PLAINTIFF'S EXHIBIT

API-146

API - BRITISH INSTITUTE OF PETROLEUM.
RESEARCH GROUP NO. 2 -
Meetings and Minutes

I believe we have this material in our file already — ~~Discard for~~

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

P. O. BOX 51, LINDEN, N. J.

MEDICAL RESEARCH DIVISION

ROBERT E. ECKARDT, PH.D., M.D.
DIRECTOR

NATHAN V. HENDRICKS, CH.E.
HEAD INDUSTRIAL HYGIENIST

HORACE W. GERARDE, PH.D., M.D.
HEAD TOXICOLOGIST

BERTRAND E. BENNISON, M.D.
CHIEF CLINICAL RESEARCH PHYSICIAN

November 15, 1955

Mr. D. V. Stroop
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Stroop:

Enclosed is copy of the Minutes of the Meeting of Research No. 2 Committee of the Institute of Petroleum, and copy of the Progress Report of the Committee on the Carcinogenic Action of Mineral Oils of the Medical Research Council. Please have these duplicated for distribution to members of the Subcommittee on Carcinogenicity of the API, and to the interested individuals at the Kettering Laboratory.

Very truly yours,

R. E. Eckardt /mak

R. E. ECKARDT, M. D.

REE:mak

Encs. 2

Copy From D. V. Stroop For Information
Of Members Of Medical Advisory Committee
11/17/55

MEDICAL RESEARCH COUNCIL

"Unclassified"
For Specified Circulation
(Members of Committee)

MRC.55/840
CAMO.55/10

Committee on the Carcinogenic Action of Mineral Oils

Progress Report
by
H.N. Green and M.J. Redcliffe
Department of Experimental Pathology and Cancer Research
School of Medicine, Leeds.

Mouse Experiment.

Fractions used:-

L_4S_2A = a

L_4S_6A = b

L_4S_1A = c

0.01%D.M.B. = d($^1/10$)

L_4S_5A = e

0.05%D.M.B. = d($\frac{1}{2}$) = f

L_4SR = g

L_4S_4A = h

L_4S_7A = i

L_4S_3A = j

L_4S_8A = k

The experiment was begun on 7.6.55.

Tumours have been produced as follows:-

b (L_4S_6A) 3 tumours, at 9, 13 and 18 weeks.

c (L_4S_1A) 2 tumours, at 13 and 14 weeks

d($^1/10$) (0.01%D.M.B.) 1 tumour at 14 weeks

f (0.05%D.M.B.) 9 tumours, 1 at 11 weeks, 2 at 14 weeks, 2 at 16 weeks and 4 at 18 weeks

i (L_4S_7A) 3 tumours, 1 at 17 weeks and 2 at 18 weeks

k (L_4S_8A) 1 tumour at 18 weeks.

Survivors at 18 weeks are as follows:-

a 45 b 32 c 39 d 35 e 20 f 44

g 29 h 29 i 21 j 19 k 17

SC-API-2223

Rabbit Experiment

This was begun on 23.8.55. The beginning of the experiment was delayed owing to an epidemic of pneumonia amongst the rabbits.

Five rabbits have died since the experiment began.

So far no tumours have been produced.

(The dilutions of D.M.B. for rabbits are 0.2% and 0.05%).

SC-API-2223

INSTITUTE OF PETROLEUM RES-2-15

RESEARCH NO 2 COMMITTEE

Minutes of Meeting No.15 held at 26 Portland Place
On Tuesday,20-9-55

ATTENDANCE

IN THE CHAIR:	F.Morton.
GUEST:	P.H.S. Trasler
MEMBERS:	W.S. Ault J.N. Haresnape R.B. Killingsworth W. Pohl H.W. Stevenson
APOLOGIES FOR ABSENCE:	E.J. Boorman J.W. Cook E.J. Dunstan A. McLean
SECRETARY:	E. Herbert

1. MINUTES OF MEETING NO.12

These were signed as correct, subject to the addition of "Shell Petroleum, Co.," at the beginning of line 3 of the last paragraph of Minute 3 - I (Page 2)

2. MEMBERSHIP

The meeting accepted the resignation of Mr. W. Pohl with regret and desired to record its thanks to him for his valuable services. The nomination of Mr. H.W. Stevenson was approved by the Committee, who were glad to welcome him back as a Member.

3. WORK AT BIRMINGHAM UNIVERSITY

The Chairman reported on the progress of work at Birmingham University and drew attention to the paper "The Constitution of Petroleum Fractions; Structural Group Analysis of a Kuwait Heavy Gas Oil" which was presented at the recent World Petroleum Congress, and to various documents circulated by the MRC, in particular MRC 55/510 CAMO Minute 18 and CAMO 55/5

The Chairman distributed tables (dated September 20th 1955) of Analytical Data on Kuwait, Lagunillas and Oklahoma Crude Oils and mentioned that work in connection with Table 5, Sub-fractions from aromatic extracts, was now being carried out at Glasgow. The results indicated a very considerable potency concentrated in residues. There were small quantities of Kuwait Crude left over; it was proposed to blend fractions K4S2Aa/K4S5Ab and K4S6Aa/K4S8Ab and then redistil the blends for further test.

SC-API-2223

There would also be available some samples prepared from a Commercial Extract supplied by the Isle Grain Refinery. The Chairman mentioned that the Langunillas tests were a year behind owing to the withdrawal of the Chester Beatty Institute. This work was being carried on by Professor Green of Sheffield/Leeds University, but no medical school had been found to take an interest in the Oklahoma Crudes, which were therefore, being stored in their fractionated state.

Professor Morton added that he would prepare for publication in the IP journal a further paper in continuation of that presented at the World Petroleum Congress.

4. DIESEL GASES FUMES

The Chairman thanked Dr. Killingsworth for the material which he had provided for the report to the MRC and referred to a letter dated the 14th December 1954 from Mr. A. Fogg of MIRA in which, on behalf of the Diesel Engine Manufacturers, the IP were asked to draw attention of the major petroleum companies to the necessity to carry out investigations with the object of eliminating any exhaust smoke which might be attributable to fuel variations.

It was agreed that a general interest in the problem, which was separate from the medical aspect of smoke, was indicated but that it was a matter more for the Research Committee. Mr. Ault who promised to raise the subject at the meeting of MIRA which he was attending the next day was invited by Professor Morten to the next meeting of the Research Committee.

5. ANY OTHER BUSINESS

The Chairman intimated that he would shortly provide for circulation a copy of Mr. A.W.E. Pell's Thesis.

6. DATE OF NEXT MEETING

To be arranged by the Chairman.

AMERICAN PETROLEUM INSTITUTE
50 WEST 50TH STREET
NEW YORK 20, N. Y.

DEPARTMENT OF TECHNICAL SERVICES
DAVID V. STROOP, DIRECTOR

March 30, 1953

To Members and Associates
Medical Advisory Committee

Gentlemen:

You may be interested in Page 2 of these minutes of the Institute of Petroleum Research Committee and in its attachment "Report of Research Group No. 2" which have been sent to us by the Institute of Petroleum in London.

Very truly yours,

DVS: jb
Attachment

INSTITUTE OF PETROLEUM RES-18

RESEARCH COMMITTEE

Minutes of Meeting No. 18 held at 26, Portland Place, 6.1.53

	ATTENDANCE
IN THE CHAIR:	F. Morton
GUEST:	E. A. Coulson
MEMBERS:	B. W. Bradford W. B. Heaton H. N. Short T. Tait
APOLOGIES FOR ABSENCE:	S. J. M. Auld R. Holroyd W. S. Ault C. S. Windebank
SECRETARY:	E. Herbert

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1. MINUTES OF MEETING NO. 17, 5.6.52.

These were signed as correct.

2. MATTERS ARISING.

(i) Minute No. 5. The Chairman reported that the research work by Dr. Cullis had been sponsored by the Shell Petroleum Co.

(ii) Minute No. 6. It was noted that Council had approved the exchange of papers with A.P.I.

3. HYDROCARBON RESEARCH GROUP.

In the enforced absence of the Group Chairman, the Chairman reported that:-

(i) The National Coal Board had become a sponsoring member and Dr. R. R. Gordon had been nominated as representative.

(ii) Mr. D. Morten had found it necessary to relinquish the Chair. This had been offered to, and accepted by Dr. J. W. Barrett. Council are asked to approve.

(iii) Circumstances had caused the Manchester Oil Refinery to withdraw from the Group.

It was agreed to inform Council of the following minute of the Group's meeting held on 22nd October.

"Mass Spectrometry. The Panel Chairman suggested that a Mass Spectrometry Symposium should be held in Manchester in November 1953, and said that his company would be glad to act as hosts. Dr. Bradford raised the question of such a meeting being held in London but as there would be insufficient facilities for displaying apparatus it was thought desirable to accept Metropolitan Vickers Ltd's generous offer. It was left to the Panel to make the necessary detailed arrangements. It was agreed that the proposal should be brought to the notice of Council and that the Publications Committee should later be given an opportunity to decide whether they would like tp publish the papers."

4. RESEARCH GROUP NO. 2.

In the absence of Colonel Auld, the Chairman drew attention to the attached report of Research Group No. 2, which had been circulated. The meeting complimented Colonel Auld on the active work of the Group and expressed gratitude on behalf of the Committee, and of Group No. 2 to all the companies which had provided background material for the detailed chemical and biological examinations.

It was noted that the I.P. is represented by Colonel Auld on the Medical Research Council Committee, recently formed to study the Possible Carcinogenicity of Detergents.

5. FINANCIAL ASSISTANCE.

The question of financial assistance for the North Staffordshire University was deferred until the Chairman had been able to circulate the relevant correspondence.

6. EUROPEAN CENTRE FOR THE CORRELATION OF HYDROCARBON DATA.

Dr. Bradford explained that the subject of a European Centre for the Correlation of Hydrocarbon Data had been raised at a meeting of Section 5 (of which he was Chairman) of the IIIrd World Petroleum Congress at the Hague in 1951. This Congress had agreed that the exchange of data should be encouraged and recently Dutch and French representatives had enquired what had been done. Dr. Bradford said that he was unable, himself, to organize the necessary mechanism which was a matter for a representative body. The work which should undoubtably be undertaken, would entail much clerical work. He felt that the Chemical Research Laboratory might be the appropriate organization to summon a meeting of representatives of interested countries and to speak for this country.

Dr. Coulson was doubtful if the C.R.L. were the right body. There was much unpublished data to be collected, entered and circulated and C.R.L. had not the necessary staff. He suggested that the matter could be handled better by a section of the Department of Industrial Research than by the C.R.L., though the latter could provide technical assistance and he was prepared to act as agent of the Hydrocarbon Research Group if asked to do so. I.P. might provide some clerical assistance. He recommended that the Department of Industrial Research should be approached.

It was agreed:-

To ask Council to take steps to implement the following resolution adopted by the IIIrd World Petroleum Congress.

"The IIIrd World Petroleum Congress considers the possibility of further international exchange of fundamental data on hydrocarbons and other compounds of importance in petroleum science should be explored."

by convening a meeting of European Societies interested in Petroleum.

To approach the Department of Scientific and Industrial Research to ascertain if the Department would be prepared to undertake the necessary work in the event of the European representatives agreeing to the work being organized in this country.

7. ANY OTHER BUSINESS.

It was noted that since the resignation of Dr. Linstead, the Chemical Research Laboratory had not been represented on the Committee. As the meeting would welcome a representative of C.R.L. it was agreed to request Council to invite Dr. Pratt, Director, Chemical Research Laboratory, to become a member of the Research Committee.

8. DATE OF NEXT MEETING.

To be arranged by Chairman.

23.1.53. KBF.

For the confidential information of members and associates of the Medical Advisory Committee from M. N. Newquist, M.D., Chairman, Subcommittee on Carcinogenicity (2 copies to Dr. R. A. Kehoe).

August 28, 1952

INSTITUTE OF PETROLEUM RES-2-11

RESEARCH GROUP NO. 2

Minutes of Meeting No. 11 held at 26, Portland Place, 25.7.52.

ATTENDANCE

IN THE CHAIR:	S. J. M. Auld
GUESTS:	J. W. Barrett (Monsanto) M. Goldblatt M.D. (I.C.I.) J. A. Hill (Shell Chemicals) C. Laird M.D. (Shell, Stanlow) A. McLean (A.I.O.C.)
MEMBERS:	W. S. Ault E. J. Boorman A. L. Down F. Morton H. W. Stevenson
APOLOGIES FOR ABSENCE:	J. W. Cook E. J. Dunstan
SECRETARY:	E. Herbert

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1. MINUTES OF MEETING NO. 10, 28.4.52.

These were signed as correct.

2. MEMBERSHIP.

The acceptance of membership by Dr. A. L. Down was noted with pleasure. Dr. J. W. Barrett had already accepted the Chairman's invitation to membership, and this was confirmed. The Chairman on behalf of the Group extended an invitation to Dr. A. McLean to become a member. This he was glad to accept.

3. DETERGENTS.

The Chairman welcomed the visitors and pointed out that the non-members present would not be committed in any way during the discussion. He explained the main purpose of the meeting, which was to consider the "Possible Carcinogenic Action of Detergents" and the questionnaire set out in Mr. Cohen's letter to the Chairman of the 6th May 1952. This had been circulated.

Dr. Goldblatt described the origin of the enquiry, and how Professor G. S. Wilson, head of the Laboratories of the Ministry of Health, had called a meeting of interested parties. The Brewers had

already shown interest from the point of view of bottle-washing. The Medical Research Council are much concerned in Detergents in general, their primary interest being in possible carcinogenicity, then in possible toxic effects after long periods of very small doses. Dr. Hill undertook, with the collaboration of Dr. Goldblatt and other members, to write a note on the chemistry of the problem in order to assist the Chairman in his reply to Professor Haddow's letter, dated 23rd July, copy attached.

There was a feeling expressed by some members that Research Group No. 2 was perhaps not the appropriate body to deal with this question as it should not be limited to the petroleum industry but should be also a concern of the Soap and Chemical Manufacturers; it might be left to individual groups of manufacturers and the Medical Research Council. It was agreed that although the final responsibility rested with the Medical Research Council and the Ministry of Health, the Institute of Petroleum should assist wherever it can and watch the interests of its member-companies by ensuring that information is correctly provided. The Institute should not sponsor Research and Investigation work but should pass on advice.

A draft reply to Mr. Cohen's letter of the 6th May was tabled. After some discussion, it was agreed that it forms a suitable basis for the reply to be sent by the Chairman, who will add a brief list of the principal known manufacturers of detergents, and references to text books. He will point out that the Institute does not represent the main body of Detergent Manufacturers and will suggest that the question be handled by a broad committee, on which the Institute would wish to be represented. The attention of the Medical Research Council will be drawn to any work on this subject being done in America which comes to the knowledge of the Institute.

4. REPORT ON WORK IN PROGRESS.

Referring to Minute No. 5(a) of Meeting No. 10, Professor Morton reported that the Column at Birmingham has been completely erected and tested. Operations commenced on 1st June. Five samples of each of the 3 crudes have been distributed for biological tests. 500 cc. are being held in reserve at Birmingham and the residue has been forwarded to Professor Cook. Further samples of Lagunillas crude have been shipped. Professor Morton also stated that Dr. Mackenzie had visited Birmingham and had seen Professor Squire and Dr. Woodhouse.

Regarding Minute No. 7 of Meeting No. 10, it was reported that Leeds University had been approached.

5. DATE OF NEXT MEETING.

To be arranged by the Chairman.

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18.8.52. KBF.

RES-2-11

Copy of letter from:

THE CHESTER BEATTY RESEARCH INSTITUTE,
The Royal Cancer Hospital,
Fulham Road,
London, S.W.3.

23rd July, 1952

from: Professor Alex. Haddow, M.D. D.Sc.Ph.D.
(Director)

Dear Colonel Auld,

Very many thanks for your letter of yesterday. I need not say that the Committee of the Medical Research Council concerned will indeed be grateful for any help which can be given by the Institute of Petroleum Research Group or the individual companies.

The so-called "dialkyl disulphonates" refer in fact to the following series:

$$CH_3SO_2O\ (CH_2)_n\ O\ SO_2CH_3$$

$$n = 2 \text{ to } 10$$

These were originally synthesised by G. M. Timmis here as agents which might alkylate genetic protein, either for example through a process of ring formation or by cross-linkage. Whatever the mechanism, there is no question these substances are capable of bringing about cytotoxic effects very largely indistinguishable from those due to the nitrogen mustards, bis epoxides and polyethyleneimines. In addition, we now find them to be carcinogenic.

I have no doubt that the chance of compounds of this type occurring in detergent mixtures is exceedingly small. On the other hand their biological potency is marked, and we thought it well at least to draw attention to them.

If I can help in any other way, please let me know. Should there be any other point to deal with before your meeting on Friday, perhaps we can settle it by phone.

With kind regards,

Yours sincerely,

(Sgd) Alex. Haddow

Colonel S. J. M. Auld,
25, Victoria Street, S.W.1.

AMERICAN PETROLEUM INSTITUTE
50 WEST 50TH STREET
NEW YORK 20, N. Y.

July 7, 1952

To Members and Associates,
Subcommittee on Carcinogenicity

Gentlemen:

For your confidential information I am attaching a copy of the minutes of Meeting No. 10 of Research Group 2 of the Institute of Petroleum, London.

These minutes were sent to me by Colonel S. J. M. Auld.

Very truly yours,

M. N. Newquist

M. N. NEWQUIST, M.D.
Chairman, Subcommittee
on Carcinogenicity

MNN:JKF
Enclosure

mc: Members and Associates, Medical Advisory Committee
Dr. R. A. Kehoe (3)
Mr. D. V. Stroop

COPY ----- CONFIDENTIAL

INSTITUTE OF PETROLEUM RES-2-10

RESEARCH GROUP NO. 2.

Minutes of Meeting No. 10 held at the Chemical Engineering Department of the Birmingham University, 28.4.52.

ATTENDANCE

IN THE CHAIR:	S. J. M. Auld
GUESTS:	W. Carruthers D. L. Woodhouse J. R. Squire
MEMBERS:	W. S. Ault E. J. Boorman J. W. Cook F. H. Garner F. Morton H. W. Stevenson
APOLOGIES FOR ABSENCE:	E. J. Dunstan D. W. Hill (guest)
SECRETARY:	E. Herbert

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The Chairman expressed the thanks of the members of the Committee to Professor Garner and Professor Morton for having invited the Group to Birmingham, and for the hospitality extended to the members. The Chairman welcomed to the meeting Professor Cook, Professor Squire, Dr. Carruthers and Dr. Woodhouse.

1. MINUTES OF MEETING NO. 9. 28.1.52

These were signed as correct.

2. GROUP REPORT

The preparation of a Report by the Group was discussed; it was felt that a report to the sponsoring bodies was both due and desirable, particularly if further financial contributions might be required. Although Research Group No. 2 is a branch of the Research Committee, the £2,000 originally given to it came direct from the Petroleum Board which is now defunct. There were two aspects to consider: (1) the work on Mule Spinners Cancer carried out in conjunction with the "Joint Committee" of the Ministry of Labour and National Service, and (2) the work on the carcinogenic action of Petroleum carried out in conjunction with the Medical Research Council. In both cases nominations to the bodies concerned were made by the Ministry of Fuel and Power. The Chief Inspector of Factories has been given a confidential interim report on (1) and Sir D. Ferguson, in his recent reply to the Chairman, has signified that his Ministry would be most interested in receiving a further report in due course.

It was agreed that a report should be rendered, through Research Committee, to Council who would make it available, as it thought fit, to such bodies as the sponsors, and Ministry of Fuel and Power at highest level, and to those likely to help the finances. It should not clash with the Medical Research Council and therefore should avoid medical aspects. In this connection Professor Cook emphasised that M.R.C. investigations were only in the early stages and M.R.C. is adverse to issuing general publications until it had something definite to say.

It was recommended that the report should cover what has been done, what the Group is proposing to do, and the Group's relationship with the Ministry of Labour and the M.R.C.; and it might contain, in correct perspective, a brief summary of the work done in America.

Mr. Stevenson was asked to prepare a draft of about the length of four pages of the I.P. Journal for submission to the Research Committee in June. The Report should be fairly full for the Research Committee who could decide on suitable extracts for the I.P. Journal.

Professor Garner pointed out that the Hydrocarbon Group was automonous as it had not been appointed by Council. He therefore considered that it was for the Group to decide what should be passed on through Research Group to M.R.C. and the Ministry of Fuel & Power, and how much A.P.I. information should be used.

3. ARTICLE IN "MUNICIPAL ENGINEERING"

Mr. Stevenson's letter dated 15th April regarding the extract from "Municipal Engineering" on Synthetic Detergents and Cancer was mentioned.

It was agreed that this was more a matter for M.R.C. and did not call for immediate action by the Group.

4. INFORMATION FROM AMERICA

The Chairman referred to Dr. Newquist's letter dated 13th March appreciative of information supplied, and was glad to be able to record that we are receiving valuable information from Cincinnati.

5. REPORTS ON WORK PROGRESSING

(a) Professor Morton stated that he had been promised sufficient additional packing to complete his Unit which should be fully operative in July; it will work on the Kuwait samples; 300°/410° material will be fractionated. Professor Morton remarked that he was satisfied that the present Kuwait Crude Oil was very similar to that used originally. Professor Cook pointed out the need for the crude to be consistent and for ensuring that there is no cracking when distillation is carried out on a commercial scale. In reply to Professor Morton's enquiry concerning the laboratory fractionation of the Stanlow samples, he said he would prefer the whole of the sample to be fractionated before any part was distributed, even if the biological tests were held up for a short time. The meeting agreed. The question of further supplies of materials was discussed, and it was generally agreed to keep to the three crudes, Oklahoma, Lagunillas and Kuwait. Professor Morton promised

to advise the Chairman the quantities which may be required.

(b) Professor Squire summarised the biological work so far undertaken. He mentioned ideas originating in Dr. Woodhouse's war-time tests on oil extracts and in the field and laboratory work carried out by Dr. Cruickshank and himself with reference to cutting oils. It was evident in November 1948, when the M.R.C. Committee on the Carcinogenic Action of Mineral Oils was set up, that the investigations would be lengthy. Substantial progress had now been made proving that the biological tests could be regarded as quantitative and emphasising that the rabbit could be included in all such tests. The grading of the five different fractions from three crude oils was an important step forward. Professor Squire mentioned that the co-operation between the chemists and biologists on the Committee had been good, and that all the work had been greatly furthered by the help given by the British oil industry. The biological research work in this country might seem to be moving a little more slowly than that in the United States, but in some ways on a more certain basis (e.g. in the use of larger groups of animals). We had confined our studies so far to non-cracked oils. The Americans working with cracked oils find them to be much more potent carcinogens.

Professor Squire enquired as to whether the introduction of cracking processes into this country implied that the refiners were now likely to market chemicals which might be potent carcinogens. If so, he felt that the refining firms should themselves set up small biological testing stations to ensure that highly dangerous materials were not passed on to an unsuspecting public. It was pointed out that Oil Companies are already taking steps to safeguard their own employees who handle such oils or products.

6. INVESTIGATION WORK ON PRODUCTS

The Chairman remarked that it seemed important to work on certain actual products such as Cutting Oils. It was generally felt that this is a problem to be tackled not by the Petroleum Industry alone, but by some central body. It was more for the M.R.C. to view it from the overall big picture. He agreed with Mr. Ault that, in the case of Cutting Oils, not only should the oil side be examined, but consideration should be given to such factors as the hot end of the cutting tools, the effect of the workman inserting his arm in the machine, and the general hygienic conditions under which employees work. Moreover, if cutting oils were carcinogenic, the trouble, as Professor Squire has pointed out, might come from additives, or other components, and not from the base oil. The aim should be to obtain a line on which to work so as to determine the origin of the trouble and how to stop it. It was not clear under whose aegis this would come.

7. LABORATORY FACILITIES

Professor Morton expressed the opinion that as the existing facilities were fully employed, it was advisable to find additional facilities, particularly as it is almost certain the Chester Beatty work is likely to cease. He suggested the Manchester University might be approached. It was agreed that enquiries in this direction should be made.

8. DATE OF NEXT MEETING

To be held in London, on Friday, 25th July, at 2:30 p.m.

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3.6.52. RS.

C O P Y C O P Y C O P Y

INSTITUTE OF PETROLEUM RE3-2-9

RESEARCH GROUP NO.2.

Minutes of Meeting No. 9 held at 26, Portland Place, on 28.1.52.

ATTENDANCE

IN THE CHAIR:	S.J.M. Auld
GUEST:	W.S. Ault
MEMBERS:	E.J. Boorman F. Morton H.W. Stevenson
APOLOGIES FOR ABSENCE:	J.W. Cook E.J. Dunstan F.H. Garner
SECRETARY:	W.H. Crouch

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1. MINUTES OF MEETING NO. 8 held 26.6.51.

These were signed as correct.

2. MULE SPINNING OIL.

Regret was expressed that Dr. Hill was not present to explain what had happened to the specification. According to Dr. Hill's letter to Colonel Auld of 15th January, the specification has now been passed by the Joint Advisory Committee of the Cotton Industry and has been sent to the Chief Inspector of Factories in London for implementation. It was not clear from Dr. Hill's letter that compounded oils had been dropped, but Mr. Stevenson explained that the Technical Sub-Committee of the Joint Committee was unable to write a specification covering the fatty components of a mule spindle oil, and in recommending the adoption of the specification for the straight mineral oil, they also expressed the opinion that no harm should result from using compounded oils and, did in fact, suggest a list of about six suitable fats which might be used. This recommendation was not included in the specification. It was agreed if nothing further were done in implementing the specification that after a further period of three months the Chairman would take the matter up with appropriate bodies. In the meantime, he proposed bringing the position that had been reached, to the notice of the Ministry of Fuel and Power and the Medical Research Council.

3. FRACTIONATION OF MINERAL OILS.

Professor Morton stated that the results of Dr. Woodhouse and Professor Squire had shown that all the fractions tested (from Oklahoma, Lagunillas and Kuwait crudes) were carcinogenic, but that fraction 4 of each crude had the highest concentration of carcinogen. Fractions 4 of Lagunillas and Kuwait crudes had greater effect than the corresponding fraction from Oklahoma.

It was now necessary to fractionate fraction 4 (of each crude) into smaller fractions for further biological testing, but the quantity of each fraction available (from 60 to 90 kg) was insufficient to charge the vacuum unit erected for the purpose at Birmingham. Professor Morton stated he had discussed this problem with Dr. Vickery of Thornton Research Centre. There was insufficient of the original crude oils left to provide additional material and the further distillation of the residues (which would provide adequate quantities of a cut slightly heavier than fraction 4) was not recommended because of the tendency to crack. The use of an inert "chase" was not considered advisable.

Mr. Ault commenting on these points said that the distillation of further quantities of crude oil at Thornton would be expensive and not very desirable from the Companies point of view. He asked what the minimum amount of material required by the biological group would be. Professor Morton stated that he thought 200-300 mls. of each fraction to be tested would be enough for biological tests, but that larger quantities were required for chemical work at Glasgow and Birmingham.

Mr. Ault then suggested that the 60 kg. of Kuwait fraction 4 should be refractionated through a 1-4 gal. still, or failing such a still being available, sufficient for biological testing should be fractionated in a laboratory column. Professor Morton agreed that if this were done the needs of the chemical group might be met by fractionation of a commercially available cut corresponding to Kuwait 4.

It was agreed,

1. That the Chairman would ascertain whether Kuwait Company had any further supplies of the original crude oil, and if not to ask for analytical details of the present production for comparison with the original sample. The position concerning Oklahoma and Lagunillas crude was also to be investigated.

2. Professor Morton to find out from Professor Squire the quantity of sub-fractions required and to arrange for sufficient for all needs to be prepared.

3. Enquiries to be made concerning the availability of a small vacuum column, suitable for a charge of 10-15 gals.

4. Enquiries to be made concerning the availability of a commercial fraction similar to K_4 produced by vacuum fractionation without cracking.

4. NON-CARCINOGENIC MINERAL OILS.

The Chairman referred to Dr. L. Beard's letter of 9.10.51, relative to Dr. Saunder's work for the Socony-Vacuum Co. New York, on cancer of the skin through contact with cutting oils. The Chairman also drew attention to the Minutes of the 8th meeting of the M.R.C. Committee on the carcinogenic action of mineral oils, copies of which were distributed. Discussion was made covering these and the information given in Squire and Cruickshank's paper, etc.

The meeting considered that in view of the present information it would be undesirable to emphasize in any way the possible hazards of cutting oils, and that the results of work done for the group should be, as formerly, for the use of the participating Companies and the Medical Research Council only.

5. MEDICINAL WHITE OILS.

Mr. Stevenson briefly outlined the present position. The problems being handled by the White Oils Association do not directly touch on carcinogenicity. They are:-

1) Absorption of Medicinal paraffin from the intestine

2) Absorption of vitamins by Medicinal paraffin.

Item 1 arose from a paper written by Professor A.C. Frazer in 1943. Fortunately contact has been established with Professor Frazer who points out that in his work absorption only took place at a particle size below 0.5 . At particle sizes over this, absorption did not take place. It follows that commercial emulsions of Medicinal paraffins (of particle size 10-20 .) and straight Medicinal paraffin would not be absorbed. Frazer has stated quite definitely that on present evidence he regards Medicinal paraffin as a safe therapeutic agent provided it is taken at the proper dosage and at the proper times.

On item 2, absorption of vitamins by Medicinal paraffin, a report is awaited from the Hektoen Institute in America. Mr. Stevenson, who is Chairman of the White Oils Association Technical Committee, stated there was no overlapping of their work with that of the I.P. Research Group 2 and M.R.C.

6. The Chairman thanked Mr. Ault for attending the meeting and for the views he had expressed on the various matters, and invited him to become a member of the Committee. Mr. Ault said he had great pleasure in being present and would later inform the Chairman whether he would be able to accept membership.

7. NEXT MEETING.

It was decided to hold the next meeting at Birmingham, the tentative date being 28th April, next.

25.3.52. KBF.

Copy from D. V. Stroop for information only (Not For Publication) of members and associates of API Medical Advisory Committee

September 14, 1951

INSTITUTE OF PETROLEUM RES-2-7

RESEARCH GROUP NO. 2

Minutes of Meeting No. 7, held at 25, Victoria Street, 22. 6.51.

ATTENDANCE

IN THE CHAIR:	S. J. M. Auld
GUEST:	K. G. Mackenzie (Texas Oil Co.)
MEMBERS:	E. J. Boorman H. W. Stevenson

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1. This meeting was held for the purpose of discussing with Mr. Mackenzie the more strictly physical and chemical aspects of petroleum carcinogenicity. He was advised in detail of the work which has been done by the Institute of Petroleum and the Petroleum Board in relationship with His Majesty's Government (Ministry of Labour), the Manchester Committee, the White Oils Association, the Medical Research Council and others.

Mr. Mackenzie explained the interest in the subject which was developing amongst petroleum technologists in the U.S.A. There was a growing desire to ensure that the oil chemists should be cognizant of the work going on and this would certainly be aided in his own case by his association with Dr. Newquist of the Texas Company who had succeeded Dr. McIver Woody as Chairman of the A.P.I. Medical Advisory Committee.

2. The chief point which emerged from this interesting discussion was Mr. Mackenzie's agreement that any participation of the American petroleum chemists in this work should be in collaboration with the A.P.I. Medical Advisory Committee and the latter's association with the Kettering Laboratory in the University of Cincinnati. The A.P.I would therefore remain the official channel for interchange of information with the Institute of Petroleum. This would not in any way interfere with individual contacts which might be made and which would prove helpful in developing the subject.

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20. 7.51. KBH.

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of members and associates of API Medical Advisory Committee
September 14, 1951

INSTITUTE OF PETROLEUM RES-2-8

RESEARCH GROUP NO. 2.

Minutes of Meeting No. 8, held at 26, Portland Place, 25. 6.51.

ATTENDANCE

IN THE CHAIR:	S. J. M. Auld
GUEST:	Dr. J. J. Phair
MEMBERS:	E. J. Boorman E. J. Dunstan F. Morton H. W. Stevenson
APOLOGIES FOR ABSENCE:	J. W. Cook F. H. Garner
SECRETARY:	W. H. Crouch

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1. The Chairman introduced Dr. J. J. Phair to all members and on their behalf expressed the great pleasure they all had in Dr. Phair's presence at this meeting.

2. The minutes of the 6th Meeting, held on 27. 4.50 were signed as correct after the elmination of the word "medicinally" before the word "beneficial" in the eighth line in Minute 3.

3. WHITE OILS FOR MULE SPINNING

Mr. Stevenson reported that the signed report had been submitted to the Superintending Inspector of Factories, and when considered by the Ministry of Labour Main Committee on Conditions of Work in the Cotton Trade; the latter decided before coming to a final agreement, to make a further trial on white oil to the proposed specification in respect of its lubricating value. He had therefore forwarded samples of such an oil of approximately 17 centistokes viscosity to the four Mills nominated by Dr. Hill.

The Chairman expressed the disappointment of the Research Group at this further delay in the implementation of the recommended Specification. He proposed that if there were still further delay the matter be brought to the attention of the Ministry of Fuel and Power and the Medical Research Council.

4. A.P.I. and I.P. REPORTS

The Chairman raised the question of exchange of information and reports, stating that the number of Medical Technical Reports received were not sufficient. He asked Dr. Phair if this matter could be remedied and stated he had already written to Dr. Newquist and asked for a total of six copies to be sent to the Institute of Petroleum of which four would be sent to the Medical Research Council and two copies to Research Group No. 2. Dr. Phair said he would support the request. The Chairman stated that he would arrange reciprocal action on the part of the Medical Research Council and the Institute of Petroleum.

