

SUMMARY OF INVESTIGATIONS SPONSORED BY
THE COMMITTEE ON THE CARCINOGENIC ACTION
OF MINERAL OILS OF THE MEDICAL RESEARCH
COUNCIL OF GREAT BRITAIN IN COOPERATION
WITH THE INSTITUTE OF PETROLEUM

for the

API Medical Advisory Committee

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THE KETTERING LABORATORY

in the

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College of Medicine

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SUMMARY OF INVESTIGATIONS SPONSORED BY THE COMMITTEE ON
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This investigation was undertaken because of the epidemiological indication, verified by animal experiments, that contact with certain lubricating and cutting oils or with unrefined paraffin wax could cause skin cancer (e.g., mule-spinners' cancer). Since the oils involved in these problems were derived from uncracked stocks, a fundamental investigation of the activity of crude petroleum and of the identity and properties of its carcinogenic constituents was desirable.

Three crude oils from Kuwait, Lagunillas (Venezuela), and Oklahoma (U.S.A.), were fractionated by distillation (the distillations were carried out with special precautions to prevent cracking). From each crude, four distilled fractions and the residuum were investigated for their carcinogenicity upon the skin of rabbits and of mice. Since relatively few tumors could be produced by the fractions in the stock mice used, most of the results to be discussed were obtained from the tests on rabbits.

In all cases the highest-boiling distillates (660-770°F.) were the most potent. The potencies of the whole crude, of the more volatile fractions, and of the distillation residues, were insignificant. Details are

given in Appendix 2. (The results of a test on the residue L-5 from Lagunillas crude at the Kettering Laboratory indicate that it contains almost as much carcinogen as the very potent distillate L-4, the difference in potency being due to the catalytic activity of L-4.)

The potency of fractions of the Oklahoma Bright (O) oil was found to be much lower than that of corresponding fractions of the Kuwait (K) and the Lagunillas (L) oils; for this reason O has not been studied as intensively as the others.

Further subdivision of each of the two most potent distillates, one from K and one from L (the latter has also been tested on C3H mice as API-111-4), was carried on by vacuum fractionation through a Stedman Distillation Column into sixteen narrow cuts and a residue. Most of these subfractions proved to be about equally potent. Their ultraviolet spectra were likewise virtually identical.

Chemical separations by use of maleic anhydride and picric acid (discussed in detail in Appendix 3) were carried out on the fractions from Kuwait crude. Relatively small proportions of the carcinogens of the Kuwait distillates were removed by reaction with maleic anhydride. The subsequent separations with picric acid also tended to leave the major portions of the carcinogens in the residue, although this was not always the case.

A variety of pure compounds have been isolated and many of them identified. The ring systems involved include anthracene, phenanthrene, fluorene, benzo(c)fluorene, pyrene, carbazole, dibenzothiophene, and naphtho(1,2-b)thiophene. Details about the compounds isolated are given in Appendix 3.

APPENDIX 1

Symbols Used in Nomenclature of Fractions

- L - Lagunillas (L-1 through L-5, cuts from original distillations)
- K - Kuwait (K-1 through K-5)
- O - Oklahoma (O-1 through O-5)
- S - Stedman Distillations of L-4 and K-4 (S1a, S1b, S2a.... through S8b were narrow cuts ($4\frac{1}{2}^{\circ}$) collected in the range 662-734^o)
- A - Aromatic extract (aqueous acetone) from Stedman distillates
- P - Non-extract (aqueous acetone) from Stedman distillates
- m - Compounds extracted from A by maleic anhydride (anthracenes, etc.)
- p - Compounds extracted by picric acid from fraction which did not react with maleic anhydride
- r - residue not extracted by picric acid.
- pi - piv - Fractions from chromatography of p (eluants: petroleum ether, benzene, methanol)

For example, K4S7bAp111 stands for the 3rd chromatographic fraction obtained from the picrate-forming compounds (non-anthracenic) extracted by aqueous acetone from the Kuwait distillate, obtained from the Stedman column at an overhead temperature of 720.5-725° (corrected from the observed temperature during vacuum distillation). The charge to the Stedman column was the 4th overhead fraction obtained in the original large scale distillation.

