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RELATIONSHIPS BETWEEN THE COMPOSITION AND CARCINOGENIC PROPERTIES OF VARIOUS REFINERY STREAMS

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For a long time, cancer of the skin and particularly that of the scrotum, has been known to occur with relatively high frequency in association with certain occupations (1). The reported instances in which this problem has developed in the American petroleum industry have been rare (2). The majority resulted from exposure to uncracked waxy distillates during their filtration in hydraulic presses.

Historically, most of our information on occupational cancers of the skin has derived from Great Britain and Germany. Chemical research in these countries on active fractions of coal tar centered interest on polycyclic aromatic hydrocarbons. In parallel investigations, organic synthesis made available a large number of 3-6 ringed compounds, many of which proved to be carcinogenic for the skin of mice (3). Isolation of one of these, the 5-ringed benzo[a]pyrene from coal tar appeared to round out the picture (4). It was shown that polycyclic aromatic hydrocarbons were indeed present in each of the various industrial materials which had been associated with a significant increase in the incidence of cancer of the skin of the exposed workers(e.g.,5). Fractions of these materials free of the

3-6 ringed aromatic compounds, when applied upon the skin of mice, produced no tumors (6).

Because of the demands of the Air Force for high-octane fuels in World War II, the practice of catalytic cracking grew rapidly in American oil refineries to large proportions. Individual research programs of certain of the oil companies showed that the concentration of polycyclic aromatics was markedly higher in heavy catalytic gas oils than in any stocks previously handled in the refinery. Preliminary tests on the skin of mice confirmed the suspicion that the relative potencies of catalytically cracked residua might be much higher than that of straight run products or thermal tars. As a result the industry initiated API Research Project MC-1 at The Kettering Laboratory to investigate fully any potential hazards of cancer which might result from contact with these or other intermediates or products of petroleum refineries.

The major objectives of the experimental phases of this project are, 1. to determine the relative carcinogenic potencies of these oils and tars for the skin of mice, and 2. to develop chemical or physical procedures for estimating the relative carcinogenic activity of such intermediates and products. Other goals, such as the development of methods for removing oils from the skin after contact, will not be referred to again in this paper.

Technique of Bioassay

The biological testing involved the repeated application of the oil in question upon the skin of mice under selected conditions

with respect to dosage and frequency of application (7). Under suitable conditions the average period of exposure required to induce visible tumors furnished a satisfactory means of comparing the potencies of different oils. Examples of the average duration of tumor-free life in experiments of this type on selected refinery streams are shown in Tables 1 and 2. For convenience in thinking of the biological result in terms of the concentration of a standard carcinogen in a standard medium required to produce it, the corresponding values of the relative potency, P_{MC}, are shown. The potency, P_{MC}, is defined as that concentration of 3-methylcholanthrene in benzene which will produce tumors at the same rate as the oil in question under comparable experimental conditions. The value of P_{MC} for a given oil is related to the average expectancy of tumor-free life, e, by the following equation:

$$P_{MC} = \frac{1}{f}\left(\frac{8}{e-3} - 0.1\right),$$

where f is equal to the number of applications each week.

Composition vs Relative Carcinogenic Potency of Complex Oils

The initial approach to this problem, for reasons apparent from the introduction, was to examine the polycyclic fractions of various carcinogenic oils. It was soon apparent that there were numerous exceptions to any attempted correlations of relative biological activity with either total content of polycyclic aromatics or with the physical properties of selected fractions thereof. Parallel experiments involving solutions of pure carcinogens in solvents of lower volatility than benzene suggested a reason for this lack of correlation.

By the use of n-dodecane, dodecylbenzene and other solvents of similar molecular length, the rate of tumor production was accelerated to a level as high as that displayed by much more concentrated solutions in benzene (8). The attempt was made therefore to determine the amounts and types of both the carcinogens and the accelerators in the oils, and to measure their relative contributions to the carcinogenic potency of various refinery streams.

