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INTERIM REPORT

API RESEARCH PROJECT MC-1

OCTOBER 1, 1955

THE KETTERING LABORATORY

in the

Department of Preventive Medicine and Industrial Health

College of Medicine

UNIVERSITY OF CINCINNATI, CINCINNATI, OHIO

ERRATA

for

Interim Report
API Research Project MC-1
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Page Number	Line Number	Suggested Change
1	4	Insert "scrotal and other" between "of" and "cutaneous"
1	5	Delete "especially scrotal"
3	3	Replace "satisfactory" with "one-to-one"
3	20	Add "in benzene." after "carcinogen"
6	19	Replace "II" with "VII"
7	18	Insert "(the contribution to potency due to accelerators)" between "M_a" and "may"
10	1	Delete "," after "far"
10	15	Replace "after each application failed to prevent the" with "accomplished a"
10	16	Delete "development of tumors. A"
10	17	Delete "could be accomplished"
11	21	Insert "(See Table X for experimental details)" after "applied"
11	24	Replace "III" with "IX"
12	2	Replace "indicated" with "suggested"

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The potential hazards deriving from carcinogenic materials in the intermediates and products of the refining of petroleum are being subjected to systematic investigation. The initiation of this work by the sponsors was stimulated in part by historical and recurrent instances of cutaneous, especially scrotal, cancer among wax pressmen. The immediate impetus came, however, with the recognition of the highly increased aromaticity of certain high boiling fractions produced in the newer catalytic processes. Further, pointed evidence from several centers of investigation showed that certain of these fractions possessed the capacity for the induction of cancers in the skin of experimental animals at a considerably higher level than that of the uncracked distillates associated with the problem among the pressmen.

Attention was directed in 1949 to products of the following processes, in which polycyclic aromatics might be synthesized and/or concentrated:

STRAIGHT THERMAL CRACKING PROCESSES

Non-catalytic cracking of virgin gas oil
Non-catalytic cracking of catalytic gas oil
Viscosity breaking
Naphtha reforming
Coking
Polyforming
Steam cracking

CATALYTIC CRACKING PROCESSES

Houdry
Thermofor catalytic cracking
Fluid catalytic cracking
Cycloversion
Catalytic reforming

SPECIFIC PRODUCTS

Slack waxes and press oils
Raw lube stocks from straight run distillation
Solvent extracts
Acid oils from acid sludge

With the full cooperation of the research and refining departments of the companies represented on the Medical Advisory Committee, detailed tables of information were assembled in the Laboratory, showing the variations in operating conditions and feed stocks of some 200 different units involved in the above processes. From this comparison of available materials, it was possible to select a series of about 600 samples to cover the extremes of the ranges of each important variable. Each company then obtained certain analytical data on a selected group of its samples and made this additional information available to responsible personnel in the Laboratory.

One item among these data was the absorption spectrum of a fraction representative of the heart-cut of the polycyclic hydrocarbons regarded as potentially carcinogenic. Estimates of the relative potency of each sample were based upon certain characteristics of these spectra. Although this empirical technique proved subsequently to be applicable mainly to residual fractions from catalytic cracking, the approach served a useful purpose. It pointed up one salient characteristic of the group of materials which, when tested biologically, were found to have potencies much higher than those predicted. Almost invariably, these samples contained high percentages of components boiling below 700°F. This observation, coupled with the results of parallel experiments on solutions of 3-methylcholanthrene in certain non-volatile solvents, led to the recognition of the contribution made to the potency of many petroleum products by a group of non-carcinogenic hydrocarbons, with boiling points in the range, 500-750°F. These materials, termed "accelerators" for reasons of convenience, will be referred to in some detail later.

From the series of samples which had been collected and analyzed in this manner, approximately 120 were subjected to tests of their relative carcinogenic potency when applied upon the skin of C3H mice. This inbred

strain has proved particularly useful, in that the results of tests made to differentiate non-carcinogenic from carcinogenic materials by means of application of these materials upon the skin, have shown a satisfactory correlation thus far with the results of prolonged contact of human skin in those instances (admittedly limited in number) in which occupational exposure has been documented properly. Since the 120 samples represented a wide range of potencies for the skin, certain modifications of the time-honored experimental procedure (for the application of such materials on the skin of animals) were developed in the course of the research. As described in an article which has been circulated and will appear soon in Cancer Research, these improvements have made this experimental method more flexible and more readily subject to quantitative interpretation.

