 times more active than the heavy distillate, while 2-aminoanthracene was 100 times more active.

The materials encountered in coal liquefaction processes are generally complex organic mixtures, so identification of the biologically active components is essential. This was accomplished by chemical and physical fractionation of the mutagenically active products, followed by additional mutagenicity

testing. Fractionation of heavy distillate by a solvent-extraction procedure yielded acidic, basic, and neutral fractions, as well as basic and neutral tar fractions. When these fractions were tested for mutagenicity by the Ames system, the basic fraction showed the highest number of revertants per mg of material. The basic and neutral tar fractions were 0.125 and 0.5 as active as the basic fraction. The acidic and neutral fractions were nonmutagenic. Basic fractions from shale oil and other materials also showed high specific activity. These results suggested that the polar nitrogen-containing components might be responsible for the mutagenic activity of the heavy distillate and other oils. Separation of mutagenic compounds from the heavy distillate and the process solvent was followed by gas chromatographic/mass spectral (GC/MS) analyses of the specific components. The results indicated that 3- and 4-ring primary aromatic amines were responsible for a large fraction of the mutagenic activity of the heavy distillate and the process solvent. The 2-ring aminonapthalenes contributed little to the mutagenic activity of these products.

Indications that the aromatic amines were responsible for mutagenic activity were further confirmed by mutagenicity testing of materials eluted by thin-layer chromatography (TLC) of the basic fraction of heavy distillate. Testing was conducted in strain TA 98 S typhimurium, utilizing mixed-fraction amine oxidase (MFAO) or a mixture of hepatic enzymes (S9). MFAO is specific for the metabolic transformation of primary aromatic amines to mutagenically active compounds; it is inactive with PAH's. The mutagenic responses obtained with the TLC components of heavy distillate using both MFAO and S9 were comparable, yielding direct evidence of the presence of aromatic amines in heavy distillate. Additional evidence of the mutagenicity of the aromatic amines was provided when the mutagenic activity of the heavy distillate, the process solvent, and their basic and tar fractions was reduced by 90% after nitrosation.

In all of these studies, the PAH's, the ether-soluble bases and weak acids of COED syncrude and Synfuels A and B, and the insoluble bases and neutral portions of Synfuels A and B are mutagenic when tested in S typhimurium, but studies in higher organisms (human leukocytes and Drosophila) indicate negative results. Both SRC-II heavy distillate and SRC-I process solvents are mutagenic in S typhimurium. Further testing indicates that this activity is caused by their aromatic amine components.

Reproductive Effects and Other Studies

Andrew and Mahlum [14,15] also evaluated reproductive effects by exposing pregnant rats to SRC-I light oil, wash solvent, and process solvent [47,52]. These substances were given either undiluted or in corn oil by gavage once daily for either days 7-11 or 12-16 of gestation. Corn oil alone was administered to vehicle control groups, and 2.5% Aroclor 1254 in corn oil was used for positive control groups. Rats were killed at 21 days of gestation for evaluation of embryotoxicity or were permitted to deliver offspring for postnatal monitoring of growth, physical maturation, and reflex ontogeny.

Maternal lethality and embryolethality of ≥50% were seen in groups dosed on days 7-11 of gestation with light oil, wash solvent, or process solvent at 3.0, 1.4, or 0.7 g/kg/d, respectively. Similar results were seen after the same dosing on days 12-16 of gestation. Malformations, consisting of cleft palate and brachydactyly, and low fetal weights were seen after light oil dosing at 3.0 g/kg/d on days 7-11 of gestation or after process solvent dosing at 0.7 g/kg/d on days 12-16. No effects on postnatal maturation were seen.

Andrew and Mahlum [15] reported the results of studies on reproductive effects of SRC-II light, medium, and heavy distillates. Pregnant rats were administered unspecified doses once daily for 5 consecutive days during the gestation periods of either 7-11 days (early period of organogenesis) or 12-16 days (late period of organogenesis). Embryolethality, malformations, and fetal weights were determined after killing the rats at 21 days of gestation. Fetal growth and survival were decreased by all three materials administered during either period. Fetal effects for all three materials were more severe at 12-16 days of gestation than at 7-11 days of gestation. None of the materials increased the incidence of malformation over that in controls at 7-11 days of gestation. Increased incidence of malformations, principally cleft palate, diaphragmatic hernia, and hypoplastic lungs, was produced by the heavy distillate when administered at 12-16 days of gestation. In most cases, doses of materials that produced prenatal toxicity also produced some indications of maternal toxicity.