The following equation is being used as an expression of a tentative hypothesis to orient the collection of pertinent information:

$$\text{Relative potency, } P_{MC} = M_a(C_1 + C_2 + C_3),$$

where M_a is a multiplication factor due to the contribution of accelerators. This factor is not only a function of the concentration and activity of the accelerators but also a function of the concentration of the carcinogens and of the quantity of oil applied upon the skin (See Table 3). Obviously, the concentration of carcinogen may reach such a high level that maximal activity obtains without acceleration (average time of appearance of tumors approximately 6 weeks in C3H mice). The concentrations, C_1, C_2, and C_3, of the important types of carcinogens must be expressed in terms of methylcholanthrene equivalents.

The validity of the concept of the summation of the potencies of the different classes of carcinogens, so long as the maximum activity for any given class has not been reached, is supported by a certain amount of experimental evidence such as that shown in Table 4. In previous experiments benzo[a]pyrene has been found to

be about 60 per cent as active as methylcholanthrene. Comparison of the results of Experiments 623 and 624 indicates that the potencies of these two strong carcinogens summate satisfactorily.

Benz[a]anthracene (BA) is a very weak carcinogen which shows significant potency only when tested in a powerful accelerator. There have been reports in the literature that this polycyclic hydrocarbon inhibits the potency of certain strong carcinogens, particularly 7,12-dimethylbenz[a]anthracene (DMBA),(e.g.,9). However, in Experiments 625 and 626 it was found that BA does not influence the potency of methylcholanthrene to C3H mice significantly. Similar indications were obtained by extracting a distillate, Sample 8-12, rich in BA, with maleic anhydride and testing the non-adduct.

Some carcinogens are relatively susceptible to oxidation, DMBA being particularly reactive from this standpoint. It may be that this compound's extremely high level of potency is related to this chemical property. By inhibiting the oxidation under certain circumstances BA might be able to reduce the potency of DMBA to some basic level.

Some of the available evidence on straight run products has been interpreted as suggestive of the presence of inhibitors in the high boiling fractions of crude oils. Thus, although most raw lubricating stocks which we have tested have shown some capacity to produce tumors of the skin of mice, the crude oils and reduced crudes, such as those in Table 1, rarely showed any activity. The possibility that some of the high boiling non-hydrocarbons might act as inhibitors is intriguing, but no direct evidence has yet been

developed on this point. The lack of potency of the reduced crudes, even though they contain carcinogens, might be explained partly on the basis of dilution of the carcinogens, and partly on the basis of the fact that accelerators, which are so important to the potency of lube distillates, are usually present in reduced crudes in concentrations too low to be effective.

Composition of Pertinent Polycyclic Fractions

Research directed toward the characterization of the important classes of polycyclic carcinogens has been carried out primarily on catalytically cracked residua. Although some of these cracked oils are not much more potent on the skin of mice than certain straight run distillates, the more active samples have produced tumors in an average time of less than two months. It was expected that the cracking operation would greatly reduce the complexity of the aromatic fraction of these oils as compared to uncracked materials, and further that the concentration of carcinogens might be significantly higher.

Analyses for one specific carcinogen, benzopyrene (BP), by a method developed in the course of this investigation (10), confirmed this expectation. As shown in Table 1, the content of this compound in uncracked streams is usually insignificant, although the potencies, in some cases, are moderately high. In the case of tars from thermal cracking of virgin feeds, the concentration of BP reaches an average level of 0.05 per cent. In residua from catalytic cracking (moving bed), the content of this carcinogen is significantly greater, 0.30 per cent being an average value.

There is some evidence from biological tests that BP may be more refractory to thermal cracking than are the carcinogens of lower molecular weight in catalytically cracked oils. A series of such comparisons are shown in Table 5, in which the feed stock and the tar produced from a thermal cracking operation were collected at the same time and then tested on comparable groups of mice. From the analytical data it will be noted that in each case the concentration of BP was higher in the tar than in the feed. Further, the ratio of the concentraion of BP to the relative potency was increased in this process.

However, one cannot interpret these data as proof that the concentration of other carcinogens was reduced. The potency of most of the feed streams depended to some extent on long-chain accelerators. Extensive splitting of these chains by the thermal cracking would be expected.

