INTERIM REPORT

API RESEARCH PROJECT MC-1

SEPTEMBER 26, 1957

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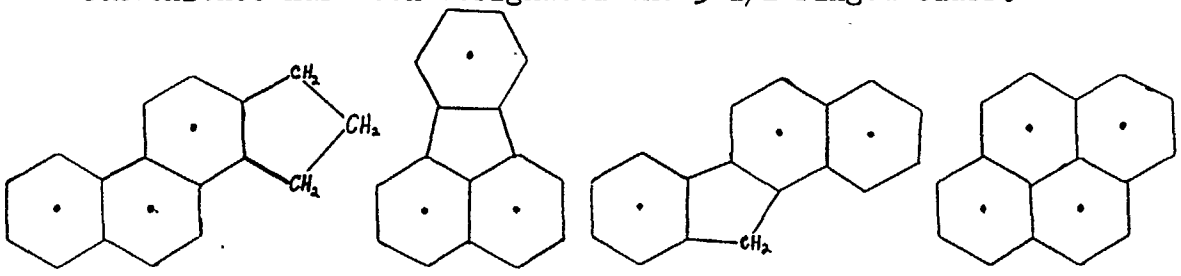
Current experimental work is being directed entirely towards the second of the objectives of this project, which were reviewed in detail in the major progress report submitted April 10, 1957. This particular phase is the development of an analytical method whereby the carcinogenic capacity of various oils can be detected and, ultimately, assessed quantitatively by physicochemical procedures.

As indicated in the previous report, the specific experimental efforts during 1957-58 are centered in the following areas:

1. The characterization of the most volatile class of carcinogens in petroleum oils.

This group is based structurally upon an aromatic nucleus of 16 or 17 carbon atoms, e.g. cyclopentanophenanthrene I, fluoranthene II, benzofluorene III, or pyrene IV, and for convenience has been designated the 3 1/2-ringed class.

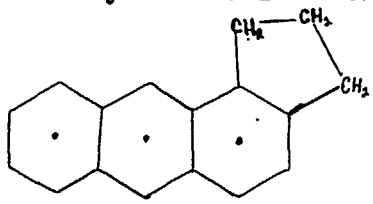


I II III IV

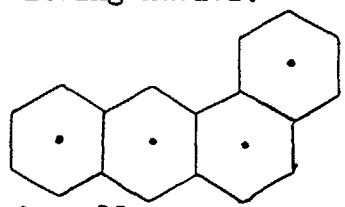
There is evidence that almost all petroleum oils that are carcinogenic contain significant quantities of this group and, further, that in some important distillate oils the 3 1/2 ringed group is the only type of carcinogen present.

2. The determination of the part of the basic potency of an oil that is contributed by carcinogens that may be extracted by reaction with maleic anhydride.

In general, these compounds are derivatives of anthracene, but it should be noted that the experimental evidence indicates that, structurally, a fourth ring is required if a significant contribution to the potency of a petroleum oil is to be effected. The carcinogens in this class are probably derivatives of the following nuclei:



cyclopentanoanthracene


benz [a] anthracene

3. The accelerating activity of the main body of the oil, with particular emphasis on the tendency of certain non-accelerating components to counteract the effect of the long-chain accelerators.

Since the period of exposure to the average high-boiling oil required for the development of cancer of the skin depends so heavily upon the accelerating activity of the oil (the onset of the disease may be brought about by a

strongly accelerating oil in as little as one-quarter of the time which would be required if it were non-accelerating), the ability to relate this activity to composition will be essential if the goal of a generally applicable analytical method for estimating potency is to be reached. An important lead developed from experiments in which the active accelerators, n-dodecane and t-dodecylbenzene, were diluted with various liquid hydrocarbons and the blends produced then tested for their accelerating activity. It was found that either of the two white oils used as diluents were unusually effective in reducing the accelerating activity to a relatively low level. Mass spectrometric analysis of these oils and of various wax distillates and lubricants led to the tentative assignment of this inhibiting property to the condensed polycycloparaffins with three or more rings. (This assignment is strengthened by the published findings that the induction of sarcomas by methylcholanthrene in ethyl laurate is inhibited partially by inclusion of hydrogenated derivatives of methylcholanthrene in the solution injected).

During the five month period since the issuance of the previous report, methods have been perfected for the separation of the various 3 1/2 ringed classes of aromatic hydrocarbons (see appendix). These methods have been applied to the most volatile of the distillate fractions having significant carcinogenic potency from the catalytically cracked oil, API-8.

Biological tests of a preliminary series of chromatographic fractions (Table 1 of the Appendix) demonstrated that the major part of the potency in this boiling range, 730 - 760° F., is contributed by fractions in which cyclopentanophenanthrenes, fluoranthenes, and pyrenes are the dominant factors. These classes have now been separated from each other to a large extent by a more efficient fractionation and are being subjected to biological test. (In each case a series of concentrations in benzene is being tested in order to establish at the same time the shape of the dose-response curve for the potent class).