It was agreed that samples of the fifteen fractions of each of the typical crude oils should be sent for Dr. Phair's use at the Kettering Laboratory. Mr. Stevenson would arrange.

A copy of the Minutes of the 6th Research Group 2 Meeting, of the report on White Oils for Mule Spinning, and of the Specification of the White oil for lubrication of Mule Spindles were handed to Dr. Phair.

5. The Chairman briefly outlined the I.P. research activities, particularly those relating to the crude oil used, their distillation into fractions and their testing, at various centres, for their biological activities, and the work being done on white oils for mule spinning in connection with mule spinner's cancer.

Dr. Phair described at some length the A.P.I. research organization with respect to the Medical Advisory Committee, the Safety Committee, a sub-division of the Technical Committee and their interrelations, the A.P.I. sponsored Kettering Laboratory, its history and organization. He outlined the epedemiological study of hazards and the research results recorded in the two reports which had been circulated to the M.R.C. Committee and I.P. Research 2 Committee members. He described Dr. Horton's method for determining the carcinogenetic potency number of the materials tested. The method uses ultraviolet absorption at 430 μ and Methylcholanthrene is used as a standard.

Dr. Phair discussed the petroleum fractions used, particularly the samples of A.P.I. 8 and 12 from fluid catalytic cracking, their preparation and testing for carcinogenetic propensities. These contained high contents of carcinogens. Samples showing high intensities in the u-v were the most potent.

The potency of products from various types of cracking operation and solvent extraction were discussed and emphasis was placed on recommended practice of dilution of heavy catalytic bottoms so that not more than 10% would be present. Companies differed in the disposal methods they employed, e.g. burning the whole bottoms made, carbon black manufacture, etc.

The potency of high sulphur fractions A.P.I. No. 8 was unaffected by catalytic hydrogenation even when the sulphur content was reduced to 0.15%.

Slack wax and pressing operation hazards were discussed. Slack wax was more potent than most other products. Operators in wax pressing work, mule spinning and shale oil operations were more prone to be affected than other workers. Maintenance workers and firemen came more in contact with dangerous products than other workers.

Dr. Horton had found that white oils were skin irritants, but were not carcinogenic. Solvent extracts were toxic. Sulphur dioxide is poor in removing carcinogens. He had recently found potent oils in fractions from thermal cracking operations.

Oils of high viscosity were thought less likely to cause cancer than low viscosity products, as the latter more easily penetrated the skin.

6. In thanking Dr. Phair for his informative and important talk the Chairman expressed the views of the Group that it was gratifying to know the fine work being done on both sides of the Atlantic dovetailed so well. The American approach was specific, whilst that of the British was fundamental to the crude oil. This meant there would be little overlapping and that the ground would be covered. It was likely to be a very long job.

7. On the supply of Extracts, as used by Dr. Woodhouse, for Dr. Squire, both Dr. Boorman and Mr. Stevenson replied that they had not been able to locate further quantities.

8. RESEARCH GRANT REQUEST.

Professor Morton stated that the Trinidad Leaseholds fractionating equipment and the building to contain it would be ready about September for the next stage of the crude oil fractionation for the middle distillate investigations and requested that a grant of L500 for one year be made for the establishment of a research fellowship at Birmingham University. The stipend to be L240 per annum, the balance for expenses.

The meeting gave full support to this application. The Chairman would report to the Research Committee and ask that this decision be brought before Council at their next meeting.

9. DATE OF NEXT MEETING.

It was decided that the next meeting should be held early in October, by which time Dr. Woodhouse's results would be available, and deliberations between Professor Morton and Professor Cook would have taken place. The exact date would be advised by the Chairman.

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20. 7.51. KBH.

June 22, 1953

Dear Dr. Horton,

We received your letter last week and the one this week and enjoyed them very much. We were very glad to hear that your transportation and accommodations were the best. (Maybe now you'll appreciate the wonderful service of American Airlines and the excellent hotel accommodations in the U.S Despite all this it sounds like you're having a grand time. The various men you're meeting sound like a bunch of characters. Could that possibly be characteristic of Cancer research workers?

Since you left we have added two members to our department. The girl Rus knew about, Ann Rush, from Florida came up and was interested in the job so she's helping in the lab. Then we thought that since you were going to be gone for three months we really ought to get a replacement for you. Do you remember Jeanne Rehm, the girl I ride home with? We wanted to assure a ride during the summer and your office was vacant so she consented to take your place for a couple of months. There was just one condition that she insisted on, however. She wants the API to send her to Europe next summer. I thought that would be easy to arrange so everything, is set. Seriously though, Jeanne is working with us. She is supposedly helping Dottie, but she is camped in your office because of lack of space on the fourth floor. She is doing statistics right now and it makes it real convenient because there are many questions that have come up and I don't think Dottie could help on all of them since she has never completely done one by our present method.

Dr. Schiessler called about two weeks ago. He wouldn't talk to anyone else so we don't know what he wanted. After the call I wrote him telling him that Ruth was working on accelerators and was interested in receiving the list of samples available in the quantity we needed that you had written to him for.

Dr. Samuelson from Columbia Carbide came to see you last week. He was interested in seeing the chem labs and talking to Ruth and Rus when he found you were out of town.

Dr. Kehoe frightened me last week. He came in and ask to see the API report. For a minute all I could think of was the table, and that it wasn't near completion. He was interested only in the text. I don't know why he thought it was ready for him to see, but he evidently thought you wrote it before you left.

Speaking of the report one question I have concerns the table, "Concentration Standards with Synthetic Carcinogens in Various Solvents", the one I made up for you before you left. In accumulating the experiments for this table I found a lot of experiments involve benzpyrene and most of these are at a concentration of 0.15 g/100 ml solvent. As you can see (enclosed sheet - ex. 1) by the spacing (which is as it would appear in the report) the columns can not be too big. In most of the experiments the solvent will fit in one or two lines but some of them, e.g., 267-1,3,5, and 8 and 80-1 thru 80-6 and 71-4a thru e are distillation cuts in which boiling ranges should be given(?) to describe the solvent. I was wondering whether the carcinogen(BP) could be used in the title and the above mentioned experiments put on a separate page. See second example, on attached seet. Or all the BP in various solvents could be segregated - see ex. 3 on the attached sheet. You will notice on example 3 we still must have a concentration column because it varies - in ex. 2 the concentration is 0.15 in all cases. I will wait with this table until I hear from you. You may have noticed - the concentration is given in g/100 ml on this table because of the number of different solvents - some of which it is impossible to get gravities on. All the other tables completed so far have conc. given in g./100 g.

Rus said to tell you he found that most Diels-Alder compounds and most pyrene cpds. react with iodine. Those that do not are the exception rather than the rule. Rus leaves on vacation next week when Ruth returns. The odds have the Tye's heading Colorado way.

No sample of croton oil or word have come from Shubik or Schoental since you left - they must have gotten caught in one of the tornadoes we've had recently.

Dottie doesn't remember a session of Tyzzer's disease during the summer of 1951 but she will check. Dan said to tell you he was catching his limit in bass each week-end at Sharon Woods.

Dottie and I are both getting the travel bug. Dottie plans to take off about the second week in July for NewYork, Boston, Cape Cod and all points east. We're planning the same route but we can't quite swing it to leave at the same time so that we could make it an eightsome instead of two foursomes. I'm afraid we're going to be stuck with Dottie for at least 2 more years. It seems she's signing most of her life away to the finance company in exchange for a new Chev. By the way mother and I invested in a new car - another plymouth. New I can say the back left fender is all mine - that is it will be in about a year and a half when I pay off the loan from my mother.

Helen (Dr. Phair's secretary) is engaged and will be married November 21.

Enclosed you will find a copy of an invoice from Scandinavian Airlines. What is it and what should I do with it or about it?

Dottie said she spent the day "shaving, stretching mouse skins and pipetting solvents on freshly killed mouse skin. I was fairly well satisfied with results of different solvents on films of anhydrous lanolin but I've got a long way to go with skin. Concerning the former - I prepared films by heating the lanolin and spreading 3-4 drops on slides on a hot plate (about 60°C) and allowing these to cool on a flat surface. Then I pipetted the solvents (using no dye)and a micr-pipette calibrated to hold 13.78 ml. on these slides and measured the amount of spread after 10 min. With 7-8 slides for each solvent I had good results and found the average spread to be: Benzene 7.6 mm, DDN 8.3 mm., sec.-AB 10.0 mm., white oil - API-80 8.5 mm., PDB, cut 3 8.1, 1-ø-dodecane 7.3 mm., API-56 7.4 mm.,. Both Sec.-AB and Benzene dissolved the lanolin but using a thick film the edges remained circular.

The results of todays play are frustrating. Even when the skin was shaved with a fine razor the solvents spread out into very irregular areas. My chief consultant - Mary Lou Eisele (Dr. Suskind's 1st assistant) claims she gets nice circular areas with solvents on guinea pigs so we shall put our heads together and find the answer.

After a firm letter in reply to a slight misunderstanding with Bar Harbor concerning orders running out everything is fine and we have received 160 healthy mice with 160 more on the way Monday. We started some tests on mono and diamylnaphthalene, amylbenzene from dist'n of C-24, the residue of API-80 b. more than 720°F. and triisopropylbenzene. "

I don't want to bankrupt Kettering with the postage bill for this letter so this will have to be all for right now. One thing though I checked with Dr. Kehoe about the coal-tar project and he said he hasn't heard a word. He is planning to write someone about it though.

Sincerely,

P.S. We may have to have an ice tea seminar to discuss the possibility of your taking more vacation. We'll let you know the outcome at a later date.

RESEARCH COMMITTEE

Report of Research Group No. 2.

Since the last meeting of the Committee there has been one meeting of Group No. 2, most of the work having been done by personal meetings and correspondence.

Recent activities fall under three headings:

1. Medical Research Council - Committee on the Carcinogenic Action of Mineral Oils.

This association is progressing satisfactorily. The work at Birmingham under Professor Morton has arrived at a stage where redistillation of fractions O_4, L_4 and K_4 has been completed on the small scale. The distillates have been reblended to give 5 agreed fractions from each crude for chemical and biological testing.

In order to provide a sufficient reserve of active material the following steps have been taken:

Langunillas Distillate. N. V. Curacaosche Petroleum Industrie Maatschappij have provided 10 drums of distillate 300-400°C prepared by commercial vacuum distillation.

Oklahoma Bright Crude Distillate. 10 drums of No. 70 lubricating oil distillate have been provided by Socony-Vacuum Oil Co., the bulk of the distillate being concentrated in the 350-400°C range.

Kuwait Distillate. Esso Petroleum Co. Fawley have supplied 3 drums each of a vacuum overhead and a No. 1 side stream fraction which together cover the boiling range in which we are interested.

With the erection of the Stedman vacuum distillate unit at Birmingham it should be possible from these sources to obtain all the necessary fractions in sufficient quantity for detailed chemical and biological examination.

An interim Report by the Medical Research Council committee to the Council has now taken shape and has been accepted in principle. Final distribution will probably be restricted but will enable copies to be provided to those directly concerned in the Petroleum world including, of course, the Ministry of Fuel and Power.

2. Medical Research Council - Committee on the Possible Carcinogenicity of Detergents.

The M.R.C. have formed a committee under Professor A. Haddow of the Chester Beatty Research Institute to study this subject. Whilst Group No. 2 is by no means certain that the problem is one in which they or the Petroleum Industry should become too closely involved it was agreed that help should be given as far as possible to the M.R.C. in their investigation. For this purpose meetings of the Group and individuals were held to discuss the situation. These included representation from chemical manufacturing firms concerned notably

Shell Chemicals and I.C.I. A summary of the information thus collected and the views expressed was passed to Professor Haddow who has expressed his gratitude for the great help and interest displayed. In order (it is hoped) to bring this investigation to a close four chosen commercial detergents will be subjected to biological testing at the Chester Beatty Laboratories.

3. White Oils.

A second report of the Joint Advisory Committee, Technical Oils Sub-Committee has been issued by the Ministry of Labour and National Service - Factory Department. This report contains the specification developed by the Technical Oils Sub-Committee of the Joint Committee of which two members are from the Petroleum Industry - S. J. M. Auld and H. W. Stevenson. The question of making the adoption of the specification a statutory obligation is at present under consideration by the Ministry.

4. Other Matters.

Since the last meeting of the Research Committee the Group has been approached by Dr. Brandon Lush of the M.R.C. and by The Pedestrians' Association both raising the question of the potential carcinogenicity of heavy oils and of exhaust fumes particularly those from diesel engines. In both cases certain information has been communicated to the parties concerned; but the opinion was expressed that the Industry had no information which would lead it to believe there was any likelihood of the causes referred to producing pathological effects.

S. J. AULD.

Example 1

API SAMPLE NUMBER	STARTING DATE	CARCINOGEN[1]	SOLVENT	CONCENTRATION (g./100 ml.)	STRAIN OF MICE AND NUMBER OF APPLICATIONS PER WEEK	DOSAGE PER APPLICATION (mg.)[6]	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./wks.)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (5% F.L. in weeks	RELATIVE CARCINOGENIC POTENCY, P_{MC}

Example 2

API SAMPLE NUMBER	STARTING DATE	SOLVENT	STRAIN OF MICE AND NUMBER OF APPLICATIONS PER WEEK	DOSAGE PER APPLICATION (mg.)	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./wks.	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (5% F.L. in wks.)	RELATIVE CARCINOGENIC POTENCY, P_{MC}

Example 3

API SAMPLE NUMBER	STARTING DATE	SOLVENT	CONCENTRATION (g./100 ml.)	These columns would be the same as Example 1 and 2.

5. Before I forget, have them dilute the L-4 as much ~~equally~~ with B as they did L-5 so the results can be compared directly.

6. If DMPh has produced no tumors, do not include it in this report.

7. The experiments on BP -- don't include any under "Conc Standards" Table I.

8. Some of the 252's were discontinued because of poor health -- omit them unless Dottie thinks they are good enuf for the Table on Limited applications.

9. In the BP table, experiments do not need to be in numerical order; better group them according to type of solvent. I might suggest the following order: 244, 252 - space - 258, 264, 270, 271 (assign nos. in 200 series) 80-1, 80-2 etc. -- 80-6, and C-24) alkylbenzene, - space - 262, 245, 259, 260, 267's (call PDDR "alkylate from benzene and propylene polymer") - space - 265, 272, 273, 274's, 275 - space - 263, 269, 257 (if essentially free of aromatics from U.V. spectrum), 266 - space - 71-4's (give these nos. in 200 series when used as solvents for BP).

10. Omit API-9 from Limited Applications.

11. Leave 71-1 and -2 where you have them.

12. Skip 8-23gh

13. Re equations relating d and $\bar{x}$, your new conc. in wt % should be used to calculate them, thus redefining the "Standard Area" as that covered by 100 mg. of benzene. P_{MC}'s should be calc. from the new equations thus obtained for C3H and CFW mice.

14. Can we get enough pure cetane to do a control experiment at 100 mg? - to see if it produces any pops.

15. Please ask Miss Shyppard to hold my Aug. 1 check until I get back.

16. Let's try n-decane as solvent for BP. Also we should try the rabbit for crust tests - may be more sensitive to accelerators than mouse skin - can probably use 5 diff sites on each rabbit.

17. Have a good holiday and don't take any worries about the report with you. Seriously, it just isn't that serious a matter. Best regards, WH

First fold here

BY AIR MAIL

AIR LETTER

IF ANYTHING IS ENCLOSED THIS LETTER WILL BE SENT BY ORDINARY MAIL.

SEATHAM 12 AUG 7 JUL 1953 S.W.16

POSTAGE ON LETTERS

EUROPE

POSTAGE

Miss Mary Jane Graf
Kettering Laboratory
Eden & Bethesda
Cincinnati 19, Ohio
U.S.A.

Second fold here

To open cut here

Sender's name and address:-

A W Horton
112 Sanley Rd.
Hanbury
London S.W. 16

To open cut here

SC-API-2223



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June 14
(No birthday card yesterday, tsk, tsk)

Dear Jane,

Having been grazed by two of these buzzwagons as I stepped off the curb looking to the left during my first week in Glasgow and, more recently, experiencing the rude awakenings that come when behind the wheel of an Austin touring the right side of a main 2-way London thoroughfare, I figured I'd better take time out this weekend and scribble a few notes of semi-practical interest just in case 9 lives are only allotted to certain experimental animals.

Ff 2 (sent. 2, même chose). Dr. Peacock (R. Ca. Hosp., Glasgow) is convinced that overheated fats are carcinogenic to the squamous fore-stomach of mice. Humans don't have such a unit in their g. i. tracts, so he did not admit to me his conviction that his data could be extrapolated to us. However, Dr Cook privately advised me that Peacock is quite sure that any of the comforts and conveniences of modern living, such as the cooking of food, is probably in the end harmful to the human body in some way.

SC-API-2223

Curiously enough, he does drive a car. Dr Cook does, too, - but like he was frustrated in some childhood ambition to some day pilot a hot rod in a Memorial Day race at Indianapolis. Don't know what practical use you can make of this, unless one day you happen to be invited by the professor to take a leisurely Sat. afternoon ride thru the Scottish highlands, in which case there would seem to be 2 alternatives to suggest:

1. Make sure your life insurance premiums are paid and your will notarized,
or 2. Get out of the country before Saturday.

Incidentally, Dottie, Peacock said Tiger's disease as supposed to recur every 18 months. There's nothing in our records to indicate summer, 1951, was hard on the liver, was there? Of the mice, that is. As far as humans are concerned, a recent note in my diary tells me to never again comment to Dr ERA Merewether, at least not as early in the day as 1300, that if the Ohio River were made of beer, it would stop at Cincinnati. By 1900, he was making like T. McCarthy about fluoridation, only not on the toxicity basis but that they should be spending the money in something more important like statistics on bladder and lung Ca incidence rates in industry. Anyway, I shall see him again on the 29th just after I return to London and hope to get some of the actual numbers on these rates in Britain, assuming we can stay off the track of relative potencies of Worthington draught vs Milwaukee's finest. Seems a shame that such an interesting topic should be wasted on me, a bona fide member of WCTJ.

"<0.0001% BP in overheated fats" - that's the sensitivity they (Peacock's group) claimed for a 5 min. exposure of a plate to the fluorescence of the test sample. However, they're waiting for Schoental to come home to make the method more sensitive with the moving wedge. Did she ever come to Cincy? Did we ever get a sample of croton oil or bear from Schubik again?

I'll be on the move until June 26 - settle down then at 112 Semley Rd., Norbury, London S.W.16. Let me hear how things are going -

Best regards to all - Wes

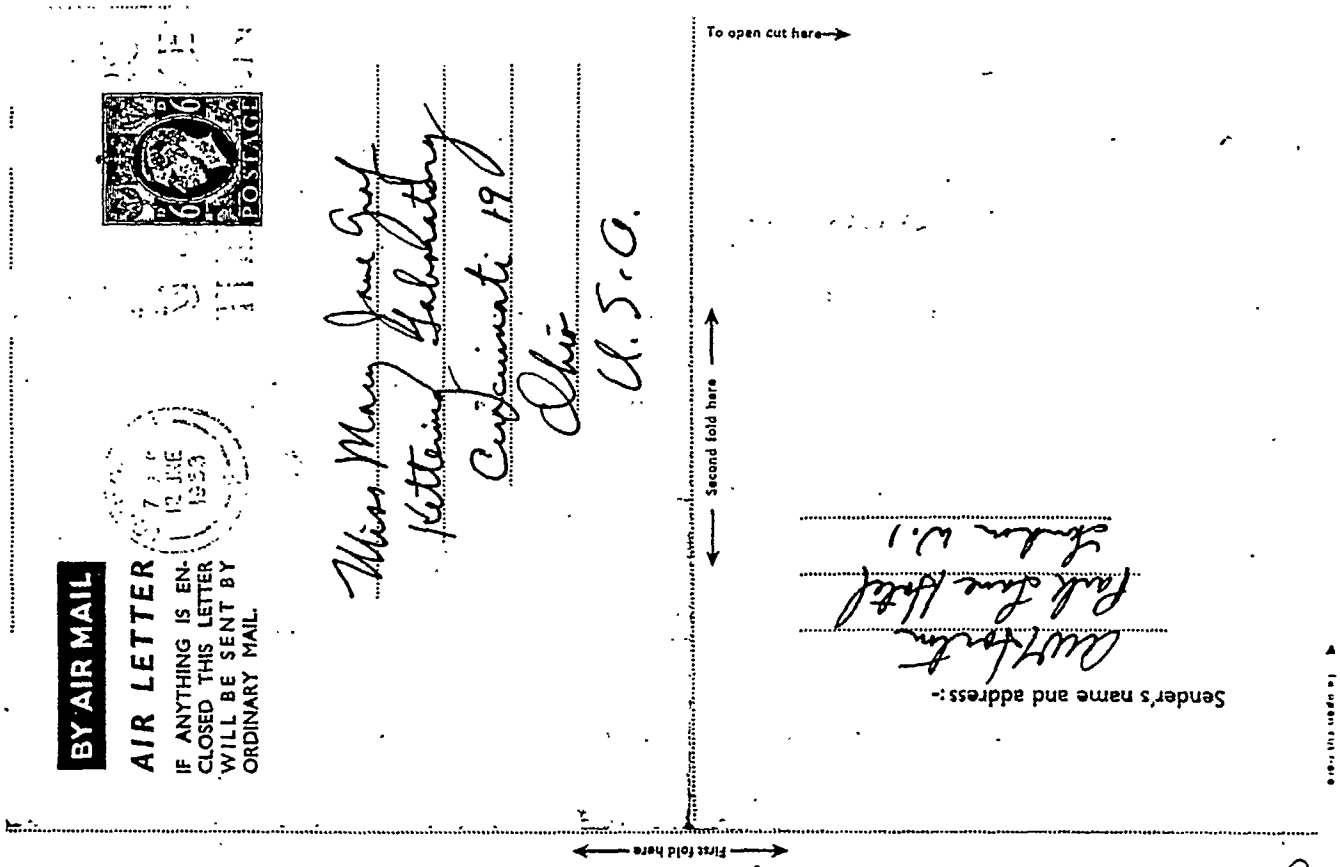
Many June - how soon may I have your permission to take some more vacation? The way Haddow + Auld are scheduling my July dates, I won't get more than the week I pick up the family in Ireland over here -

June 12

Dear Jane,

Don't believe any thing you hear about my having landed either in the brink or in the Bastille. For one thing I haven't reached Paris yet. Though about next Tues. noon I expect to sight the Eiffel Tower.

Life has been just a bit hectic since I landed at Prestwick a couple weeks back - The first couple hours in Glasgow were the worst though. I thought I was going to freeze or starve, - didn't know which first. Some fresh air fiend had been at work with the windows in my hotel room. When the rickety old bus rolled into a railroad station with ancient coal burners puffing up and down and making that eerie whistle you've heard in the movies and the driver said, "Well, here's your hotel", I thought my plane must have taken the wrong turn somewhere and just skidded off the 20th



century into antiquity. This concept was lightened by the fact I hadn't had a wink of sleep on the flight from New York, perhaps because dawn arrived only about 2 hours after the sun went down. However, the scene to date has the St Enoch in Glasgow down as the best, or at least most comfortable, hotel I've been in in Britain so far.

If my room reservation arrives for Stockholm, I'll be receiving mail at 112 Tenley Rd, Norbury, London SW. 16 from 20 June on. I don't suppose any questions have arisen, but di

Tell Dan I haven't wet a line yet, but still figure to baptize his rod case with holy water from the R. Thames in a couple w

Best regards, W.H

Say hi to all the gang for me.

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Send the following message, subject to the terms on back hereof, which are hereby agreed to

Dr. A. W. Horton
Hotel Statler
New York, New York

Mr. Frondorf suggests you contact Henry Diot, International Travel Department, American Automobile Association, 250 Park Ave. He is making all overseas arrangements and may know more about hotel reservations. I am forwarding to Dr. Cook a program for the symposium from Dr. Lacassagne.

Mary Jane Graf

May 23, 1953

Dear Dr. Horton:

In the process of clearing your office of journals and reprints from the library I came across a reprint by A. F. Sladden of Swansea, South Wales, on "Pitch Cancer". It was a paper given at the First International Conference on Cancer, London, 1928. He did an epidemiological survey of patent-fuel workers of Swansea. The chief form of occupational cancer in Swansea is epithelioma occurring among these workers and ascribed it to the influence of gas-works pitch. Incidence per year was 1/2 to 1%. The article did not give any other information on the authors address or the industry on which the survey was made. From the British Medical Directory I got his name and address as Arthur F. Sladden, Cheltenham House, Eaton Grove, Swansea. I know you have one other person on your list in this town and I thought you might be interested if you decide to make the trip to South Wales.

Enclosed is the information you were interested in on the British samples.

Friday a letter came from Professor Haddow, Director of the Chester Beatty Research Institute. He says he will be happy to see you during the week of June 1st, and asks "will you perhaps be good enough to ring me on arrival"? His phone number is Flaxman 1484-5 and 4188.

To review - Dr. Lacassagne will be pleased to see you June 8-12. He and his associates are going to have a seminar or symposium June 17-20 to discuss subjects in which you are concerned or interested and you are invited if you can make it. His phone no. is Odeon 45-75. Dr. Scott will also be pleased to see you on the 28th of May. Suggests 10 or 10:30 AM as a time for a meeting and that you call him, Broxburn 115, upon your arrival in Scotland. The best time to call him is from 9:30 to 10 AM any morning. He has been in contact with Mr. Crichton. Mr. Crichton says that if you advise him in time He will arrange for a car to meet you. He thought that Edinburgh would be your best destination. His phone no. is Broxburn 35 and 34.

Sincerely yours,

P.S. There was an article in the paper this morning about a man who caught 54 bass at Dale Hallow in 5 days. Want to change your mind and come back,

SC-API-2223

also have to check one of our saturated accelerators without BP and prove that they don't really produce pops by themselves when pure — if so.

Cook is sending a sample of L-4-4 among others — I wanted it specially for an "iodometric" BP analysis. Cook, and especially Carruthers, find it hard to believe there's any BP present.

The latent period for 0.15 BP in many of the cuts seems to be 15-18 weeks — the same as we got for L-4. Ask them to start some lower concentrations of BP in a saturated accelerator to see how low a conc. of carcinogen still gives this latent period — Might suggest what the minimum conc. of carcinogen (MC or BP equivalents) is in L-4 and the like.

Kennaway's chemist says that 4-Me and 4,9-Me_2-pyrene are ~~less~~ more strongly adsorbed on Al_2O_3 than pyrene. Have we been led astray by the properties of a warped 9-Me in 9,10-DMBA? Wes

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LETTER
EUROPE

1953
CORONATION

Dr. Ruth Pirle
Kettering Laboratory
Cincinnati 19,
Ohio
U.S.A.

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Sender's name and address: A W Horton
112 Semley Rd.
London S.W. 16

IF ANYTHING IS ENCLOSED THIS LETTER MAY BE SENT BY ORDINARY MAIL

To open cut here

SC-API-2223

SC-API-2223

The London Philharmonic sounds as good in the Park as any July evening as on a Hi-Fi recording. It'll need another visit for such worthwhile things. There've been just too damn many meetings. July 24

Dear Ruth,

Since the latest results I May have sent on the test on cetane seem to confirm our early suspicions about its accelerating activity, it certainly seems, as you suggest, that we should test only one of Schiessler's samples — perhaps the cyclohexane deriv. if we can assume that nighthenes are the most common type of HC in crude distillates b. ~ 600°F. The position of the ring at the middle of the alkane chain may make a big difference in its irritating properties from those we have previously tested. Hope not — imagine devising an analyt. method to differentiate the 2 types in complex mix;

I shall try to drive thru State College, Pa. on my way home from N.Y. Will particularly want to see what S. has in pure HC's corresponding to the cut-off point (the max. MW or b.p. with of accelerating activity) as far as we can guess that point from mouse tests on the fractions you've distilled from 71, 80, etc. Would you, along about Aug. 7, prompt me with a brief summary of the latest results of the mouse tests on your samples at that time? General Delivery, State College, or % Schiessler, should reach me OK.

Has polypropylene been started yet? We might as well cover the HC horizon while we're at it. We'll

time between the 12th and 17th depending on what, if anything, I hear from Schlesser. I dropped him a note last week -

Thanks for the birthday card - we all got a kick out of it. Actually, even the ones my kids sent I didn't get until I reached the Institut de Radium a week later than my original plans had it.

And so to work; - I'll wade thru these questions and try to answer them.

1. In general, use "colorless fraction" instead of "white oil" for the chromatographic cuts from various refining streams such as 8,71, 8-13, etc. Refer only to 80 and 95 as white oils.

2. OK, OK, leave the conc. as γ/100 ml in the cases mentioned.

3. Don't include any OMBA's we didn't include in the Sept '52 table. Some of them were spidiged.

4. Under "Conc. Standards", perhaps we can simplify our current problems by using only the 15 or so experiments with 14C in B (plus the extra ones on 0.15 γ 14C/100 ml B completed in '53) with which our standard curve is calculated, including the column "(mg./standard area/week)" Design of week city. These will show something about reproducibility.

Might be a good notion to drop Mrs Stopp a note advising that the stencils will be forwarded in September -

16 July

Dear Jane,

Sounds like I'll have to buy a hat just to have something to toss into the office to make sure it doesn't come sailing out, before I dare enter, come Aug 15 or thereabouts. Anyway, please don't take the possible "errata" on these interim reports so seriously. You've had far less corrections to make on past ones than most authors who have half as many tables in books they are printing for publication.

Re vacationing - do finish out that week ending Aug 16, - on your trip, I mean. I certainly wouldn't want you to spoil the last part of your holiday - just to work Thurs + Fri before the weekend or anything like that. I'll arrive some

SC-API-2223

July 13, 1953

Dear Bes,

Dr. Schiessler called today. Apparently he had just had a letter from you. I don't know why he didn't just write - he had selected three compounds which he was offering to send us. It seemed rather a take it or leave it proposition, so of course I took it. He started to spell out the names, and was surprised and relieved to find that I was a chemist. I thought we had explained that when we wrote to him.

He is sending 25g. each of 7-n-hexyltridecane, 7-phenyltridecane, and 7-cyclohexyltridecane. These all boil at about 600 F. He mentioned that you had requested at least one of them, and he seemed to feel that they were quite widely different compound types, though closely related in size and shape. After the results on phenyl- and cyclohexyldecane, since the compounds are so precious and so scarce (he gave me a lecture on that), do you think it is necessary to test all three?

Dr. Schiessler said he would be there in August, but hoped you would not come the 14th, as he will have other visitors. Mary Jane therefore did not write to him as she told you she would.

I am not sure of this yet, but it looks as if 4,5-dimethylchrysene is isomerized or otherwise changed by sulfuric extraction. This is one of the sterically hindered compounds like 4,5-dimethylphenanthrene. The methylbenzphenanthrenes were apparently unchanged under the same conditions.

Ruth

July 9, 1953

Dear Dr. Horton,

Things are getting a little hectic here at the lab. The report is not finished, vacation time is coming soon and to top it all off Dr. Princi's inaugurating an orientation program for the students. Each new student (there are five) will spend the afternoons of one week in each department (our first student came Monday, July 6th). In addition the department head is to talk to them about what all is going on in the department during one morning period. You're not here so that throws it on the rest of us. Dottie's little nephew died last week so she was out a few days: Ruth has her hands full with Ann and Jim in the lab since Rus is away (by the way his plans changed again and he headed for the eastern shore rather than out West); and I'm going crazy with this report. I thought for once I'd be able to take my time and really have it correct - well, don't you believe it. The further I get into it the more doubtful I become. The tables on accelerators (using BP) are mixed with the BP standards. Enclosed you'll find a copy of the table as it will appear in the report (except for the * on side and the one footnote about b. ranges for PDD fractions). This is everything on BP. API-245 also appears in the table using DDS as solvent. You will notice the concentrations are still g/100 ml. Ruth made a ~~calibrated~~ micro gravity tube as you suggested and we now have a lot of gravities but if we use them it will throw the whole table off. Instead of most of the concentrations being .15 g/100 ml. they will all vary with the solvent and that would require a column for concentration and they would not be able to see that they were set up with comparable concentrations. So for this report unless you want it the other way I'll leave it in g/100 ml for this table. Also you may want the BP standards duplicated on the table "Concentration Standards with Synthetic Carcinogens". This table will also have MC, DMBA, DMPh in various solvents on it. This table will be like my example 1 in my last letter. SO if you want the BP stds. duplicated, or the enclosed table should have some changes as far as descriptions, footnotes, etc. let me know as soon as possible - I leave the 24th of July.

One of the many other things that are bothering me are the descriptions of lab samples. These are far too many to ask about in a letter. They'll have to wait. I think the table for crusting experiments is set and the one employing a limited number of applications has just 73-2, and the 6 new experiments Dottie just set up. They are 43-1 to test for constant P_{50} and 71 to test for variable P_{50}. There is another ~~xxxx~~ which was discontinued, 73I-9, just one cage after the 34 week. 5 out of the 5 tumors came ~~B~~ before the 34th week. There are 4 deaths in the beginning but the weight curve does not look too bad except for that. Should this be included on this table too?