APPENDIX 2

Carcinogenic Potencies of the Crudes and of Various Fractions

The two species of animals upon which the materials were tested, mice and rabbits, behaved quite differently. The former gave high tumor yields with solutions of the known carcinogens dimethylbenzanthracene and methylcholanthrene, but the oils and fractions tested produced either no tumors or such a low number that no significant information as to the relative potency of the fractions could be obtained. Therefore, the statistician evaluating the results, Dr. J. O. Irwin, has recently questioned further use of these stock mice. The rabbits, on the other hand, gave high tumor yields both with the standards and with many of the fractions.

Differences in the strains of animals used, and variations in their general health, must be held responsible for the fact that one of the fractions (L-4) (which in British tests over the period of one year had produced only three tumors among fifty mice), when tested as API-111-4 at the Kettering Laboratory on C3H mice, yielded fifteen tumors among nineteen animals, of which the last tumor-free survivor developed a tumor by the 28th week.

The results of the tests on the rabbits are expressed in terms of the probability that a given animal would develop a tumor in one year, on being painted twice weekly for this period.

The crudes, lower boiling fractions, and the distillation residues showed a potential tumor-producing

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ability ranging from zero to 6 per cent. The higher boiling fractions from Oklahoma crude had potencies only in the range of 1 to 6 per cent, whereas those from Kuwait and Lagunillas were more potent, varying from 5 to 47 per cent. (K-3 distilled mostly between 578 and 707°F., K-4 between 653 and 752°, L-3 between 574 and 738°, and L-4 between 640 and 752°.) The results obtained in duplicate tests show an unfortunately wide scatter; the relative potencies of corresponding fractions of K and L are inverted in the two series.

	<u>K-3</u>	<u>K-4</u>	<u>L-3</u>	<u>L-4</u>
Series 1	9%	26%	32%	47%
Series 2	5%	17%	5%	10%

Since in all cases, fraction 4 was more potent than fraction 3 from the same crude, further work was concentrated on K-4 and L-4.

Further fractional distillation (Stedman column) and biological testing showed that K-4S2 to K-4S8 were more potent than K-4S1, and, more strikingly, that L-4S2 to L-4S8 were more potent (57 to 64%) than L-4S1 (30%). No secure differentiation between the fractions 2 to 8 emerged.

The separation into an aromatic extract (A) and a paraffinic raffinate (P) by solvent extraction with aqueous acetone was, predictably, not a sharp one. Therefore, the fact that K-4SP, a blend of equal volumes of K-4S1P to K-4S8P,

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had a potency of 8% may be taken in stride, but the potency of 29% found for L-4SP raises some questions. The reviewer suggests that either one or more of the extractions was not as effective as supposed, or else that the non-aromatics of Lagunillas crude are accelerators of very high activity. The experience at the Kettering Laboratory with a 650 to 690° fraction (distilled from L-4) lends some weight to the second alternative. This fraction was non-carcinogenic to C3H mice per se, but was among the strongest accelerators for added carcinogens found so far.

In the separation of the Kuwait aromatics into m, p, and r subfractions, p and r were generally potent, the r fractions usually exceeding the corresponding p fractions in potency. On the other hand, the m fractions from distillates below 710° were non-potent. Slight potency (3 to 8%) was shown by these obtained from higher boiling distillates. As pointed out in Appendix 3 the latter yielded tri- and tetramethyl-anthracenes, among them one with apparently a meso substituent (9- or 10- position). This is perhaps the closest approach of the British investigation to the identification of a carcinogen from a crude, since 9,10-dimethylanthracene has elsewhere been reported to be a weak carcinogen.

