THE CONCENTRATION OF CARCINOGENIC MATERIALS IN MINERAL OILS BY DISTILLATION PROCESSES

By J. M. TWORT and R. LYTH

Twelve refined lubricating cuts of a topped crude, Borneo, mineral oil (our no. 138) were kindly prepared for us by Messrs Lobitos Oilfields, Ltd. The crude oil was distilled under partial vacuum (approx. 40 mm.), each cut consisting of about 5% by volume of the original oil.

After distillation, the twelve cuts were treated with from 5–15 of 95–80% sulphuric acid, and were further refined by treatment with clay. Unfortunately, the residuum left after distillation was not sent to us, but particulars as regards its specific gravity (0·996 at 60° F.) and viscosity (3935 Redwood no. 1 at 200° F.) proved it to be very viscous and of high specific gravity.

Our first procedure was to carry out twelve painting experiments with the cuts to find out, as far as possible, their action on the skin. Each experiment consisted of painting 100 mice with the oils, five times per week for 45 weeks on the interscapular region. All surviving animals were then killed. The carcinogenic potency and physical constants of the control oil 138 had been determined previously. As is often the case with toxic mineral oils, the death rate was very high in most of the experiments, the less toxic more viscous cuts, however, permitting the animals to live fairly well.

While these experiments were in progress physical constants as regards refractive index, density and viscosity of the cuts were determined at 25° C.; also, following our usual procedure, small quantities of each cut were injected into the peritoneal cavities of mice in order to find out whether the refractive index of the oil recovered after a standard period of 7 days differed from that of the oil before injection (Twort & Lyth, 1935). In point of fact all eight mice injected with the first seven cuts and the control oil 138 were found dead, the following morning and therefore data as regards the indices of the oil recovered from the peritoneal cavities of these animals were useless for purposes of comparison. The remaining five mice lived until the seventh day and on being killed, the indices of the recovered oils were determined.

In Table I are set out the particulars as regards physical constants of the twelve cuts and control before injection into mice, together with the indices of the oils recovered from the peritoneal cavities after injection. In the table d = density; n = refractive index; n^2 = refractive index of recovered oil; RC = refractivity constant calculated from the formula $\frac{n-1}{d}$; RIF = refractive index fall of the recovered oil ($n-n^2$); KV = kinematic viscosity and T = toxicity (Twort & Lyth, 1933*b*), (Twort & Twort 1938).

https://doi.org/10.1017/S0022172400011785 Published online by Cambridge University Press

Table I

Sample	*d*	*n*	n^2	*RC*	*KV*	*T*	*RIF*
Oil 138	0·9552	—	—	—	1351·0	—	Kill
Cut no. 1	0·8885	1·4970	—	5594	3·7	2109	,,
2	0·8997	1·5038	—	5600	6·8	2105	,,
3	0·9088	1·5070	—	5579	10·5	2082	,,
4	0·9191	1·5126	—	5577	18·3	2077	,,
5	0·9260	1·5167	—	5581	34·7	2050	,,
6	0·9297	1·5189	—	5581	62·7	1994	,,
7	0·9362	1·5214	—	5569	167·8	1871	,,
8	0·9380	1·5220	1·5131	5565	303·8	1755	0·0089
9	0·9409	1·5229	1·5182	5557	726·7	1588	0·0047
10	0·9465	1·5268	1·5223	5566	1561·0	1539	0·0045
11	0·9486	1·5283	1·5254	5569	2342·0	1493	0·0036
12	0·9498	1·5300	1·5262	5580	4033·0	1418	0·0027

We see from the table the usual happening of a gradual increase in density, index and viscosity from cut no. 1 onwards while the refractivities of all the cuts are very similar. We were unable to obtain an index reading of 138 as the oil was too dirty, but in any case an exact comparison of oil 138 with the cuts is impossible since, as we have remarked previously, the cuts were to some extent refined before we received them and exact data regarding the residuum were not available. From data provided, however, we know that the densities of all the cuts and their viscosities, with the exception of the first three or four, were somewhat reduced by the refining process. We may assume that the indices were also reduced but these were apparently not determined.

The variation in viscosities which will be readily observed in the table, may be considered in relation to the approximate similarity of the refractivity constants of the cuts. This analysis was of great interest to us, especially as it was found later on that the cuts had a very different carcinogenic action on the skin (Table II). It supported previous statements of ours, namely that the viscosity of oils has usually to be taken into account when estimating their relative carcinogenic and dermatitic action on the skin (Twort & Lyth, 1933*b*; Twort & Twort, 1938). The carcinogenic potencies were calculated by our ordinary methods (mean of methods 1 and 2), (Twort & Twort, 1933).