In 1978, MacFarland [46] reported the results of several short-term toxicity studies in rats and rabbits exposed to dry mineral residue (DMR) and to solvent-refined coal products. The lethal dose for 50% survival of group (LD_{50}) for both materials tested was >15,380 mg/kg in short-term oral studies on rats. The short-term dermal LD_{50} for both compounds in rabbits was >10,250 mg/kg. For short-term vapor inhalation in rats, lethal concentration for 50% survival of group (LC_{50}'s) were determined for the process solvent (>1.69 mg/liter), coal slurry (>0.44 mg/liter), heated filter feed (>1.14 mg/liter), wet mineral residue (3.94 mg/liter), light oil (>71.6 mg/liter), and wash solvent (>7.91 mg/liter). The adult rats that received lethal doses of light oil or wash solvent showed signs of distress within 30 minutes, including convulsions and twitching of extremities. Because of their low volatility, the process solvent and wash solvent were tested as aerosols in short-term inhalation studies using rats, and the LC_{50}'s were determined to be >7.6 and 16.7 mg/liter, respectively. Tests for acute eye irritation in rabbits identified light oil and wash solvent to be severely irritating, wet mineral residue extremely irritating, coal slurry and filter feed moderately irritating, process solvent mildly irritating, and dry mineral residue and solvent-refined coal minimally irritating [46]. The three materials identified to be severely or extremely irritating were tested in 14-day eye irritation studies. Only with light oil was there a noticeable improvement after 14 days. Indications of fetotoxicity were reported in rats and rabbits in pilot teratogenic testing with filter feed and wet mineral residue applied dermally [46]. However, no additional data were included. In addition, the

number of animals used in the short-term studies was not mentioned, except for the statement that a small number of animals was used.

Mahlum and Andrew [47,58] observed short-term toxicities in fasted adult, female Wistar rats following administration of SRC-I and SRC-II liquids by gavage. Ten to 25 rats per dose per material in two to four replicates were used. Adult LD_{50}'s were determined for SRC-I light oil, wash solvent, and process solvent and for SRC-II light, medium, and heavy distillates. The process solvent was also tested in newborn and weanling rats. Acute adult LD_{50}'s of 0.57, 2.9, and 2.8 g/kg were obtained for undiluted wash solvent, light oil, and process solvent, respectively. Dilution in corn oil increased the LD_{50} for wash solvent to 1.7 g/kg but did not alter values for light oil and process solvent. LD_{50} values for light and heavy distillates (2.3 and 3.0 g/kg, respectively) were similar to those for light oil and process solvent, while the value for distillates of 3.7 g/kg was five times the value for wash solvent. The lethal dose (LD) of process solvent for weanling and adult rats was similar but about twice as high as that for newborn rats. Subchronic LD_{50}'s for light oil, wash solvent, and process solvent diluted in corn oil were 2.4, 1.5, and 1.0 g/kg/d, respectively. Subchronic toxicity data for light, middle, and heavy distillates were 0.96, 1.48, and 1.19 g/kg/d, respectively. All materials were administered once a day for 5 consecutive days. For all materials except light oil and wash solvent, the subchronic values were significantly lower than the acute values. These results indicate that the cumulative effects are low for light oil and wash solvent, but significant for process solvent and light, middle, and heavy distillates.

Frazier and coworkers [77,78] examined the in vitro cytotoxicity of materials from the SRC-I and SRC-II processes and compared the results with those from studies with other fossil fuel products. The clonal growth assay and Syrian hamster embryo (SHE) cell transformation assay were used. The SRC-I process solvent, the shale oil, and the SRC-II heavy distillate caused a 50% reduction in the relative plating efficiency (RPE_{50}) of Vero African green monkey kidney cells at concentrations between 30 and 50 µg/ml. Other materials, including other SRC byproducts, diesel oil, and several crude oils, were slightly less toxic and produced RPE_{50}'s at concentrations between 50 and 500 µg/ml.