Less emphasis is being given to the 4-ringed class of carcinogens, since it appears that the only type of oil in which this class makes a major contribution to the carcinogenic potency of the whole is the recycle gas oil from coking operations. Two tests in this area, which were reported as incomplete in April, are now nearing completion. These involved the fractions extracted by maleic anhydride from a feed stock, API-44, and the tar produced, API-45, in a thermal cracking operation (API-44 was the product of catalytic cracking.) The preliminary results show that the specific activity of the fraction from API-45 is not appreciably less than that of the fraction from API-44. Since the latter contained less of this fraction and also a lesser amount of benzopyrenes, its greater potency must be attributed either to other classes of carcinogens, such as the 3 1/2 ringed class, or to accelerators.

Current work on the non-carcinogenic bulk of petroleum oils, which in many cases contributes so importantly to the speed with which tumors can be induced, is largely chemical in nature. By a combination of several procedures of fractionation, including formation of adducts with thiourea, distillation, and liquid thermal diffusion (or alternately the use of molecular sieves), the various classes of saturated hydrocarbons (and the napthenoaromatics with 1 benzene ring) from certain oils, especially the technical white oil, API-80, are being separated for subsequent biological test as inhibitors of acceleration. Analyses will be handled primarily by mass spectrometry (fragment peak analysis). A suitable instrument has been designed for our purposes by the Consolidated Electrodynamics Corporation and should be completed in December of this year.

APPENDIX

The use of cold concentrated sulfuric acid for the separation of the 3 1/2 ringed aromatic hydrocarbons

The earlier use of the technique of partitioning mixtures of polycyclic aromatic hydrocarbons between concentrated (90 - 100 °/₀) sulfuric acid and isooctane at 5-10° C. resulted in losses due to oxidation and sulfonation. Since in the current application of this fractionation procedure it was essential to avoid these losses because of the possibility of selective loss of carcinogens, the use of lower temperatures was investigated. The concentration of the acid was maintained as high (95 - 96 °/₀) as was practicable with respect to solidification at the temperatures selected, -10 to -30°F (at the lower end of this range the acid was essentially a supercooled liquid). Under these experimental conditions a satisfactory separation of the pyrenes from the fluoranthenes and phenanthenes was accomplished without measurable loss. In this particular operation a small fraction of the fluoranthenes accompanied the pyrenes into the acid extract. However, it was found that most of this contamination of the pyrenes could be eliminated by subsequent chromatography on activated alumina.

TABLE 1

Experiments to Determine the Relative Content of Carcinogens in a Series of Chromatographic Fractions from the Most Volatile of the Carcinogenic Distillates from the Catalytically Cracked Oil, API-8

API Experiment Number [1]	Fraction From Chromatography (% Eluted)	Concentration of Fraction in Solution Tested (%)	Solvent	Schedule of Applications (no./week - mg.)	Mean Time of Appearance of Tumors (weeks)	Content of Carcinogens,[2] C_{rc}
8-26-7	26-64	33.4	Benzene	3-15	35.9	.07
8-26-8	64-80	32.6	Benzene	3-15	22.4	.13
8-26-9	80-85	9.6	Benzene	3-15	23.8	.08
8-26-10	85-88	6.2	Benzene	3-15	28.5	.05
8-26-11	88-91	4.1	Dodecane	3-50	25.0	.01
8-26-12	91-93	3.8	Benzene	3-15	30.4	.04
8-26-14	93-94	0.9	Dodecane	3-50	-	.00
8-26-15	94-100	10.4	Dodecane	3-50	-	.00
						.38
8-26-1	Distillate	50	Benzene	3-10	34.1	.05
8-26-m	"	50	Dodecylbenzene	3-40	12.7	.15
8-26-n	"	50	Benzene	3-10	21.4	.21
						.41
8-26-n-1[3]		2	50% Benzene 50% Dodecylbenzene	3-50	23.5	.03

[1] The major components of fractions 7 through 10 were pyrenes, phenanthrenes, and fluoranthenes. Benzofluorenes occurred in fractions 10 through 14 and 4-ringed hydrocarbons, such as chrysene, in fractions 12 and 14. Fraction 15 was composed of non-hydrocarbons.

[2] In units of the "reference carcinogen".

[3] Residue removed from distillate 8-26-n prior to chromatography

TABLE OF CONTENTS OF REPORT TO BE PREPARED
FOR DISTRIBUTION UPON COMPLETION OF THE TECHNICAL
PHASE OF API RESEARCH PROJECT MC-1
(PROJECTED DATE OF COMPLETION, JUNE 30, 1959)

A. Introduction

1. Background
2. Objectives
   a. Pinpointing of carcinogenic oils in refining operations.
   b. Determination of the relative potency of these oils for the skin of mice.
   c. Experimental estimate of efficacy of washing procedures for removal of oils from the skin of mice.
   d. Development of analytical tools for the estimation of the relative potency of any refinery stream.