Table II

Sample	Cumulative tumours at 25 weeks	Cumulative tumours at 35 weeks	Cumulative malignant tumours at 45 weeks	Survivors	Carcinogenic potency at 45 weeks
Oil 138	3	35	35	4	20
Cut no. 1	0	0	0	10	+*
2	0	0	0	7	0
3	0	0	0	5	0
4	0	5	0	22	1
5	4	6	0	16	3
6	14	22	23	7	18
7	48	78	50	7	56
8	32	57	67	5	46
9	11	19	47	6	25
10	6	39	44	29	31
11	0	10	10	36	6
12	1	3	2	45	3

* One small sebaceous benign tumour, seen at 45 weeks.

https://doi.org/10.1017/S0022172400011785 Published online by Cambridge University Press

In the table cumulative frequency at 25 and 35 weeks is not the actual number of living animals which bore tumours at that time, but the estimated number which would have borne tumours at that period had all the animals survived. Cumulative malignant tumours in the same way refers to the number of mice in which it is assumed the tumours would have become malignant had all the animals survived. Survivors = no. of mice still living at the 45th week of the experiment.

An examination of the table reveals that no tumours were produced in the first three cuts by the 25th week. As a matter of fact only one small sebaceous wart, in cut no. 1 was produced by the 45th week. All the cuts induced, however, definite hyperplasia of the skin and some dermatitis in most if not all, survivors. Cuts nos. 7 and 8 would appear to be the most carcinogenic whether we consider their potency figures, the number of tumours, or those which became malignant at various periods, nos. 9 and 10 are out of place as regards their carcinogenic potency and cumulative figures. As these cuts gave refractive index falls of 0·0047 and 0·0045 respectively it might have been presumed that they had a very similar carcinogenic potency, but in view of the fact that no. 10 had twice the viscosity of no. 9 it is not surprising that the former gave indications of having a higher carcinogenicity with consequently a lower dermaticity than its fellow cut with an approximately similar refractive index fall.

We can see from the table, however, that cuts nos. 1–5, 11 and 12 are definitely less carcinogenic than the others. We can thus conclude that when oil 138 is distilled in this manner the first 25% which comes over will have relatively a very low viscosity, will be very toxic for the animal, but contains little material which is carcinogenic for the skin. The second 25% will have a viscosity appreciably lower than the original oil, will be more carcinogenic for the skin, but apparently will be somewhat less toxic for the animal. The residue will have a very high viscosity, a relatively low toxicity for the animal and little cancer-producing properties.

MOLECULAR DISTILLATION

By way of comparison with the foregoing distillates of a Borneo topped crude it may be of interest to report some preliminary results with five fractions obtained from the same crude by molecular distillation (Lyth, 1937). Owing to technical difficulties only enough of these fractions was obtained to determine their physical characteristics and the animal reaction on injection, but more complete experiments to be described later have been carried out with molecular distillates of shale oil.

The following table shows the physical characteristics of these molecular distillates of oil 138. All the fractions were distilled off at the same pressure (less than 1×10^{-4} mm.) but at gradually increasing temperatures. Abbreviations as before, in addition T° C. = approximate distillation temperature Centigrade.

https://doi.org/10.1017/S0022172400011785 Published online by Cambridge University Press

Table III

Sample	*d*	*n*	*RC*	*KV*	*T*	*RIF*	*TC*
Oil 138	0·9552	—	—	1351·0	—	—	—
Fraction 1	0·9148	1·5095	5569	13·05	2085	Kill	34
2	—	1·5134	—	18·95	—	,,	36
3	0·9260	1·5168	5581	23·09	2114	,,	38
4	0·9334	1·5201	5572	35·78	2104	,,	52
5	0·9387	1·5223	5564	75·65	2039	,,	70

On injection into animals all these fractions killed within 24 hr., so that no figures for reduction in index could be obtained. From a study of this table and Table I it is apparent that these five fractions correspond fairly well to cuts nos. 3, 4, 5, 7 and 8 with regard to density and index but their viscosity range is nothing like so wide. No density determination of fraction 2 could be made owing to lack of material. The absence of any low density early fractions is due to the fact that by molecular distillation a spiritous fraction can be distilled off and is condensed in the liquid air trap of the pumping system, but this fact alone will not account for the gradual divergence in viscosity, of the two series. The molecular distillation has not yet been carried far enough to see whether the divergence will appear in the density and refractive index but in all probability it will do so owing to the greater efficiency of molecular distillation compared with ordinary distillation in separating the constituent of an oil.

With shale oil the animal experiments have been carried a stage further, and a concentration of the carcinogenic factor in certain fractions is clearly indicated. The animal painting experiments will, however, be dealt with later on, but for the moment it is interesting to compare the molecular distillation of one shale oil with distillation of another one at 10 mm. pressure.