The relative potencies of these samples are shown in Table I. The scale on which they are expressed is based upon a series of parallel tests on solutions of 3-methylcholanthrene in benzene. The conventionalized expression (P_{MC}) for the capacity of a material to induce cancer in the skin of the mouse, simply indicates that the oil in question induces tumors in the skin of mice at the same rate, under comparable experimental conditions, as a solution of that level of concentration, in percent by weight, of the synthetic carcinogen.

It may be pertinent at this point to glance at the relative potencies of certain products of another industry which has experienced an increased incidence of skin cancer among specific groups of its employees. Crude coal tars from by-product coke ovens have shown potencies in the P_{MC} range, 0.08 to 0.4, there being some evidence that the majority of these would be included in the 0.10 to 0.15 part of this range. Thus, it will be noted that some uncracked distillates from petroleum (e.g., API-79 and 105) have approxiamtely the same potency for mice as these coal tars. The average

duration of the exposure of workmen who developed cancer of the skin in association with contact with tar was comparable to that of wax pressmen, about 15 to 20 years.

It is apparent from the data in Table I that thermal cracking of catalytic gas oils, as it is usually practiced, does not reduce their carcinogenic potency. Indeed, when one notes the relative increase in the content of benzo [a] pyrene (BP) which often results from this process, it might be expected that potency would increase significantly, were it not for the fact that the probable accompanying destruction of long-chain accelerators operates in the opposite direction.

Such correlations between the relative potencies of different oils and their chemical composition have been among the major aims of this investigation. It was recognized from the outset that the needs of such a dynamic industry would not be met by determining and listing the potencies of samples selected more or less arbitrarily at any particular time. The changes in refining methods which have accompanied the competitive technological developments of the past decade have had a profound effect upon the composition of many fuels and other petroleum products. Since continued biological testing is time-consuming and costly, the need for chemical-analytical methods for the estimation of carcinogenic potency of complex mixtures has been evident.

To these ends an intensive program of fractionation and analysis of selected carcinogenic oils was initiated in 1949. In its early stages this work yielded suggestive evidence of a relationship between the carcinogenic potency of cracked residua for mice and the intensity of a band in the absorption spectra of certain distillates prepared from these oils in the laboratory. The most intensive work has been carried out with a sample of slurry from Fluid Catalytic Cracking, API-8. Spectrographic evidence of

the presence in this oil of the well known carcinogenic hydrocarbon, benzo[a]-pyrene, led eventually to its isolation and its identification with a synthetic sample of the pure compound. Further evidence that the concentration of this particular family of carcinogens in many residual oils was of such a level as to contribute to their carcinogenic potency to a major extent furnished the stimulus for the development of a generally applicable method of analysis for this family of compounds. The method has been published in Analytical Chemistry, 27, 248 (1955).

The content of benzo[a]pyrene has been determined in each of the oils which have been tested by application on the skin of mice. The results are shown on Table I. With certain striking exceptions, it will be noted that there is a rough correlation between the content of BP and the relative potency of the residual products from any given process. One of the exceptions is sample API-71 from Thermofor Catalytic Cracking. A detailed examination of this oil, and of certain of the distillates (sidestreams) which have been tested, has been made in order to learn more about other classes of compounds which contribute significantly to the carcinogenic potency of these oils.

In parallel with the program of fractionation of samples obtained from refining operations, solutions of synthetic carcinogens in various solvents and in mixtures of known composition have been investigated. Generally, it has been found that the replacement of benzene by progressively less volatile solvents has resulted in progressive retardation of the rate at which a given concentration of carcinogen would induce tumors. Comparison of the relative areas of skin wetted by given quantities of the solution in benzene with those involved in contact with the less volatile solvents has suggested a very plausible explanation for the slower induction of tumors by the latter. It could be shown that, so long as the dosage in milligrams of the carcinogenic solute per unit area of skin was kept constant,

the rate of induction of tumors was unchanged. For example, since solutions of methylcholanthrene (MC) in amylbenzene spread about twice as far over the skin as solutions in benzene, it is necessary to use almost 0.2 percent MC in the alkylbenzene to obtain the same dose per unit area and rate of carcinogenesis as were obtained with 0.1 percent MC in benzene.

