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**CARCINOGENICITY OF RESIDUAL FUEL OILS BY
NONBIOLOGICAL LABORATORY METHODS:
ANNOTATED BIBLIOGRAPHY**

Part I. Laboratory Methods of Analysis

Part II. Analysis Results

Roger S. Cichorz

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Oils
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Chemical Composition
Chemical Analysis
Characterization

ROCKWELL INTERNATIONAL
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CARCINOGENICITY OF RESIDUAL FUEL OILS BY NONBIOLOGICAL LABORATORY METHODS: ANNOTATED BIBLIOGRAPHY

Roger S. Cichorz

Abstract. Recent emphases have been directed by Federal government regulatory agencies and other research groups on the carcinogenic effects of certain aromatic hydrocarbon components in naturally occurring petroleum products. These are used in plant operations, and underline the importance of evaluating environments. Since Rocky Flats Plant uses large quantities of fuel oil, the author was prompted to undertake a search of the chemical literature. Articles and accounts of studies were reviewed on nonbiological laboratory methods for determining the carcinogenicity of residual fuel oils and related high-boiling petroleum fractions.

The physical and chemical methods involve the separation or measurement (or both) of polynuclear aromatic constituents which generally are responsible for the carcinogenic effects. Thus, the author suggests that the total carcinogenic activity of any petroleum product may not be due to a specific potent carcinogen, but rather to the cumulative effect of several individually weak carcinogens.

The literature search is presented as an annotated bibliography, current as of January 1, 1975, and includes significant parts of the studies along with the total number of other references found when the citation was examined in its entirety.

Part I deals with laboratory chemical and physical methods of determining carcinogenicity or polynuclear aromatic hydrocarbons (or both) in residual fuel oils and contains ten entries.

Part II includes the results of testing specific fuel oils for carcinogenic constituents and contains

eleven entries. An author index and subject categories are included.

INTRODUCTION

Recent emphases by the National Institute for Occupational Safety and Health (NIOSH), the Environmental Protection Agency (EPA), and other Federal government regulatory agencies on the carcinogenic nature of industrial chemicals have served to focus interest at the Rocky Flats Plant (Hazardous Materials Committee and Industrial Hygiene) in the area of the potential carcinogenicity of petroleum products used in plant operations. Specifically, interest was directed to a No. 6 fuel oil used in large quantities (700,000 gallons per year mean over the past seven years)¹ to heat the buildings during the winter months.

The American Society for Testing and Materials (ASTM) has developed specifications for six grades of fuel oils. The No. 6 fuel oil, often referred to as bunker fuel oil or residual fuel oil, has a minimum set of specifications. In general, these heavy fuel oils begin distilling at 650 °F, with 30 to 70 weight percent distilling from 650 to 1000 °F and the end boiling point in the 1200 to 1500 °F range. The American Petroleum Institute (API) arbitrarily defined the specific gravity specification (API gravity) in the 10 to 16 range, which means the oil is heavily naphthenic (cycloparaffinic) and aromatic in character. Several classes of potential carcinogenic² chemicals are known constituents of

¹ Nathan H. Miller of Rocky Flats Plant. Private Communication. August 7, 1975.

² *Carcinogenic* as related to petroleum products generally applies to those materials which produce a significant tumor or cancer response (or both) when repeatedly applied to the skin of certain experimental mice under specified conditions.

residual fuel oils, most notably polynuclear aromatic hydrocarbons and polynuclear heterocyclic (S, N, O) hydrocarbons.

Conclusions from various investigations concerning the carcinogenic activity of petroleum products have been reached: (1) The major part of the carcinogenic activity resides in the portion boiling from 700 to 1000 °F. Little carcinogenic activity is associated with portions boiling below 700 °F. (2) No carcinogenic activity is associated with the nonaromatic fractions (paraffins, olefins, and naphthenes) of petroleum oils. (3) Most of the carcinogenic activity is due to polynuclear aromatic hydrocarbons and to their derivatives containing nitrogen, sulfur, and oxygen. (4) Current evidence suggests that some of the oxidized material in high-boiling petroleum samples may act to reduce the carcinogenic potency which the sample would have in the absence of the oxidized material.

Since early 1950, physical-chemical methods for determining the carcinogenicity of petroleum products have been based on measurements of certain types of polynuclear aromatic hydrocarbons. Since these methods appear to give good correlations with biological data, they obviate the necessity of isolating and analyzing the actual carcinogenic constituents. Previously, the only methods for predicting carcinogenic activity had been limited to series of pure aromatic compounds or to highly specialized types of petroleum products.