The analysis for BP furnished one of the parameters, C_1, of the general potency equation above. Information about other important classes of carcinogens, symbolized by C_2 and C_3, was required. A catalytically cracked residuum of high potency, Oil No. 8, was fractionated in a 25-plate Stedman type of column at 0.03 mm. pressure at the head. From the data in Table 6, it will be noted that the fractions boiling below 670°F. (corr. to 760 mm) showed no activity, confirming the reports of Fischer and coworkers (11). A high level of activity was found in the fractions in the range, 750-800°F. estimated, although no significant amount of the benzopyrene of this oil (0.4 per cent in the original) distilled until higher temperatures

were reached. The highest boiling distillate, 8-14, and the residuum, 8-15, contained sufficient BP, 0.9 and 2.0 percent, respectively, to account for all of their carcinogenic potency.

It is not surprising that none of the fractions proved to be more potent than the original oil. Most of the non-carcinogenic but important accelerators of this oil distil below the point at which significant levels of concentration of polycyclic carcinogen are obtained.

The carcinogenic distillates were subjected to further fractionation by chromatography on alumina, partition between certain volatile hydrocarbons and concentrated sulfuric acid at 5° to 10°C., and reaction with maleic anhydride. Most of these techniques are standard practice. One of them, that involving the solution of certain classes of polycyclics in concentrated sulfuric acid without sulfonation (10,12), may be relatively unfamiliar. In general, pericondensed molecules, such as pyrene, BP, and perylene, as well as anthracene and BA dissolve. Phenanthrene, fluoranthene, chrysene, benzo(c)phenanthrene, and dibenz(a,h)anthracene do not, though some of their alkyl derivates do. The utility of this method of fractionation is shown by the absorption spectra of Figure 1, representing the separation of dimethylpyrenes from a mixture also containing alkylphenanthrenes and fluoranthenes.

Unsubstituted benz(a)anthracene (BA) was found to be concentrated chiefly in the distillate 8-12. Since only one of the known carcinogenic hydrocarbons has a lower molecular weight than BA, i.e., 9,10-dimethylanthracene, and its relative potency is very low, considerable interest has centered in the identification of the active

components of fractions 8-10 and -11. Furthermore, these carcinogens are probably the same as those responsible for the potency of sidestreams from catalytic cracking and delayed coking, and possibly even those in certain raw lubricating stocks.

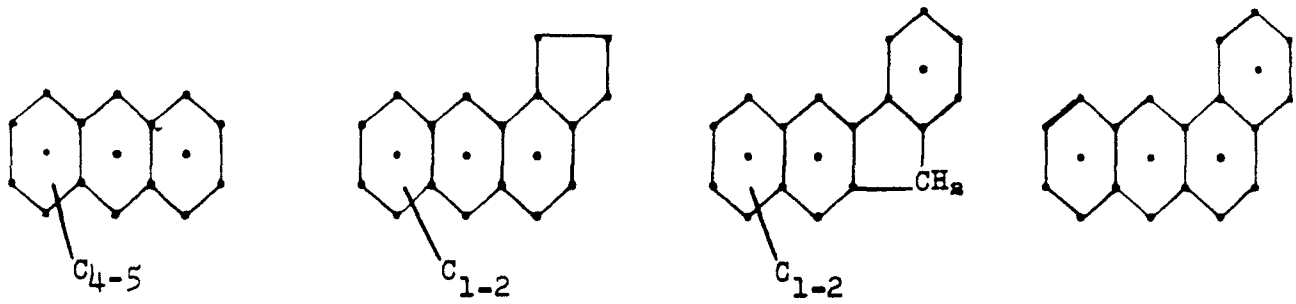
The absorption spectra of these distillates indicated the presence of pyrene and its methyl and dimethyl derivatives. The 4-methyl and 4,9-dimethyl homologues were isolated. In view of the importance of benzopyrene in higher boiling cuts, the possibility that some dimethylpyrene might be responsible for the potency of fractions 8-10 and -11 seemed plausible. However, such does not seem to be the case. The combined distillates were partitioned between cold concentrated H_2SO_4 and n-heptane. The raffinate, which was essentially free of pyrenes after 5 extractions, proved to have most of the potency of the original distillates. Its spectrum indicated that alkylphenanthrenes were among the major components.