In the following table are shown temperature ranges over which fractions were distilled off, and their physical characteristics, etc., as far as they have been determined. No painting experiments were performed with the distillates of oil 8 (2).

Table IV. *Oil* 8 (2) *at* 10 *mm.*

Sample	*T*° C.	*d* 25° C.	*n*	*RC*	*KV*	*T*	*RIF*
Oil 8 (2)	—	0·8935	1·5034	5635	52·70	1729	0·0076
Fraction 1	110–230	0·8716	1·4882	5601	11·37	1801	*K*
2	230–240	0·8878	1·4979	5608	25·34	1790	0·0135
3	240–248	0·8895	1·4994	5614	30·84	1785	0·0107
4	248–256	0·8912	1·5003	5614	—	—	0·0075
5	256–264	0·8926	1·5013	5616	43·91	1734	0·0061
6	264–272	0·8936	1·5022	5620	52·19	1714	0·0054
7	272–280	0·8959	1·5038	5623	74·68	1666	—
8	280–288	0·9027	1·5057	5602	90·51	1661	—
9	288–296	0·9047	1·5085	5621	—	—	—
10	296–304	0·9100	1·5123	5630	—	—	—

Scrutiny of the tables, as regards toxicity, viscosity, and index figures, reveals that fractions 2 and 3 in Table V are likely to be more carcinogenic for the skin than any of the fractions set out in Table IV or either of the controls. Fraction 1 in Table IV will probably contain relatively more

Table V. *Oil* 55, *molecular distillation*

Sample	*T*° C.	*d*	*n*	*RC*	*KV*	*T*	*RIF*
Oil 55	—	0·8935	1·5039	5640	51·86	1747	0·0079
Fraction 2	30–58	0·9061	1·5136	5668	31·8	2013	0·0186
3	58–62	0·9025	1·5025	5649	36·74	1921	0·0128
4	62–70	0·8967	1·5054	5636	48·33	1789	0·0080
5	70–90	0·8880	1·4983	5611	60·16	1608	0·0042
6	91–105	0·8812	1·4949	5616	71·41	1503	0·0019
Residue 7	—	0·9004	1·5068	5629	208·5	1408	0·0016

material likely to cause dermatitis, because of its low viscosity. The most striking physical feature is the fact that there is a complete inversion in the order of the values for density and refractive index in the two series. This feature is not shown by any distillation carried out at relatively high pressures of 3–10 mm. and is, we believe, peculiar to molecular distillations. It is exhibited by several other oils that have been tried, but not at all by Borneo oils. As these other oils have not been tested on animals we do not propose to include tables of their physical characteristics in the present paper, but append herewith one more table for a Persian oil for which the animal injection experiments have been previously reported (Lyth & Twort, 1938).

Table VI. *Oil* 102, *molecular distillation*

Sample	*T*° C.	*d*	*n*	*RC*	*KV*	*T*	*RIF*
Oil 102	—	0·8939	4990	5582	34·82	—	0·0140
Fraction 1	30–32	0·9113	5120	5618	14·89	2103	*K*
2	32–35	0·9027	5063	5609	17·61	1990	0·0258
3	35–36	0·8964	5019	5609	21·17	1888	0·0216
4	36–37	0·8902	4978	5592	25·28	1787	0·0153
5	37–38	0·8907	4974	5587	25·28	1780	0·0146
6	38–40	0·8859	4945	5582	29·69	1698	0·0107
7	40–41	0·8845	4931	5575	32·84	1652	0·0091
8	41–45	0·8841	4922	5567	37·17	1607	0·0074
9	46–47	0·8835	4921	5570	40·28	1573	0·0064
10	50–53	0·8830	4913	5564	43·46	1563	0·0055
Residue	—	0·9183	5101	5555	—	—	0·0033

In this distillation the range is much narrower and the cuts proportionately so, but the progressive decrease in density and refractive index and the relatively narrow range of viscosities is well shown. It is hoped that subsequently animal experiments will show a definite concentration of the carcinogenic factor at some point in this range, as in the case of shale oil. Further distillation experiments are now in progress, with this end in view.

PAINTING EXPERIMENTS WITH DISTILLATES OF A SHALE OIL

Unfortunately, as mentioned previously, animal painting tests were not performed with the distillates of oil 8 (2) mentioned in Table IV but a number of painting experiments were completed about 8 years ago with another similar grade shale oil—oil 8 (1) and its distillates.

No physical data are available as regards these distillates except the temperature ranges at which they were distilled. The painting was performed

twice weekly and 100 mice were utilized in each experiment. In Table VII is given data regarding the animal experiments together with the temperature ranges at which they were distilled.