B. Materials

1. Intermediates and products of refining operations.
   a. List of materials considered for investigation.
   b. Methods of selection of samples for test.
2. Reference blends of pure compounds.
3. Fractions of carcinogenic oils

C. Biological Methods

1. Development of biological methods for quantitative estimation of relative carcinogenic potencies of complex materials; reference, Cancer Research, 15, 701 (1955).

a. Significance of rate of induction of tumors.

b. Influence of intercurrent disease among mice.

c. Use of reference standards and definition of Relative Carcinogenic Potency, P_{MC} and P_{rc}.

2. Method of estimation of relative content of carcinogens in oils (in units of the reference carcinogen).

3. Determination of relative accelerating activity of pure compounds and fractions of oils.

4. Measurement of preconditioning and promoting effects of accelerators.

5. Methods used to test various washing procedures.

D. Physicochemical Methods

1. Fractionation procedures

a. Methods of concentrating carcinogens. Reference - isolation of benzopyrene from catalytically cracked oil, Anal. Chem., 27, 248 (1955).

b. Methods of concentrating accelerators. Reference - Cancer Research (Sep. 1957).

c. Methods of concentrating inhibitors of acceleration.

2. Analytical procedures

a. Absorption spectrophotometry

(1) Use of band at 430 mμ.

(2) Special applications of difference spectra. Reference - Analysis for benzopyrene, Anal. Chem., 27, 248 (1955).

(3) Solvent effects - application in qualitative determination of nitrogen compounds.

b. High mass spectrometry

(1) Parent peak analysis of low voltage spectra for aromatic carcinogens.

(2) Fragment peak analysis for inhibitors of acceleration.

3. Combination of procedures to estimate the effective carcinogenic potency of petroleum intermediates and products.

E. Results

1. Relative carcinogenic potencies of various cracked and uncracked oils for the skin of mice.

a. Dependence upon area of skin exposed when oil contains accelerators.

b. Comparison of the experimental potencies of waxy distillates and lubricants for the skin of mice, with published epidemiologic information on cancer of the skin of men subjected to contact with similar materials.

c. Comparison of experimental results on certain lubricating distillates with those reported from England.

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of the individual oils.

3. Comparison of biological potencies of various oils with those calculated from analytical data.
4. Relative activities of various pure compounds and combinations thereof.
   a. Relative potencies of polycyclic aromatic hydrocarbons as carcinogens.
   b. Summation of potencies in mixtures of aromatic carcinogens.
   c. Relative accelerating activity of liquid hydrocarbons - reference, Cancer Research, September 1957.
   d. Relative ability of various hydrocarbons to counteract acceleration.
5. Retardation of rate of induction of tumors by use of soap or detergent and water.
   a. Relation of efficiency of washing to frequency of contact with the skin.
   b. Deleterious effects of rinsing contaminated skin with solvents containing accelerators prior to washing with detergent and water.
6. Effect of contact of the skin of mice with accelerators prior to application of carcinogenic oils or solutions.
   a. Dependency upon composition of carcinogenic material.
7. Effect of contact of the skin of mice with accelerators subsequent to a single application of a strong carcinogen.

8. Increase in carcinogenic potency of raw lubricating stocks under the influence of added alkylpolystyrene (viscosity improver).

F. Discussion

1. On the value of information on the carcinogenic potency of oils for the skin of C3H mice in estimating the hazard of the development of occupational cancer of the skin of men.
   a. The relative rate of onset of cancer of the skin of wax pressmen and "mule spinners" as compared with the rate exhibited by C3H mice subjected to contact with the different types of oils involved in these occupations.
   b. The lack of carcinogenic activity for C3H mice of highly refined oils traditionally used in cosmetics and as laxatives.
2. Apparent differences in the susceptibility of mice and rabbits to lubricating oils (as reported by British investigators.)
   a. Importance of experimental conditions that do not interfere with normal growth and survival of mice.
   b. Potential difficulties resulting from the toxicity of alkylbenzenes and naphthalenes for mice.

3. General relationships between the composition of complex mixtures such as petroleum oils and the relative rate of induction of skin tumors under various conditions of exposure.
   a. Basic stimulus by polycyclic carcinogens
   b. Postulated role of accelerators in growth of latent cancer.
   c. Classes of compounds in petroleum oils that accelerate the gross appearance of cancer.
   d. Classes of compounds that counteract the effects of long-chain accelerators.
4. The shift in the relative importance of accelerators and carcinogens in high boiling oils as they are converted from virgin oils to recycle gas oils and slurry oils or residual tars in cracking operations.
5. The influence of refining practices in lubricant manufacture on composition, as the latter relates to carcinogenic potency.
6. Consideration of any cases of oils the actual biologic potency of which is significantly different from that calculated from analytical data.

G. Summary
   1. Extent to which objectives have been fulfilled.
   2. Problems requiring further elucidation.
   3. Specific contributions of the program to a more basic understanding of the mechanism of the chemical induction of cancer.

H. Bibliography

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Date: September 26, 1957