Now table I! Thats a mess too. One thing I want to know - why do you plan all of these long trips during the time the tables have to be done? Once again the questions are too numerous to mention here. One thing - how important is it to have 71-1 and 71-2 in the front with API-71? Last time it was in the back and I got all the way to the last page before I found the note I wrote to myself about puting them in the front part under TCC. Another - should 0-23gh be included - the one started as 0-23g and then switched to 0-23h so it's now 0-23gh. The new tests you wanted on L-4 and L-5 were started yesterday at 5 mg. (L-5 is 75% in benzene).

Shortly after you left I calculated the new equation for CFW experiments. Since then Joanne has been doing statistics on all the experiments and we found a few little differences in the experiments used for the C3H experiments. The enclosed graph has the new C3H and also the latest CFW (recalculated based on new C3H). Shall we use the 2 new equations to calculate potency or still use the C3H equation that was done before you left. None of the P_{50} for the report are calculated.

e haven't heard a word from State College, Pa as yet, but I will write to Dr. Schlessler to see if he'll be home around the time you plan to pass thru there.

Dottie has postponed her vacation for two weeks, so it looks like we'll all be heading east together. We're leaving Friday, July 24th from work. That will leave only Ruth because I us left the 3rd of July for a month. I'll be back around the 12th or 13th unless you're planning to stay away longer than the 17th. Let me know if possible.

Mary Jane

Dear Wes,

The boss (Mary Jane, of course) has instructed me to use an abs. min. of paper to go into this already bulky mailing, so I shall use C.A. style and no margins to tell you that one of your chemists is hard at work. I came back so Russ could leave. Ellen was ill, so they didn't leave till last week, then went East! I have bought an air-conditioner for my bedroom, hoping it will help my hay fever. So they have ragweed in Europe?? Maybe the API could send me over there during the 1954 season! Things here have been most hectic - I have been frantically busy, but there doesn't seem to be much to show for it. The latest is a scheme whereby each of the new Fellows is assigned to each department for a week to get acquainted with the people and the work.- an excellent idea, but just one more thing to be done. It seems to be almost a full-time job to keep Ann and Jim busy, at least until I get my work rearranged so that they can be useful. They both work well alone, once they know what to do. Jim has been doing all the messy viscosities and gravities, and Diels-Alders for Russ. Ann is doing sulfuric extns. on all our known compds. - at long last! I am trying to distil n-decane, but the good stillhead won't work at atm. pressure, so I shall have to start all over. I expect to distil a big batch of DDB through the 4-ft. column for Dottie to use in her next batch of tests on DDB. Do you think that will be sufficient purification, or can you suggest something else to do to it? The distn. of C-24 surprised me a bit - roughly there was 20% below 400F.; 20-30% at 400-500; 30-50% at 500-550; 50-80% at 550-600; 80-90% at 600-650. We are testing the 450-500 and 555-560 cuts with BPY (not an "amylbenzene" cut as it appeared in the last letter to you). Also I chromatographed the distn. res. from API-80 (50%, b. >720), hoping it will be a non-accelerating solvent for some of the diln. tests. We both seem to have had the same idea about the little pycnometer. It is made, but not yet calibrated. I haven't had a chance to look at L-4 and 5 and the others you mentioned. We are testing L-5 as a 75% soln. in benzene, as it was too viscous for a 5 mg. dosage straight. The Org. Symposium at Ann Arbor turned out to be most informative as well as a lot of fun. Everyone I wanted to see was there, including Schoental, who was in the next room! She is a darling - I hope we can get her to the lab when you are here. She said there are two reasons for using fluorescence spectra: (1) once the app. is set up and there is someone to run it, you can get a permanent record of 5 spectra in 1 hr.: (2) a characteristic BPY spectrum can be obtained from a very small and relatively impure sample. She said flatly that if the sample is pure enough to give a good uv spectrum (and she was surprised that we could get them that pure), there is no advantage in fluorescence spectra. The latter are much more specific than the literature suggests, but still not as characteristic as uv. Guess I have used up my allotted space - hope you are finding a few spare moments for vacationing in your busy schedule!

Ruth

TABLE II(

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS

OF BENZPYRENE IN VARIOUS SOLVENTS TO THE SKIN OF C3H MICE

	API SAMPLE NUMBER	STARTING DATE	SOLVENT	STRAIN OF MICE AND NUMBER OF APPLICATIONS PER WEEK	DOSAGE OF SOLUTION[1] PER APPLICATION (mg.)	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./wks.)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (5% F.L. in weeks)	RELATIVE CARCINOGENIC POTENCY, P_{MC}
	244	9/14/51	Benzene	C3H 3	100	7 (20)	7 (20)	100/33 (100/33)	22.1±5.8	
	245	12/3/51	Dodecylbenzene	C3H 3	100	20	18	100/19	11.3±2.0	
*	245	11/21/52	Dodecylbenzene	C3H 3	20	15	13	100/31	16.3	
*	245	11/21/52	Dodecylbenzene	C3H 3	5	15	11	100/23	15.3	
*	252	10/9/52	Benzene	C3H 1	20[2]	30	25	80/38	35.0	
*	252	10/11/52	Benzene	C3H 2	20[3]	18	18	78/38	30.7	
*	252	10/10/52	Benzene	C3H 3	20[4]	20	20	100/27	23.1	
*	252	10/10/52	Benzene	C3H 3	100[4]	20	18	100/27	20.1	
*	257	11/6/52	API 8-24[5]	C3H 1	50[4]	20	16	25/31		
*	258	11/6/52	White Oil, API-80	C3H 1	50[4]	30	21	38/34		
*	259	11/21/52	20% DBB in API-80	C3H 3	100	15	13	100/32	26.8	
*	260	11/21/52	5% DBB in API-80	C3H 3	100	15	15	93/32	28.3	
*	262	12/19/52	1-phenyldecane, API-255[5]	C3H 3	20	19	19	95/16	9.1	

[1] Concentration of benzpyrene in the solution was .15 g./100 ml. solvent except where otherwise noted.
[2] Concentration of benzpyrene in the solution was .20 g./100 ml. solvent.
[3] White oil from API-8 by chromatography on alumina; see page of Table I for description.
[4] Concentration of benzpyrene in the solution was .20 g./150 ml. solvent.
[5] See page of Table V for description.

* Experiment not completed. The average latent periods for these experiments are estimated.

TABLE II

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS

OF BENZPYRENE IN VARIOUS SOLVENTS TO THE SKIN OF C3H MICE

	API SAMPLE NUMBER	STARTING DATE	SOLVENT	STRAIN OF MICE AND NUMBER OF APPLICATIONS PER WEEK	DOSAGE OF SOLUTION[1] PER APPLICATION (mg.)	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./wks.	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (5% F.L. in weeks)	RELATIVE CARCINOGENIC POTENCY, P_{MC}
*	263	12/19/52	cyclohexyldecane, API-256[2]	C3H 3	20	20	19	95/20	13.1	
*	264	12/19/52	White oil, API-80	C3H 3	100	20	19	79/28	25.3	
*	264	12/19/52	White oil, API-80	C3H 3	20	20	18	83/28	25.5	
*	265	2/5/53	Dibutylmethyl-naphthalene	C3H 3	20	20	16	69/20	18.0	
*	266	1/16/53	White oil of API 8-13 50% benzene	C3H 3	100	15	13	0/24		
*	267-1	1/31/53	Fraction b.599-642 (603) from distillation of Polydodecylbenzene	C3H 3	20	15	11	18/19		
*	267-3	1/31/53	Fraction b.625-683 (655) from distillation of Polydodecylbenzene	C3H 3	20	15	12	17/22		
*	267-5	1/31/53	Fraction b.664-730 from distillation of polydodecylbenzene	C3H 3	20	15	13	0/22		
*	267-8	1/31/53	Fraction b.659-760 from distillation of polydodecylbenzene	C3H 3	20	15	13	0/22		

[1] Concentration of benzpyrene in solution was .15 g/100 ml. solvent.

[2] See page of Table V for description.

(b.ranges are ... nalene, ... than TBP.)

TABLE II (page 1)

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS

OF BENZPYRENE IN VARIOUS SOLVENTS TO THE SKIN OF C3H MICE

	API SAMPLE NUMBER	STARTING DATE	SOLVENT	STRAIN OF MICE AND NUMBER OF APPLICATIONS PER WEEK	DOSAGE OF SOLUTION[1] PER APPLICATION (mg.)	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./wks.)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (5% F.L. in weeks)	RELATIVE CARCINOGENIC POTENCY, P_{MC}
*	269	3/9/53	Cetane (chromatography on silica gel)	C3H 3	50	15	11	36/10		
*	270	5/1/53	White oil, API-80	C3H 3	20[2]	20	20	0/9		
*	271	5/1/53	White oil, API-80	C3H 3	20[3]	20	20	0/9		
*	272	6/12/53	Monoamylnaphthalenes b. 570°F	C3H 3	40	6	6	0/3		
*	273	6/12/53	diamylnaphthalenes, b. 630 - 690°F	C3H 3	50	9	7	0/3		
*	274-1	6/12/53	Fraction b.450-500°F from distillation of alkylbenzene	C3H 3	40	8	8	0/3		
*	274-2	6/12/53	Fraction b.555-560°F from distillation of alkylbenzene	C3H 3	50	9	9	0/3		
*	275	6/12/53	Triisopropylbenzene	C3H 3	50	11	10	0/3		
*	71-4a	3/9/53	Fraction b.430-500°F from distillation of white oil of API-71[4]	C3H 3	30	8	8	38/17	14.1	
*	71-4c	3/9/53	Fraction b.550-565°F from distillation of white oil of API-71[4]	C3H 3	20	8	6	67/16		

[1] Concentration of benzpyrene in solution was .15 g./100 ml. except where otherwise noted.

[2] Concentration of benzpyrene in solution was .30 g./100 ml. solvent.

[3] Concentration of benzpyrene in solutions was .3 and .1 g./100 ml. solvent painted alternately.

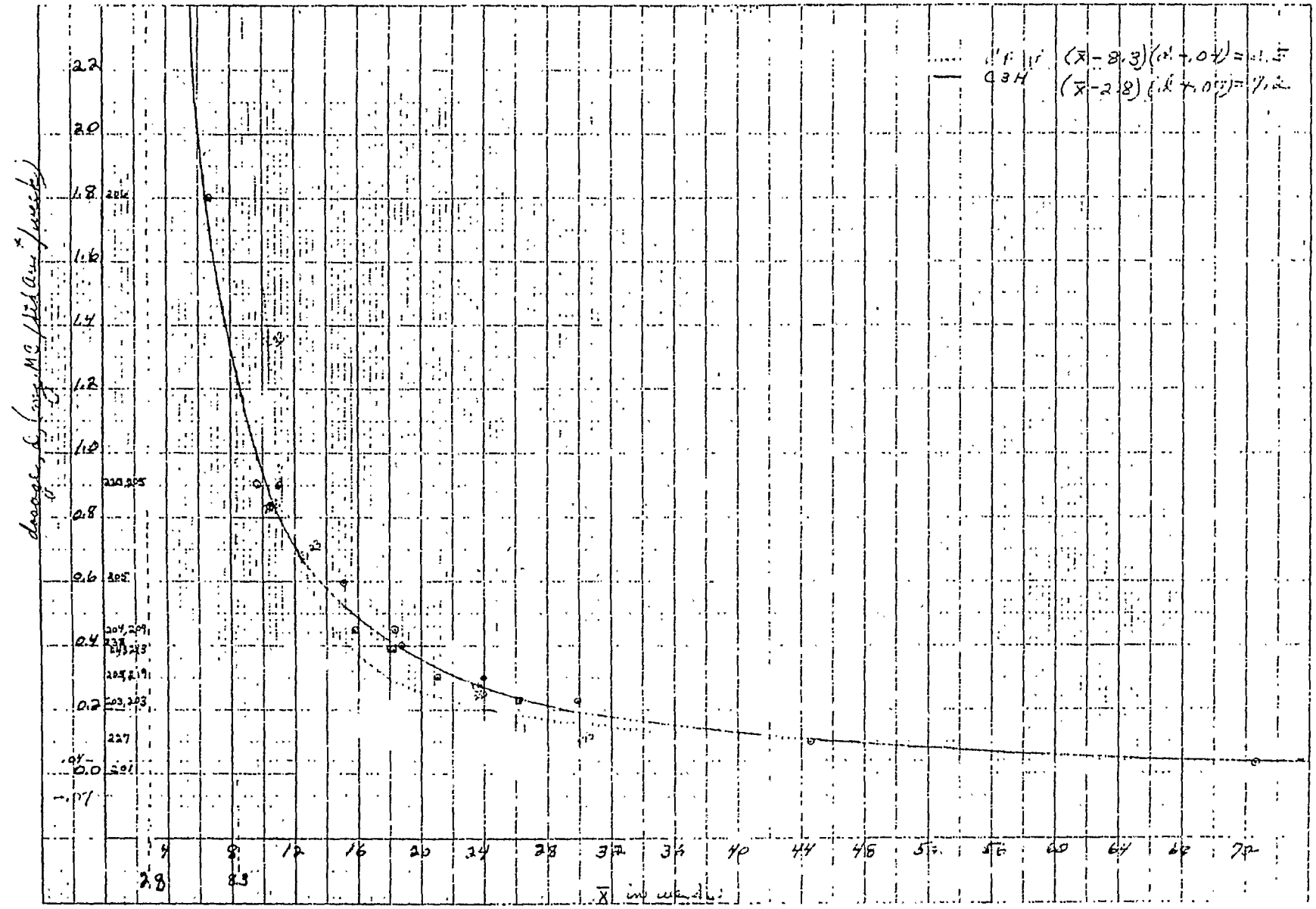
[4] White oil of API-71 obtained by chromatography on silica gel.

TABLE II (page 1)

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS OF BENZPYRENE IN VARIOUS SOLVENTS TO THE SKIN OF C3H MICE

	API SAMPLE NUMBER	STARTING DATE	SOLVENT	STRAIN OF MICE AND NUMBER OF APPLICATIONS PER WEEK	DOSAGE OF SOLUTION[1] PER APPLICATION (mg.)	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./wks.)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (5% F.L. in weeks)	RELATIVE CARCINOGENIC POTENCY, P_{BC}
*	71-4d	3/9/53	Fraction b.580-600°F from distillation of white oil of API-71[2]	C3H 3	40	8	8	50/17		
*	71-4e	3/9/53	Fraction b.600-620°F from distillation of white oil of API-71[2]	C3H 3	50	8	8	13/15		
*	80-1	2/13/53	Fraction b.520-600°F from distillation of API-80	C3H 3	(20-50)[3]	(12-6)[3]	6	83/19	16.5	
*	80-2	2/13/53	Fraction b.600-630°F etc.	C3H 3	(20-50)[3]	(12-6)[3]	6	67/19	17.5	
*	80-3	2/13/53	Fraction b.630-645°F etc.	C3H 3	(30-50)[3]	(12-6)[3]	5	60/20	18.7	
*	80-4	2/13/53	Fraction b.645-655°F etc.	C3H 3	(30-50)[3]	(12-6)[3]	6	0/20		
*	80-5	2/13/53	Fraction b.655-700°F etc.	C3H 3	100	15	15	20/20		
*	80-6	6/12/53	Silica gel chromatography of distn residue b.> 720°F. of API-80	C3H 3	50	15	15	0/3		

[1] Concentration of benzpyrene in solution was .15 g./100 ml. solvent.
[2] White oil of API-71 obtained by chromatography on silica gel.
[3] After 4 weeks the dosage was increased to 50 mg. and the number of mice was reduced to 6.



SC-API-2223

Dear Dottie,

The English biologists are somewhat agog at your 85% T.I. on L-4. But Woolhouse's cattle had terrific mortality rates — he doesn't know for sure but thinks his dosage is ~100 mg. Let's start tests on L-4 and L-5 at about the lowest dose we can use and still be sure of hitting the same spot (not lower than 5 mg.)

Snake like lanolin is not a useful pseudo-mouse skin. At least the relative areas you mentioned in the ltr of 22 June don't sound at all like the little Table we set up 2 months ago. Does mouse skin have creases like the palms of our hands?

If you get that far East, be sure to visit Jackson Memorial Lab. They need a needling at reasonable intervals and besides, Mt Desert is a beautiful island.

Dear Ruth and/or Russ (or anent) any of our practicioners declamic home this month? — not that I would blame them, considering the reports on the weather there),

Is the potency of L-4 on C3H due to a high potency accelerator or a new superactive low MW carcinogen? What kind of alkylhydrazines, b. 500-650°F., does AFL-9 have?

And what's this business about Colorado? Did the bears in the East Fork quit biting or is the middle valley of America just too hot? Best to all Wes

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A.P.L. Research Proj. MC-1
c/o Miss M.J. Graf
Kettering Laboratory
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U.S.A.

First fold here

Second fold here

Sender's name and address: A.W. Horton
112 Semley Rd
Norbury
London S.W. 16

IF ANYTHING IS ENCLOSED THIS LETTER MAY BE SENT BY ORDINARY MAIL

Fold here first

SC-API-2223

Glasgow
July 3

Dear Mary Jane,

If the surface of stone could proliferate, the buildings of this city would certainly be as decorated with neoplasms as some of the shale oil workers. To get as very black as they are, these walls must have been accumulating their quota of BP-filled coal soot since the first mine started producing the stuff.

Me for London + Paree - I'm catching the evening plane "home", - Norbury, that is. The English statistitians are slated to have a go at our data on L-4 Tuesday. I'll bet they'll be unhappy about calculating T.I.'s. Too much weight on the early tumors.

Re the tables, etc:

1. If feasible at the present time, let's call the concentrations {"Percent by weight"} rather than

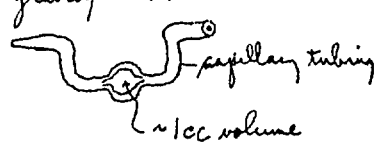
Ohyes - please send me latest data on these "BP in various solvents" deals

"g/100g", where applicable. If stencils are already typed, don't bother.

2. Titles of tables on accel. solvents should probably not start out "Conc. Standards ...." Let's title them "Expts Involving the Appp. of Solns of BP in Various Solvents to the Skin of C3H Mice". Your Example 2 seems the preferred one. In the dosage column of tables on the synthetic carcinogens, we should probably say "Dosage of Solution[1] per Appp."

[1] Conc of BP in the soln was 0.15g/100ml of solvent. (This, of course, only where applicable.)

3. Can't we get gravities on either the solvents & the BP solns (the 0.15g. BP wouldn't affect the density significantly) with a sort of calibrated micro gravity tube?



4. I sent a check to SAS for the $50.

5. The reference to bisepoxides is Brit. J. Pharmacol. 6, 235 (1951).

6. If you hear from Schiessler again by phone or letter, ask him if he'll be in State College, Pa around Aug 12 or 13. I'll be driving thru those hills heading West about then towards Cinti.

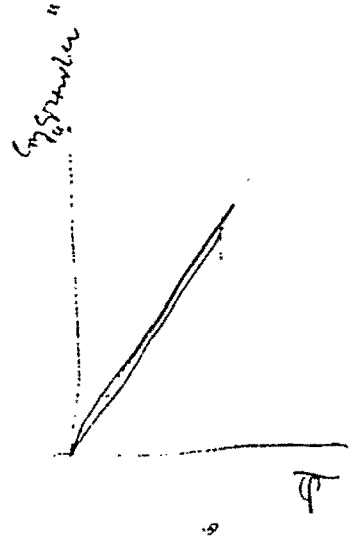
TABLE 1

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS OF METHYLCHOLANTHRENE IN BENZENE

UPON THE SKIN OF C3H MICE

API SAMPLE NUMBER	CONCENTRATION OF METHYLCHOLANTHRENE IN SOLUTION (g./100 ml. of Benzene)	DOSAGE OF SOLUTION PER APPLICATION (mg.)	NUMBER OF APPLICATIONS PER WEEK	DOSAGE OF METHYLCHOLANTHRENE (mg./Standard Area*/week)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (weeks)
227	0.10	100	1	0.10	44.6
205	0.30	100	1	0.30	21.0
209	0.45	20	1	0.45	18.3
220	0.90	100	1	0.90	9.8
238	0.20	20	2	0.40	18.0
205	0.30	100	2	0.60	15.1
201	0.01	100	3	0.03	72.6
203	0.075	100	3	0.225	26.2
203	0.075	100	3	0.225	30.2
219	0.10	100	3	0.30	24.0
243	0.13	20	3	0.39	18.2
243	0.13	100	3	0.39	17.9
204	0.15	100	3	0.45	15.7
205	0.30	100	3	0.90	11.0
206	0.60	100	3	1.80	6.7

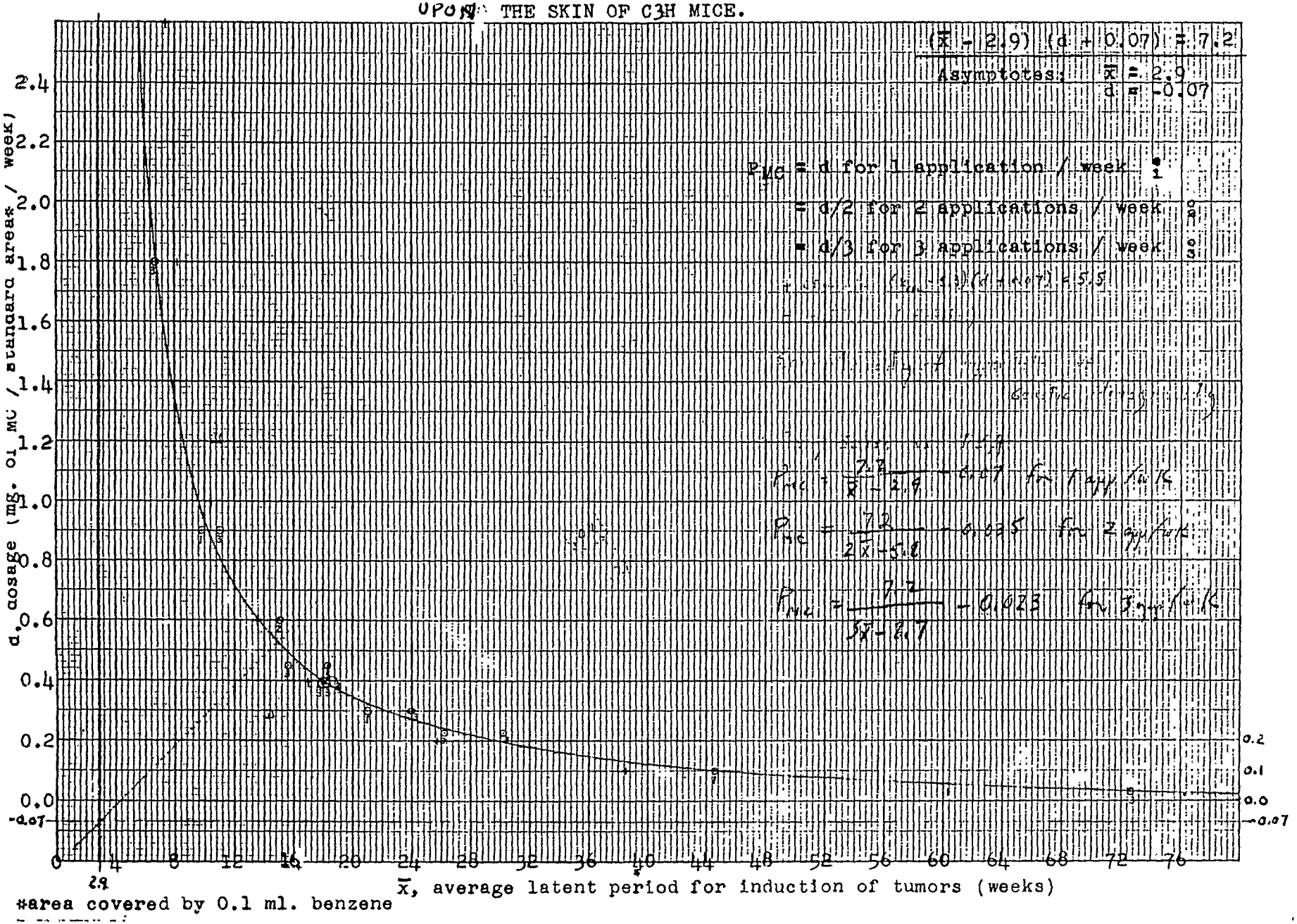
* Standard Area is that covered by 0.10 ml. of benzene.



10 × 10 to the ½ inch. 6th lines accented.

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS OF METHYLCHOLANTHRENE IN BENZE.

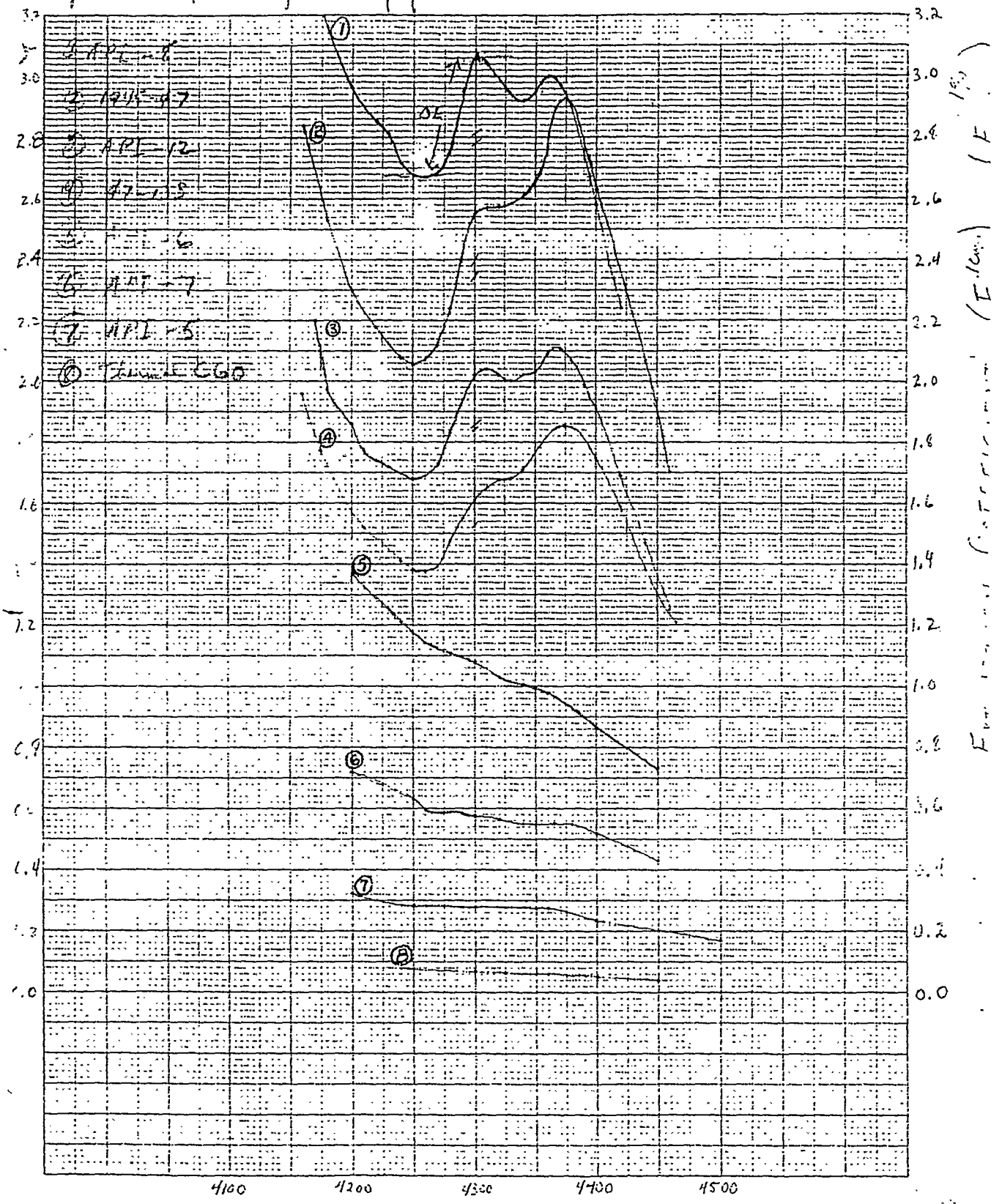
UPON THE SKIN OF C3H MICE.



*area covered by 0.1 ml. benzene

SC-API-2223

Fig 5. Absorption Spectra of fractions b. 200-275° C. / 2 mm.



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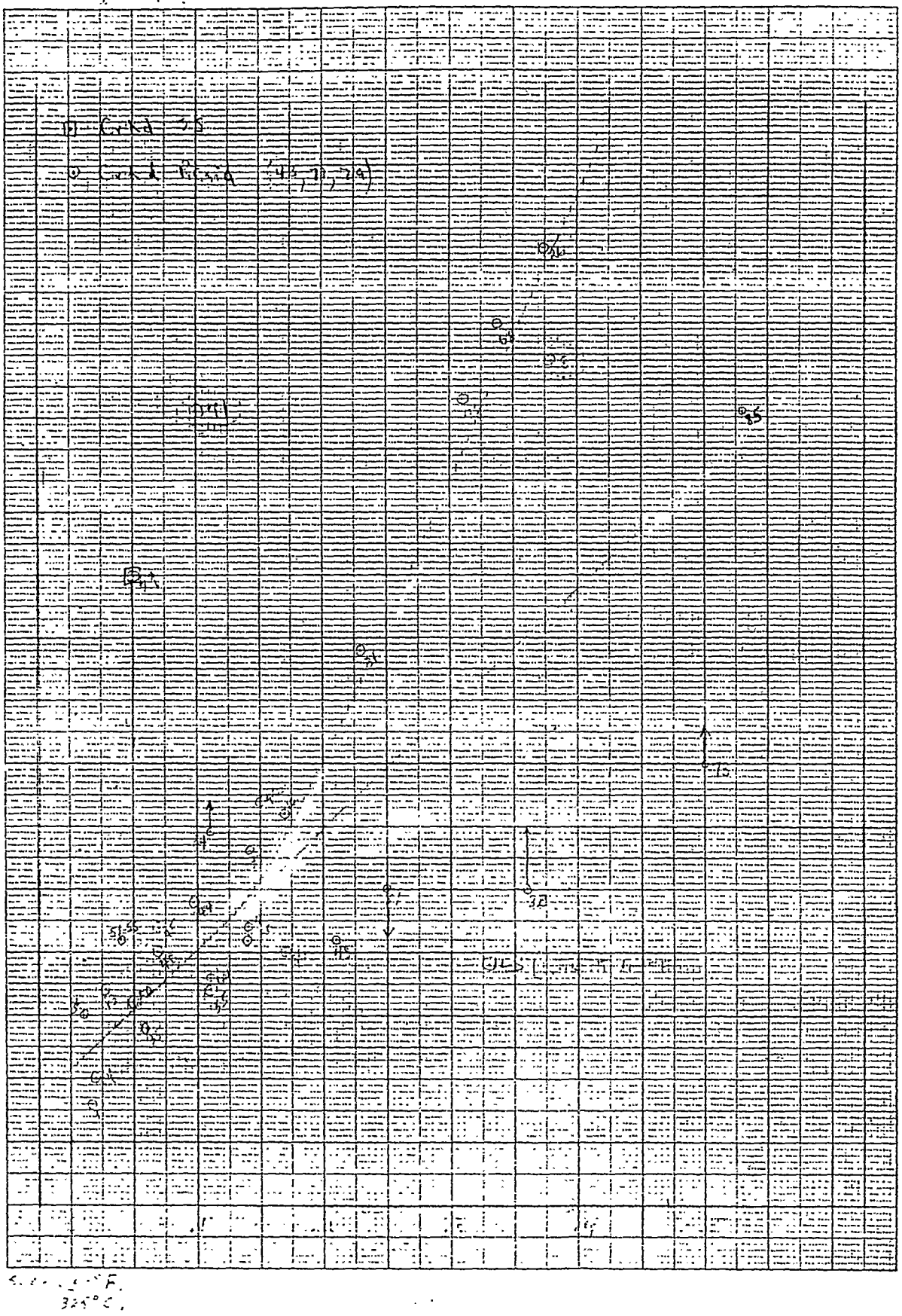

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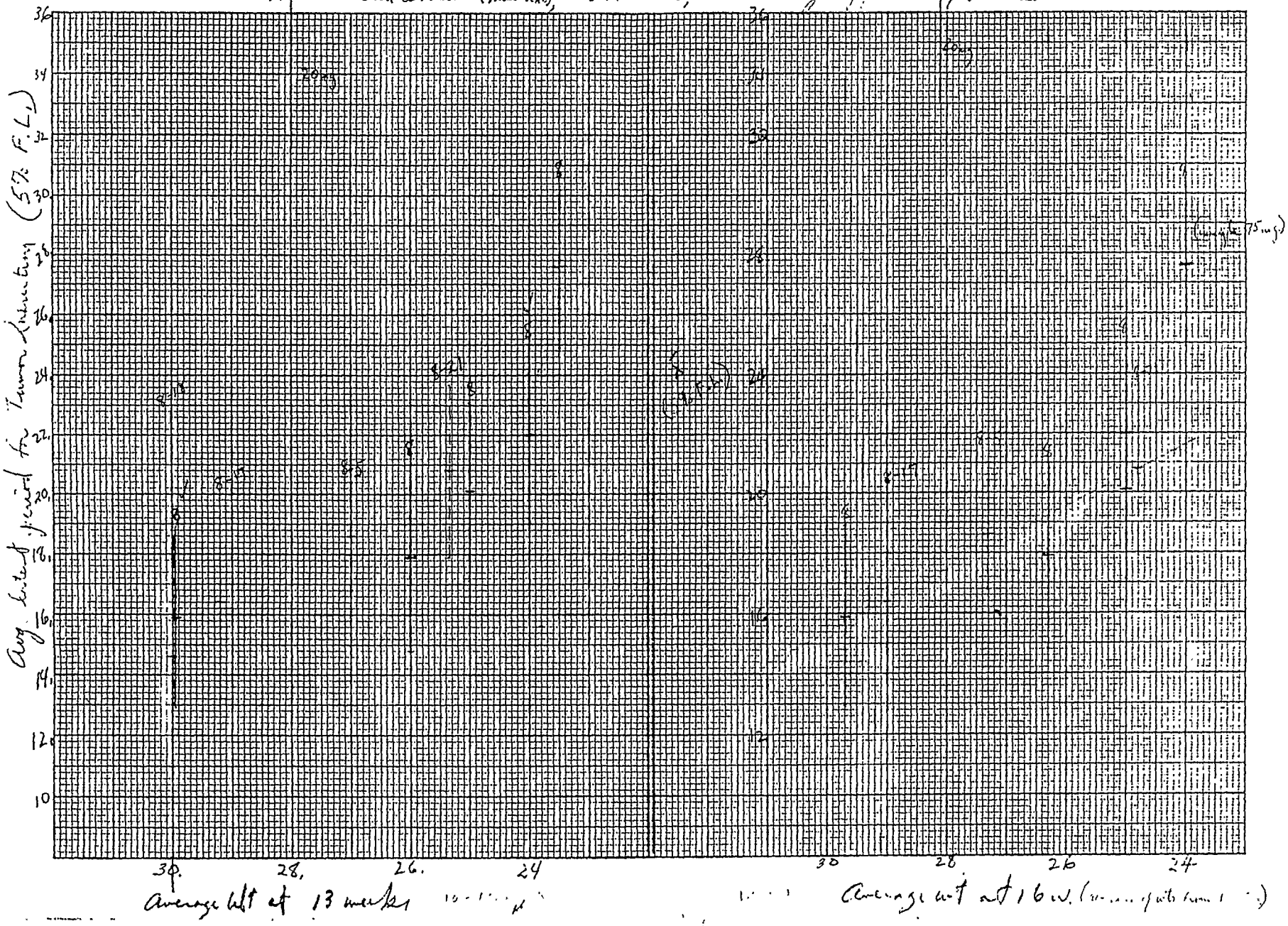
358-11 KEUFFEL & ESSER CO.
10 X 10 to the ½ inch, 5th lines accented.
MADE IN U.S.A.