In order to have an adequate quantity of this material for detailed examination, a sample of 3.5 kg. of the aromatics from chromatography of Oil No. 8 has been subjected to a more efficient fractional distillation (column rated at 80 plates at total reflux). The fractions of lowest boiling range which showed significant carcinogenic activity, 8-26-l,-m, and -n in Table 7, were extracted with aqueous acid and base and then subjected to the reaction with maleic anhydride. It was found that the compounds regenerated from the Diels-Alder adduct from 8-26-n had contributed about 1/6th of the potency of the original distillate. The following composition was estimated from the mass

spectrum of this mixture:

n	C_nH_{2n-18}	C_nH_{2n-20}	C_nH_{2n-22}	C_nH_{2n-24}
17	1.8%	1.0%	0.6%	-
18	7.5	8.7	6.1	29.3%
19	7.4	10.4	7.6	3.9
20	3.0	4.9	2.6	1.1
21	1.2	1.5	0.8	0.4
Total	21%	26%	18%	35%

Suggested structures of principal components:



The cyclopentano substitution is considered the most likely structure for the C_nH_{2n-20} hydrocarbons. A six-membered ring would be expected (on the basis of the spectrum of tetralin) to yield a strong fragment peak at m/e of 204 by loss of $-CH_2-CH_2-$. The intensity of this ion in the spectrum of 8-26-n-1 is negligible.

Similar extractions of 8-26-l and -m with maleic anhydride were carried out. The pooled non-adducts were then subjected to a systematic fractionation on alumina (Alcoa F-20). The components in successive fractions, the presence of which was most likely on the basis of UV

and IR absorption spectra, were as follows:

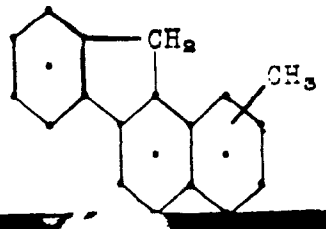
Eluted	Apparent Components
0 - 22%	Saturated hydrocarbons
21 - 27%	Alkylnaphthalenes
25 - 55%	Alkylphenanthrenes
35 - 86%	Pyrenes, chiefly dimethyl
78 - 87%	Fluoranthenes, chiefly methyl
82 - 91%	Benzofluorenes, chiefly methyl
87 - 91	Chrysene and triphenylene
91 - 100%	Non-hydrocarbons, including carbazoles

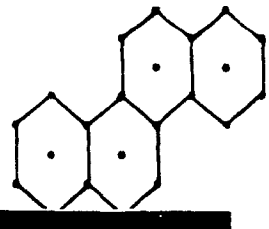
No satisfactory evidence for the presence of any significant amount of the carcinogenic benzo(c)phenanthrene has been found. Further, its highly strained, non-planar structure would seem to militate against its presence in a severely cracked oil of this type.

Biologic tests of certain of the more strongly adsorbed fractions have been initiated. The approximate composition of selected cuts has been estimated from absorption and mass spectra as follows:

	85.0 - 88.4% Fraction		88.4 - 90.5% Fraction	
n	C_nH_{2n-22}	C_nH_{2n-24}	C_nH_{2n-22}	C_nH_{2n-24}
17	2.0%		1.4%	
18	79.8%	3%	68.6%	21.1%
19	14.8%		8.3%	0.2%
20	0.8%		0.7%	
Total	97%	3%	79%	21%

Suggested structures of principal components:





Conclusions

Various classes of hydrocarbons, including the saturates, contribute to the carcinogenic potency of certain refinery streams for the skin of mice. A useful hypothesis about the relationships between these contributions may be represented by the general equation:

$$P_{MC} = M_a \ (C_1 + C_2 + C_3 \ )$$

The relative potency, P_{MC}, expresses the results of a given bioassay in units of concentration of standard polycyclic carcinogen, 3-methylcholanthrene.

The multiplication factor, M_a, is a complex function describing the catalytic influence of certain non-carcinogenic hydrocarbons, such as n-hexadecane and amylnaphthalene. Factors up to 10 may be introduced into the potency equation by such accelerators.