Extension of this work to include solutions of MC in the detergent intermediate, dodecylbenzene, yielded results which were surprising at first, but subsequently rewarding in that they provided the explanation for the potency of oils such as API-71. Solutions of MC in this 18-carbon solvent spread more widely upon the skin then did those in amylbenzene, and yet induced tumors much more rapidly than did the corresponding solutions in benzene. The presence of fractions with comparable "accelerating"properties in a number of petroleum oils was demonstrated subsequently. It soon became apparent that these materials are derived from the crude petroleum, that they may increase in concentration to some extent under the conditions of catalytic cracking, and that they may eventually be reduced significantly by very drastic cracking.

Further investigation of solutions of BP in various pure solvents (Table II) has shown that this property of acceleration is shared by at least some members of almost all classes of hydrocarbons containing 10 to 20 carbon atoms per molecule. The shape of the molecule, as well as its overall length, seems to be an important characteristic, since 7-cyclohexyltridecane (Experiment API-279) proved to be rather weak as an accelerator. The finding that normal paraffins, such as cetane, may contribute significantly to the carcinogenic potency of a mixture, although they themselves are non-carcinogenic, has thrown some light, it seems, upon the apparent paradox involved in the occurrence of scrotal cancer, under certain circumstances, among men engaged in wax-pressing operations, despite the low aromaticity of the distillates which enter into these operations.

The current systematic study of the properties of distillates from a variety of cracked and straight run oils represents an effort to quantitate the knowledge which has been accumulated on accelerators. The goal is an analytical method by which the factor introduced into the potency equation by these fractions in any given oil may be determined.

The knowledge accumulated thus far from numerous experiments on solutions of certain polycyclic hydrocarbons in solvents of well defined composition suggests that a realistic technique for estimating the potency of any given oil on the basis of its physical and chemical characteristics may involve a summation of four separate analyses. In algebraic terms

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

The summation of C_1, C_2, and C_3 represents the effective potency contributed by three major categories of polycyclic carcinogens, expressed in methylcholanthrene equivalents. Included in these three classes are (1) the benzopyrenes previously discussed, (2) carcinogenic substances extractable by maleic anhydride (probably derivatives of benzanthracene), and (3) carcinogenic substances insoluble in concentrated sulfuric acid (sometimes referred to as low-boiling carcinogenic compounds). The factor M_a may vary, apparently, from 1 to 8, and thus in certain instances, such as catalytic cycle gas oils and light lube distillates, may provide the major contribution to potency. Hence, the quantitation of the effects of accelerators becomes a matter of consideraole practical importance.

The available evidence indicates that a significant fraction of the carcinogens may be extracted from catalytically cracked oils by maleic anhydride. In the case of API-8, approximately 40 percent of the carcinogenic material (in terms of methylcholanthrene equivalents) may be separated from the oil by this technique. Experiments 8-25a and 8-25b have shown that there

is no significant difference in potency between the fractions obtained by successive extractions of API-8 with maleic anhydride, even though the mass spectra of the different fractions indicate that there are gross differences in their chemical composition.

Similarly, when such an extraction is applied to a cracked sidestream, such as API-89, a small amount of carcinogenic material may be extracted. Because of the uncertainty about the multiplication factor due to accelerators in API-89, it cannot be said yet what portion of the carcinogens were removed by maleic anhydride. However, separation of the extract from API-89 by chromatography yielded valuable information to the effect that the large proportion of the fraction which was desorbed more easily than benzanthracenes, approximately 90 percent of the total Diels-Alder adduct, contained practically none of the carcinogenic materials. Further, to resolve some of these questions a series of chromatographic fractionations are being applied to a series of straight-run and cracked distillates, similar in boiling range to API-89 (600 to 750°F.), to produce accelerator-free concentrates of carcinogens, which may then be tested directly for their relative potency. This should then provide values for the summation ($C_1 + C_2 + C_3$) in the potency equation. A parallel series of extractions of the oils by maleic anhydride will furnish quantitative information regarding the relative contribution of this class of carcinogens to the effective sum. Some evidence has been collected which indicates that these compounds extracted by maleic anhydride contribute relatively less to the potency of sidestreams than to that of residual oils.

From the practical standpoint of the occupational hazard of skin cancer, perhaps the most important oils are the distillates obtained in the range 550 to 800°F. Such materials as wax distillates, diesel fuels, and base stocks for cutting oils are found within this range. It is probable

that the first two major classes of carcinogenic substances, C_1 and C_2 in the potency equation, may contribute relatively little to these distillates. Both of these classes would be extracted in fairly large part by concentrated sulfuric acid. It has been noted previously that the raffinate, API-8-20, from such an extraction of the lower boiling carcinogenic distillates of API-8 contains most of the carcinogens of these fractions. A large batch of polycyclic aromatics concentrated from API-8 by chromatography has been subjected to careful distillation through a column having a theoretical efficiency of 80 plates. Selected fractions are being tested under the code number 8-26.