API-8 and associated materials, C3H mice, 1 100mg application/week



SC-API-2223

TABLE II

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS OF METHYLCHOLANTHRENE IN VARIOUS SOLVENTS UPON THE SKIN OF C3H AND CFW MICE

SOLVENT	CONCENTRATION OF METHYLCHOLANTHRENE (g./100 ml. of Solution)	DOSAGE OF SOLUTION PER APPLICATION	NUMBER OF APPLICATIONS PER WEEK	ORIGINAL NUMBER OF MICE[1]		FINAL EFFECTIVE NUMBER OF MICE[1]		AVERAGE LATENT PERIOD FOR TUMOR[2] $\bar{x}$ (5% F.L., weeks)	RELATIVE CARCINOGENIC POTENCY, P_{MC}
				C3H	CFW	C3H	CFW		
sec.-Amylbenzene	0 (Control)	100 mg.	3		30		23	-[3]	-
sec.-Amylbenzene	0.075	100 mg.	3	10 (20)		9 (16)		$40.2^{+}_{-}7.8$	$0.04^{+.02}_{-.01}$
sec.-Amylbenzene	0.075	100 mg.	3		10 (20)		9 (18)	$32.3^{+}_{-}11.1$	$0.04^{+.05}_{-.02}$
sec.-Amylbenzene	0.15	100 mg.	3	10 (20)		10 (20)		$23.9^{+}_{-}2.5$	$0.09^{+.02}_{-.01}$
sec.-Amylbenzene	0.15	100 mg.	3		20		19	$23.6^{+}_{-}2.2$	$0.08^{+}_{-}.01$
Cottonseed Oil	0.038	100 mg.	3	31 (38)		30 (37)		$49.0^{+}_{-}4.4$	$0.029^{+}_{-}.005$

[1] Numbers in parentheses indicate total mice involved, some groups of which failed to maintain normal weight.

[2] Calculated by method of least squares, using only those cages of animals which showed normal average growth.

[3] Four mice developed suspected lesions after 44 weeks. Only one of these proved to have a neoplastic growth on microscopic examination of a section (classified as squamous cell papilloma) taken by biopsy 16 weeks after the appearance of the papilloma.

TABLE III

EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS OF METHYLCHOLANTHRENE (MC) AND BENZPYRENE (BP) IN DODECYLBENZENE UPON THE SKIN OF C3H AND CFW MICE

CARCINOGEN	CONCENTRATION OF CARCINOGEN IN SOLUTION (g./100 ml.)	DOSAGE OF SOLUTION PER APPLICATION	NUMBER OF APPLICATIONS PER WEEK	ORIGINAL NUMBER OF MICE		FINAL EFFECTIVE NUMBER OF MICE		AVERAGE LATENT PERIOD FOR TUMOR INDUCTION, $\bar{x}$ (5% F.L., weeks)	RELATIVE CARCINOGENIC POTENCY, P_{MC}
				C3H	CFW	C3H	CFW		
MC	0 (Control) 0.005	100 mg.	3	20		18		-[1]	-
MC	0.01	100 mg.	3	20		15		34.0±4.0	$0.05^{+.02}_{-.01}$
MC	0.01	100 mg.	3		10 (20)		8 (14)	43.3±16.1	$0.02^{+.04}_{-.01}$
MC	0.04	100 mg.	3		20		15	10.5±1.7	$0.33^{+.17}_{-.09}$
MC	0.10	100 mg.	3	20		20		10.9±2.0	$0.28^{+.10}_{-.06}$
MC	0.10	100 mg.	3		10		7	11.5±2.9	$0.27^{+.26}_{-.09}$
MC	0.13	100 mg.	3	30		30		10.3±.74	0.30±.03
MC	0.15	100 mg.	3	13 (20)		13 (16)		9.1±1.2	$0.36^{+.08}_{-.06}$
MC	0.15	20 mg.	3	20		18		10.4±1.4	$0.29^{+.07}_{-.05}$
MC	0.30	100 mg.	3	10 (20)		10 (19)		10.2±2.1	$0.31^{+.13}_{-.08}$
BP	0.15	100 mg.	3	20		18		11.3±2.0	$0.26^{+.09}_{-.05}$

[1] Six tumors induced, two of which regressed; no gross characteristics of malignancy.

TABLE II

CONCENTRATION STANDARDS WITH SYNTHETIC CARCINOGENS IN VARIOUS SOLVENTS

API SAMPLE NUMBER	START-ING DATE	CARCIN-OGEN[1]	SOLVENT	CONCEN-TRATION (g/100 ml)	STRAIN OF MICE	NUMBER OF APPLICA-TIONS PER WEEK	ORIGINAL NUMBER OF MICE	FINAL EFFECT-IVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./weeks)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (weeks)	RELATIVE CARCIN-OGENIC POTENCY, P_{MC}
257[3]	11/6/52	BP	API-8-24[2]	0.2	C3H-h	1	20	16	0/27		
259	11/21/52	BP	20% DDB in API-80	0.15	C3H-h	3	15	13	19/25		
260	11/21/52	BP	5% DDB in API-80	0.15	C3H-h	3	15	15	13/24		
262[4]	12/20/52	BP	1-phenyl-decane	0.15	C3H-h	3	19	19	92/16	~ 9.1	
263[4]	12/20/52	BP	1-cyclo-hexyl-decane	0.15	C3H-h	3	20	19	92/20	~13.1	
264	12/20/52	BP	API-80	0.15	C3H-h	3	20	20	2/21		
264[4]	12/20/52	BP	API-80	0.15	C3H-h	3	20	18	6/21		
265[4]	2/4/53	BP	Di-bu-Me-Naphtha-lene	0.15	C3H-h	3	20	11	18/14		
269[3]	3/9/53	BP	Cetane	0.15	C3H-h	3	15	11	23/10		

1. 3,4-benzpyrene (BP)
2. Non-aromatics from API-8 by chromatography on alumina.
3. Dosage per application was 50 mg.
4. Dosage per application was 20 mg.

TABLE II

CONCENTRATION STANDARDS WITH SYNTHETIC CARCINOGENS IN VARIOUS SOLVENTS

API SAMPLE NUMBER	STARTING DATE	CARCINOGEN[1]	SOLVENT	CONCENTRATION (g/100 ml)	STRAIN OF MICE	NUMBER OF APPLICATIONS PER WEEK	ORIGINAL NUMBER OF MICE	FINAL EFFECTIVE NUMBER OF MICE	MAXIMUM INCIDENCE OF TUMORS (T.I./weeks)	AVERAGE LATENT PERIOD FOR TUMOR INDUCTION (weeks)	RELATIVE CARCINOGENIC POTENCY, P_{MC}
	2/17/47	MC	API-80[2]	Sat. Sol.	C3H	1	20	16	81/49	39.2±4.2	
218	2/3/50	MC	API-5[3]	0.30	CFW	3	10 (20)	9 (16)	89/25 (81/49)	(21.6±3.8)	
218	2/3/50	MC	API-5[3]	0.30	C3H	3	20	17	100/30	(24.9±1.4)	0.09±.01
244	9/14/51	BP	Benzene	0.15	C3H	3	7 (20)	7 (20)	100/33 (100/33)	22.1±5.8	$0.10^{+.06}_{-.03}$
245	12/3/51	BP	DDB	0.15	C3H	3	20	18	100/19	(11.3±2.0)	$0.26^{+.09}_{-.05}$
246[4]	3/22/52	MC	API-112-1[5]	0.15	C3H	3	20	20	100/22	15.1±1.2	$0.17^{+.02}_{-.01}$
246[4]	4/11/52	MC	API-112-1[5]	0.15	C3H	3	20	18	100/21	15.0±1.4	$0.18^{+.02}_{-.03}$
248	4/11/52	MC	API-1101[6]	0.15	C3H	3	20	20	100/22	(16.7±1.2)	$0.15^{+.02}_{-.01}$
252[4]	10/9/52	BP	Benzene	0.28	C3H-h	1	30	26	8/27		
252[4]	10/11/52	BP	Benzene	0.28	C3H-h	2	18	18	50/31		
252[4]	10/10/52	BP	Benzene	0.28	C3H-h	3	20	20	95/27		
252	10/10/52	BP	Benzene	0.28	C3H-h	3	20	18	97/27		

1. 20- Methylcholanthrene (MC) and 3,4-Benzpyrene (BP).
2. Technical white oil.
3. Wilmington residuum.
4. Dosage per application was 20 mg.
5. Fraction 1, Oklahoma Bright crude feedstock.
6. Fraction 1, Kuwait crude feedstock.

C O P Y C O P Y C O P Y

MEDICAL RESEARCH COUNCIL

MRC.52/812
CAMO. Ag. 12

Committee on the Carcinogenic Action of Mineral Oils

A G E N D A

for the Twelfth Meeting to
be held at 38 Old Queen Street,
London, S.W.1. on Wednesday,
17th December, 1952 at 11.15 am.

1. Minutes of the last meeting. (MRC.52/675. CAMO. Min. 11 - already circulated).

2. Committee's Report to Council. (CAMO. 52/12 - enclosure A).

3. Discussion on progress since the last meeting:

   (a) Chemical studies. (CAMO. 52/13 - enclosure B).

   (b) Biological studies. (CAMO. 52/14 - enclosure C).

4. Any other business.

5. Date of the next meeting.

SC-API-2223

C O P Y C O P Y C O P Y

MEDICAL RESEARCH COUNCIL

For Specified Circulation
(Committee members only)

MRC.52/793
CAMO.52/12

Committee on the Carcinogenic Action of Mineral Oils

REPORT TO THE COUNCIL BY THE COMMITTEE ON THEIR FIRST THREE YEARS' WORK

Section I

INDUSTRY AND RAW MATERIALS

The petroleum industry and skin cancer

The petroleum industry has been concerned with occupational skin cancer caused in the following ways:

(1) In industry

(a) From long-term contact of lubricating oils with the skin of workers (e.g. mule-spinners' cancer).

(b) From long-term contact of constituents of paraffin wax with the skin of workers (e.g. shale workers' cancer).

(c) From long-term contact of cutting oils with the skin of workers (e.g. skin cancer in the light engineering industry).

(2) Experimentally

(a) From skin contact with selective solvent extracts.

(b) From skin contact with cracked oil of low volatility.

However, in comparison with many other factors in industry, petroleum is of small importance in the occupational causation of cancer.

The light oils and wax of shale have long been known as causes of skin cancer. It is most likely that this effect is due to carcinogens accompanying the waxy fractions, and not to the wax itself, which is paraffinic in nature. Recognition of the factors concerned has led to greatly improved conditions of work in the shale oil refineries and has brought the disease largely under control. There is no corresponding occurrence of skin cancer or of dermatitis amongst mineral oil refinery workers.

The occupational disease of mule-spinners' cancer has attracted much attention in this country over the last thirty or so years and from early on was ascribed to the lubricating oil used. Investigation by a committee set up by the Manchester Corporation in 1928 confirmed this view and resulted in an attempt on their behalf by C. C. Twort six years later to distinguish, by physical means, carcinogenic from non-carcinogenic oils, using a formulation based upon a relationship involving specific gravity and specific refractivity. The formula was wrongly accepted in many quarters as a solution

of the problem and lead to a false sense of security until Auld in 1934 pointed out its weaknesses. Between 1934 and 1939 an independent examination of C. C. and J. M. Twort's data was carried out by Irwin*, who while finding considerable correlation between carcinogenic potency and refractivity, did not confirm all the conclusions in C. C. and J. M. Twort's published papers. The Manchester formula had also lead, unfortunately, in the minds of many petroleum scientists, to insufficient attention being paid in the textile industry to cleanliness, working conditions, medical inspection, change of occupation for susceptible workers and so on; that is to say, the measures which had brought the paraffin dermatitis and paraffin cancer of the shale industry under control. It also lead to insufficient attention being paid to the development of truly non-carcinogenic oils. It had been apparent in the petroleum industry that more drastic refining, such as solvent extraction or sulphuric acid treatment, which might be expected to destroy or eliminate possible carcinogens, was the most logical approach to the problem as far as lubricating oils were concerned. In addition, acceptance of the formula delayed consideration of systematic examination of petroleum for the purpose of separating possible carcinogens and study of their structure and properties.

In 1943-44 attention was focussed on the lubricating oil extracts which were being used as substitutes for extenders, plasticisers, etc., which could no longer be obtained under war-time conditions. It was suggested that these extracts might have carcinogenic or other very noxious properties and, as a result, work was initiated at Birmingham University by the Petroleum Board for the biological testing of extracts. This work is referred to later, and more fully, in Section III of this report. If the supposition that by solvent refining oil stocks from which lubricating oils are made, the extracts are made more carcinogenic than the original stocks, then the refined oils must be less carcinogenic, which leads to the thought that intensive refining to the stage where white oils are produced would be a logical means of obtaining a non-carcinogenic oil. In 1946 the Petroleum Board took the step of recommending that if a prima facie case could be made out for the non-carcinogenicity of such white oils, they should be made available to the textile trade as mule spindle oils. At a meeting convened by the Ministry of Labour, the decision of the Ministry of Fuel and Power to make white oils available as textile lubricants was confirmed, despite the meagre evidence available and despite their scarcity and dollar cost at that time. Simultaneously, the Petroleum Board announced its further financial subsidy for research work at Birmingham to supplement the very limited information on which the decision had been made.

In 1948, the master spinners and the cotton operatives recalled the ad hoo committee of 1946 to consider the matter, by which time there was corroboration from Birmingham of the biological inactivity of white oil samples submitted by the Petroleum Board. Decision was thereupon made to standarize on white oils, subject to practical tests showing them to have satisfactory lubricating properties. Mill trials carried out with plain white oils and white oils

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* J. O. Irwin & Nancy Goodman. (1946) J. Hyg., Camb. 44, 362

blended with fatty oils, all of comparable viscosity, showed satisfactory performance and very little difference between the oils in power consumption. The ad hoo committee which achieved an official basis by being brought under the control of the Factory Department of the Ministry of Labour and National Service, then asked the Petroleum Industry representatives to submit a specification to cover suitable white oils for adoption by the Trade. This was done and the specification is referred to in the Ministry of Labour and National Service publication: "Mule Spinners' Cancer" - Second Interim Report of the Joint Advisory Committee of the Cotton Industry (H. M. Stationery Office). One of the recommendations in this report is that appropriate action should be taken to make the specification as effective as possible at the earliest possible date and it is understood that consideration is being given to its enforcement by means of a Statutory Instrument.

The work at Birmingham on behalf of the Petroleum Board to evaluate the carcinogenicity of solvent extracts is dealt with in Section III of this report. The results varied quantitatively from one material to another, but showed that solvent refining tended to concentrate carcinogens, where they existed, in the extract. The most active were lower in biological activity than the corresponding coal tar products and less noxious than certain materials already in use for similar purposes. Further work involving biological testing was envisaged and the Board set aside money for this purpose. When the Board was dissolved in 1948, it approached the Institute of Petroleum, with the concurrence of the Ministry of Fuel and Power, to accept responsibility for continuing the work, and offered a substantial sum to continue the research. The Institute of Petroleum accepted the responsibility and implemented it by forming (under its Research Committee) the Research Group No. 2, whose Chairman is Colonel Auld. The Research Group No. 2 felt that a fundamental investigation was required in an endeavour to separate the active components present in petroleum and to determine their properties. As there are many different oil-fields and many different crude oils, it was necessary to be realistic and make a limited choice. At about the same time the Medical Research Council's Committee on the Carcinogenic Action of Mineral Oils was set up and it was natural that the activities of the Institute of Petroleum Research Group No. 2 should be dovetailed with those of the Medical Research Council's Committee.

Raw materials

As a result of the co-operation between the Medical Research Council Committee and Research Group No. 2 of the Institute of Petroleum, three crude oils, Kuwait, Lagunillas and Oklahoma were selected as being either typical of crude oils likely to be much in use in the United Kingdom, or alternatively typical sources of lubricating oil fractions, bright stocks and residual lubricating oils. Substantial supplied of these oils were arranged by the oil companies concerned, i.e. Kuwait Oil Co. (Kuwait), Shell Petroleum Co. (Lagunillas) and Socony-Vacuum Oil Co. (Oklahoma). Each oil was initially fractionated by controlled distillation at the Shell Research Centre at Thornton-le-Moors into four fractions and a residue. This fractionation is described in Appendix II at the end of Section IV. The fractions were numbered for convenience O_1, O_2, O_3, O_4, O_5 (Oklahoma), and similarly

K_{1-5} and L_{1-5} for Kuwait and Lagunillas respectively. Later, the fourth fraction of each oil was selected for further study.(1) A description of the progressive fractionation of the three chosen fractions, carried out by Professor F. Morton at Birmingham University, follows.

Redistillation of fractions O_4, L_4, and K_4(2) Three crude oils, representative of typical stocks, from Oklahoma, Lagunillas and Kuwait respectively, had been fractionated at the Thornton Research Centre into four overhead fractions and a residue. The distillation had been conducted with open steam to avoid the use of temperatures in excess of 250°C. In each case fraction 4 (boiling range approximately 300-400°C. at 750 mm. Hg.) was selected for further investigation.

The further fractional distillation of each of the three samples O_4, L_4 and K_4 was carried out in the following manner.

Apparatus. The fractionating column and auxillary apparatus is shown in the accompanying diagram (Fig. 1). The column (18 in. high x 1 in. diameter) was packed with 1/8 Fonske helices. Adiabatic conditions were maintained by a heating jacket and the flask was heated by isomantle to avoid local overheating.

The still was charged with 1000 ml. of the sample (previously dried over anhydrous sodium sulphate) and operated under total reflux at a controlled pressure of 1.0 mm. Hg. for 1 hr. The take off cock was then opened to give an effective reflux ratio of 10-1. This distillate was collected to 100 ml. fractions, temperature and pressure being recorded throughout. The distillation was stopped at a still pot temerature of 250°C.

The distillation was repeated with further charges of 1000 ml. until 9000 ml. of each fraction had been distilled. Data for the first distillation of each of the three fractions are given in Tables I, II, and III.

Blending. After discussion with Professor Squire it was agreed to blend the distillates according to the following scheme shown in Table I.

The arrangement gives five fractions from each crude charge, with a minimum column of 900 ml. for any fraction. The final blends and volumes are given in the attached Table I, II, III, IV.

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(1) Further description of this work is given in Sections II and III of this report.

(2) This is the text of a report (B1/52) by Professor Morton to Research Group No. 2 of the Institute of Petroleum and is reproduced by their permission.

Report on samples of Lagunillas, Oklahoma and Kuwait distillates received (3). At the Tenth meeting of the Research Group No. 2 it was agreed that future work on the examination of crude oils should be restricted to the original three crude oils from Lagunillas, Oklahoma and Kuwait. Accordingly, samples of commercially prepared distillates approximating to the No. 4 fraction prepared at Thornton Research Centre have been obtained and are stored at the University of Birmingham.

Lagunillas distillate. Through the kindness of Mr. H. W. Stevenson and the courtesy of N. V. Curacaosche Petroleum Industrie Maatschappij we have been fortunate to obtain 10 drums of Lagunillas distillate 300-400°C. prepared by commercial vacuum distillation from a charge stock consisting of almost 100% Lagunillas crude oil. The inspection data on this distillate is given in Table V; for comparison, the distillation data on fraction No. 4 prepared at Thornton Research Centre is included.

Oklahoma Bright Stock. Through the kindness of Colonel Auld and the courtesy of Dr. L. C. Beard Jr., and the Socony-Vacuum Oil Co. 10 drums of No. 70 lubricating oil distillate ex Oklahoma Bright Stock have been received and are stored at the University of Birmingham. Inspection data on this material is given in Table VI with the corresponding distillation data from Thornton Report. It will be seen that in this case the new material is a somewhat high boiling fraction than the original O_4, the bulk of the distillate being concentrated in the 350-400°C. range. There is, however, sufficient of the material available to prepare large samples in the 300-350°C. range should this prove to be necessary.

Kuwait Vacuum Distillate. It proved impossible to obtain, as a single stream, a sample of Kuwait Vacuum Distillate boiling in the range 300-400°C. Through the kindness of Mr. E. J.Boorman and the courtesy of Esso Petroleum Co., Fawley, we have obtained three drums of each of a vacuum overhead and No. 1 side stream fraction which together cover the boiling range in which we are interested. The distillation data for the two fractions are shown in Fig. 2 with a similar distillation for the Lagunillas distillate for comparison.

Future programme

The erection of the Stedman Vacuum distillation unit is now completed. Thermocouples and manometers etc. are now being calibrated and the adiabatic jacket winding has been fitted in place and is under test. On completion of these tests the unit will be charged with the first of the Kuwait samples and the distillate collected in appropriate size samples. In the first case it is proposed to collect the samples in accordance with the boiling range distribution $K_4.1$, $K_4.2$, $K_4.3$, $K_4.4$ and $K_4.5$ (cf. Report No. B1/52). Material boiling outside this range will be collected separately and stored.

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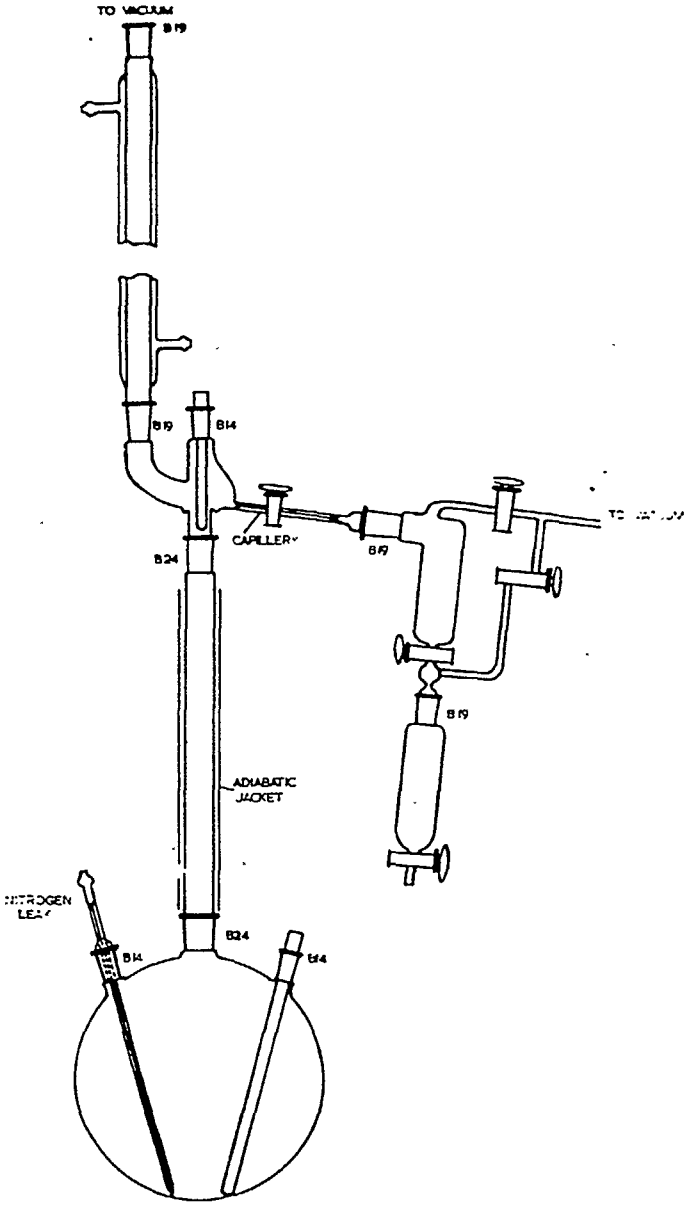
(3) This is the text of a report (B.2/52) by Professor Morton to Research Group No. 2 of the Institute of Petroleum and is reproduced by their permission.

A full report on the design etc. of the Stedman unit will be issued in due course. The unit consists of a still pot capable of a working charge of 75 gals, a fractionating column 10 ft. high, 6 ins. diameter packed with circular Stedman packing, and the necessary condensers, heaters, reflux accumulation and control instruments. The unit is the gift of Messrs. Trinidad Leaseholds Ltd. and the new packing has been supplied by courtesy of Foster Wheeler Corp. and the Midland Tar Distillers Co. Ltd.

FIG. I


TO VACUUM
B19
B19
B14
CAPILLERY
B19
B24
B19
ADIABATIC
JACKET
B24
B14
B14

Section II

THE RESULTS OF CHEMICAL STUDIES

Objective

The ultimate objective of the chemical part of the investigation is the identification of the constituent(s) responsible for the carcinogenic action of the petroleum oils and the utilisation of this knowledge in devising chemical or physical methods for the detection and estimation of the carcinogenic constituents of commercial oil fractions. This would furnish a rapid means of evaluating the carcinogenic potency of a given material, thus obviating the need for lengthy and costly biological examinations. Furthermore, a knowledge of the chemical nature of the active material might well lead to the elaboration of refining procedures which would result in detoxification of the oil.

The oil fractions

Three representative crude oils were selected (see Section I). Each was separated by distillation at the Thornton Research Centre into four fractions and a residue. As it is known that very high temperatures (ca 800-1000°C) may transform such materials into carcinogenic polycyclic hydrocarbons the conditions of distillation were carefully controlled so as to eliminate any risk of producing carcinogens by pyrolytic treatment. By distilling under reduced pressure and by introducing steam it was possible to maintain the temperature of the liquid below 250°C during the distillation. The later stages of sub-fractionation are all being planned so as to avoid high-temperature treatment, and in this way ensure that any compounds which may be isolated are reasonably certainly present in the original crude oil. The first stage of sub-fractionation of selected fractions prepared by the Thornton Research Centre is being carried out under the supervision of Professor F. Morton in the Department of Chemical Engineering of the University of Birmingham. The 15 sub-fractions (5 from each of the three crudes) are undergoing biological test in the Cancer Research Laboratories and are also being subjected to chemical examination by Dr. W. Carruthers working in the Chemistry Department of the University of Glasgow under the general direction of Professor J. W. Cook. This section of the Report is concerned with the work of Dr. Carruthers.

The hypothetical chemical structure of the unknown carcinogen

It has long been known that mineral lubricating oils are liable to contain carcinogenic constituents. This is undoubtedly responsible for the high incidence of skin cancer among cotton spinners, and the carcinogenicity of the lubricating oils used for the mules has been demonstrated repeatedly by experiments on mice, but no pure carcinogenic compound has hitherto been isolated from such a source. A similar carcinogenic industrial material is coal tar, and from this a potent carcinogenic constituent has been isolated and shown to be a fused-ring aromatic hydrocarbon, 3:4-benzpyrene. Other hydrocarbons

of comparable potency, belonging to the same class, have been prepared synthetically. Using as a working hypothesis, therefore, the assumption that the active ingredients of mineral oils may be of the same type, attention is being focussed in the first instance on the aromatic fractions of the oils, and particularly on the polycyclic aromatic materials, which have certain characteristic properties by which they may be recognized. This may help to simplify the course of the investigation, but it is recognized that the active constituents of the oils may be of quite other chemical types and the non-aromatic fractions will certainly not be neglected. There is some justification for devoting more attention initially to the polycyclic aromatic constituents of the oils, for not only do the most potent compounds known belong to this class, but it has been our experience that all of the carcinogenic oils which we have examined contain polycyclic aromatic fractions.

Chemical analysis of the fractions

Up to the present the chemical examination of the fractions has inevitably lagged far behind the other phases of the work, for not only have the crude oils had to be selected, procured and fractionated, but the carcinogenic potencies of these fractions have had to be evaluated by laborious biological tests extending over more than a year. Consequently it has been possible only recently to make a start on the main chemical task of attempting the isolation of pure constituents of the oils selected for systematic investigation. Before this start became possible attention was devoted to shorter term projects of examining some oil extracts which had already been shown by Dr. Woodhouse to be carcinogenic and also some related materials separated from petroleum oils. A description of the results of this work is included in this Report. It has proved well worthwhile, not so much for the intrinsic value of the results obtained, by chiefly for the experience which has been gained in devising and studying the methods of separation which will be applied in the main task confronting us in this research. Among the methods studied were fractional distillation, chromatography, extraction with concentrated sulphuric acid, and formation and fraction crystallisation of molecular compounds with polynitro compounds such as picric acid and s-trinitrobenzene.

The extracts examined were commercial products obtained by extracting certain petroleum distillates with liquid sulphur dioxide, a solvent which selectively dissolves the aromatic components. The extracts were generally dark viscous oils of high boiling-point, containing only small amounts of material distilling below 200° C/0.2 mm. Higher-boiling material was distilled at temperatures up to 250° C on the mercury pump (10^{-3} - 10^{-5} mm.), affording viscous orange or yellow distillates. Because of the high boiling points fractional distillation could not be employed to achieve an initial sub-division of the extracts. For this purpose use was made of chromatography.

The viscous distillates obtained as above were chromatographed on a large scale on columns containing large amounts of alumina, with the object of obtaining less complex fractions which might serve as the basis for further separations by crystallisation of molecular complexes or other means. By elution with petroleum ether containing increasing proportions of benzene, a large number of

arbitary fractions was collected of increasing aromaticity. These fractions were purely arbitary; there was no clear demarcation between them. In general, elution with petroleum ether afforded a considerable amount (50-60 per cent of the charge) of almost colourless eluate, representing the least aromatic portion of the fraction. Continued elution with petroleum - benzene then afforded fractions of increasing refractive index and deepening colour, containing more aromatic material. The last eluates were usually dark orange viscous gums. From many of these later fractions highly coloured crystalline picrates could be obtained by treatment with picric acid in alcohol. These were generally dark red or brown in colour. They could be crystallised from alcohol or benzene, but generally were obtained in poor yield, and none has yet been obtained in a pure state. On decomposition only small amounts of oily material were obtained. These picrates must represent a considerable concentration of aromatic material over that present in the original extract, but it is clear that they are still complex mixtures. Only by working on a very large scale will it be possible to obtain sufficiently large amounts to offer any prospect of achieving purification of the picrates by crystallisation or other means.

In some cases, the fractions obtained by chromatography were further subdivided by repeated chromatography on alumina. From one of the extracts treated in this way (Edmor 33) four crystalline solids were isolated. These were readily obtained homogeneous by crystallisation. One of them has been identified as the pentacyclic aromatic hydrocarbon perylene. The others have not been completely identified. One appears to be an aromatic hydrocarbon of the formula $C_{24}H_{24}$, $C_{25}H_{24}$, or $C_{25}H_{26}$. The ultra-violet absorption spectrum suggests that it is a derivative of chrysene with a large alkyl substituent of six or seven carbon atoms possibly located at position 3 or 4 of the nucleus. The two other compounds isolated are not aromatic. They have the approximate formulae $C_{21}H_{31}O$ and $C_{29}H_{46}O_2$, and are extremely resistant to chemical reagents. The latter compound has been more extensively investigated. What evidence there is of its structure seems to indicate a saturated naphthenic structure containing seven rings. The function of the oxygen atoms is unknown.