PROCEDURES

Various sources through 1974 were searched for journal articles and reports dealing with the subject of carcinogenicity as related to residual fuel oils and similar high-boiling petroleum products. The author attempted to be thorough in his search but makes no claims for the completeness of the search and suggests the citations be used as a general starting guide prior to any laboratory investigations. In addition to the sources, a

CHEMCON³ computer retrieval search was made using the following keywords: FUEL OIL, OIL, CARCINOGEN, CARCINO-. Fifty-four literature citations were generated, but all were outside the scope of this report.

The literature citations covering the subject of residual fuel-oil carcinogenicity are cited chronologically in the text and have been arbitrarily classified into two areas. The first deals with laboratory methods of analysis of petroleum products for carcinogens (chemical analyses and tests based on physical properties of known carcinogenic constituents) or laboratory methods (or both) for characterizing polynuclear aromatic hydrocarbon constituents in high-boiling petroleum products. The second includes the results of testing specific petroleum products for carcinogenic constituents. Biological testing (e.g., administering petroleum products or carcinogenic compounds or both to laboratory test animals) is beyond the scope of this survey, and articles pertaining to these investigations have been intentionally excluded.

CONCLUSIONS

The bibliography will serve as a valuable source since it incorporates a number of referenced studies relating to the determination of carcinogenic constituents. The various physical and chemical laboratory methods are described, and results are given of applying these methods to varied petroleum products.

Since Rocky Flats operations require use of large quantities of No. 6 fuel oil, it is important to recognize that certain constituents may be carcinogenic. In general, the total carcinogenic activity of any petroleum product may be due to the combined effect of several individually weak carcinogens rather than an abundance of a particularly potent individual carcinogen such as 3,4-benzopyrene.

³CHEMCON is the nationwide interactive retrieval service offered by System Development Corporation, Santa Monica, California.

PART I

Laboratory Methods of Analysis

Nonbiological laboratory methods for the separation and measurement of these polynuclear aromatic hydrocarbon constituents include refractometry of chromatographic fractions, ultraviolet absorption characteristics, extraction with aqueous caffeine solutions, reactions with maleic anhydride and various oxidizing agents, silica-gel column chromatography separations, polarographic reduction, ultraviolet spectrophotometry, fluorescence spectrometry, complex formation with acridine, thin layer chromatography, gel permeation chromatography, and mass spectrometry.

The articles cover chemical analyses and physical tests based on properties of known carcinogenic constituents of residual fuel oils and related high-boiling petroleum products. Included are articles relating to the characterization of polycyclic aromatic hydrocarbons that are known carcinogenic constituents.

1. G. G. Wanless, L. T. Eby, and J. Rehner, Jr. "Properties of High Boiling Petroleum Products. Physical and Chemical Characterization Studies with Relation to Polynuclear Aromatic Components." *Analytical Chemistry* 23:563. April 1951.

The paper covers a variety of physical and chemical studies that have been carried out in search of improved methods for characterizing the polynuclear aromatic components of high-boiling petroleum products. The authors maintain that the usual measures of petroleum aromaticity—based on simple determinations of refractive index, aniline point, density, boiling point, and combinations of these properties—do not meet the then current needs. The purpose was not to undertake the Herculean task of unambiguously determining all of the classes of compounds known or expected to occur in high-boiling refinery products, but rather to explore new and more complex methods for characterizing the

aromaticity for process and product evaluation. The investigations include the following areas: refractometry of chromatographic fractions, ultraviolet absorption characteristics, behavior in high frequency fields (abandoned as unpromising), extraction with aqueous caffeine solutions, reactions with maleic anhydride, and reactions with various oxidizing agents. (21 references pertaining to analytical methods for characterizing aromatic compounds.)

2. L. T. Eby. "Properties of High Boiling Petroleum Products. High Aromatics Characterization by Chromatography." *Analytical Chemistry* 25:1057. July 1953.

A rapid, column chromatographic separation method was developed for characterizing the aromatic and other fractions of petroleum products boiling above 316 °C (600 °F) for the purpose of predicting carcinogenic potencies. The procedure employs an elution method with silica gel adsorbent and three selective solvents (n-hexane, benzene, and pyridine) and allows nonaromatic, aromatic, and oxy fractions to be obtained. Typical results are described for 63 refinery products including 14 bunker fuel oils. Data obtained by this procedure have been found useful in various processing studies involving high-boiling petroleum products. (21 references pertaining to chromatographic procedures for the analysis of petroleum products.)