The fractions which were distilled below 720°F. showed at most a very low order of carcinogenic potency. The behavior of the fractions between 675 and 710°F. suggested the presence of a trace of carcinogen in an active accelerating medium. This was confirmed by experiment 8-26-2, which demonstrated that the accelerators in these fractions had a multiplication factor, with respect to potency, of about two. The alkylanthracenes removed during the same chromatography were tested for potential acceleration in experiment 8-26-3. Their lack of significant activity in this respect is compatible with the general idea that more alkylation of such a nucleus would be required then would be found in a cut of this boiling range.

The major components of the carcinogenic fractions boiling between 720 and 760°F. are derivatives of pyrene, benzfluorene, and fluoranthene, together with a substantial amount of non-hydrocarbons, particularly derivatives of carbazole. A separation of these classes is being carried out in order to determine their relative contribution to the potency of straight-run and cracked distillates.

This report thus far, has concerned itself with the problem of the relationships between chemical composition and carcinogenic potency. Considerations of industrial hygiene involved in the general problem have also been investigated in a number of ways. Experimental work on the possibility of reducing the hazard by washing the materials from the skin, and by the application of so-called barrier creams, has been carried out. Generally speaking, it has been found that the development of tumors could be retarded significantly by washing the skin with soap and water at an appropriate interval after the application of a carcinogenic material, so long as the frequency of application did not exceed once per week. For example, in an experiment in which the oil API-8 was removed by washing within 10 minutes after its application, no tumors developed during the entire life span of the mice. However, in most of the experiments involving three applications of a material per week, the washing of the skin with an apparently effective detergent after each application failed to prevent the development of tumors. A significant retardation of the rate at which the tumors developed could be accomplished, but, as a general rule, half or more of the animals developed neoplastic growths. The use of barrier creams prior to the application of a carcinogenic oil had no significant effect upon the outcome of the experiment.

The use of various hydrocarbon solvents to rinse off the excess of oil prior to washing the skin with a detergent has been referred to in some detail in a previous report and in verbal discussions. Briefly to summarize these observations, these procedures in some instances, have augmented the carcinogenic effect instead of contributing to the efficiency of the cleansing of the skin by the detergent and water. Solvents containing accelerators have proved to be particularly disadvantageous in this respect. In short, it appears that such rinsing with solvents does not improve the efficiency

of the washing, and may accelerate the induction of experimental cancers. As a measure of occupational hygiene, such a procedure does not appear to be desirable.

In the course of the general experimental program, a number of other questions raised withing the Laboratory and by representatives of the Medical Advisory Committee have been answered. For example, numerous experiments have shown that if the applications of a carcinogenic oil from catalytic cracking be discontinued at the time the first evidence of neoplastic growth of the skin of the mouse appears, the likelihood of the further development of the lesion into a malignant tumor is the same as it would be if the applications had been continued. On the other hand, if the applications upon the skin of all of the numbers of a group of mice terminated at the time of the earliest appearance of a papilloma on the skin of the first member of its group, widely variant results are obtained in different experiments. In the case of a test with API-71, the majority of the animals subjected to no further applications went on to develop tumors at almost the same rate as that which characterized a parallel group which was the subject of uninterrupted applications. On the other hand, in an experiment with API-43-1, very few additional animals developed tumors after the interruption of the applications until at a much later time, after a second period during which the carcinogenic oil was applied.

The action of accelerators upon skin previously subjected to contact with carcinogenic materials, has also been investigated. A number of experiments referred to in Table III have demonstrated that accelerators such as dodecylbenzene and dodecane are capable of eliciting tumors in skin previously subjected to single applications of catalytically cracked oil.

Other experiments have shown that contact with the accelerating alkylbenzenes may so alter the skin as to augment the effects of its subsequen contact with carcinogenic oils. The nature of this alteration is speculative,

but the phenomenon, of itself, suggests certain approaches in investigating the mechanisms of acceleration. That these are multiple is indicated by the indication (Experiments 600 and 620) that dodecane seems to lack the capacity to condition the skin in this manner.

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