There was no absorption of hydrogen over a palladium catalyst and the compound was unchanged after attempted selenium dehydrogenation and after heating under pressure with aqueous or alcoholic hydrochloric acid. Reactions for carbonyl and hydroxyl groups were negative.

We also investigated the possibility of effecting a concentration of some of the aromatic components in the extracts by utilising the selective solvent action of concentrated suphuric acid. This is known to dissolve certain polycyclic aromatic hydrocarbons (e.g. 3:4 benzpyrene) without sulphonation, regenerating them on dilution with water. It was found that generally extraction of a petroleum ether solution of the distillates with concentrated sulphuric acid did indeed lead to the separation of a small proportion of the original fraction, but this was still complex in nature. This was shown by the ultra-violet absorption spectra of fractions prepared in this way. These showed only general absorption and were completely devoid of any characteristic features. The bulk of oils was completely

unattacked by cold concentrated sulphuric acid. Highly coloured crystalline picrates could be readily isolated if these extracts were further broken down by chromatography on alumina, and the fractions thus obtained treated with alcoholic picric acid. These picrates were far from homogeneous, and again the amounts obtained were too small to allow of much separation by crystallisation.

Parallel with this work, we also examined some samples of oleum sludge and solid sodium sulphonates, supplied to us by Manchester Oil Refinery and the Stanlow refinery of Shell. These are produced in the refinery by treatment with oleum of the undissolved material (the farrinate) from the liquid sulphur dioxide treatment. Any aromatic hydrocarbons present either dissolve in the oleum (partly as sulphonic acids) or form sulphonic acids soluble in the oil. The former solution comprises the oleum sludge, and solid sodium sulphonates are obtained from the oil-soluble sulphonic acids. It seemed possible that these materials might contain some of the polycyclic aromatic hydrocarbons of the original oil and an attempt was made to isolate such compounds from them. The solid sodium sulphonates were hydrolysed with hydrochloric acid under pressure, and the recovered oil distilled and chromatographed. The fractions obtained gave only very pale colourations with picric acid, however, and in no case was a solid complex isolated. Similar results were obtained with the oil recovered from the oleum sludge. It seems probable, therefore that these materials do not contain any appreciable amount of polycyclic aromatic compounds, and further examination of them was therefore postponed.

The ultra-violet absorption spectra of four carcinogenic extracts supplied by Dr. Woodhouse (F, H, I, K*) were measured to determine if they exhibited any common feature which might be related to their biological activity. In this connection it is noteworthy that American workers at the Kettering Laboratory of the University of Cincinnati have reported a relationship between the carcinogenic potencies of certain cracked oils and the intensity of an absorption band at 430 mμ. In the present case the spectra of fractions prepared from the four crude oil extracts by distillation were examined over the range 300-450 mμ in cyclohexane solution. Generally, they showed a gradual fall of intensity towards the long wavelengths. The spectra of I and K showed no maxima or points of inflection; the higher boiling fractions of F and the fractions of H showed weak maxima in the region 430-440 mμ , but were otherwise smooth. There was, however, no evident relationship between the spectra and the biological properties of the fractions (see Figs. II-V).

Biological testing at Birmingham and at London of the five fractions prepared at the Thornton Research Centre from the three crude oils (Kuwait, Lagunillas and Oklahoma) being used for the main systematic investigation has indicated that fraction 4 is the most active in each case (designated $K\text{-}_4$, $L\text{-}_4$ and $O\text{-}_4$ respectively). These are dark coloured fairly mobile oils, boiling in the range 330-400° C, 317-384° C and 296-400° C respectively. These active fractions have been further subdivided by Professor Morton into four sub-fractions

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* For properties and methods of preparation see Section IV, Tables VII(a) and VII(b).

and a residue (designated K.4-1 up to K.4-5 etc.) and these are now being tested biologically. Samples of all of these sub-fractions (except K.4-4 and L.4-1) have been made available to us, and we have carried out some preliminary experiments with them. For purposes of comparison, the ultra-violet absorption spectra of the three main fraction (K.4, L.4 and O.4) and the sub-fractions were measured in cyclohexane solution over the range 240-440 mμ. The three main fractions were very similar and showed only general absorption with a decrease of intensity towards the long wave length. With most of the sub-fractions, however, there were distinct maxima or points of inflection at about 325 mμ and 385 mμ (See Figs. VI, VII, VIII). The Oklahoma sub-fractions O.4-1, 2, 3 also showed maxima at about 255mμ. Some of these maxima may be due to anthracene hydrocarbons. Only the three residues, K.4-5, L.4-5 and O.4-5, and the fractions O.4-4 gave smooth curves similar to those of the main fractions. The similarity of the spectra of the other sub-fractions is note-worthy.

The main fraction K.4 has been examined in more detail. The aromatic components were concentrated by selective absorption on a column of silica gel, and the concentrate distilled and collected in 2-3° fractions. Forty-one fractions were taken. These fractions readily yielded orange-red crystalline picrates with picric acid, some of which were crystallised and analysed, although they were not homogeneous. The analyses indicated that the aromatics present consisted of compounds of the C_{15} to C_{19} range, suggesting three or four rings in their molecules. These picrates gave positive tests for sulphur, but the analysis showed that it was not present in significant amount. The ultra-violet absorption spectra of the oils recovered from these picrates were examined in cyclohexane solution. The oils recovered from the earlier picrates of the earlier fractions of the distillation (b.p. <170°/0.4 mm.) gave spectra resembling each other closely, and showing distinct maxima in the region of 375-380 mμ, and others of less intensity at about 360 mμ and 325 mμ. The spectra of the fractions from which these picrates were obtained were also measured and found to be similar to those of the material recovered from the picrates. The spectra are consistent with the presence of anthracene hydrocarbons. This view is supported by the fact that there is a radical alteration of the spectrum after treatment of the oil with maleic anhydride; the bands at 375-380 mμ and 360 mμ are completely eliminated (cf. Fig. IX). (Fig. X gives the absorption spectrum of 1:2 benzanthracene and its maleic anhydride addition compound. It will be noted that formation of the latter is attended by a complete change in the character of the spectrum.) The presence of anthracene hydrocarbons in K.4 has now been confirmed by the isolation from the fractions K.4-1, -2 and -3 (see above), through maleic anhydride adducts, of small amounts of crystalline materials, the spectra of which show clearly that they are derivatives of anthracene (Fig. XI). The spectra of the higher boiling fractions from the distillation (b.p. 170°/0.4 mm.) - and of the material recovered from the corresponding picrates - showed more general absorption, with no maxima or pronounced points of inflection. By chromatography of the oil from the picrate of one of these fractions, a solid was isolated whose spectrum (after crystallisation) is clearly that of a chrysene derivative (Fig. XII). These results seem to indicate the presence of three- and four-ring aromatics in K.4, and this is consistent with the boiling point, 330-400° C of the fraction. Anthracene boils at 340° C, pyrene at 404° C and chrysene at 436° C.

Before the results of the biological tests on the main fractions became available, some preliminary work was carried out with the residues from the initial distillation of the three crudes (i.e. K.5, L.5, and 0.5). These were black viscous oils, and contained much high boiling material, only a very small amount distilling below 200° C/0.5. They could be partly distilled up to about 250° C/10^{-5}., affording viscous orange distillates. Fractional distillation was therefore out of the question. As in the case of the extracts already studied, use was made of chromatography to achieve some initial fractionation of these materials. It was found an advantage first to separate the aromatic compounds from the naphthenes and paraffins also present. For large scale work, this was best accomplished by extraction of the distillates with furfural, a process used industrially for this purpose. The aromatic extracts thus obtained were chromatographed on alumina as described before. The results obtained were similar to those in the experiments with the extracts. Many of the fractions readily yielded highly coloured crystalline picrates, but in amounts too small to allow purification by crystallisation. The dark red or brown colour of these picrates suggests their formation from complex aromatic material. One deep red picrate from 0.5 analysed for a hydrocarbon of approximate formula $C_{26}H_{18}$. Work with these residues was discontinued when the biological tests showed them to be without activity.

Summary

Taken as a whole, the results obtained indicate that it should be possible eventually to separate and identify a number of the polycyclic aromatic compounds present in the actively carcinogenic fractions of the oils. It is clear, however, that this will be a difficult and laborious task and a rapid conclusion of the investigation is not to be expected. This is especially so as the successive stages in the chemical separation of the constituents of the fractions will need to be controlled by very time-consuming biological tests. There is always the possibility, however, that the work may be accelerated by the isolation from some of the sub-fractions at present under examination of known carcinogenic hydrocarbons or closely related compounds.

FIG. II

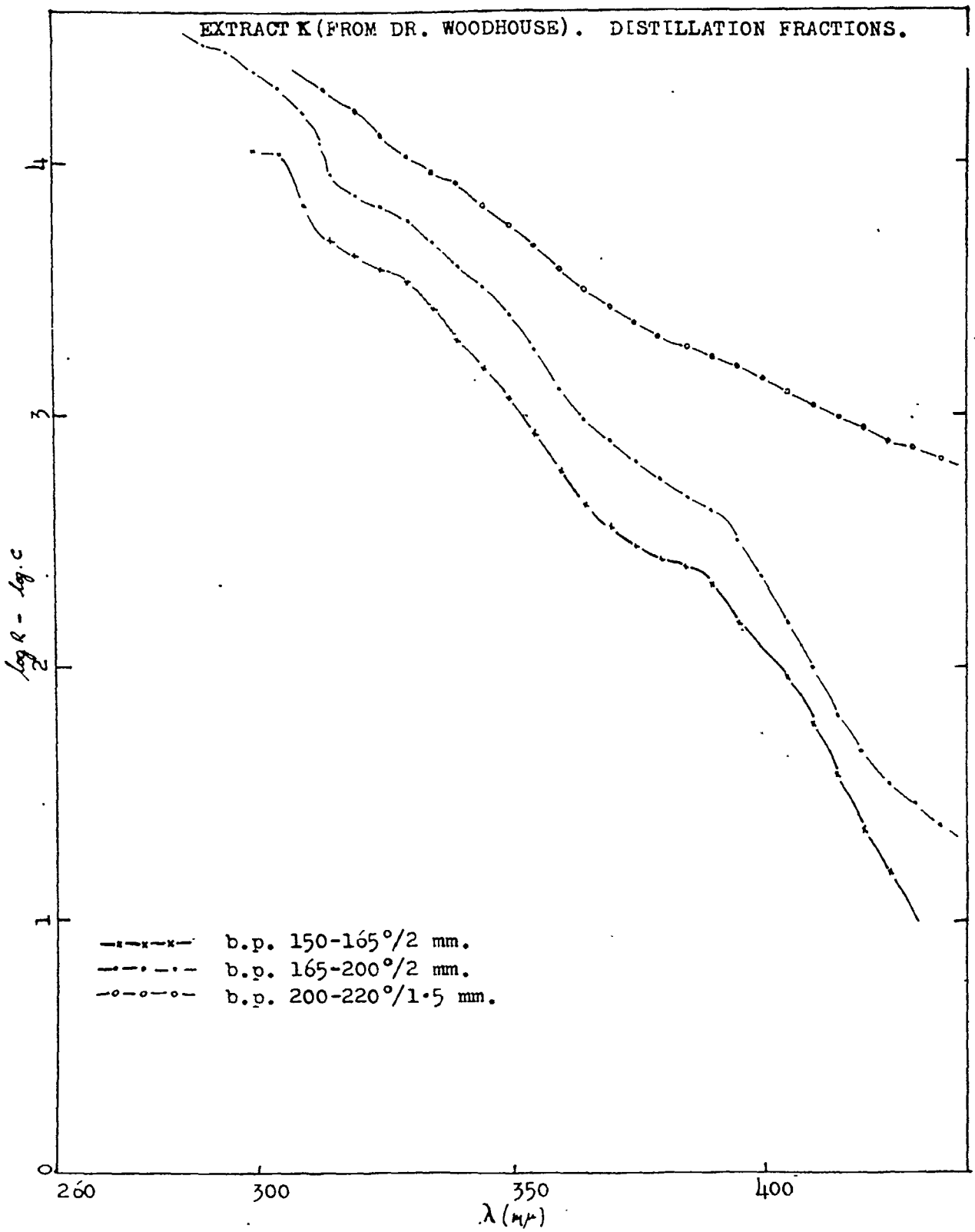

EXTRACT K (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
log ε - log c
4
3
2
1
0
260
300
350
400
λ (mμ)
b.p. 150-165°/2 mm.
b.p. 165-200°/2 mm.
b.p. 200-220°/1·5 mm.

FIG. III

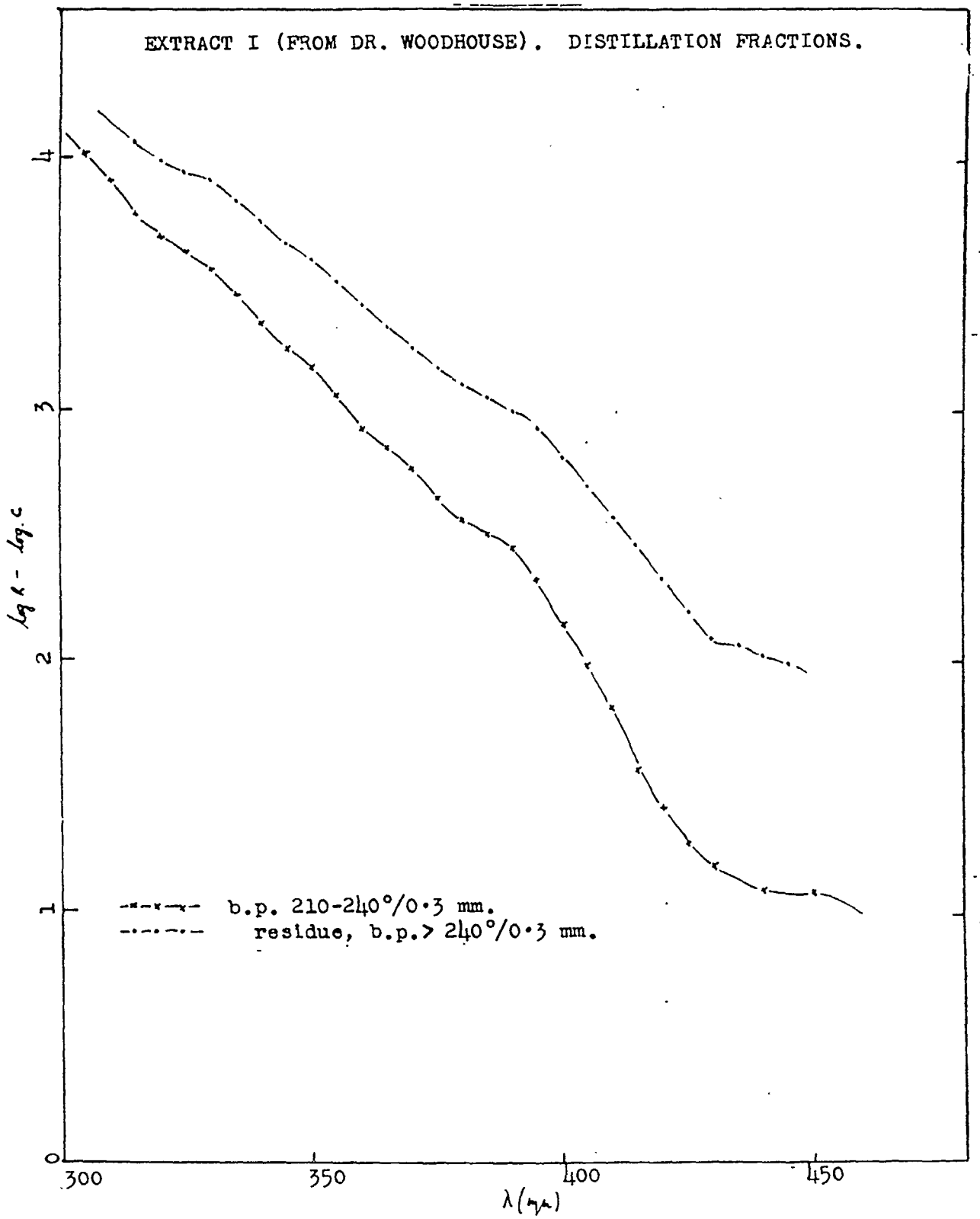

EXTRACT I (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
log ε - log c
0
1
2
3
4
300
350
400
450
λ (mμ)
b.p. 210-240°/0·3 mm.
residue, b.p.> 240°/0·3 mm.

FIG. IV

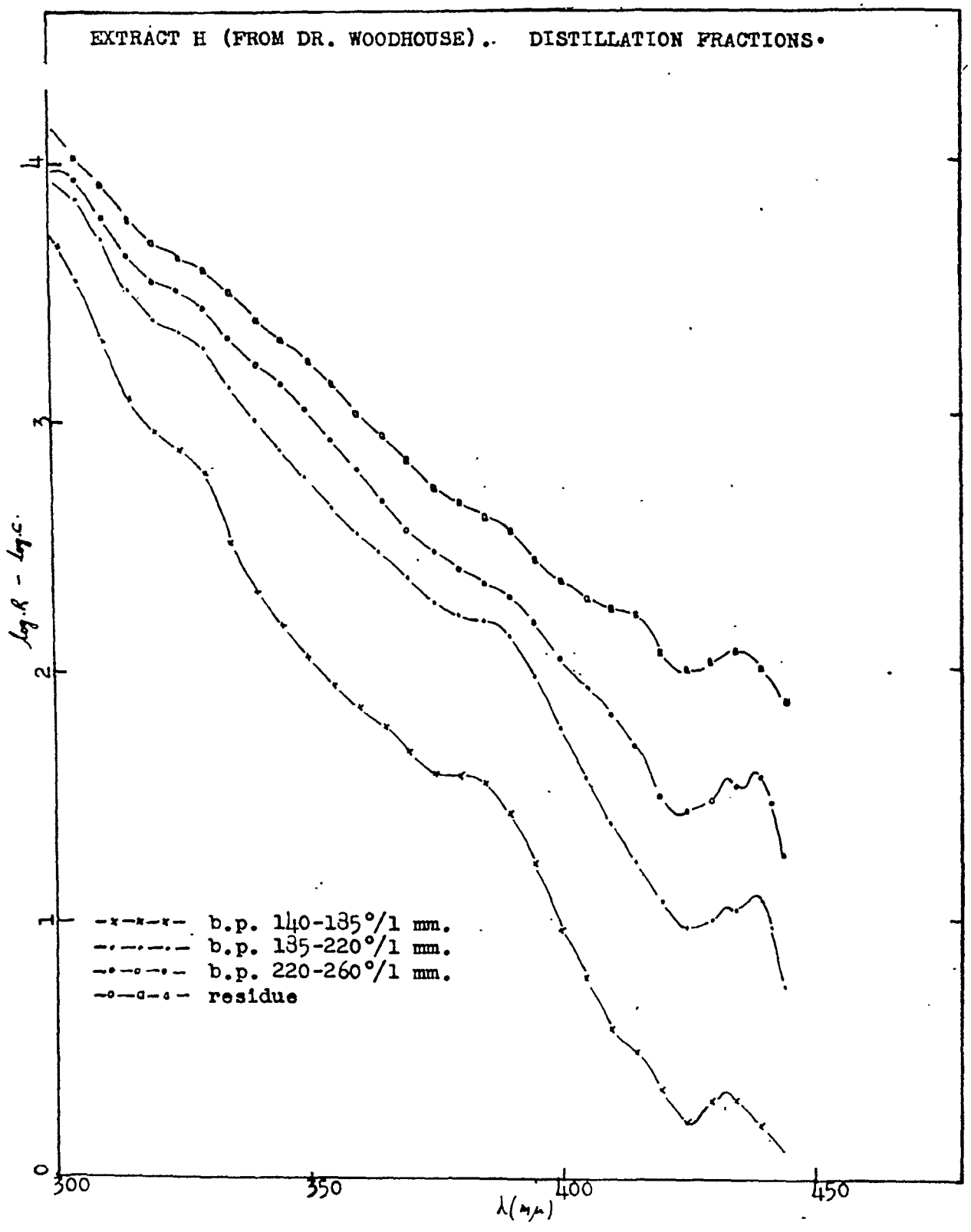

EXTRACT H (FROM DR. WOODHOUSE).. DISTILLATION FRACTIONS.
log.R - log.c.
λ(mμ)
0
1
2
3
4
300
350
400
450
b.p. 140-185°/1 mm.
b.p. 185-220°/1 mm.
b.p. 220-260°/1 mm.
residue

FIG. V

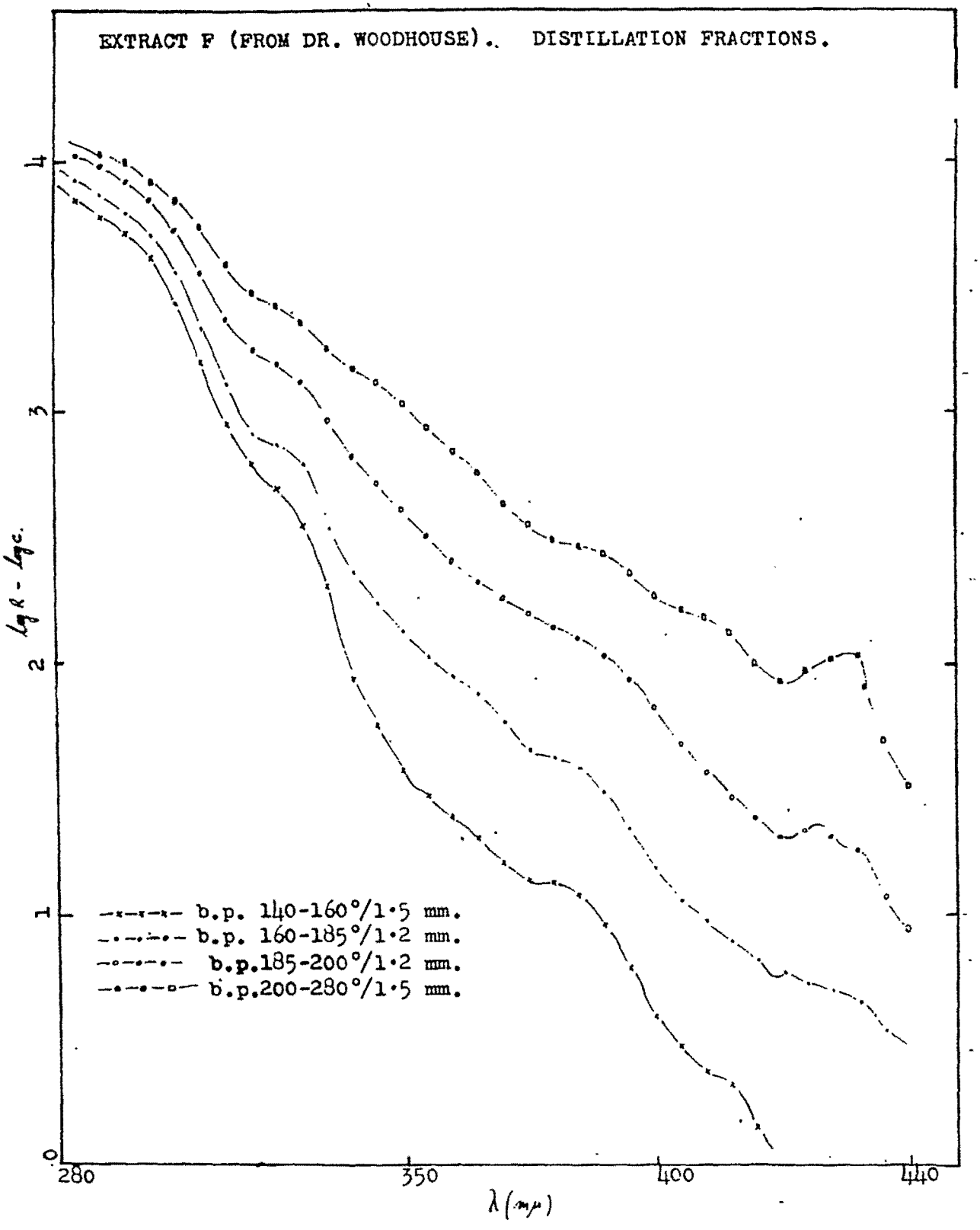

EXTRACT F (FROM DR. WOODHOUSE). DISTILLATION FRACTIONS.
4
3
2
1
0
log R - log c.
280
350
400
440
λ (mμ)
b.p. 140-160°/1·5 mm.
b.p. 160-185°/1·2 mm.
b.p.185-200°/1·2 mm.
b.p.200-280°/1·5 mm.

FIG. VI

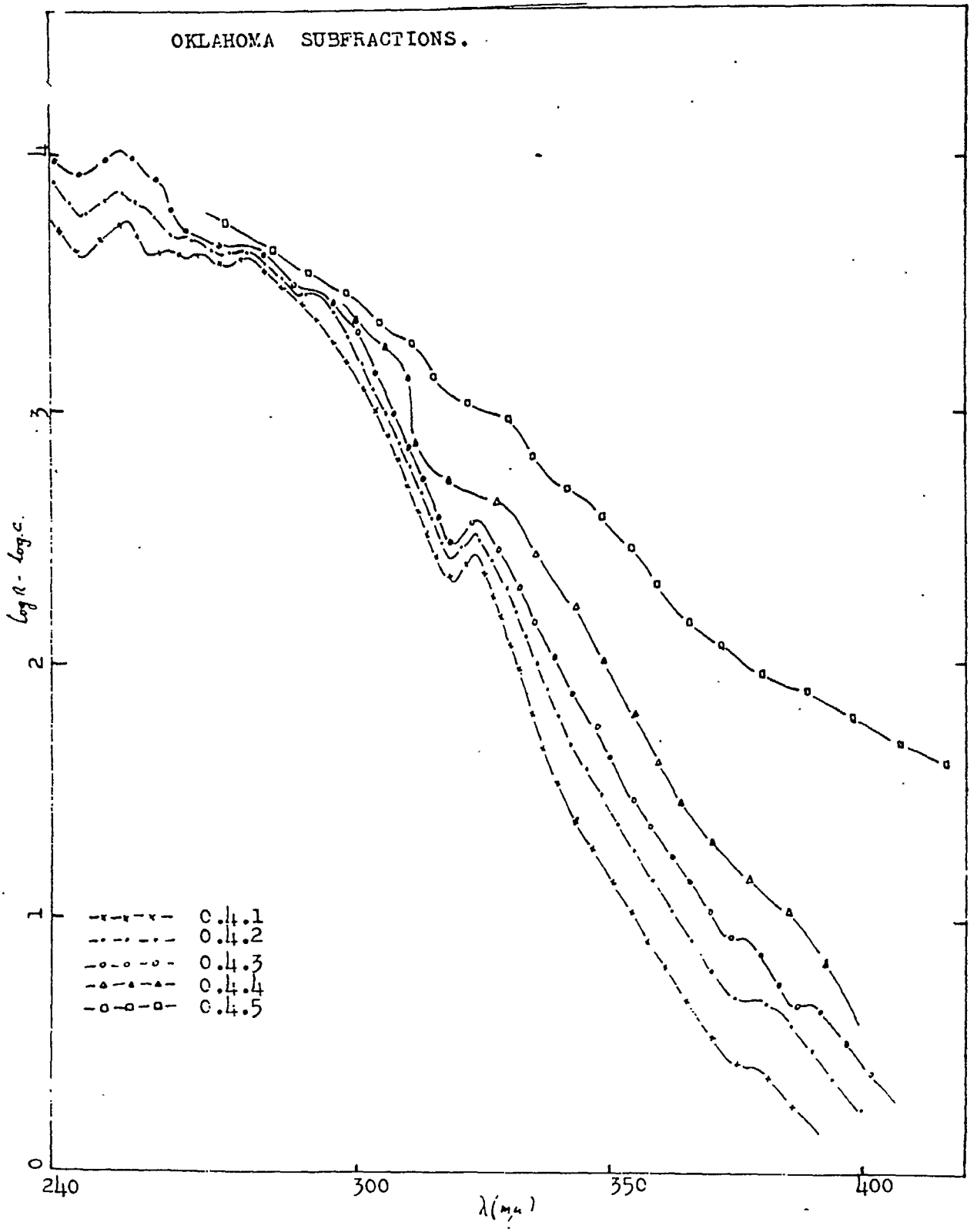

OKLAHOMA SUBFRACTIONS.
Log α - Log c.
0
1
2
3
4
240
300
350
400
λ(mμ)
0.4.1
0.4.2
0.4.3
0.4.4
0.4.5

FIG. VII

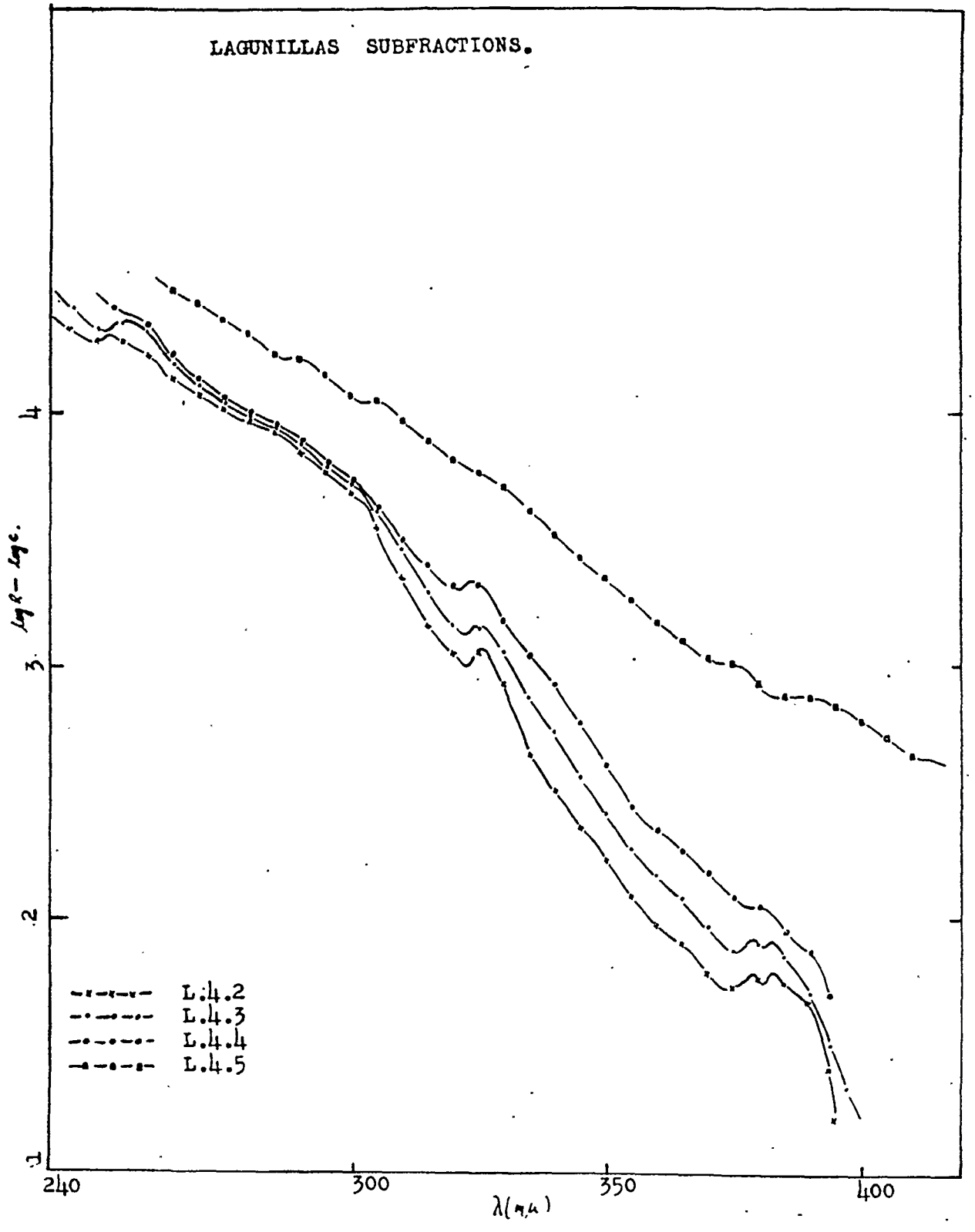

LAGUNILLAS SUBFRACTIONS.
Log ε - Log c.
4
3
2
1
240
300
350
400
λ(mμ)
L.4.2
L.4.3
L.4.4
L.4.5