Transformation studies were also performed in SHE cells in the presence and absence of S9 [77,78]. Cells that were preincubated for 16-24 hours were treated with the test materials. The results of the transformation assays were in general agreement with those of the microbial mutagenesis studies. Heavy distillate and process solvent produced 6.8 and 10% transformed colonies, respectively, compared with 0.2-0.4% for petroleum crudes and 3% for shale oil. Basic fractions were more active than the unfractionated crudes. Transformation frequency was higher for all the materials when they were metabolically activated. Petroleum crudes and shale oil exhibited low levels of activity in the cell transformation assays.

The authors concluded that these data demonstrate that certain fossil fuel components are toxic and are capable of transforming mammalian cells. However, the authors also stressed that considerable variability in these assays, due to solubility differences, may arise, and therefore these data represent only potential results and should not be used to establish the carcinogenic potential of these compounds [77,78].

In the same series of tests, Burton and Schirmer [79,80] examined by gas chromatography (GC) the tissue distribution of SRC process solvent components in two rats administered 90% process solvent in corn oil (1 ml/300 g) by gavage. The rats died within 2 days. Small, unspecified amounts of process solvent were found in the kidneys, liver, lungs, and fat; larger amounts were found in the gut and gut contents, and in the stomach and stomach contents. Total amounts recovered were 10-40% of the administered dose.

A second group of 10 rats was administered 0.5 ml of process solvent by gavage. The animals were killed 2, 4, 8, 24, and 48 hours after the dose, and tissues, urine, and feces were collected. In addition, blood samples were taken at 0.5, 1, 1.5, and 16 hours as well as after the animals were killed. Significant levels of phenanthrene (17 μg/g), biphenyl (3 μg/g); and 2-methylnaphthalene (7 μg/g) were found in the livers within 1 hour. Significant levels were also found in red blood cells (RBC's) after 1 hour. Concentrations were highest during the first 8 hours and significantly lower through 48 hours.

In the same series of experiments, the pulmonary resistance, dynamic pulmonary compliance, respiratory rate, tidal volume, and minute volume were also determined in guinea pigs that inhaled 100 mg/m^3 light oil from solvent-refined coal [49]. Preexposure values were recorded for 15 minutes prior to exposing the animals. Animals were then exposed either to air or to light oil for 30 minutes, followed by a 15-minute recovery period. No effects were noted, indicating that inhalation of 100 mg/m^3 of light oil did not affect pulmonary resistance, dynamic compliance, or breathing patterns in guinea pigs.

By measuring fluorescence intensity, Holland et al [9,81] developed an assay system to determine the time-integrated dose of material that interacts with epidermal deoxyribonucleic acid (DNA) after topical application in vivo. Although a relationship between fluorescence intensity and carcinogenicity exists for certain materials, with the exception of coal liquid A, the synthetic petroleums actually exhibited lower specific fluorescence than did the reference blend of natural petroleum, thus exhibiting little or no correlation with carcinogenicity. The authors also compared in vitro and in vivo fluorescence intensity with carcinogenicity for coal liquid A, coal liquid B, shale oil, and composite crude. A positive correlation between tissue fluorescence and carcinogenicity was observed for both of the coal liquids but not for shale oil. Nonfluorescing constituents of shale oil may have been responsible for these differences, which indicate limitations in using this technique for complex organic mixtures.

Data on the effects of exposing animals to coal liquefaction materials are summarized in Table III-8.