The summation of C's represents the basic potency contributed by certain polycyclic aromatic compounds, the most important of these being 4- and 5-ringed hydrocarbons. Benzo(a)pyrenes are probably the chief class of carcinogens of catalytically cracked residua. The data presented suggest that the most important carcinogens of sidestreams from catalytic cracking or coking operations may be derivatives of the benzofluorenes or possibly of cyclopentanophenanthrenes.

Certain uncracked lubricating distillates also demonstrate significant carcinogenic activity for the skin of mice and men. Acceleration of the effects of a relatively low concentration of carcinogens by long-chain saturates and alkylnaphthalenes is of primary importance for such oils.

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Figure 1

SEPARATION OF POLYCYCLIC AROMATICS BY PARTITION BETWEEN ISOOCTANE AND CONCENTRATED SULFURIC ACID

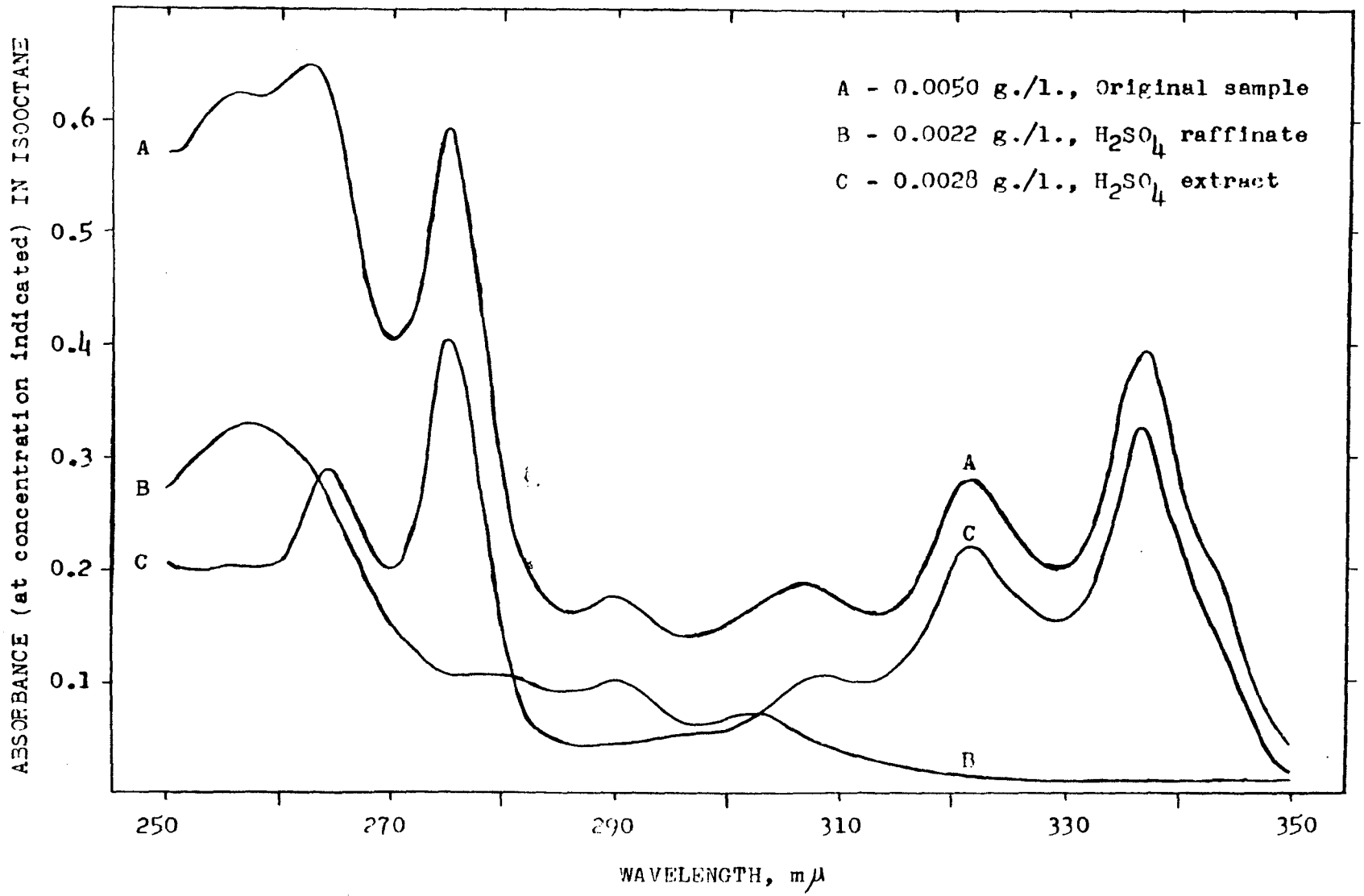

Table 1

Relative Carcinogenic Potencies of Various Uncracked Refinery Streams

Sample Number	Description	Fraction Below 750°F. (%)	Benzo[a]-pyrene (%)	Average Duration of Tumor-Free Life,[1] ℓ (weeks)	Relative Carcinogenic Potency, P/ℓ
[illegible]	Paraffinic distillate	73	0.0003	22	0.11
[illegible]	Lube distillate	50	≦0.002[2]	50	0.02
[illegible]	Asphaltic crude oil	40	~0.0005	-	0.00
[illegible]	West Texas reduced crude	4	~0.002	-	0.00
[illegible]	Phenol extract from naphthenic distillate	10	≦0.003[2]	79	0.01
[illegible]	Phenol extract from naphthenic distillate	30	≦0.002[2]	11	0.30

[1] For tests involving three applications each week.

[2] Uncertainty due to interference from perylenes.

Table 2

Relative Carcinogenic Potencies of Various Cracked Refinery Streams