FIG. VIII

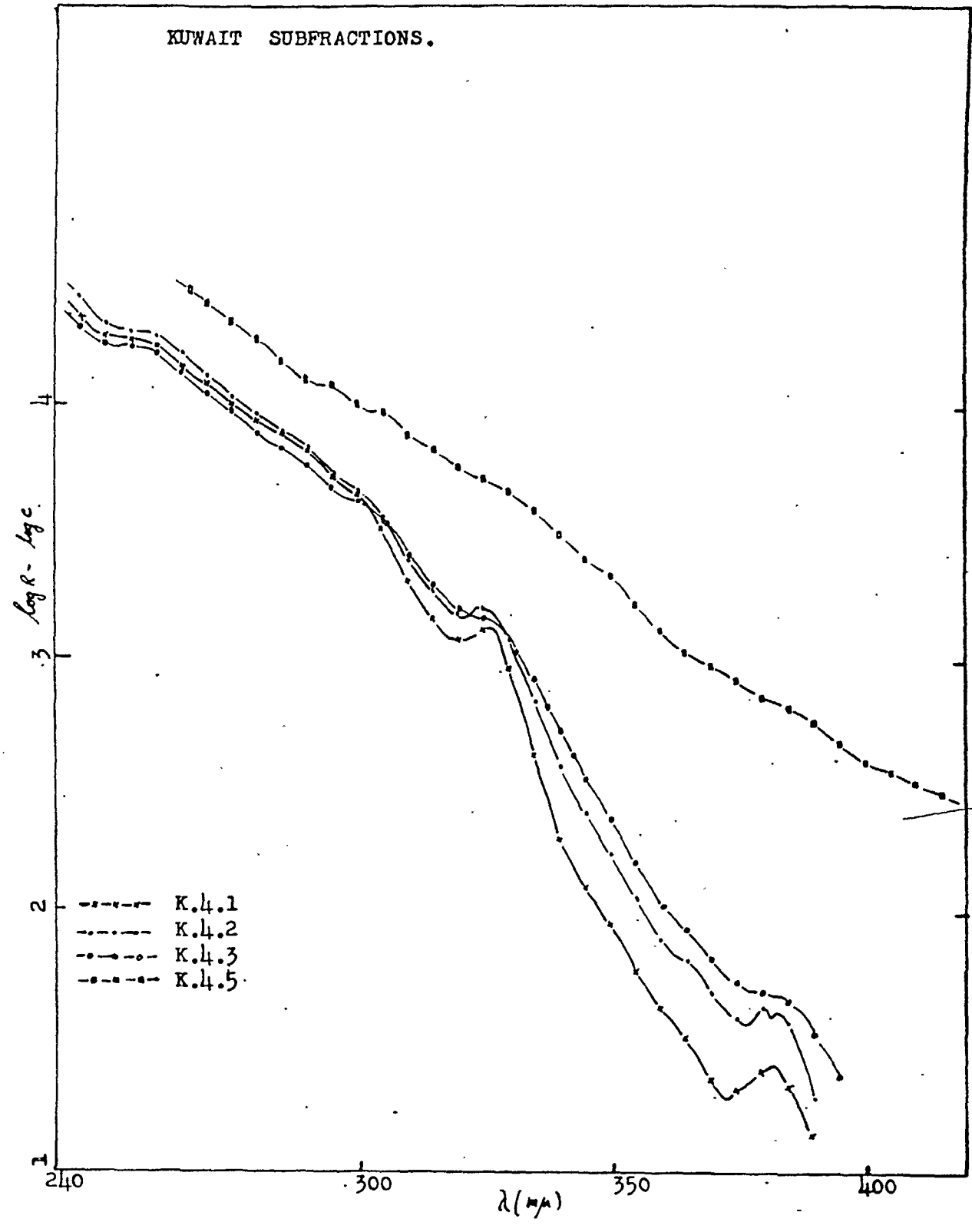

KUWAIT SUBFRACTIONS.
log ε - log c
4
3
2
1
240
300
350
400
λ(mμ)
K.4.1
K.4.2
K.4.3
K.4.5

FIG. IX

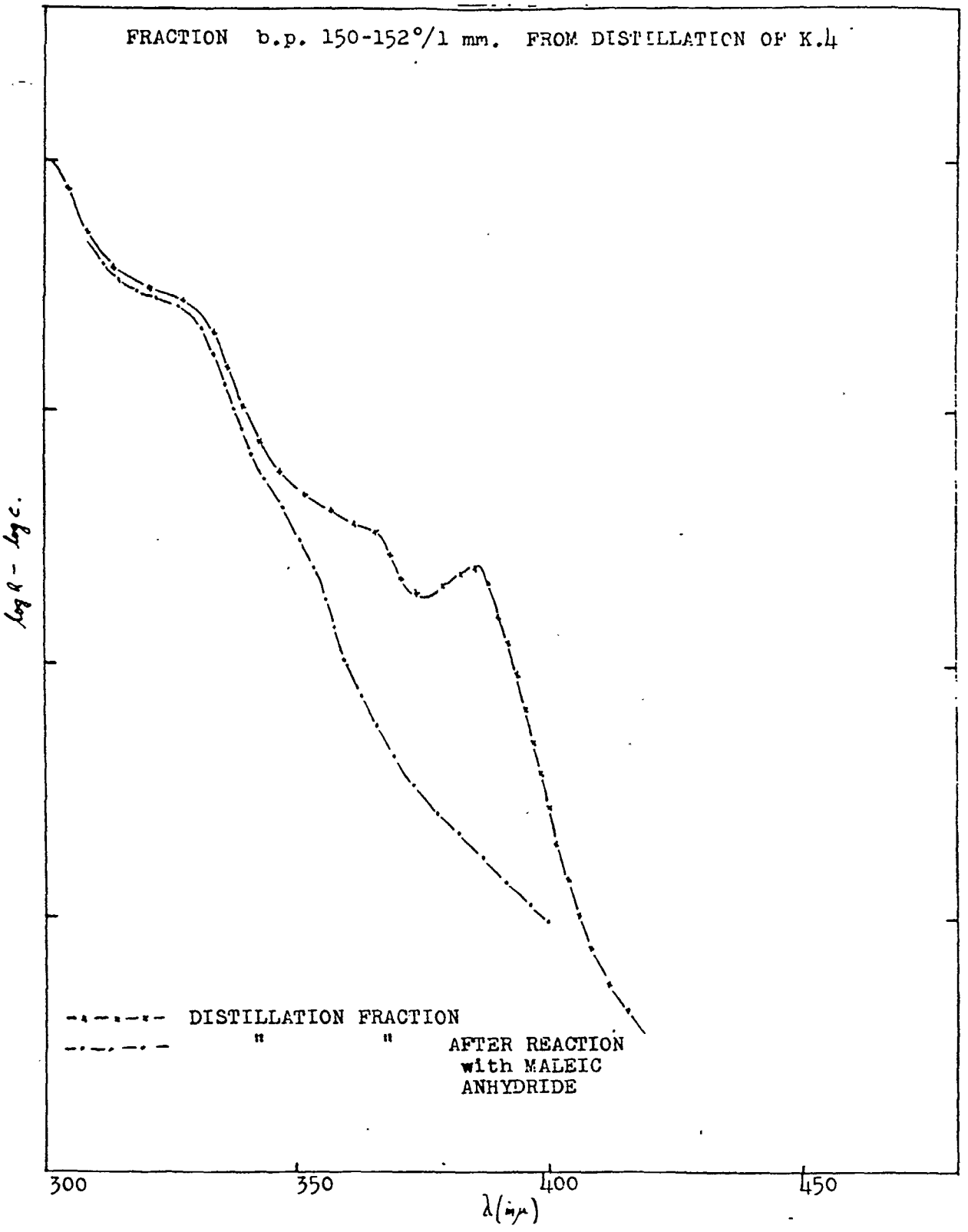

FRACTION b.p. 150-152°/1 mm. FROM DISTILLATION OF K.4
log ε - log c.
DISTILLATION FRACTION
" "
AFTER REACTION with MALEIC ANHYDRIDE
300
350
400
450
λ(mμ)

FIG. X

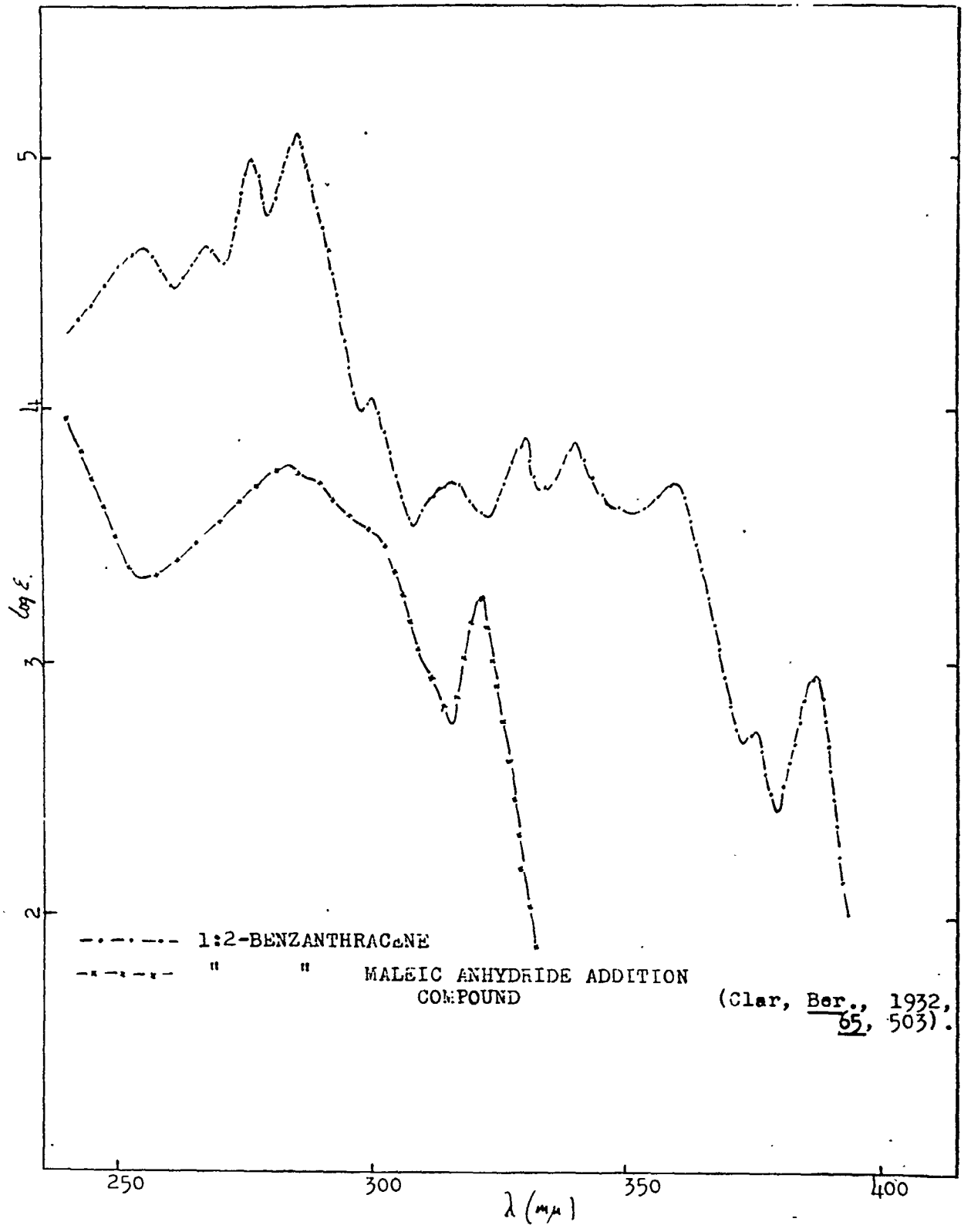
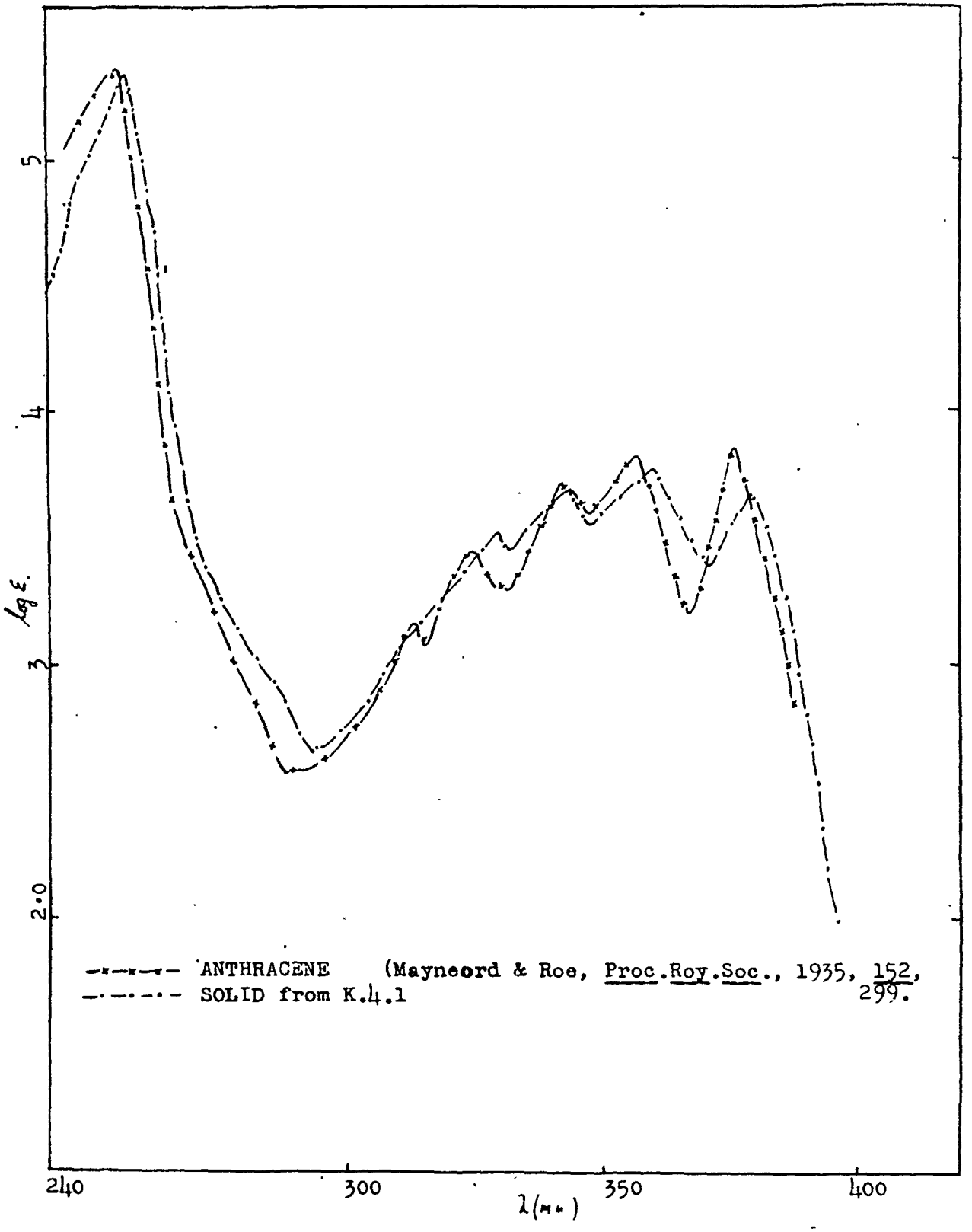
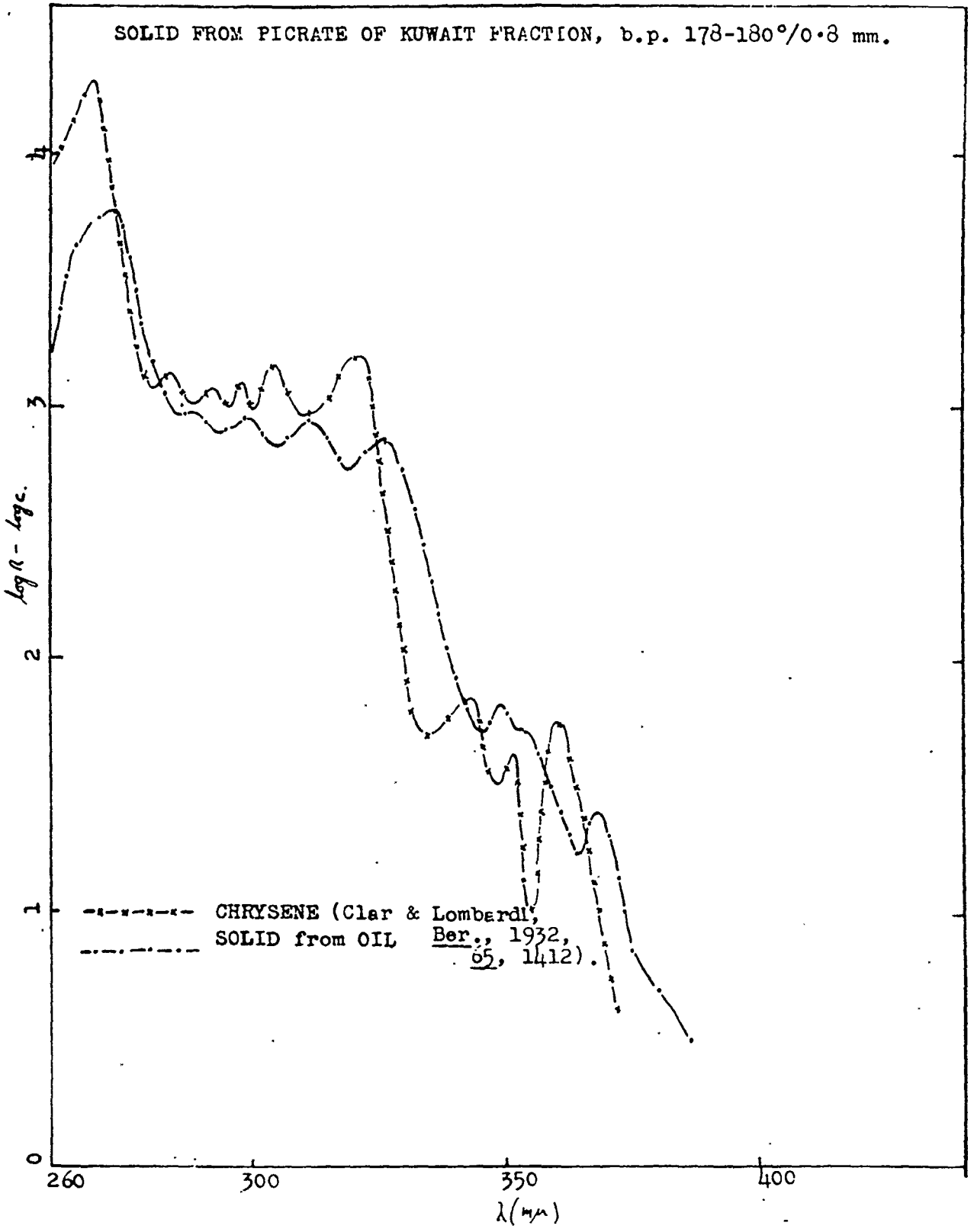

5
4
3
2
log ε.
250
300
350
400
λ (mμ)
1:2-BENZANTHRACENE
" "
MALEIC ANHYDRIDE ADDITION COMPOUND
(Clar, Ber., 1932, 65, 503).

FIG. XI



SC-API-2223

FIG. XII


SOLID FROM PICRATE OF KUWAIT FRACTION, b.p. 178-180°/0·8 mm.
log ε - log c.
0
1
2
3
4
260
300
350
400
λ(mμ)
CHRYSENE (Clar & Lombardi, Ber., 1932, 65, 1412).
SOLID from OIL

Section III

THE RESULTS OF BIOLOGICAL TESTS

The technique employed in the biological tests on behalf of the Medical Research Council were based to a considerable extent on the experience gained during work previously carried out for the Petroleum Board. A part of the work during the period under review dealt with extracts utilised in the earlier work. A short outline of this will therefore be given.

Tests of mineral oil fractions carried out at Birmingham for the Petroleum Board

A. Birmingham. Tests on 16 fractions and residues. During 1944-1945 a series of 16 fractions, A-P, were tested on groups of 50 mice. These consisted of lube distillates and residues from crude oils originating in U.S.A., Venezuela, Equador, Colombia, Iran and U.K., processed by Duosol, Edeleanu and Furfural methods. Their action on animals, including the production of benign and malignant tumours, was noted and an estimate of their relative carcinogenic potency was made.

During the same period 6 materials, not of mineral oil origin, were tested either as controls or on account of their use industrially. These were Creosote oil, Anthracene oil, Linseed oil, Pine Tar oil, Butyl phthalate, and Tricresyl phosphate. (The last was so toxic that long term experiments with it were abandoned.)

The creosote and anthracene oils were potent carcinogens. The 'vegetable' oils did not produce any tumours. The characteristics of the materials and a summary of the results of these tests are given in Tables VII(a) and VII(b).

B. Birmingham. Tests on 'Spindle' oils and 'White' oils. During 1946-1947, using similar techniques, the carcinogenicity of 7 spindle oils designated R, S, T, U, V, W, X, and 3 samples of 'white' oils designated A, B, Pl, were tested. (These symbols have no connection with the mineral oils mentioned in A.) The spindle oils in every instance had some carcinogenic activity and the general results are given in Table VIII.

The 'white' oils, however, caused no skin reaction, no epilation and no tumours on the mice during the painting and observation period of 50 weeks or more. The complete absence of any reaction indicated that such substances might be employed safely in industry and also in subsequent animal tests as a solvent for standard carcinogens.

C. Birmingham. Duplicate experiments. During the same period (1946-1947) a repetition with 10 fractions selected from those tested during 1944-1945 was undertaken in order to ascertain how close the results would be to one another and what degree of uncertainty might be introduced by unavoidable variations in technique under such circumstances.

The results of these tests were analysed statistcally by Irwin and the findings were published (Woodhouse & Irwin, 1950).* The following summary of technique, observations and results are taken from the published paper. Subsequently some slight modifications in technique were introduced following discussion with Professor Haddow, Dr. Carr and Dr. Hieger, so that tests which were to be carried out in collaboration with the Chester Beatty Research Institute should be made as far as possible under standard conditions. These changes are noted below.

General arrangements for the tests

Animals

Each fraction was tested on 50 albino mice obtained from one source. They were approximately 10 weeks old when the experiments commenced and 15-20 g. in weight; males and females were used in about equal numbers for each fraction. They were housed in wooden boxes 10" x $4\frac{1}{2}$" x $4\frac{1}{2}$", two animals per box, bedded with sawdust and 'cellulose wool'. A diet of wholemeal bread and rusks with water and fresh milk was given daily, with the addition of crushed oats and a little bran two or three times a week.

Experimental technique

The oils were taken from bottles by means of short glass rods and applied to the skin in the interscapular area from which the hair was removed with dilute sodium sulphide some days before commencing the paintings. An area about $1\frac{1}{2}$ cm. in diameter was covered, and the amount of oil used was kept as constant as possible, though it was difficult to do this exactly with samples of widely different viscosity. With few exceptions, applications were made twice weekly for a period of 25 weeks; on the death of any animal which had survived a period of more than 12 weeks the skin of the treated area was removed for microscopic examination.

It was thought desirable to use the substances neat whenever possible, but in some instances they were so viscous that it was necessary to add a 'solvent'. Acetone has been favoured by many investigators for making solutions of carcinogenic hydrocarbons, but some of the specimens were not sufficiently miscible in this medium; benzene or toluene, however, yielded homogeneous solutions. It is known that both of these solvents are somewhat toxic, but the latter has been shown to be less injurious to animals. The amount added to individual fractions is given in Table 7b. This also described the general characters of the fractions and their general effects on the mice during the experiments.

Results

The number of animals surviving 25 weeks or more and the total number of tumours produced by the various fractions in the experiments are given in Table IX. Fuller analysis of the results by

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* Woodhouse, D. L. & Irwin, J. O., (1950). J. Hyg., Camb. 47, 121.

statistical methods are given in the paper cited above. Two illustrative examples of data from which these statistical calculations were made are also set out in Tables X and XI:- (for Expt. 1, fraction K, a moderately carcinogenic sample and Expt. 1, fraction L, a weak carcinogen. The complete data are available.)

Conclusions

From a general study of the data in Table IX and the 'life sheets', of which Tables X and XI are examples, it is possible to draw some general conclusions.

1. There was a fair agreement in the results of the two series, though some distinct differences may be noted.

2. On the whole, the survival rate in Experiment 2 was consistently less than in Experiment 1.

3. There appeared to be a slightly greater tumour response in a number of tests in Experiment 2.

4. Several fractions had a very similar, moderate degree of activity (F, H, I, K,), while fraction M was very weak or of negligible activity.

5. The investigation indicated that in order to obtain a general estimate of the carcinogenic properties of this type of material at least 50 animals should be employed.

For reliable comparison of certain carcinogenic potencies, it is desirable that fractions should always be compared concurrently in an experiment carried out on randomised batches of mice. The paintings in all the tests necessary on any one day should be carried out by a single individual. Under these conditions it appears possible to achieve reproducible results.

Summary of Irwin's findings

Woodhouse's experiments on the carcinogenicity of 10 different fractions for the skin of mice have been analysed statistically. Two experiments separated by a considerable period of time were performed on each fraction. A number of different measures of carcinogenicity were used. All agree in showing that:

1. The apparent carcinogenicity was greater in the second series of experiments.

2. There were significant differences of carcinogenicity. The fractions can be divided into three groups within which the carcinogenicities were about the same, F, H, I, K; B, D, C, L, E; M. The groups are in order of decreasing activity.

D. Research co-ordinated by the Council's Committee on the Carcinogenic Activity of Mineral Oils

Period 1949-1950 - 'Dilution experiments'

During this period work for the development of a standard method of expressing carcinogenic potency was carried out and, following on the observations of Professor Squire and Dr. Cruickshank and the advice of Dr. Berenblum, experiments to test the suitability of rabbits for assays of carcinogenicity were also conducted. The mouse dilution experiments were carried out in duplicate, at Birmingham and at the Chester Beatty Research Institute. The rabbit dilution experiments were carried out at Birmingham only.

The objects in these tests were:-

1. To ascertain the effect of diluting the material to be tested on the carcinogenic response of the skin of mice, with a view to providing data which will serve as a basis for future tests on fractions or isolated compounds separated from the crudes or distillates by physical or chemical methods.

2. To compare the effects of the same dilutions of these materials on the response of rabbits when they are applied at a number of sites on each animal.

Standardised technique agreed upon by the workers at Birmingham and London in the light of the experiments outlined above and the design for the rabbit tests devised by Irwin were followed. The chief points respecting the experiments are as follows.

Mice

White albino mice, strain Clarke 0.1, 12 weeks old at the start were used, 25 males and 25 females on each fraction. The sexes were separated and the animals housed in metal boxes 5 in a box. Food for the mice consisted of proprietary cubes with supplement of cod-liver oil (on bread), milk, etc., with water ad libitum. The applications of the samples were made twice weekly by glass rod applicators to the areas from which hair had been removed by clipping. The results are given in Tables XIIa-g, XIIIa-c, XIV, XV and have been analysed statistically by Irwin.

Rabbits

Short haired males of 'Old English' type with erect ears chiefly white with some coloured patches, 4 months old at the start were used. They were housed in separate cages with a painting chart for each animal. Food consisted of oats and bran with a small amount of cabbage or roots. Applications to the designated sites were made after hair had been removed by clipping.

All deaths, papillomata etc. were recorded to the nearest half week. The mice experiments commenced on 14th March, 1949, the rabbit experiment on 12th May, 1949 and both series were continued for 12 months.

The following materials were used in these tests:-

1. 9:10-dimethyl-1:2-benzanthracene in dilutions of 0.1% (D/1)*; 0.025% (D/4); 0.00625% (D/16); 0.00156% (D/64).

2. Oil fraction I, neat; diluted 1/4; diluted 1/16; diluted 1/64.

3. Oil fraction F; diluted as for I.

The solvent diluting medium was colourless B.P. grade of liquid paraffin. F and I were fractions similar to those used in the experiments described above and had been proved to produce epitheliomata in mice. The fractions and solutions were stored in amber coloured bottles and in all the experiments recorded in this report precautions were taken to avoid exposure to light or any accidental contamination.

36 rabbits were used, divided into 12 randomised groups of 3. Each rabbit was painted on 6 sites: - I, II, - both ears, outer surface near base; III, IV, - lateral thoracic; V, VI, - lateral abdominal. The four dilutions of each of the three oils were assigned to the groups and sites as shown in Part IV, Appendix I, p.i. Some results are given in Table XVI. They have been further analysed statistically by Irwin (Part IV). Sections of skin from the mice which received more than 12 weeks' application, and the treated patches from the rabbits were cut and stained for examination of histological changes including classification of tumours.

Tests on 'experimentally derived' fractions (Birmingham only)

Some tests on materials derived from oil fractions during Professor Cook's and Dr. Carruther's researches were also made by application to the skin of mice and by subcutaneous injection.

1. An homogenous crystalline solid (shown to be a homologue of chrysene) which was isolated from the extract Edmor 33 and designated Edmor 33A. This was injected subcutaneously into 24 mice; one sarcoma occurred after 14 months.

2. A viscous oil which was obtained by decomposition of a crystalline picrate prepared from an extract of a Lagunillas (Venezuela) crude. This was applied to the skin of 40 mice, 1% in benzene, and produced papillomata in 3 mice after 52, 56, and 60 weeks, respectively.

Period 1950-1951 - Tests on 3 selected crudes and 15 derived fractions

These tests were carried out in duplicate, at Birmingham and the Chester Beatty Research Institute. The source, quantities required and the scope of the initial fractionation of the crudes to be examined were considered in committee from all aspects (see also Part IV, Appendix I).

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* The letters and numbers in brackets are the short form of reference to each dilution.

The crudes were (1) an asphaltic, paraffinic type (Kuwait), (2) a naphthenic type (Lagunillas (Venezuela), and (3) a paraffinic type (Oklahoma, U.S.A. Mid Continental). Each of these was separated into 5 fractions at the Thornton Research Centre.

The tests for carcinogenic activity were carried out as before on groups of 50 mice and on a total of 105 rabbits. For the 3 crudes a total of 30 rabbits were used with 6 skin patches on each and for the 15 derived fractions a total of 75 rabbits with 7 application sites. These were the same as those described in the section on 'Dilution Experiments' with the addition of one 'interscapular' site.

The allocation of the animals and sites is set out in Part IV, Appendix I, p. i., where for Birmingham, A = Oklahoma, B = Lagunillas, C = Kuwait, and for London A = Kuwait, B = Lagunillas and C = Oklahoma. (see also Table XIX).

Results

Mouse tests

The number of tumours induced by the crudes and fractions are given in Table XVII and the time of appearance of all the tumours is given in Table XVIII. The total numbers of tumours obtained were 33 and 6 respectively (in 900 mice) for Birmingham and London, and the greatest number for any one fraction was 5 (in 50 mice). The results suggest that the Lagunillas derived fractions were somewhat more active than the Kuwait and that the Oklahoma fractions were essentially inert.

At Birmingham only two tumours were obtained after continued application of the crudes; at London they yielded no tumours after 50 weeks.

Rabbit tests

In contrast with the tests on mice, there was a total of 61 tumour-bearing sites in the Birmingham series and 39 in the London series, on a possible 705 sites (105 rabbits), or, excluding the crudes and residues (fraction 5), the remaining 12 fractions produced 61 and 35 tumours respectively on a possible 420 sites (60 rabbits) (Table XVI). Thus the skin of the rabbit was distinctly more sensitive to these fractions than that of the mouse. This observation was confirmed by the type and rate of growth of the papillomata. In a number of instances, at both laboratories, several tumours occurred on one rabbit site. In general the grading of the carcinogenic potency ran similarly in the Birmingham and London tests. Sections of skin from the treated areas have been prepared for histological examination.

Irwin's statistical analysis brings out the following points. Fractions L1, L5; O1, O2, O5; K1, K2, K5 can be regarded as of negligible carcinogenic potency. According to the Birmingham figures the order of potency for the fractions 4, the most potent, is L4, K4, O4; and the London results are not inconsistent with this.

A striking feature of the tests is the special sensitivity of rabbit skin compared with mouse skin for these mineral oil fractions. The response of different species of animals to different carcinogens has been investigated by a number of workers including Berenblum; some pure chemical carcinogens have selective activity towards certain species. It may be no more than a conjecture to suggest that the results indicate the presence of carcinogens differing in chemical structure from the better known polycyclic hydrocarbons. However, they reinforce the opinion that the rabbit should be included in tests for 'carcinogenicity' on this type of material.

1952 - Work in progress

After comparing the results of the Dilution Experiments in which the 9:10-dimethyl-1:2-benzanthracene was used at 0.1% - 0.0015% in liquid paraffin, with those obtained in the experiments on the derived fraction of O, K, and L, Professor Squire suggested that some idea of the content of carcinogen of the various fractions in terms of 'equivalent' of that polycyclic hydrocarbon could be computed. Similarly, the equivalent concentration in the original crude could be estimated and thus an idea obtained of the degree of concentration achieved in the derived fractions. Thus K4 and L4 appear to have about 7 and 9 times the dimethylbenzanthracene 'equivalent' of the original crude.

Since it was possible that further considerable 'concentration' of carcinogen might be expected to be attained by separating these active fractions into cuts of narrower temperature limits, a scheme was devised for testing 5 sub-fractions from each and incorporating two dilutions of three possible reference standards.

The 15 sub-fractions separated under the supervision of Professor Morton are shown in Table I. The reference standards comprise:-

9:10-dimethyl-1:2-benzanthracene, 0.2% and 0.05% in liquid paraffin;

Methylcholanthrene 0.2% and 0.5% in liquid paraffin;

Kuwait fraction 4, neat and diluted 4 times with liquid paraffin.

These 21 specimens are now being tested on a total of 1050 mice and 105 rabbits.

The allocation of sites for the rabbit applications, devised by Dr. Irwin and Dr. Armitage is given in Table XIX, together with the appropriate key. As will be noted, 5 rabbit sites are involved, the 2 ears, left and right throacic, and interscapular. The experimental procedure previously used is being followed.