TABLE III-8

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
Mice (225 male and 225 female)	Bergius oil fractions: Centrifuge residue* Heavy oil let-down Light oil bottoms Middle oil Cold catchpot liquid Cold catchpot vapor Raw gasoline Finished gasoline Pitch flash distillation residue*	Undiluted, 2x/wk/lifetime, cutaneous	Carcinoma (10), papilloma (6), leukemia (6)	5 (1956)
(450)	Bergius oils: Heavy oil Light oil Centrifuge residue	Progressive dilutions, 1x/wk/15 mo, cutaneous	Carcinoma (28), papilloma (45), leukemia (4), lung adenoma (3)	8 (1953)
(375)	Fischer-Tropsch oil: Light oil (undiluted) Heavy oil (diluted 1:2) Reaction water (diluted 1:4)	1x/wk/18 mo, cutaneous	Papilloma (1), carcinoma (5), adenoma (4), leukemia (1), hepatoma (4)	8 (1953)
(25 male and 25 female)	Fischer-Tropsch oil: Synthesis condensate Cracking stock Diesel oil Raw gasoline Coolant oil	Undiluted, 2x/wk/lifetime, cutaneous	Papilloma (2), sarcoma (1)	6 (1956)
(450)	15 coal hydrogenation chemicals	Varying dilutions, 3x/wk/1 yr, cutaneous	Carcinogenic**, tumorigenic**	7 (1960)
(120)	Product oils: Synthoil COED Shale oil Composite petroleum	25 mg 3x/wk/22 wk, cutaneous	Carcinoma (44)	9 (1979)

TABLE III-8 (CONTINUED)

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
Mice (continued)				
(320)	Product oils: Synthoil COED Shale oil Single source petroleum	25 mg 2x/wk/30 wk, cutaneous	Carcinoma (36)	9 (1979)
(800)	Product oils: Synthoil COED Shale oil Composite petroleum	2.5 mg 3x/wk/24 mo, cutaneous	Carcinoma (124)	9 (1979)
Guinea Pigs	Light oil from solvent-refined coal	100 mg/m^3 for 30 min, inhalation	No pulmonary effects	49 (1979)
Rats (100)	Bergius oil fractions: Centrifugation residue Heavy oil let-down Light oil bottoms Middle oil Cold catchpot liquid Cold catchpot vapor Raw gasoline Finished gasoline Pitch flash distillation residue	0.02 g for 1x/wk/3 wk, then 1x/wk/3 wk after 6 mo, im	Sarcoma (7), carcinoma (2), fibroma (1)	5 (1956)
(75)	Fischer-Tropsch oil: Synthesis consensate Cracking stock Diesel oil Raw gasoline Coolant oil	Dose unknown, 6 doses in 7 mo, im	Sarcoma (15), carcinoma (2)	6 (1956)
	Dry mineral residue Solvent-refined coal	LD_{50}> 15.4 g/kg, oral		46 (1978)

TABLE III-8 (CONTINUED)

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
Rats (continued)				
	Process solvent Wash solvent	LC_{50} >7.6 mg/liter LC_{50} >16.7 mg/liter, inhalation		46 (1978)
		LC_{50}'s, vapor inhalation:		46 (1978)
	Process solvent	>1.69 mg/liter		
	Coal slurry	>0.44 mg/liter		
	Heated filter feed	>1.14 mg/liter		
	Wet mineral residue	3.94 mg/liter		
	Light oil	>71.6 mg/liter		
	Wash solvent	>7.91 mg/liter		
	SRC-I light oil	Acute LD_{50}, = 2.9 g/kg		47 (1979)
	Wash solvent	oral = 0.7 g/kg		
	Process solvent	= 2.8 g/kg		
	"	0.7, 1.4, and 3.0 g/kg to pregnant rats, oral	Fetal malformations, low fetal weights	14 (1979)
	SRC-I light oil	Subchronic = 2.4 g/kg/d		52 (1979)
	Wash solvent	LD_{50}, oral = 1.5 g/kg/d		
	Process solvent	= 1.0 g/kg/d		
	SRC-II light distillate	Acute LD_{50}, = 2.3 g/kg		52 (1979)
	Middle distillate	oral = 3.7 g/kg		
	Heavy distillate	= 3.0 g/kg		
	SRC-II light distillate	Subchronic = 0.96 g/kg/d		52 (1979)
	Middle distillate	LD_{50}, oral = 1.48 g/kg/d		
	Heavy distillate	= 1.19 g/kg/d		

TABLE III-8 (CONTINUED)