Sample Number	Description	Benzo[a]-pyrene (%)	Average Duration of Tumor-Free Life, e (weeks)[1]	Relative Carcinogenic Potency, P/C
[illegible]	Delayed Coker gas oil	0.006	9 est.[2]	0.39
[illegible]	Delayed Coker gas oil	0.003	23	0.10
[illegible]	Catalytically cracked sidestream, F.B.P. 750° F.	0.001	12	0.26
[illegible]	Catalytically cracked residuum	0.42	5.6 - 8	0.5 - 1.
[illegible]	Catalytically cracked residuum	0.10	8 est.[2]	0.52
[illegible]	Catalytically cracked residuum	0.53	11 est.[2]	0.30
[illegible]	Thermal tar (no catalytically cracked components in feed)	0.04	22	≥0.11[3]

1. For tests involving three 100 mg. applications each week.
2. Based on comparable tests involving 1 or 2 applications each week.
3. Uncertainty due to poor health of mice.

Table 3

Relative Activity of Various Solvents
to Accelerate Carcinogenesis

Experiment Number	Solvent	Relative Accelerating Activity
302	n-Octane	1
278	n-Decane	3
295	n-Dodecane	5
269b	n-Hexadecane	≥3[1]
298	Hexadecene-1	4
263	1-Cyclohexyldecane	5
262	1-Phenyldecane	8
273	Diamylnaphthalenes	7
325	Technical White Oil, 95 S.S.U./100°F.	2

1. Uncertainty due to poor health of mice.

Table 4

Experiments to Determine the Potency of Mixtures of Polycyclic Hydrocarbons for the Skin of Mice

Experiment Number	Hydrocarbon	Solvent	Concentration of Carcinogen (% by weight)	Relative Carcinogenic Potency, P_{MC} [1]
624	3-Methylcholanthrene	Technical white oil, 95 S.S.U./100°F.	0.20	$0.15^{+.01}_{-.02}$
623	3-Methylcholanthrene Benzo[a]pyrene	Technical white oil	0.10 0.20	0.17±.02
625	3-Methylcholanthrene	Technical white oil	0.10	0.10±.01
626	3-Methylcholanthrene Benz[a]anthracene	Technical white oil	0.10	≧0.03 [2]
8-12	50%, Distillate from catalytically cracked oil No. 8; 50% benzene			0.21±.03
8-12a	50%, Non-adduct from Diels-Alder on 8-12; 50% benzene			0.23±.04