3. L. T. Eby, W. Priestley, Jr., J. Rehner, Jr., and M. E. Hall. "Properties of High-Boiling Petroleum Products. Nonbiological Laboratory Methods for Predicting Carcinogenicity." *Analytical Chemistry* 25:1500. October 1953.

The paper is the first to describe methods for predicting carcinogenic activity of petroleum products other than pure aromatic compounds or highly specialized types of petroleum products. After examining a number of different reactions and properties of polycyclic aromatic compounds, the authors have

developed five nonbiological methods for the prediction of tumor potency of high-boiling petroleum products. The methods include two ultraviolet spectrophotometry determinations, caffeine number determination, high aromatics characterization by refractive index determination, and polarographic reduction. The authors examined 126 high-boiling petroleum products (9 bunker fuel oils) by all five methods and found that each method gives about the same degree of correlation with biological data. They conclude that a choice of laboratory methods is available, depending upon the particular types of petroleum products to be analyzed and the available facilities. (19 references pertaining to reactions of polycyclic aromatic hydrocarbons reactions with various laboratory reagents and other laboratory tests.)

4. Y. Hirshberg. "Full Luminescence at Liquid-Air Temperature of Methyl-1,2-benzanthracenes and Methylbenzo[c]phenanthrenes." *Analytical Chemistry* 28:1954. December 1956.

The afterglow (luminescence) of the fluorescence spectra of most carcinogenic compounds of the benzantracene series is specifically different from that of all the other members of these series. This important finding has not been mentioned by other investigators although they have discussed the correlation between biological activity and the chemical and physical properties of these series. (12 references to fluorescence spectrographic analysis of polycyclic aromatics.)

5. A. Szent-Gyorgyi and J. McLaughlin. "Reaction of Carcinogens with Acridine." *Proceedings of the National Academy of Sciences of the United States* 47:1397. August 1961.

Chloroform solutions of acridine form complexes with classical carcinogens (including methylcholanthrene, 3,4-benzopyrene, 9,10-dimethylbenzantracene, and

4-dimethylaminostilbene) which give a strong yellow color when dried on a filter paper.

6. H. Woggon and D. Juhle. "Determination of Carcinogenic Hydrocarbons in Paraffins and Waxes by Thin-Film Chromatography." *Plaste und Kautschuk* 13:460. August 1966.

Two thin-layer chromatographic (TLC) methods are described for determining certain carcinogenic aromatic hydrocarbons present in paraffin and microcrystalline waxes. The first method involves dissolving the sample in cyclohexane, extracting with nitromethane, separating the concentrated solution on a N,N-dimethylformamide-impregnated silica gel paper, and determining the fluorescence in ultraviolet light. In the more-sensitive second method, the TLC medium is eluted with 3:1 methylcyclohexane:cyclohexane. The R_f values, fluorescence data, and detection limits are given for various carcinogenic compounds.

7. C. J. Robinson. "Low-Resolution Mass Spectrometric Determination of Aromatics and Saturates in Petroleum Fractions." *Analytical Chemistry* 43:1425. September 1971.

The paper describes a new mass spectrometric procedure for determining up to 25 saturated and aromatic compound types in petroleum fractions. The procedure eliminates the need for a variety of methods to cover wide ranges of boiling points and composition, and a base-line technique to resolve the mass spectrum into saturates and aromatics spectra permits analysis of any sample boiling within the range of 93 to 593 °C (200 to 1100 °F) without need for physical separation or a high-resolution mass spectrum. The entire composition is accounted for in terms of 4 saturated hydrocarbon types, 12 aromatic types, 3 thiopheno types, and 6 unidentified aromatic groups. This mass spectrometric procedure gives reasonable and consistent

results on a wide variety of petroleum samples. (10 references including the author's earlier work and other high-resolution mass spectrometric methods.)

8. J. F. McKay and D. R. Latham. "Polyaromatic Hydrocarbons in High-Boiling Petroleum Distillates. Isolation by Gel Permeation Chromatography and Identification by Fluorescence Spectrometry." *Analytical Chemistry* 45:1050. June 1973.

The authors report the identification of 7 polyaromatic hydrocarbons not previously found in virgin petroleum in addition to 6 polyaromatic ring systems known to be constituents of petroleum. These condensed aromatic hydrocarbons were isolated from a 335 to 550 °C (635 to 1022 °F) petroleum distillate by a separation scheme involving ion-exchange chromatography, gel permeation chromatography, and thin-layer chromatography, and were identified by fluorescence spectroscopy. Gel permeation chromatography separated the pericondensed aromatic ring systems from catacondensed ring systems and other components of an acid concentrate. The isolated compounds were present in trace quantities in the acid concentrate, but may represent a significant percentage of the total crude oil. (17 references to investigations of polyaromatic ring systems.)