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
Rabbits (5)	Fischer-Tropsch Fractions: Synthesis condensate Cracking stock Diesel oil Raw gasoline Used coolant oil	2x/wk/25 mo, cutaneous	Noncarcinogenic effects	6 (1956)
	Bergius oil fractions: Centrifuge residue Heavy oil let-down Light oil bottoms Middle oil Cold catchpot liquid Cold catchpot vapor Raw gasoline Finished gasoline Pitch flash distillation residue	2x/wk/22 mo, cutaneous	Carcinoma (10), papilloma (18), leukemia (1)	5 (1956)
	Dry mineral residue Solvent-refined coal	LD_{50} >10.3 g/kg, cutaneous		46 (1978)
S typhimurium	Fractions of Synfuels A and B: Sodium hydroxide, insoluble Weak acids, diethyl ether-soluble Bases, insoluble (a) Bases, diethyl ether-soluble Neutral Weak acids, insoluble Strong acids, insoluble Strong acids, water-soluble Bases, insoluble (b)	Unspecified	Mutagenic " " " " Nonmutagenic " " "	44 (1978)

TABLE III-8 (CONTINUED)

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
S typhimurium (continued)				
	Fractions of Synthoil and COED process:	Unspecified		40 (1976)
	Hydrogen chloride, insoluble		Mutagenic	
	Bases, ether-soluble		"	
	Weak acids, ether-soluble	"		
	First cyclohexane extract (neutrals)		"	
	Second cyclohexane extract	"		
	Phenanthrene-BaA		"	
	BaA		"	
	BaP		"	
	Sodium hydroxide, insoluble		Nonmutagenic	
	Weak acids, insoluble		"	
	Strong acids, insoluble		"	
	Bases, insoluble		"	
	Strong acids, ether-soluble		"	
	Neutrals		"	
	Polar compounds in water		"	
	Dimethyl sulfoxide residuals		"	
	Phenanthrene		"	
	Fractions of Syncrude from COED process:	Unspecified		42 (1976)
	Sodium hydroxide, insoluble		Nonmutagenic**	
	Weak acids, insoluble		"	
	Weak acids, diethyl ether-soluble		"	
	Strong acids, insoluble		"	
	Strong acids, diethyl ether-soluble		"	
	Strong acids, water-soluble		Mutagenic**	
	Bases, insoluble (a)		Nonmutagenic	

TABLE III-8 (CONTINUED)

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
S typhimurium (continued)				
	Bases, insoluble (b)		Nonmutagenic	
	Bases, diethyl ether-soluble		Mutagenic	
	Bases, water-soluble		Nonmutagenic	
	Hexane		Mutagenic	
	Hexane/benzene		"	
	Hexane/ether		"	
	Methanol		Nonmutagenic	
	Polyaromatic hydrocarbons:	50 µg/plate		41 (1975)
	Anthracene		Nonmutagenic	
	Benzo(a)pyrene		Mutagenic	
	Benzo(a)anthracene		"	
	Benzo(e)pyrene		Nonmutagenic	
	7,12 Dimethyl-benz-(a)anthracene		Mutagenic	
	Dibenzo(a,i)pyrene		"	
	Dibenzo(a,c)pyrene		Nonmutagenic	
	Dibenz(a,b)anthracene		"	
	Light oil		Mutagenic	50 (1979)
	Wash solvent		"	
	Process solvent		"	
	Light distillate		"	
	Medium distillate		"	
	Heavy distillate		"	
	Paraho 16 shale oil		"	
	Paraho 504 shale oil		"	
	Livermore L01 shale oil		"	
	Fractions of Synfuel A-3	Unspecified	Mutagenic	45 (1978)
	Fractions of Synfuels A and B	"	"	43 (1977)
E coli	"	"	"	43 (1977)

TABLE III-8 (CONTINUED)

SUMMARY OF TOXICITY STUDIES

Animal Organism (Number)	Exposed to	Maximum Dose and Route	Effects (Incidence)	Reference (Year)
Saccharomyces	Synfuel A	Unspecified	Mutagenic	43 (1977)
Human leukocyte	Synfuel B	"	Nonmutagenic	43 (1977)
Drosophila	"	"	Weakly mutagenic	43 (1977)

*Applied in diluted form
**Incidence not reported