APPENDIX 3

Preparation of Fractions and Isolation of Pure Compounds From Kuwait Crude Oil

The main work was carried out with the Kuwait fractions as follows:

Each cut from the Stedman distillation, such as K-4S2a, was extracted with aqueous acetone. The aromatic extracts (A), thus separated from the mainly paraffinic raffinates (P), were reacted with maleic anhydride and the adducts decomposed to yield subfractions, m, consisting of derivatives of anthracene. The non-adducting material was then fractionated further by means of picric acid into p, compounds forming crystalline picrates, and r, residual fractions. Some work was done on the p fractions directly; in other cases they were further subdivided by chromatography over activated alumina into (i) petroleum ether eluates, (ii) benzene eluates, and (iii) methanol eluates; in some cases four fractions were taken by subdividing (i) into (i) with n_D below 1.5000 and (ii) with n_D above 1.5000; in this case the benzene and methanol eluates become, respectively, (iii) and (iv).

From the m fractions ten anthracenes were isolated, namely 2,6-dimethyl-, 2,7-dimethyl-, 2,3,6-trimethyl-, 2,3,6,7-tetramethyl-, 1,3,6,7-tetramethyl-, 1,3,5,7-tetramethyl-,

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and four unidentified C₁₇ or C₁₈ anthracenes (J. Chem. Soc., 1956, 603). The ultraviolet spectrum of one of these last four indicates the presence of a substituent in the 9- or 10-position; this may be of special significance since the only known carcinogenic anthracene is the 9,10-dimethyl- compound.

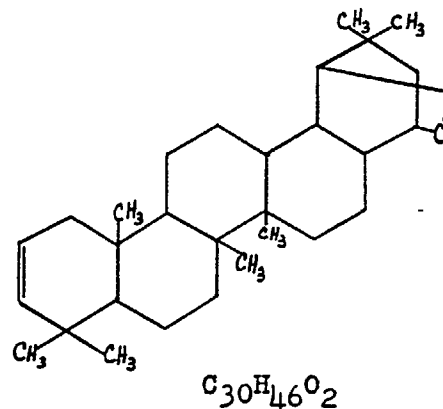
Three phenanthrenes were isolated from the picrate-forming fractions, 1,8-dimethyl-, 1,2,8-trimethyl-, and an unidentified tetramethyl-derivative. The trimethyl compound, found in relatively large amount, is a known dehydrogenation product of triterpenes and thus is suggestive evidence of a vegetable source for at least part of the crude. The following were also isolated via their picrates: an unidentified tetra- or pentamethylfluorene; benzo(c)fluorene, and its 8-methyl- derivative; 4-methylpyrene; two unidentified carbazoles, one a tetra- or pentamethyl-, the other a penta- or hexamethyl- derivative; four dibenzothiophenes, 4,6-dimethyl-, a trimethyl-, and two tetramethyl- derivatives, one of them either the 3,4,6,7- or the 2,3,6,7-compound; and a naphtho (1,2-b)thiophene, probably the 6,8-dimethyl-3-ethyl derivative (J. Chem Soc., 1957, 278). Where necessary the names have been changed to bring them into accord with the nomenclature of Chemical Abstracts.

Since the tetramethylantracenes were found only in the highest-boiling fractions, it is perhaps not surprising

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that compounds with higher boiling points, such as the 4-ringed chrysenes or benzantracenes, were not isolated from the Kuwait fractions studied.

Before these fractions from Kuwait crude became available, preliminary studies were made on two other materials. From the 518-527°F. cut of a Trinidad crude three trimethylnaphthalenes were isolated and two identified as the 1,2,8- and the 2,3,6-trimethyl derivatives (J. Chem. Soc., 1955, 1847). A commercial sulfur dioxide extract of the lubricating oil fraction of a West Beaumont crude yielded perylene, and two unidentified substances, one $C_{20}H_{28}O$, $C_{21}H_{30}O$, or $C_{22}H_{32}O$, apparently tetracyclic with one benzene and three alicyclic rings and the oxygen present as ether, the other $C_{25}H_{24}$ or $C_{26}H_{26}$, a chrysene derivative, apparently with one additional cyclopentane ring. An interesting compound, $C_{30}H_{46}O_2$, having the ring structure of typical plant triterpenes, was also isolated. The authors point out that the presence of this substance also indicates that at least a portion of this crude was of vegetable rather than of animal origin (J. Chem. Soc., 1954, 2047 and 1956, 788).



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