Section IV

STATISTICS AND METHODS

The plan of the experiments and the instructions for carrying them out are shown in Appendix I. The experiments were of two kinds:-

1. Experiments on the three whole crudes and five fractions derived from each of them.

2. Dilution experiments.

Both sets were carried out on rabbits as well as mice. The three crudes were Kuwait, Lagunillas and Oklahoma. The methods of distillation and the physical characteristics of the fractions are described in Appendix II. Fraction 5 was in each case the residue.

1. Experiments on the three whole crudes and fifteen derived fractions

In the mouse experiments a batch of 50 mice was used for each crude and fraction both at Birmingham and at the Chester Beatty Research Institute. At the Chester Beatty Institute there were only 6 tumours in 900 mice; these results were clearly not worth while analysing statistically. At Birmingham there were very few tumours and no significant differences between any of the oils (crudes or fractions). Table XX shows for each of these fractions the expectation of tumourless life (limited to 52 weeks), the dates when 10 per cent of surviving mice had tumours together with their standard errors. The total number of tumours is also given. The distribution of the number of tumours was as follows:-

No. of tumours	No. of fractions	
	Observed	Expected (Poisson)
0	6	3
1	1	5
2	6	5
3 or more	5	5
	18	18
$\chi^2 = 6.2$		2 d.f.

There is no significant departure from the frequencies expected on the basis of chance variation about a fixed mean (Poisson frequencies).

The other two measures of response also show no significant differences.

For the rabbit experiments on the three whole crudes a 6 x 6 Latin Square design was used. (see Appendix I). For the experiments on the fifteen derived fractions a more complicated design (known as a Youden square) was employed. Here there were 15 groups of five rabbits. In each group each rabbit was painted with the same 7 fractions. Each of the 15 fractions was painted on each site in one and only one group. Each fraction was used in exactly 7 groups and every pair of fractions was used in exactly 3 groups. To obtain the designs actually used at Birmingham and the Chester Beatty Research Institute we must substitute in the key of Appendix I:- for Birmingham (A = Oklahoma, B = Lagunillas, C = Kuwait) and for the Chester Beatty Research Institute (A = Kuwait, B = Lagunillas, C = Oklahoma).

In the experiments with the three whole crudes no tumours were obtained at Birmingham and only 2 tumours on 180 sites at the Chester Beatty Research Institute; thus, there was no need for statistical analysis in these two experiments. Results of great interest were, however, obtained from the experiments with the fifteen derived fractions.

The percentage of each group of five animals which got tumours on each site was calculated. The method of analysis employed was to transfer percentages to degrees by means of the angular transformation $p = \sin^2 y$ (which approximately equalises the variance at different levels) and then to perform an analysis of variance of the results. Table XXI shows the mean values of the transformed variable, and the probabilities of getting a tumour, estimated for each fraction. Fractions L_1, L_5, K_1, K_2, K_5, O_1, O_2, O_5 can be regarded as of negligible potency; so can O_3, O_4 in the Birmingham results but not in the Chester Beatty Institute's results, and L_2 in the Chester Beatty Institute's but not in the Birmingham results. If we exclude the eight fractions first mentioned we are left with seven; L_2, L_3, L_4, K_3, K_4, O_3, O_4. The Birmingham results show that fraction 4 is more potent than fraction 3 and that the order of potency is $L > K > O$. Further, these differences in potency are significant. The results from the Chester Beatty Institute for these fractions show no significant differences among themselves (except for L_2) but are not incompatible with the Birmingham results.

The analysis of variance shows that there are significant differences in sensitivity between the fifteen groups of rabbits in both sets of results. In the Birmingham experiment, site differences might be due to chance ($P = 0.12$ for greater variance ratio than that observed), in that at the Chester Beatty Institute they just pass the 5 per cent level of significance ($P = 0.04$). However, the actual results shown in Table XXIII are concordant and suggest a real effect. Both series show the greatest number of tumours on site VII (in the middle of the back nearest the ears) and sites I and II (the ears) come next on the average. There seems to be a larger number of tumours nearer the head.

2. Dilution experiments

As stated in Section II of Appendix I the pure carcinogen was 9:10-dimethyl-1:2-benzanthracene. The other two oils were the fractions I and F which had been tested before at Birmingham during 1944-6 and 1946-7. (Woodhouse and Irwin 1950). In the mouse experiments batches of 50 mice were used for each oil in each centre (Birmingham and the Chester Beatty Institute). The results are given in Table XXIV. Graded dosage response curves were obtained for all three oils at Birmingham, and for the pure carcinogen in both centres. It clearly seems best to use E.T.L. as a response in these experiments since the 10 per cent dates are not always reached. Between the undiluted oil and the 1/4 dilution the slopes of these 4 dosage-response curves do not differ significantly. At Birmingham the same is true of the slopes between the 1/4 and 1/16 dilution, for all three oils. This result is very encouraging as it suggests that dimethyl-benzanthracene might be used as a standard.

Owing to changes of staff, the rabbit dilution experiments were completed only at Birmingham.

The design for the rabbit experiments (see Appendix I (2)) consisted essentially of three 4 x 4 Latin Squares, using sites III, IV, V and VI. Sites I and II, being available, were used for some additional observations, so as to permit if necessary of direct comparisons between different fractions at the same dilution in the same rabbits. However, in the event, the statistical analysis of the three 4 x 4 Latin Squares, was sufficient to show that there were no significant differences between the dosage-response curves of the three fractions. This analysis is presented here.

Rather unexpectedly there were no significant differences between rabbits treated alike (except possibly for fraction I) in the probability of getting a tumour; nor were there significant differences between sites treated alike.

The method of analysis employed was to transfer percentages to degrees by means of the angular transformation $p = \sin^2 y$ (which approximately equalises the variance at different levels) and then to perform an analysis of variance of the results. The regression of y on log-dilution was calculated and did not differ significantly from linearity. The error variances obtained from the experimental results were in all cases in good agreement with their theoretical values.

$$\left\{ \frac{1}{12} \left(\frac{180}{\pi} \right)^2 = 273.6 \right\}$$

This justifies the method of analysis.

From the regressions an estimate of the probability of getting a tumour at the four dilution levels was estimated for each fraction. The results are:-

Dilution	Fraction			All Three	5% Fiducial limits
	D Prob.	I Prob.	F Prob.		
1	0.53	0.33	0.49	0.45	0.30-0.75
1/4	0.22	0.16	0.19	0.19	0.11-0.36
1/16	0.03	0.04	0.02	0.03	0.00-0.10
1/64	0.00	0.00	0.00	0.00	0.00-0.01

These results are not significantly different for the different oils, so that the estimates in the last two columns can be taken as applying to each of them.

The error variances per group of 3 rabbits taken from the analyses of variance are

Fraction	Error Variance (6 d.f.)
D	218.4
I	213.1
F	288.3
Theoretical value	273.6

The detailed analyses of variance are shown in Tables XXV and XXVI.

The Latin Squares are analysed in the usual manner; the 3 degrees of freedom for differences between the four dilutions have been sub-divided into 1 due to the linear regression of response (y) on the logarithm of the dilution and the remaining 2 due to departure from linear regression. This departure is not significant. These results are given in Table XXV.

To investigate whether there are any significant differences between rabbits treated alike, the results for individual rabbits must be analysed. For any one dose and site there are 2 degrees of freedom for difference between the three rabbits making 32 in all (4 sites, 4 groups of rabbits, 4 x 4 x 2 = 32). These 32 together with the 15 from the Latin Square analysis make 47 in all, clearly correct for 48 observations. The 32 degrees of freedom can be sub-divided into 8 due to differences between rabbits and 24 due to error. The analysis has been carried out by 90°, scoring for a tumour and 0° for the absence of a tumour; the results are shown in Table XXVI.

The differences between rabbits are significant only for fraction I (Variance Ratio = 2.59, 5% pt = 2.36). There are no significant differences between sites. There are no significant differences between the three fractions at the same dilution, even if the smaller standard error, based on the assumption of homogeneity of the rabbits be used. Hence, it was unnecessary to use the supplementary observations mentioned above.

TABLE I

Oklahoma (O_4)			Lagunillas (L_4)			Kuwait (K_4)		
Fraction No.	Boiling range corrected to 760 m. °C	Volume ml.	Fraction No.	Boiling range corrected to 760 m. °C	Volume ml.	Fraction No.	Boiling range corrected to 760 m. °C	Volume ml.
O_4.1	-320	1800	L_4.1	-380	900	K_4.1	-350	1770
O_4.2	320-370	1800	L_4.2	380-390	1800	K_4.2	350-380	1690
O_4.3	370-390	1800	L_4.3	390-400	1800	K_4.3	380-390	1760
O_4.4	390-410	2700	L_4.4	400-410	1800	K_4.4	390-400	1070
O_4.5	Residue > 410	900	L_4.5	Residue > 410	2700	K_4.5	Residue > 400	2500

TABLE II

OKLAHOMA, CRUDE FRACTION NO. 4.

Volume charged 1000 mls. Pressure 1 mm.Hg.

Fraction No.	Boiling Range (Corrected °C).	Volume mls.
1	0-290	100
2	290-320	100
3	320-360	100
4	360-370	100
5	370-370	100
6	370-390	100
7	390-400	100
8	400-400	100
9	400-410	100
Residue	410	100

TABLE III

LAGUNILLAS CRUDE FRACTION No.4.

Charge 1000 mls. Pressure 1 mm.Hg.

Fraction No.	Boiling Range °C(Corrected)	Volume
1	0-380	100
2	380-385	100
3	385-390	100
4	390-400	100
5	400-400	100
6	400-410	100
7	410-410	100
Residue	410	300

TABLE IV

KUWAIT CRUDE FRACTION 4.

Charge 1000 mls. Pressure 1 mm.Hg.

Fraction No.	Boiling Range °C (Corrected)	Volume
1	0-300	100
2	300-350	100
3	350-360	100
4	360-380	100
5	380-385	100
6	385-390	100
7	390-395	100
8	395-400	100
Residue		200

TABLE V

N.V. Curacaosche Petroleum Industrie Maatschappij
(Curacao N.A.)

Analysis of a sample of Lagunillas distillate(4)

Specific gravity at 15/4°C	0.9299	
Specific gravity at 60/60°F	0.9304	
Gravity A.P.I. at 60°F	20.6	deg.
Viscosity Redwood I at 140°F	55	sec.
Flashpoint P.M. cc.	330	°F.
Water	0.05	%
Pourpoint	-33	°F.
Organic acidity	5.0	mg.KOH/g.
Inorganic acidity	nil	mg.KOH/g.
Ash	nil	%
Asphaltenes	nil	%
Distillation (Vacuum 1 mm.)		Thornton Report No. C3.2/50 Table 3 fraction 4
	°C	°C
2% recovered at	310	317
10% " "	332	343
20% " "	342	350
30% " "	350	354
40% " "	357	360
50% " "	365	365
60% " "	371	370
70% " "	376	376
80% " "	383	384
90% " "	394	-
95% " "	401	-

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TABLE VI

Socony-Vacuum Laboratories

Analysis of a sample of 70 distillate(5)

Laboratory No.	934796			
Buffalo Refinery No.	192			
Product	70 distillate			
Gravity	28.2			°A.P.I.
ASTM colour	1-1/2			
Pourpoint	+30			°F
Flashpoint	335			°F
S. U. Viscosity	83			@ 100° F
Vacuum distillation (Readings adjusted to 760 mm.Hg.)	°F	°C	°F	Thornton Research Centre Report No. C3/2/50 Table II. Fraction 4 °C
	IBP	296	566	296
	10%	356	673	315
	20%	368	695	320
	30%	374	706	327
	40%	378	712	332
	50%	385	725	338
	60%	388	731	344
	70%	392	737	350
	80%	396	745	359
	90%	402	755	375
	95%	405	762	400

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Table VIIa. Birmingham Tests. Data relating to source and characteristics of fractions A - P

Sample	Petrol. Board Designation	Feed Stock	Process	Refinery
A	Group 5(71)	Colon Residue (V)	Duosol (Propane/Selecto)	Stanlow
B	Group 5(78)	Eakring Residue	" " "	Stanlow
C	Group 1(72)	Lagunillas L.O.D. (V)	Edeleanu (Duosol/SO_2)	Stanlow
D	Group 1(85)	Concepcion L.O.D. (V)	Furfural Extract	Shell Haven
E	Group 5A(S.5)	Distn. residue from D. Concepcion L.O.D. (V)	Furfural Extract	Shell Haven
F	Group 1(11)	W. Beaumont L.O.D. (T)	Edeleanu (Benzol/SO_2)	M.O.R.
G	Group 3(33)	W. Beaumont L.O.D. (T)	" " "	M.O.R.
H	Group 1A(140)	W. Beaumont L.O.D. (T)	" " " (Reprocessed at Lobitos Oilfields Ltd. Ellesmere Port)	Lobitos
I	Group 2(20CX)	Eakring L.O.D.	Edeleanu (Benzol/SO_2)	Lobitos
J	Group 4(2228)	Iranian L.O.D.	" " "	Llandarcy
K	Group 1(2241)	Colombian L.O.D.	" " "	Llandarcy
L	Group 4(2252)	Colombian L.O.D.	" " "	Llandarcy
M	Group 5A(2345)	Colombian L.O.D.	" " " (sulphur hardened)	Llandarcy
N	Group 5A(2345)	Colombian L.O.D.	Edeleanu (Benzol/SO_2) (Air blown)	Llandarcy
O	Group 1A(2419)	Tia Juana L.O.D. (V)	Edeleanu Benzol/SO_2	Llandarcy
P	Group 2(20CE)	Ecuadorain L.O.D.	" " "	Lobitos

L.O.D. = Lubricating Oil Distillate; V = Venezuela; T = Texas;
M.O.R. = Manchester Oil Refinery

Table VIIb. Birmingham Tests. Data relating to characteristics of fractions A - P

Sample	Character of Fraction	How applied	Toxicity by Survival Rate	Viscosity @ 140°F	Redwood 1 @ 200°F	Softening Point (R & B)	Penetration @ 25°C
A	Shiny black tar. Almost solid at 18°C	Solution of 50 g. in 30 ml. toluene	Moderate	-	-	51°C	65
B	Similar to A but slightly more liquid	Ditto	Slight	-	-	37°C	188
C	Very viscous oil Yellow-green fluor-escence	With 10% toluene added	Pronounced	662	-	-	-
D	Similar to C but slightly more liquid	Ditto	Pronounced	473	-	-	-
E	Black pitch	Soln. of 50 g. in 30 ml. toluene	Slight	-	-	37.5°C	160
F	Fairly thin brownish oily liquid	Without diluent	Very toxic	48	-	-	-
G	Very viscous dark brown tarry liquid	With 10% toluene	Moderate	-	450	-	-
H	Amber oil. Green fluor-escence	Without diluent	Moderate	140	-	-	-
I	Thin, dark brown tar	Ditto	Moderate	-	236	-	-
J	Viscous brown tar	Soln. of 50 g. in 30 ml. toluene	Moderate	-	824	-	-
K	Thick brown oil	With 10% toluene	Very pro-nounced	190	57	-	-

Table VIIb continued

Sample	Character of Fraction	How applied	Toxicity by Survival Rate	Viscosity @ 140°F	Redwood 1 @ 200°F	Softening Point (R & B)	Penetration @ 25°C
L	Very viscous brown tar	With 10% toluene	Pronounced	-	744	-	-
M	Solid pitch, black	50% wt./vol. "suspension" in toluene	Slight	-	-	80°C	-
N	Solid black pitch	Ditto	Very slight	-	-	80°C	-
O	Yellow oil marked green fluorescence	Without diluent	Slight	300	-	-	-
P	Very viscous brown tar	With 10% toluene	Fairly great	-	200	-	-

Table VIIc. Birmingham Tests. Carcinogenic response to samples A - P and controls

Material	Estimate of Carcinogenic Response
Extract A	15
Extract B	0
Extract C	20
Extract D	slightly positive
Extract E	0
Extract F	25
Extract G	0
Extract H	20
Extract I	25
Extract J	10
Extract K	30
Extract L	5
Extract M	0
Extract N	0
Extract O	10
Extract P	3
Creosote	50
Anthracene oil	30
Linseed oil	0
Pine Tar oil	0
Butyl phthalate	0
Tricresyl phosphate	-

This estimate is based on the total number of tumours compared with numbers previously obtained with 0.5% solution of 3:4-benzpyrene in benzene. This would be assigned a figure of 60.

Table VIII. Birmingham Tests. Spindle Oils

Spindle Oil	Animals Surviving 25 weeks	Tumours			Skin Reaction	Estimate of Carcinogenic Response
		Papillomata	Carcinomata	Total		
R	19	3	3	6	Slight roughness with epilation	15
S	21	7	2	9	Slight roughness with epilation	25
T	25	2	2	4	Slight roughness with epilation	10
U	16	3	3	6	Rough & dry with epilation	15
V	15	1	2	3	Slight epilation	5
W	19	1	0	1	Smooth & dry with epilation	5
X	25	1	0	1	No epilation	5

Table VIIIa. Birmingham Tests. Other control oils

Anthracene oil	20	8	6	14		30
Creosote oil	19	10	9	19		50
Pine oil	18	0	0	0		0
Linseed oil	44	0	0	0		0

Table IX. Birmingham:- "Duplicate Experiments"

1st Experiment

Fraction	Mice Surviving 25 weeks*	Papillomata	Carcinomata	Total Tumours
B	32	1	1	2
D	15	2	1	3
E	35	2	0	2
F	12/62	2	4	6
G	23	2	0	2
H	26	3	4	7
I	29	2	6	8
K	20/70	4	4	8
L	30/60	2	1	3
M	30	0	0	0

2nd Experiment

Fraction	Mice Surviving	Papillomata	Carcinomata	Total Tumours
B	22	2	1	3
D	12	1	3	4
E	22	1	2	3
F	15	5	3	8
G	12	2	0	2
H	16	3	5	8
I	15	5	3	8
K	20/60	5	3	8
L	20	3	1	4
M	20	1	0	1

* Survivors out of 50 unless otherwise stated

Table X. Birmingham Duplicate Experiments.
Mice Life Sheet

Fraction K - Experiment 1

Weeks from Start of Experiment	Survivors (out of 50)	Survivors with tumours	% Survivors with tumours	New tumours in previous week
16	39	0	0	0
17	38	0	0	0
18	36	0	0	0
19	36	0	0	0
20	34	2	5.9	0
21	31	3	9.7	2
22	29	4	13.8	1
23	29	4	13.8	1
24	28	5	17.8	0
25	26	6	23.1	1
26	26	6	23.1	1
27	25	7	28.0	0
28	25	7	28.0	1
29	25	7	28.0	0
30	25	7	28.0	0
31	25	7	28.0	0
32	25	7	28.0	0
33	24	7	29.2	0
34	23	7	30.4	0
35	23	7	30.4	0
36	23	7	30.4	0
37	23	7	30.4	0
38	23	7	30.4	0
39	23	7	30.4	0
40	23	6	26.1	0
41	22	6	27.3	0
42	21	5	23.8	0
43	20	5	25.0	0
44	18	5	27.8	0
45	17	4	23.5	0
46	15	4	26.6	0
47	14	3	21.4	0
48	13	3	23.1	0
49	12	2	16.6	0
50	11	2	18.2	0

Table XI. Birmingham Duplicate Experiments.
Mice Life Sheet

Fraction L - Experiment 1

Weeks from Start of Experiment	Survivors (out of 60)	Survivors with tumours	% Survivors with tumours	New tumours in previous week
30	30	0	0	0
31	30	0	0	0
32	30	0	0	0
33	28	0	0	0
34	28	1	3.6	0
35	28	1	3.6	1
36	28	1	3.6	0
37	26	1	3.8	0
38	25	2	8.0	0
39	25	2	8.0	1
40	24	3	12.5	0
41	22	3	13.6	1
42	19	3	15.8	0
43	17	3	17.7	0
44	17	3	17.7	0
45	17	3	17.7	0
46	14	3	21.4	0
47	13	3	23.1	0
48	12	2	16.6	0
49	9	1	11.1	0
50	6	1	16.6	0

Table XIIa. Birmingham Dilution Tests.
Mice Life Sheet, Solution D/1

Weeks from Start of Experiment	Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
13	36	0	0	0
14	36	0	0	0
15	36	2	5.5	0
16	36	3	8.3	0
17	36	3	8.3	0
18	36	6	16.7	0
19	35	8	22.8	0
20	35	8	22.8	0
21	34	8	23.5	0
22	34	8	23.5	0
23	34	13	38.2	0
24	33	16	48.5	1
25	33	17	51.5	1
26	32	19	59.4	1
27	29	18	62.1	3
28	28	18	64.3	4
29	25	16	64.0	6
30	24	15	64.2	7
31	24	16	64.7	7
32	23	16	72.7	8
33	21	14	66.7	10
34	19	15	78.9	11
35	18	15	83.3	12
36	15	15	100.0	15
37	15	15	100.0	15
38	12	12	100.0	18
39	11	11	100.0	19
40	11	11	100.0	19
41	10	10	100.0	20
42	9	9	100.0	21
43	3	3	100.0	27
44	2	2	100.0	28
45	2	2	100.0	28
46	1	1	100.0	29
47	1	1	100.0	29
48	1	1	100.0	29
49	1	1	100.0	29
50	1	1	100.0	29
51	0	0	-	30
52	0	0	-	30

Table XIIb. Birmingham Dilution Tests. Mice Life Sheet. Solution 1)/4

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
20	29	0	0	0
21	29	0	0	0
22	29	0	0	0
23	29	1	3.4	0
24	29	1	3.4	0
25	29	1	3.4	0
26	28	1	3.6	0
27	26	1	3.8	0
28	26	1	3.8	0
29	26	1	3.8	0
30	25	1	4.0	0
31	24	1	4.2	0
32	21	1	4.8	0
33	20	2	10.0	0
34	19	2	10.5	0
35	18	2	11.1	0
36	18	2	11.1	0
37	18	3	16.7	0
38	18	3	16.7	0
39	15	2	13.3	1
40	14	1	7.1	2
41	12	1	8.3	2
42	12	2	16.6	2
43	11	2	18.2	2
44	10	1	20.0	3
45	10	1	20.0	3
46	10	1	20.0	3
47	10	1	20.0	3
48	10	1	20.0	3
49	10	1	20.0	3
50	8	1	12.5	4
51	8	1	12.5	4
52	6	1	16.7	4
53	6	1	16.7	4
54	6	1	16.7	4
55	6	1	16.7	4
56	6	1	16.7	4
57	5	2	40.0	4
58	5	2	40.0	4
59	5	2	40.0	4
60	5	2	40.0	4
61	5	2	40.0	4
62	5	2	40.0	4
63	5	2	40.0	4
64	0	0	-	6

Table XIIc. Birmingham Dilution Tests. Mice Life Sheet. Solution D/16

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
45	15	0	0	0
46	12	0	0	0
47	11	0	0	0
48	11	0	0	0
49	11	0	0	0
50	11	0	0	0
51	11	2	18.2	0
52	10	2	20.0	0
53	10	2	20.0	0
54	8	1	12.5	1
55	8	1	12.5	1
56	6	0	0	2
57	6	0	0	2
58	6	0	0	2
59	6	0	0	2
60	4	0	0	2
61	4	0	0	2
62	4	0	0	2
63	4	0	0	2
64	0	0	-	2

Table XIId. Birmingham Dilution Tests. Mice Life Sheet. Solution D/64

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
45	16	0	0	0
46	14	0	0	0
47	12	0	0	0
48	11	0	0	0
49	11	0	0	0
50	11	0	0	0
51	11	1	16.1	0
52	9	1	11.1	0
53	9	1	11.1	0
54	9	1	11.1	0
55	8	1	12.5	0
56	8	1	12.5	0
57	8	1	12.5	0
58	8	1	12.5	0
59	7	1	14.3	0
60	7	1	14.3	0
61	7	1	14.3	0
62	7	1	14.3	0
63	5	0	0	1
64	0	0	-	1

Table XIIe. Birmingham Dilution Tests. Mice Life Sheet. Fraction I/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
15	34	0	0	0
16	33	0	0	0
17	33	0	0	0
18	33	1	3.0	0
19	33	1	3.0	0
20	33	1	3.0	0
21	33	1	3.0	0
22	32	1	3.1	0
23	32	3	9.4	0
24	32	5	15.6	0
25	31	6	19.3	0
26	31	7	23.5	0
27	31	7	23.5	0
28	30	10	33.3	1
29	29	11	37.9	2
30	28	13	46.4	2
31	27	15	55.5	3
32	27	15	55.5	3
33	27	16	59.2	3
34	26	16	61.5	4
35	26	16	61.5	4
36	24	14	58.3	6
37	24	17	70.8	6
38	24	17	70.8	6
39	24	18	75.0	6
40	19	16	84.2	11
41	17	14	82.3	13
42	17	14	82.3	13
43	13	10	76.9	17
44	12	10	83.3	18
45	10	9	90.0	20
46	6	6	100.0	24
47	5	5	100.0	25
48	5	5	100.0	25
49	5	5	100.0	25
50	5	5	100.0	25
51	4	4	100.0	26
52	3	3	100.0	27
53	3	3	100.0	27
54	2	2	100.0	28
55	2	2	100.0	28
56	2	2	100.0	28
57	2	2	100.0	28
58	2	2	100.0	28
59	2	2	100.0	28
60	2	2	100.0	28
61	2	2	100.0	28
62	2	2	100.0	28
63	2	2	100.0	28
64	2	2	100.0	28
65	2	2	100.0	28
66	1	1	100.0	29
67	0	0	-	30

Table XIIf. Birmingham Dilution Tests.
Mice Life Sheet. Fraction I/4

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
40	14	0	0	0
41	12	1	8.3	0
42	10	1	10.0	0
43	9	0	0	1
44	8	0	0	1
45	8	0	0	1
46	8	0	0	1
47	8	0	0	1
48	8	0	0	1
49	6	0	0	1
50	5	0	0	1
51	5	0	0	1
52	5	0	0	1
53	5	0	0	1
54	5	0	0	1
55	5	0	0	1
56	5	0	0	1
57	5	0	0	1
58	4	0	0	1
59	4	0	0	1
60	4	0	0	1
61	4	0	0	1
62	4	0	0	1
63	4	0	0	1
64	4	0	0	1
65	3	0	0	1
66	3	0	0	1
67	0	0	-	1

Table XIIg. Birmingham Dilution Tests.
Mice Life Sheet. Fraction F/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
20	32	0	0	0
21	32	0	0	0
22	32	0	0	0
23	31	2	6.4	0
24	31	2	6.4	0
25	31	3	9.6	0
26	27	3	11.1	1
27	22	3	13.6	2
28	22	4	18.2	3
29	18	3	16.7	3
30	17	5	29.4	4
31	14	5	35.7	4
32	14	5	35.7	4
33	11	6	54.5	5
34	11	6	54.5	5
35	11	7	63.6	5
36	9	7	77.8	5
37	9	7	77.8	5
38	9	7	77.8	5
39	9	7	77.8	5
40	7	5	71.4	7
41	6	4	66.7	8
42	6	3	50.0	9
43	5	3	60.0	9
44	5	4	80.0	9
45	4	3	75.0	10
46	4	3	75.0	10
47	3	3	100.0	11
48	2	2	100.0	12
49	1	1	100.0	13
50	1	1	100.0	13
52	0	0	-	14

Table XIIIa. London Dilution Tests.
Mice Life Sheet. Fraction D/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
12	29	0	0	0
13	29	4	13.8	0
14	29	5	17.2	0
15	29	5	17.2	0
16	29	5	17.2	0
17	29	6	20.7	0
18	29	7	24.1	0
19	27	10	37.0	2
20	26	10	38.5	2
21	24	10	41.7	4
22	24	10	41.7	4
23	24	10	41.7	4
24	21	15	71.4	7
25	19	14	73.7	8
26	16	11	68.8	11
27	16	11	68.8	11
28	12	11	91.7	15
29	12	11	91.7	15
30	11	10	90.9	16
31	11	10	90.9	16
32	10	9	90.0	17
33	9	8	88.9	18
34	5	5	100.0	22
35	5	5	100.0	22
36	4	4	100.0	23
37	3	3	100.0	24
38	3	3	100.0	24
39	2	2	100.0	25
40	2	2	100.0	25
41	2	2	100.0	25
42	2	2	100.0	25
43	2	2	100.0	25
44	2	2	100.0	25
45	2	2	100.0	25
46	0	0	0	27

Table XIIIb. London Dilution Tests.
Mice Life Sheet. Fraction D/4

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
18	41	0	0	0
19	39	1	2.6	0
20	39	1	2.6	0
21	38	4	10.5	1
22	38	4	10.5	1
23	38	4	10.5	1
24	38	4	10.5	1
25	38	5	13.2	1
26	38	5	13.2	1
27	38	5	13.2	1
28	37	8	21.6	2
29	35	10	28.6	3
30	35	12	34.3	3
31	35	12	34.3	3
32	33	10	30.3	5
33	33	11	33.3	5
34	32	16	50.0	5
35	29	13	44.8	8
36	29	13	44.8	8
37	29	13	44.8	8
38	29	13	44.8	8
39	27	17	63.0	10
40	27	17	63.0	10
41	27	22	81.5	10
42	25	20	80.0	12
43	24	19	79.2	13
44	19	14	73.7	18
45	19	14	73.7	18
46	19	14	73.7	18
47	15	12	80.0	20
48	15	12	80.0	20
49	15	14	93.3	20
50	13	12	92.3	22
51	12	12	100.0	22
52	11	11	100.0	23

Table XIIIc. London Dilution Tests.
Mice Life Sheet. Fraction D/16

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
20	39	0	0	0
21	39	2	5.1	0
22	39	2	5.1	0
23	39	2	5.1	0
24	39	2	5.1	0
25	39	2	5.1	0
26	38	1	2.6	1
27	38	1	2.6	1
28	37	1	2.7	1
29	37	1	2.7	1
30	35	0	0.0	2
31	35	0	0.0	2
32	35	0	0.0	2
33	34	0	0.0	2
34	34	0	0.0	2
35	34	0	0.0	2
36	33	2	6.1	2
37	33	2	6.1	2
38	33	2	6.1	2
39	33	2	6.1	2
40	33	2	6.1	2
41	33	2	6.1	2
42	33	2	6.1	2
43	33	2	6.1	2
44	33	2	6.1	2
45	33	2	6.1	2
46	33	2	6.1	2
47	31	2	6.5	2
48	31	2	6.5	2
49	31	3	9.7	2
50	31	3	9.7	2
51	31	3	9.7	2
52	30	8	26.7	2

Table XIIId. London Dilution Tests.
Mice Life Sheet. Fraction I/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
12	44	0	0	0
13	43	1	2.3	0
14	42	1	2.4	0
15	41	1	2.4	0
16	41	1	2.4	0
17	41	1	2.4	0
18	41	1	2.4	0
19	41	1	2.4	0
20	41	1	2.4	0
21	41	1	2.4	0
22	41	1	2.4	0
23	41	1	2.4	0
24	39	1	2.6	0
25	39	2	5.1	0
26	37	2	5.4	0
27	37	2	5.4	0
28	34	2	5.9	0
29	34	2	5.9	0
30	34	2	5.9	0
31	34	2	5.9	0
32	34	2	5.9	0
33	34	2	5.9	0
34	34	2	5.9	0
35	33	2	6.1	0
36	33	2	6.1	0
37	33	2	6.1	0
38	31	1	3.2	1
39	31	2	6.5	1
40	31	2	6.5	1
41	31	2	6.5	1
42	31	2	6.5	1
43	30	2	6.7	1
44	30	2	6.7	1
45	30	2	6.7	1
46	30	5	16.7	1
47	30	5	16.7	1
48	30	5	16.7	1
49	30	9	30.0	1
50	30	9	30.0	1
51	30	9	30.0	1
52	30	10	33.3	1

Table XIIIe. London Dilution Tests.
Mice Life Sheets. Fraction F/1

Weeks from Start of Experiment	Total Survivors	Survivors with tumours	% Survivors with tumours	Total dead with tumours
15	45	0	0	0
16	45	1	2.2	0
17	45	1	2.2	0
18	45	1	2.2	0
19	45	1	2.2	0
20	44	1	2.3	0
21	44	1	2.3	0
22	43	1	2.3	0
23	42	1	2.4	0
24	42	1	2.4	0
25	42	1	2.4	0
26	42	1	2.4	0
27	41	1	2.4	0
28	40	1	2.5	0
29	39	1	2.6	0
30	37	1	2.7	0
31	37	2	5.4	0
32	37	2	5.4	0
33	37	2	5.4	0
34	37	2	5.4	0
35	37	2	5.4	0
36	37	3	8.1	0
37	37	3	8.1	0
38	34	2	5.9	1
39	34	2	5.9	1
40	34	2	5.9	1
41	33	2	6.1	1
42	33	2	6.1	1
43	33	2	6.1	1
44	33	2	6.1	1
45	33	2	6.1	1
46	33	2	6.1	1
47	33	2	6.1	1
48	33	2	6.1	1
49	33	2	6.1	1
50	32	2	6.3	1
51	31	2	6.5	1
52	30	2	6.7	1

Table XIV. Dilution Tests. Survival Rate of Mice

Material	Number of Mice out of 50 surviving					
	12 weeks		25 weeks		50 weeks	
	B	L	B	L	B	L
D/1	36	29	33	19	0	0
D/4	34	41	29	38	8	13
D/16	34	41	30	39	11	31
D/64	48	44	42	44	11	36
I/1	36	44	31	39	5	30
I/4	36	41	33	38	5	32
I/16	34	49	25	48	6	37
I/64	34	44	30	43	8	33
F/1	35	45	31	42	5	32
F/4	44	47	44	47	17	36
F/16	40	43	36	40	19	29
F/64	26	46	17	43	9	35

B = Birmingham L = London

Table XV. Dilution Tests. Tumour Incidence in Mice

Material	Week in which 1st tumour appeared		No. of tumours elicited at 25 weeks		Total No. of tumours out of 50 possible	
	B*	L∅	B	L	B	L
D/1	15th	14th	20	22	30	27
D/4	23rd	20th	1	5	5	34
D/16	51st	22nd	0	2	2	10
D/64	51st	-	0	0	1	0
I/1	18th	14th	7	1	30	11
I/4	41st	-	0	0	1	0
I/16	?1 in 41st week, re-gressed, not visible at 44 weeks	-	0	0	0	0
I/64	0	-	0	0	0	0
F/1	23rd	17th	4	1	14	3
F/4	0	-	0	0	0	0
F/16	0	-	0	0	0	0
F/64	0	-	0	0	0	0

* B = Birmingham ∅ L = London

Table XVI. Birmingham Dilution Tests.
Tumour Incidence in Rabbits

Material	Week in which first tumour appeared	Sites with tumours at 52 weeks			Time for 4 tumours (22%)	Time for 50% incidence of tumours	
		Total	Body	Ear		Total	Body
D/1	34th	11/18	7/12	4/6	40 wks	49 wks	42 wks
D/4	34th	4	3	1	48 wks	-	-
D/16	-	0	0	0	-	-	-
D/64	-	0	0	0	-	-	-
I/1	33rd	9/18	5/12	4/6	40 wks	-	-
I/4	34th	8	4	4	42 wks	-	-
I/16	48th	2	1	1	-	-	-
I/64	-	0	0	0	-	-	-
F/1	36th	11/18	8/12	3/6	41 wks	45 wks	45 wks
F/4	46th	1	1	0	-	-	-
F/16	47th	1	0	1	-	-	-
F/64	-	0	0	0	-	-	-

Total number of rabbit sites = 216,144 on the body and 72 on the ears.
There are 18 rabbit sites to each fraction, 12 on body and 6 on ears.