9. H. J. Coleman, J. E. Dooley, D. E. Hirsch, and C. J. Thompson. "Compositional Studies of a High-Boiling 370-535 °C Distillate from Prudhoe Bay, Alaska, Crude Oil." *Analytical Chemistry* 45:1724. August 1973.

The paper presents a comprehensive analytical characterization of a high-boiling 370 to 535 °C (698 to 995 °F) petroleum distillate. The methods involved several distillation steps, separation procedures with ion-exchange resins, coordination-complex formation of neutral nitrogen compounds with ferric chloride, dual silica-alumina gel adsorption, and gel permeation chromatography. The aromatic portion, which constituted 45.5 weight percent of the distillate, was divided up into

monoaromatic, diaromatic, and polyaromatic-polar concentrates and subjected to nuclear magnetic resonance spectroscopy and mass spectrometry to further determine the number of aromatic rings, relative aromaticity, and the degree of condensation of aromatic, naphthene, and heterocyclic rings. The end result of this investigation is a scheme of sample preparation, analysis, and characterization that should be applicable to similar high-boiling distillates from other crude oils and of value to the petroleum industry in studies related to the origin of petroleum as well as its production, processing, storage, and usability. (18 references to crude oil analyses.)

10. A. H. Qazi, C. A. Nau, H. Turnex, and G. T. Taylor. "Identification of Carcinogenic and Noncarcinogenic Polycyclic Aromatic Hydrocarbons through Computer Programming." *American Industrial Hygiene Journal* 1973:555. December 1973.

A computer program has been developed for the rapid screening and identification of carcinogenic and noncarcinogenic polycyclic aromatic hydrocarbons. The program is based on the ultraviolet absorption spectra for these hydrocarbons, utilizes 3 classification schemes with tolerance limits of 1, 2, and 5 nanometres, and lists 10 possibilities at each tolerance level in decreasing order of probability. The program allows for screening and identification of a large number of polycyclic aromatic hydrocarbons in a matter of minutes and saves time and energy required in visual comparison of the unknown spectra with known spectra of more than 250 compounds. (11 references to carcinogenicity and origin of carcinogens.)

PART II

Analysis Results

Using the laboratory techniques described in Part I and others, the petroleum products have been

examined for carcinogenic constituents. The carcinogen 3,4-benzopyrene, for example, has been detected in Colorado shale oil and in fuel oil fractions from Ukrainian petroleum but is absent in heavy fuel oil residues derived from the distillation of Russian crude oil and coked coal. Some compounds isolated from petroleum fractions are members of families known to be potent carcinogens.

The articles include the results of testing residual fuel oils and related high-boiling petroleum products for carcinogenic constituents.

1. C. C. Twort and J. M. Twort. "The Carcinogenic Potency of Mineral Oils." *Journal of Industrial Hygiene* 13:204. 1931.

Although this investigation relates mainly to biological mice-painting studies, the authors recognized that the cancer-producing properties of oils are related to fluorescence, i.e., oils having a blue fluorescence seem more active than those having a green fluorescence. The study is frequently cited as a historical reference in later papers reporting ultraviolet and fluorescence spectrophotometry data.

2. J. M. Twort and R. Lyth. "The Concentration of Carcinogenic Materials in Material Oils by Distillation Processes." *Journal of Hygiene* 39:161. 1939.

Carcinogenic activity of distillates from a crude Borneo mineral oil was predicted from an examination of the physical properties which were alone sufficient to condemn their use as lubricants when likely to come into contact with the user. Carcinogenic activity was confirmed by biological testing.

3. H. J. Cahnmann. "Detection and Quantitative Determination of Benzo[a]pyrene in American Shale Oil." *Analytical Chemistry* 27:1235. August 1955.

The detection and quantitative determination of benzo[a]pyrene in a sample of Colorado shale oil is described. The methods used are distillation followed by chromatographic fractionation and ultraviolet and fluorescence spectrophotometric investigation of the chromatographic fractions. This work is the first systematic investigation of shale oil's carcinogenic constituents; the investigated shale oil sample contained between 0.003 and 0.004 weight percent benzo[a]pyrene. (37 references.)

4. V. Masek. "3,4-Benzopyrene and the Addition of Fuel Oil to Coal during Coking." *Paliva* 41:333. 1961.

Heavy fuel oil residues from the distillation of Russian crude oil and from coked coal do not contain carcinogenic 3,4-benzopyrene in measurable amounts. Method of determination was not given in the abstract.