1. Limits of Confidence (P=0.05).

2. Uncertainty due to poor health of mice.

Table 5

Relative Contribution of Benzo(a)pyrene to the Carcinogenic Potencies of the Diverse Stocks and Products of Thermal Cracking Operations

Experiment Number	Description	Benzo(a)pyrene (%)	Relative Carcinogenic Potency, Eyc	Ratio of Benzo-pyrene to Potency
23	Feed: Catalytically cracked sidestream	0.04	0.22	0.2
24	Tar produced from 23	0.24	0.4	0.6
29	Feed: Catalytically cracked gas oil	0.10	0.31	0.3
30	Tar produced	0.23	0.26	0.9
34	Feed: Catalytically cracked gas oil	0.014	0.1	0.1
35	Tar produced	0.18	0.15	1.2
46	Feed: Catalytically cracked sidestream	0.07	0.15	0.2
47	Tar produced	1.21	0.22	1.0
63	Feed: Catalytically cracked gas oil	0.05	0.17	0.3
64	Tar produced	0.11	0.17	1.6
91	Feed: Catalytically cracked sidestream	0.04	0.26	0.15
92	Tar produced	0.07	0.31	0.23

Table 6

Fractional Distillation of Catalytically Cracked Residuum, Oil No. 8

Experiment Number	Estimated Boiling Range (in °F. at 760 mm.)	Percent of Original Oil	Relative Carcinogenic Potency, P_{yc}
8-6[1]	430 - 550	0 - 9.3%	0
8-7	550 - 620	9.3 - 19.4%	0
8-8	620 - 670	19.4 - 30.3%	0
8-9	670 - 695	30.3 - 39.8%	~ 0.03
8-10	695 - 730	39.8 - 49.6%	0.10
8-11	730 - 755	49.6 - 59.2%	0.14
8-12	755 - 780	59.2 - 69.1%	0.21
8-13	780 - 805	69.1 - 78.5%	0.17
8-14[1]	805 - 830	78.5 - 80.8%	(0.15)[2]
8-15	> 830	80.8 - 100%	0.11

1. All fractions were tested as 50% solutions in benzene except 8-14, which was used as a 33% solution.
2. Uncertainty due to high mortality among mice.

Table 7

Tumor-Inducing Activity of Products of Fractional Distillation of the Polycyclic Aromatics of a Catalytically Cracked Oil

Experiment Number	Fraction Distilled	Estimated Boiling Range (In ° F. at 760 mm.)	Incidence[1] of Tumors (% - weeks)	Relative Carcinogenic Potency, P_{MC}
8-26-c[2]	32 - 34.1%	651 - 656°F.	0% - 44 wks.	-
8-26-d	34.1 - 36	656 - 662	0 - 65	-
8-26-e	36 - 38	662 - 668	0 - 66	-
8-26-f	38 - 40	668 - 674	7 - 80	-
8-26-g	40 - 43.9	674 - 686	15 - 52	-
8-26-h	43.9 - 46	686 - 693	12 - 45	-
8-26-i	46 - 50.3	693 - 709	28 - 52	~ 0.01
8-26-j	50.3 - 52.5	709 - 717	71 - 72	$0.03 \pm .01$
8-26-k[2]	52.5 - 54.8	717 - 727	83 - 19	≥ 0.21[2]
8-26-l	54.8 - 57.3	727 - 736	90 - 51	$0.09 \pm {.02 \atop .05}$
8-26-m[2]	57.3 - 60.4	736 - 747	87 - 14	0.29[2]
8-26-n	60.4 - 62.4	747 - 759	100 - 39	$0.15 \pm {.02 \atop .07}$

Contributions of accelerators to potencies of fractions above

		Accelerating Factor
8-26-2	Solution of Benzopyrene in fraction[3] of 8-26-g, -h, and -i; plus 20% benzene	5
8-26-4	Solution of Benzopyrene in fraction[3] of 8-26-l, -m, and -n; plus 30% benzene	2

1. During indicated period of exposure.
2. All fractions tested as 50% solutions in benzene except 8-26-k and -m, which were accelerated with 50% dodecylbenzene.
3. Chromatographic fractions including saturates through naphthalenes, 27% of 8-26-g, -h, and -i, 23% of 8-26-l, -m, and -n.