Table XVII. Carcinogenic activity of fractions of oils

Number of tumours at the Birmingham Laboratory = B
Number of tumours at the London Laboratory = L

Oil	(Fractions arranged in order light ⟶ heavy)		Maximum possible No. of tumours	Rabbit test Number of tumours B/L	Mouse test Number of tumours B/L	Maximum possible No. of tumours
Kuwait	crude oil	K	60	0/0	2/0	50
	light fraction	K1	35	1/0	2/0	"
	↓	K2	"	3/1	4/1	"
	↓	K3	"	5/4	3/0	"
	heavy fraction	K4	"	12/8	2/1	"
	residue	K5	"	0/0	1/0	"
Lagunillas	crude oil	L	60	0/0	0/0	"
	light fraction	L1	35	1/0	2/0	"
	↓	L2	"	4/2	5/0	"
	↓	L3	"	13/3	5/2	"
	heavy fraction	L4	"	16/6	3/1	"
	residue	L5	"	0/2	0/0	"
Oklahoma	crude oil	0	60	0/2	0/0	"
	light fraction	01	35	0/1	0/0	"
	↓	02	"	1/2	0/0	"
	↓	03	"	1/4	2/1	"
	heavy fraction	04	"	4/4	2/0	"
	residue	05	"	0/0	0/0	"

Table XVIII.
Carcinogenic activity of fractions of oils in mouse tests

Date of appearance of each tumour (weeks from start) and numbers of survivors at that time

Fraction	Birmingham		London	
	No. of tumours	Weeks from start and no. of survivors*	No. of tumours	Weeks from start & no. of survivors
K crude	2	29/27, 32/26,	0	-
K1	2	30/25, 32/29,	0	-
K2	4	24/24, 28/20, 31/18,	1	34/25
K3	3	23/23, 27/18, 28/18,	0	-
K4	2	30/24, 32/23,	1	52/36
K5 (residue)	1	30/24	0	-
L crude	0	-	0	-
L1	2	26/27, 41/20,	0	-
L2	5	28/29, 28/29, 29/27, 44/16, 48/14,	0	-
L3	5	28/24, 28/24, 31/24, 32/23, 48/13	2	26/34, 52/19
L4	3	30/21, 32/18,	1	52/32
L5 (residue)	0	-	0	-
0 crude	0	-	0	-
01	0	-	0	-
02	0	-	0	-
03	2	30/23, 30/23,	1	31/40
04	2	29/21, 38/13,	0	-
05 (residue)	0	-	0	-

*The first figure is the date of appearance (e.g. 29 means the 29th week of the experiment), the second is the number of survivors out of 50.

Table XIX. Design of experiment on 21 fractions. (1952 tests)

Rabbits	I	II	III	IV	V
1 - 5	d	p	n	u	a
6 - 10	e	q	o	a	b
11 - 15	f	r	p	b	c
16 - 20	g	s	q	c	d
21 - 25	h	t	r	d	e
26 - 30	i	u	s	e	f
31 - 35	j	a	t	f	g
36 - 40	k	b	u	g	h
41 - 45	l	c	a	h	i
46 - 50	m	d	b	i	j
51 - 55	n	e	c	j	k
56 - 60	o	f	d	k	l
61 - 65	p	g	e	l	m
66 - 70	q	h	f	m	n
71 - 75	r	i	g	n	o
76 - 80	s	j	h	o	p
81 - 85	t	k	i	p	q
86 - 90	u	l	j	q	r
91 - 96	a	m	k	r	s
97 - 100	b	n	l	s	t
100 - 105	c	o	m	t	u

Key to fractions a - u

$K_4 \cdot 1 = k$
$K_4 \cdot 2 = b$
$K_4 \cdot 3 = u$
$K_4 \cdot 4 = m$
$K_4 \cdot 5 = g$

$O_4 \cdot 1 = o$
$O_4 \cdot 2 = t$
$O_4 \cdot 3 = r$
$O_4 \cdot 4 = j$
$O_4 \cdot 5 = e$

$L_4 \cdot 1 = f$
$L_4 \cdot 2 = s$
$L_4 \cdot 3 = a$
$L_4 \cdot 4 = l$
$L_4 \cdot 5 = d$

$M_2 = h$
$M_{\frac{1}{2}} = p$
$D_2 = c$
$D_{\frac{1}{2}} = q$
$K_4 = n$
$K_{4 \cdot \frac{1}{4}} = i$

$a = L_4 \cdot 3$
$b = K_4 \cdot 2$
$c = D_2$
$d = L_4 \cdot 5$
$e = O_4 \cdot 5$
$f = L_4 \cdot 1$

$g = K_4 \cdot 5$
$h = M_2$
$i = K_{4 \cdot \frac{1}{4}}$
$j = O_4 \cdot 4$
$k = K_4 \cdot 1$
$l = L_4 \cdot 4$

$m = K_4 \cdot 4$
$n = K_4$
$o = O_4 \cdot 1$
$p = M_{\frac{1}{2}}$
$q = D_{\frac{1}{2}}$
$r = O_4 \cdot 3$

$s = L_4 \cdot 2$
$t = O_4 \cdot 2$
$u = K_4 \cdot 3$

Table XX. Experiments with mice at Birmingham.
Three crudes and fifteen derived fractions

Crude oil or fraction	Expectation of tumourless life and standard error (limited to 52 weeks)	Dates when 10% of survivors had tumours and standard error	Total number of tumours
Crude Kuwait	50.34 ± 1.16	39.4 ± 9.3	2
Kuwait 1	50.73 ± 0.89	39.5 ± 6.4	2
" 2	47.42 ± 2.11	28.9 ± 2.8	4
" 3	48.10 ± 2.13	27.8 ± 1.0	3
" 4	50.19 ± 1.26	not reached	2
" Residue	51.06 ± 0.94	not reached	1
Crude Lagunillas	52	no tumours	0
Lagunillas 1	50.48 ± 1.10	not reached	2
" 2	48.67 ± 1.48	28.7 ± 0.8	5
" 3	48.75 ± 1.85	30.4 ± 1.5	5
" 4	48.98 ± 1.66	31.8 ± 1.2	3
" Residue	52	no tumours	0
Crude Oklahoma	52	no tumours	0
Oklahoma 1	52	no tumours	0
" 2	52	no tumours	0
" 3	50.04 ± 1.35	32.5 ± 3.1	2
" 4	49.92 ± 1.43	46.5 ± 4.3	2
" Residue	52	no tumours	0

Table XXI.

Experiments on rabbits with 15 derived fractions. Mean values (corrected for group differences)

Fraction	Birmingham		Chester Beatty Institute		Total no. of tumours	
	Trans-formed Value (y)	Proba-bility	Trans-formed Value (y)	Proba-bility	Birmingham	Chester Beatty
L_1	4.27	0.007*	(-3.12)	0.000	0	0
L_2	14.89	0.066	5.52	0.010	4	2
L_3	34.58	0.323	13.52	0.055	13	3
L_4	43.51	0.473	19.38	0.101	16	6
L_5	-1.35	0.000	8.15	0.020	0	2
K_1	3.97	0.007	0.25	0.000	1	0
K_2	8.52	0.022	2.90	0.005	3	1
K_3	17.80	0.093	13.44	0.054	5	4
K_4	30.95	0.264	24.21	0.168	12	8
K_5	-0.02	0.000	2.23	0.004	0	0
O_1	1.25	0.002	3.70	0.006	0	1
O_2	2.64	0.005	5.59	0.010	1	2
O_3	5.24	0.009	14.62	0.064	1	4
O_4	8.99	0.025	13.80	0.057	4	4
O_5	1.27	0.002	0.25	0.000	0	0
SE individual means	5.15		4.16			
SE diff. bet. 2 means	7.29		5.88			

*The unbracketed fractions are of negligible carcinogenicity.

Table XXII. Experiment with rabbits on fifteen derived fractions

Analysis of variance on angular transformation of proportion of tumours out of 5

Birmingham				
	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Sites	1957.36	6	326.23	1.84 (not sign.)
Groups of rabbits	5772.04	14	412.29	2.33 (0.01<P<0.05)
Fractions	18924.47	14	1351.78	7.64 (P<0.01)
Error	12384.47	70	176.92	
	39038.81	104		

Chester Beatty Institute				
	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Sites	1613.75	6	268.96	2.36 (0.01<P<0.05)
Groups of rabbits	4980.42	14	355.74	3.12 (P<0.01)
Fractions	5561.15	14	397.23	3.48 (P<0.01)
Error	7983.01	70	114.04	

(The 'theoretical' error variance is 820.7/5 = 164.1, with which the above estimates are in good agreement.)

Table XXIII. Experiment with rabbits on fifteen derived fractions

Number of tumours out of 75 on the seven different sites

Site	Birmingham	Chester Beatty Institute	Mean
I	11	6	8.5
II	11	7	9.0
III	12	2	7.0
IV	4	4	4.0
V	3	4	3.5
VI	7	3	5.0
VII	13	11	12.0

Table XXIV. Mouse Dilution Experiments

Expectation of tumourless life and dates when 10 per cent of survivors have tumours

	Birmingham			Chester Beatty Institute		
	E.T.L. (limited to 52 wks.)	10% Date	No. of tumours	E.T.L. (limited to 52 wks.)	10% Date	No. of tumours
D/1	28.17 ± 2.04	17.2 ± 0.6	30	22.06 ± 1.58	12.70 ± 0.3	27
D/4	48.08 ± 1.71	33.0	5	34.41 ± 1.45	21.0 ± 0.7	34
D/16	51.73 ± 0.23	50.5 ± 0.4	0	49.26 ± 1.27	not reached	10
D/64	51.86 ± 0.17	50.6 ± 0.4	0	52	"	0
I/1	31.91 ± 1.37	23.1 ± 0.8	30	48.84 ± 1.22	45.6 ± 1.0	11
I/4	51.18 ± 0.83	42.0	1	52	not reached	0
I/16	52	not reached	0	52	"	0
I/64	52	"	0	52	"	0
F/1	36.39 ± 2.57	25.3 ± 3.1	14	50.16 ± 1.08	not reached	3
F/4	52	not reached	0	52	"	0
F/16	52	"	0	52	not reached	0
F/64	52	"	0	52	"	0

Table XXV. Rabbit Dilution Experiments at Birmingham.
Analysis of Variance of Latin Squares
(Angular transformation of the proportion of tumours out of 3)

Fraction D	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Groups	1184.06	3	394.08	1.80
Sites	1714.65	3	571.55	2.62
Dilutions Regression	6769.36	1	6769.36	31.00 sign.at 1%
Dilutions Remainder	1018.65	2	509.37	2.32
Error	1310.22	6	218.37	
	11996.94	15		

Fraction I	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Groups	1944.98	3	664.99	3.12
Sites	799.57	3	259.86	1.22
Dilutions Regression	2713.29	1	2713.29	12.73 sign.at 5%
Dilutions Remainder	339.60	2	169.80	<1
Error	1278.51	6	213.08	
	7105.95	15		

Fraction F	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Groups	553.30	3	184.43	<1
Sites	210.89	3	70.30	<1
Dilutions Regression	6833.90	1	6833.90	23.71 sign.at 1%
Dilutions Remainder	2692.40	2	1346.20	4.67
Error	1729.63	6	288.27	
	12020.12	15		

Table XXVI. Rabbit Dilution Experiments at Birmingham

Analysis of Variance of differences at the same sites and dilutions
(Tumour = 90° Absence of tumour = 0)
(Sums of Squares and mean squares divided by 3,
to make them comparable with Table XXV.)

Fraction D				
	Sum of Squares	Degrees of freedom	Mean Squares	Variance Ratio
Between rabbits	2700	8	337.5	1.29
Error	6300	24	262.5	
Total	9000	32		

Fraction I				
	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Between rabbits	5625	8	703.12	2.42 sign.at 5%
Error	6975	24	290.62	
Total	12600	32		

Fraction F				
	Sum of Squares	Degrees of freedom	Mean Square	Variance Ratio
Between rabbits	2250	8	281.25	1.36
Error	4950	24	206.25	
Total	7200	32		

A P P E N D I X I

THE DESIGN OF EXPERIMENTS FOR TESTING THE CARCINOGENICITY OF OIL FRACTIONS

I. Tests on 18 fractions, three whole crudes and five fractions derived from each of them.

(1) Mouse tests

The experiments are to be done in duplicate - on batches of 50 mice at Birmingham and 50 at the Chester Beatty Research Institute. These experiments should be standardised as far as possible, in particular in the following respects:

(a) Five or six animals per box.

(b) Sexes in equal numbers but separated. Age as near to 2 months as possible but in all cases less than 4 months.

(c) The 25 males and 25 females on each fraction to be selected randomly from the stock existing at the time the experiment is started. This ratio may have to be altered at the Chester Beatty Research Institute to include a higher proportion of males.

(d) Each animal in each test to be numbered and the sex and colour, including spotting if any, to be recorded. This is in order to be able to divide the animals in each test into random groups.

(e) Diet. Thorley's Rat Cubes, plus supplement as given at the Chester Beatty Research Institute, viz. bread and cod liver oil on Mondays, oats on Wednesday, and a small amount of greens on Fridays. Free water supply.

(f) Twice weekly painting with glass rod from stoppered numbered bottles, dispensed under single control at the Chester Beatty Research Institute.

(2) Rabbit tests

The following experiments are to be done in duplicate at Birmingham and the Chester Beatty Research Institute. Male or female rabbits to be used, between 3 and 6 months old. Sex and colour to be recorded as for mice. Diet as for mice. Two experiments are to be performed (a) on the three whole crudes, (b) on the fifteen fractions derived from them. Each rabbit is to be painted on six sites in (a) and on seven in (b) as follows:-



Sites I, II	Ears, outer surface near base.
III, IV	Lateral thoracic
V, VI	Lateral abdominal
VII	Interscapular

(a) Experiment on the three whole crudes. 30 rabbits are to be used and divided randomly into 6 groups of 5 rabbits. The three crudes A, B, C to be assigned to the groups and sites in accordance with the following scheme:-

SITES

Rabbits	I	II	III	IV	V	VI
1 - 5	B	B	C	C	A	A
6 - 10	C	C	A	A	B	B
11 - 15	A	A	B	B	C	C
21 - 25	C	C	B	B	A	A
26 - 30	A	A	C	C	B	B

Dates of appearance of tumours and of course any deaths are to be recorded. The experiment is to be continued for 12 months.

(b) Experiment on fifteen derived fractions

75 rabbits to be used and divided randomly into 15 groups of 5. The 15 fractions a, b, c, d.....o to be assigned to the groups and sites in accordance with the following scheme:-

Rabbits	I	II	III	IV	V	VI	VII
1 - 5	f	g	a	e	o	b	d
6 - 10	k	j	b	i	h	a	o
11 - 15	c	n	m	l	a	o	b
16 - 20	h	i	d	n	o	e	a
21 - 25	a	e	l	k	m	d	j
26 - 30	i	a	f	m	l	g	h
31 - 35	o	k	g	a	j	f	n
36 - 40	d	m	h	o	b	j	f
41 - 45	n	f	i	b	d	k	l
46 - 50	l	b	j	h	g	n	e
51 - 55	b	o	k	g	e	i	m
56 - 60	m	h	n	d	k	c	g
61 - 65	g	d	c	j	i	l	o
66 - 70	e	l	o	c	f	h	k
71 - 75	j	c	e	f	n	m	i

Key to fractions A - O

Fraction in order of light to heavy	A	B	C				
1	k	o	n	or	a = A4	f = B3	K = A1
2	h	m	i		b = B4	g = C3	l = C5
3	j	f	g		c = A5	h = A2	m = B2
4	a	b	d		d = C4	i = C2	n = C1
5	c	e	l		e = B5	j = A3	o = B1

Date of appearance of tumours and, of course, any deaths to be recorded. The experiment to be continued for 12 months.

Tests on 4 dilutions of each of three 'oils', one of which is to be a pure carcinogen, 9:10-dimethyl-1:2-benzanthracene. The pure carcinogen is to be given at dilutions

1 = (0.1)% in medicinal liquid paraffin

1/4 = 0.025% " " "

1/16 = 0.00625% " " "

1/64 = 0.0015% " " "

The other two oils to be given at dilutions:-

1 = neat

1/4 = 25% in liquid paraffin

1/16 = 6.25% " "

1/64 = 1.5625% " "

Mouse tests

The experiments are to be done in duplicate - on batches of 50 mice at Birmingham and 50 at the Chester Beatty Research Institute. This will necessitate 600 mice in each place, 50 for each of the four dilutions of the three oils. The experiments are to be standardised as in I(1).

Rabbit tests

These are also to be done in duplicate - at Birmingham and at the Chester Beatty Research Institute.

Male rabbits are to be used, white inbred strains if possible and three-quarters grown.

Each rabbit is to be painted on six sites, the same sites as in I,2(a).

36 rabbits are to be used and divided randomly into 12 groups of three rabbits. The 4 dilutions of each of the three oils are to be assigned to the groups and sites as follows:

S I T E S

Rabbits	I	II	III	IV	V	VI
1 - 3	I 1	I 1/4	D 1/4	D 1/16	D 1/64	D 1
4 - 6	I 1/16	I 1/64	D 1	D 1/64	D 1/16	D 1/4
7 - 9	F 1/4	F 1	D 1/64	D 1	D 1/4	D 1/16
10 - 12	F 1/64	F 1/16	D 1/16	D 1/4	D 1	D 1/64
13 - 15	F 1	F 1/4	I 1/4	I 1/16	I 1/64	I 1
16 - 18	F 1/16	F 1/64	I 1/64	I 1	I 1/4	I 1/16
19 - 21	D 1	D 1/4	I 1/16	I 1/4	I 1	I 1/64
22 - 24	D 1/16	D 1/64	I 1	I 1/64	I 1/16	I 1/4
25 - 27	D 1/4	D 1	F 1/4	F 1/16	F 1/64	F 1
28 - 30	D 1/64	D 1/16	F 1/16	F 1/4	F 1	F 1/64
31 - 33	I 1/4	I 1	F 1	F 1/64	F 1/16	F 1/4
34 - 36	I 1/64	I 1/16	F 1/64	F 1	F 1/4	F 1/16

Dates of appearances of tumours and deaths to be recorded. The experiments to be continued for 12 months.

(3) Recording of warts

Records will be maintained of the following observations:-

1. The date when a wart first appears.
2. The approximate date when a tumour is regarded as malignant on clinical grounds.
3. The date of death or killing.

Histological examination of all painted sites will be carried out, provided that death occurs after 12 weeks or after the appearance of the first tumour should this appear earlier.

COPY

A P P E N D I X II

(REPORT OF THE THORNTON RESEARCH CENTRE (C 3.2/50, 7th MARCH, 1950)

CRUDE OILS - Job No. 224. Distillation of crude oil for the Institute of Petroleum

Requested by: S.R. & M. Co. Technological Department Memo. No. A.T.N./IP/7L, dated 25.2.49.

Summary

One ton each of Kuwait, Oklahoma Bright, and Lagunillas crude oils have been distilled to give four overhead fractions and one residue. Cracking was avoided during the distillation by maintaining the temperature of the liquid below 250° C and reducing the effective pressure by the introduction of steam.

Discussion

1. Distillation unit

The unit employed for the distillation is illustrated diagrammatically in Fig. 1. For steam distillation, saturated steam at 30 psig was injected into the base of the vessel V1, through a perforated coil. The rate of low of steam into the vessel was measured by a Foxboro orifice-type flow meter. The crude oil is distilled from V1 through condenser C1 to either of two primary receivers V2 and V3. Uncondensed vapour then passes through condenser C2 and V4. A 2 in. mild-steel line passes from this vessel to the vacuum pump.

2. Operation

Prior to the distillation the system was thoroughly cleaned, V1 was initially cleaned by hand and then a light kerosine was distilled from V1 to V2, V3 and V4 to flush out contaminants. A long period of steaming then followed to remove the hydrocarbons. This procedure of cleaning with kerosine and steam was carried out before the distillation of each of the three crude oils.

The crude oil was charged to the distillation vessel V1 via a line entering the vessel at the base. In order to avoid contamination of the crudes which might occur when using reciprocating pumps, charging was carried out by suction, the vacuum pump in the system being utilised for this purpose. A charge of ca 350 kg. was distilled per batch, so that the distillation of three batches was necessary to obtain the required amount of crude oil distillate.

After charging, the vessel V4 was vented to atmosphere and the crude oil in V1 heated to a maximum temperature of 240° C with stirring. The temperature of the oil entering and leaving V1 jacket was between 245° and 250° C. (During this stage care was required to avoid bumping, especially with the Lagunillas crude, which contained quite large amounts of water.)

The distillate was collected in V2 until the rate of distillation had decreased. At this point the vent at V4 was closed and the pressure slowly reduced to 150 mm Hg, the distillate being collected in V3. The two fractions so obtained were bulked to give fraction 1. The pressure was again reduced (to 100 mm Hg) and steam introduced to a maximum rate of 30 lb./hr. while three further fractions were collected. To simplify the operation, the volumes to be expected for these last three fractions were determined prior to the actual distillation by a laboratory distillation of the crude oil. Cut points in the plant distillation were then taken when the predetermined volumes had been distilled.

3. Drying and storing

After the distillations the corresponding fractions were bulked and allowed to settle. The water layer was removed and the oil partially dried by filtering slowly through cotton wool pads. This method was used to avoid unnecessary contamination by chemical driers. The dry fractions were then stored in new 40-gallon mild-steel drums. The residues from the distillations were dropped, while still hot, from the distillation vessel into new 40-gallon mild-steel open topped drums.

Results

The yields of each fraction, and simple physical characteristics of Kuwait, Oklahoma Bright and Lagunillas crude distillates are given below in Tables 1 to 3. It is interesting to note that in the high-sulphur-content Kuwait crude no hydrogen sulphide or sulphur dioxide was evolved during the distillation of fractions 2, 3, and 4. This is a good indication that no cracking occurred during the distillation. The relatively high losses were due primarily to losses in handling during drying, but in the case of Lagunillas crude the high water content considerably reduced the percent weight of oil distillate.

APPENDIX II

Table 1

Kuwait crude

Weight	kg %w	Crude	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Residue
		1018 93.9	267 26.3	99 9.8	80 7.9	60 5.9	445 43.0
Distillation range	I.P.method IP.28/42						
I.B.P.		39	44	167	284	330	-
5%		82	71	231	303	345	-
10%		117	81	242	303	350	-
20%		172	99	251	314	357	-
30%		225	116	258	318	361	-
40%		285	130	263	323	365	-
50%		332	142	273	327	368	-
60%		348	160	280	333	372	-
70%		355	180	290	340	378	-
80%		372	202	303	349	384	-
90%		390	321	319	363	393	-
95%		Cracked	259	339	375	-	-
F.B.P.		"	279	350	396	400	-
Recovery %		90.5	98	98	99	98	-
Residue %		9.5	1.0	1.5	1.0	2	-
Loss %		nil	1.0	0.5	nil	nil	-
Viscosity kinematic cS	IP.71/47T	26.0/70°F	0.95/70°F	4.53/70°F	14.0/70°F	9.26/140°F	1300/140°F
Redwood I sec	IP.70/46	108/70°F	28/70°F	37/70°F	65/70°F	50/140°F	5000/140°F
Flash point Abel closed cup °F	IP.33/44	60	50	146			
Pensky-Marten closed cup °F	IP.34/47				255	345	ca 450

APPENDIX II

Table 2

Oklahoma Bright crude

Weight	kg %w	Crude	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Residue
		1250 96.5	495 39.5	119 9.5	103 8.2	91 7.3	402 32.0
Distillation range	I.B.P. method IP.28/42						
I.B.P.		40	44	211	248	296	-
5%		60	69	226	280	309	-
10%		102	81	231	285	315	-
20%		140	100	237	290	320	-
30%		175	117	245	294	327	-
40%		230	130	250	299	332	-
50%		271	144	255	305	338	-
60%		311	164	264	311	344	-
70%		350	181	273	319	350	-
80%		354	204	284	329	359	-
90%		364	233	303	346	375	-
95%		370	268	323	361	391	-
F.B.P.		372	290	343	373	400	-
Recovery %		97.5	98	98	97.5	98.0	-
Residue %		2.5	1.0	1.5	2.0	2.0	-
Loss %		nil	1.0	0.5	0.5	nil	-
Viscosity kinematic cS	IP.71/47T	4.6/70°F	0.896/70°F	1.8/140°F	3.4/140°F	5.3/140°F	8.0/140°F
Redwood I sec	IP.70/46	38/70°F	27/70°F (calc)	31/140°F (calc)	35/140°F (calc)	39/140°F	335/140°F
Flash point Abel closed cup °F	IP.33/44	50	50				
Pensky-Marten closed cup °F	IP.34/47			185	250	285	420

APPENDIX II

Table 3

Lagunillas crude

Weight	kg %w	Crude 1120 92.0	Fraction 1 92.5 8.2	Fraction 2 94.8 8.4	Fraction 3 58.8 5.3	Fraction 4 51.4 4.6	Residue 734.8 65.5
Distillation range	I.B.P. method IP.28/42						
I.B.P.		100	97	223	262	317	-
5%		200	126	248	301	338	-
10%		262	139	251	310	343	-
20%		318	153	261	317	350	-
30%		334	166	269	322	354	-
40%		345	181	277	329	360	-
50%		350	195	287	336	365	-
60%		368	209	294	342	370	-
70%		384	222	302	352	376	-
80%		Cracking	239	317	363	384	-
90%		"	265	335	378	Cracked	-
95%		"	291	351	392	"	-
F.B.P.		"	300	359	397	"	-
Recovery %		90	98	97	98	93	-
Residue %		3.0	2.0	3.0	3.0	7.0	-
Loss %		nil	nil	nil	nil	nil	-
Viscosity kinematic cS	IP.71/47T	165/140°F	1.605/70°F	2.9/140°F	7.3/140°F	16.7/140°F	-
Redwood I sec	IP.70/46	668/140°F	30/70°F	33/140°F	45/140°F	74/140°F	20,000/140°F (calc.)
Flash point Abel closed cup °F	IP.33/44	50	50				
Pensky-Marten closed cup °F	IP.34/47			214	270	350	460

A. Parr
J.N.L. Thomas

D. VICKERY

for Director of Research
Thornton Research Centre

C O P Y C O P Y C O P Y

MEDICAL RESEARCH COUNCIL

MRC.52/813
CAMO.52/13

Committee on the Carcinogenic Action of Mineral Oils

PROGRESS REPORT BY DR. W. CARRUTHERS, GLASGOW UNIVERSITY

Exploratory experiments are continuing with the sub-fractions prepared by Professor Morton from the three crudes. So far, attention has been confined to the fractions $K_{4.1}$-$K_{4.5}$ from the Kuwait crude. Extracts enriched in aromatic material (amounting to 25-30 per cent of the whole fraction) have been prepared from these fractions by treatment with furfural. In the case of two of these extracts from fractions $K_{4.1}$ and $K_{4.2}$, the increased aromaticity has been shown by comparison of their elementary analyses with those of the original fractions. Further experiments have centred on these furfural-soluble extracts.

Some of these extracts have been further broken down by crystallisation of molecular complexes with picric acid and trinitrobenzene. A small amount of an impure crystalline solid was obtained in this way from the extract of $K_{4.1}$. This is tentatively regarded as a phenanthrene derivative on the basis of its absorption spectrum. The result is interesting as an indication that phenanthrene hydrocarbons are probably present in some of these fractions.

By chromatography of oily material, recovered from a picrate prepared from the extract of $K_{4.1}$, an apparently homogeneous sulphur-containing compound has been isolated in small amount. Elementary analysis suggests the molecular formula $C_{14}H_{14}S$ or $C_{15}H_{15}S$. There are indications that the sulphur is present in a thiophene nucleus. Investigation of the structure is proceeding.

Elementary analysis of all the sub-fractions at our disposal shows that the Kuwait, Lagunillas and Oklahoma sub-fractions contain, generally speaking, 3-4, 2-3 and 0-1 per cent respectively of elements other than carbon and hydrogen.

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CAMO.52/14

Committee on the Carcinogenic Action of Mineral Oils

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The applications to mice and rabbits of the 21 fractions have now been in progress for 25 weeks.

Mouse tests.

The data regarding the mouse tests is collected in the following Table 1. This is subject to confirmation microscopically and alteration should papillomata regress.

Table 1. (at 25 weeks)

Fraction	Survivors	No. of Papillomata
D(2)	0 (28 killed)	38 in 20 weeks (100%) 1st at 10 weeks
D($\frac{1}{2}$)	24	16, 1st at 17 weeks
M(2)	35	8, 1st at 20 weeks
M($\frac{1}{2}$)	30	1? at 22 weeks
K_4	30	1? at 20 weeks
$K_4(\frac{1}{4})$	31	0
$K_4.1$	26	0
$K_4.2$	36	0
$K_4.3$	20	1 at 24 weeks
$K_4.4$	28	0
$K_4.5$	29	0
$L_4.1$	41	0
$L_4.2$	38	0
$L_4.3$	25	0
$L_4.4$	19	2 both at 20 weeks
$L_4.5$	27	3 at 18, 20, and 24 weeks

Table 1 (continued)

Fraction	Survivors	No. of Papillomata
$O_{4.1}$	32	0
$O_{4.2}$	38	0
$O_{4.3}$	36	0
$O_{4.4}$	41	0
$O_{4.5}$	36	0

Rabbit Tests.

Up to the present only one rabbit has been lost. Nothing was visible microscopically on the treated sites. On the other rabbits, the following papillomata have now arisen. Table 2 gives in order the rabbit, site and oil fraction where papillomata are evident at present. The earliest tumour arose at 12 weeks; rabbit 97, site 3, with $L_{4.4}$.

Table 2. Tumours on Rabbits at 25 weeks

Rabbit	Site	Fraction
42	2	D(2)
44	2	D(2)
42	1	$L_{4.4}$
43	1	$L_{4.4}$
56	5	$L_{4.4}$
57	5	$L_{4.4}$
59	5	$L_{4.4}$
96	3	$L_{4.4}$
97	3	$L_{4.4}$
99	3	$L_{4.4}$
103/	5	$K_{4.3}$
104	5	$K_{4.3}$
103/	3	$K_{4.4}$

Comments

The number of tumours which have arisen and the induction time in the mice series with the standard solutions, appear to be consistent with the accepted potency of these substances for this species e.g. 100% incidence with 0.2% 9:10-dimethyl-1:2-benzanthracene at 20 weeks. The rapid development of these tumours necessitated sacrificing the animals. Also the occurrence of 16 papillomata with 0.05% 9:10-dimethyl-1:2-benzanthracene and 8 papillomata with the 0.2% methylcholanthrene appears to be in line with previous data.

The occurrence of 3 papillomata with $L_{4.5}$ and 2 papillomata with $L_{4.4}$ around the 20th week suggest that we may expect that

these fractions will prove to be more potent to mice than the original L_4. As far as 1 papilloma may afford evidence, its occurrence with $K4.3$ at 24 weeks suggests that this fraction is more active than K_4 of the previous experiment in which the first papilloma arose at 30 weeks ($K4$ has not yet induced a tumour in this experiment).

As far as the results up to date may be interpreted, 0.2% 9:10-dimethyl-1:2-benzanthracene in liquid paraffin seems to be much less effective against rabbits than mice, and the fraction $L_4.4$, conversely, appears to be more active toward rabbits than towards mice.