5. G. G. Kikodze, T. A. Eliava, A. A. Dzamukashvili, and M. A. Machabeli. "Study of the Carcinogenic Properties of High Molecular Weight Aromatic Hydrocarbons from Norii Petroleum by a Color Reaction with Acridine." *Soobshcheniya Akademii Nauk Gruzinski SSR (Bulletin of the Academy of Sciences of the Georgian Soviet Socialist Republics)* 45:595. March 1967.

None of the Norii petroleum sampled fractions yielded a positive Szent-Gyorgyi acridine reaction, indicating that they may not possess carcinogenic properties.

6. W. Carruthers, J. F. Cook, A. G. Douglas, H. N. M. Stewart, and D. A. M. Watkins. "Carcinogenic Action of Mineral Oils. Chemical Studies." *Medical Research Council (Great Britain) Special Report Series No. 306*, 45. 1968.

Three crude oil fractions in the distillation range 350 to 390 °C (662 to 734 °F) contained the bulk of the carcinogenic

compounds identified. A large number of compounds not previously recognized as mineral oil constituents were isolated:

1,2-benzanthracene derivatives, 4-methyl- and 7-methylbenzanthracene, 6,7-dimethylbenzanthracene, crystalline mixtures of alkylchrysenes, and a large number of sulfur compounds.

oil from Russian petroleum stock) indicates about 75 compounds, including 34 hydrocarbons, were present. The main groups included derivatives of naphthalene, pyrene, perylene, acenaphthalene, and diphenyl. A few of the other compounds identified (acridines, phenols, quinones) contribute towards establishing relations between the type of combustible matter and the method of combustion.

7. N. Y. Rudakova, T. A. Kvyatkovskaya, E. A. Germash, A. P. Lizogub, R. D. Novoded, and V. A. Soroka. "Ukrainian Petroleums Studied in Order to Determine 3,4-Benzopyrene in Them." *Neftepererabotka i Neftekhimiya (Kiev) (Petroleum Refining and Petrochemistry)* 1969:51. March 1969.

The carcinogen 3,4-benzopyrene was determined in fuel oil fractions from Ukrainian petroleums. The method involved distilling the samples into 10-degree fractions, separating the fractions via silica gel column chromatography according to refractive index ranges, and further dividing these fractions by thin-layer chromatography.

8. T. S. Bruevich, A. Y. Khesina, and I. A. Ogloblina. "Carcinogenic Activity of Petroleum-Refining Products." *Gigiena Truda i Professional'nye Zabolevaniya (Labor Hygiene and Occupational Diseases)* 13:53. March 1969.

Petroleum-refining products were analyzed for 3,4-benzopyrene by a spectral luminescence method. Polycyclic hydrocarbons were generated from oils at relatively low temperatures [300 °C (572 °F)], and high concentrations of benzopyrene were observed in several of the petroleums investigated.

9. M. Chiagneau, L. Giry, and L. P. Richard. "Analytical Investigation of some Types of Soot Using Mass Spectrometry." *Chimie Analytique (Paris)* 51:187. April 1969.

A mass spectrometric characterization of soot derived from combusting mazut (residual fuel

10. P. J. King. "Carcinogenic Action of Mineral Oils." *Industrial Lubrication and Tribology* 51:187. August 1969.

Crude oils were fractionated, analyzed, and tested biologically. The carcinogenic materials boiled in fractions above 350 °C (662 °F) in oils with mixed asphaltic-paraffinic, naphthenic, and paraffinic bases. By the processes of chromatography, complexing with picric acid and trinitrobenzene, and fractional crystallization, more than 40 compounds were isolated from mineral oil. Several of these compounds were members of families known to be potent carcinogens. The total carcinogenic activity of the oil may be due to the combined effect of several individually weak carcinogens: 9,10-dimethylanthracene; 1,2-benzanthracene; 7-methyl-1,2-benzanthracene; and 1,3,6,7-tetramethyldibenzthiophene. An appendix listing precautions is included.

11. J. Mohay, M. Gotz, J. Mohay-Farkas. "Detection of Carcinogenic Substances in Hydrocarbons." *Acta Pharmaceutica Hungarica* 41:20. January 1971.

Thin-layer chromatographic analysis of solid paraffin, yellow petrolatum, and gasoline indicates the presence of carcinogenic polycyclic aromatic hydrocarbons. The highest concentrations were isolated from the yellow petrolatum: benzo[a]pyrene, 200 to 300 micrograms per kilogram (µg/kg); 1,2,5,6-dibenzanthracene, 300 µg/kg; perylene, 100 µg/kg; and 1,2-benzopyrene, 50 µg/kg.

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