Table VII

Sample	*T*	*M*	*S*	*X*	*P*
Oil 8 (1)	22	10	3	25·4	27
140–220° C.	12	1	2	22·5	27
220–240° C.	27	6	9	28·4	22
240–260° C.	15	9	8	37·2	21
260–280° C.	4	0	0	27·5	10
280–315° C.	1	1	1	46·0	3

In the tables T = no. of mice in each group which bore tumours, M = no. which were obviously malignant, S = no. of survivors after 48 weeks; X average no. of weeks when tumours arose; P = approximate potency.

In Table VIII are set out the animal results with the molecular distillates of oil 55 mentioned in Table V. Abbreviations as in Table VII.

Table VIII

Sample	*T*	*M*	*S*	*X*	*P*
Oil 55	1	1	7	35·0	1
Fraction 2	5	2	1	23·8	32
3	5	1	4	32·6	13
4	5	1	3	31·6	25
5	1	0	2	37·0	2
6	0	0	7	—	0
Residue	0	0	4	—	0

In these experiments with the molecular distillates and oil 55 only very small quantities were applied twice weekly as very little of the distillates were available and fraction 2 especially was very toxic for the animal. Only ten mice were utilized for each experiment. The paintings were discontinued after 38 weeks as no more material was available in some of the fractions.

A comparison of the two tables reveals that the molecular distillation process was much more efficaceous in concentrating the carcinogenic material than was the case with ordinary distillation under a pressure of 3 mm. Of the distillates 140–220° C. gave indication of being somewhat more carcinogenic than the control, whereas the first three molecular fractions were definitely more carcinogenic than oil 55. It will be noted that only one tumour was produced in the mice painted with oil 55, the oil controlling the molecular fractions, although seven out of the ten mice survived for 48 weeks. Moreover, the skins of most of the mice which died during the experiment were examined microscopically, and hyperplasia of the epithelium was usually much more pronounced in the mice painted with fractions 2 and 3 than in either the control or the other fractions.

Examination of internal organs

Very few of the mice which died during the course of these experiments were examined except when they bore tumours which showed some clinical

evidence of malignancy. The skins, spleens, livers and on occasion other organs of all the mice treated with the twelve cuts of the Borneo oil which survived to the end of the experiments were examined microscopically. It was of interest to note that although there were relatively so few survivors in many of these experiments, there was definite evidence of group differences as regards hyaline changes in the spleen and fatty infiltrative changes of the liver.

Because female mice are more susceptible than males to fatty infiltrative changes of the liver following prolonged application of mineral oils, and pigmented mice more susceptible than albinos (Twort & Twort, 1933*a*) our mice were segregated into their appropriate groups before our findings in the various experiments were completed. As the number of pigmented mice was small these were omitted, the various groups being divided into two classes—male and female. Owing to the high death rate it was necessary to include the experiments with the first three cuts as one group; similarly for the second and third three cuts, the last three cuts being considered separately. In the experiment with the control (oil 138) only four animals survived, all of them males. The liver of one showed an early stage of fatty infiltration, and the spleen of two showed some hyaline changes.

In Table IX are set out the results of these investigations, the abbreviations being as follows: M. = male; F. = female. No. = number of animals considered. *FI* = number which showed obvious fatty infiltration of the liver. *HD* = number which showed obvious hyaline changes in the spleen.

Table IX

Cut no.	Sex	No.	*FI*	%	*HD*	%
1, 2, 3	M.	15	0	0	2	13
1, 2, 3	F.	4	0	0	0	0
4, 5, 6	M.	20	4	20	9	45
4, 5, 6	F.	15	2	13	5	33
7, 8, 9	M.	15	11	73	4	27
7, 8, 9	F.	1	1	100	0	0
10	M.	18	11	61	5	28
10	F.	5	3	60	1	20
11	M.	13	7	54	5	38
11	F.	18	13	72	2	11
12	M.	34	9	26	6	18
12	F.	6	6	100	1	17

Scrutiny of the table reveals that there was little difference in the number of animals which showed hyaline changes in the spleens in the different groups, with the exception of the first and last groups. As regards fatty infiltration of the liver, however, there is obviously considerably less in groups 1 and 2 comprising the first six cuts than there is in the remaining groups. There was some evidence to show that the fatty infiltration tended to be less pronounced in cut no. 12 than in the three preceding groups for a few of the mice in each of the last mentioned groups showed the condition in a fairly advanced state, whereas when present in the livers of the mice in the last group, the condition

was only in an early stage of development. Moreover, if the males only in the last three groups are compared, there is a significant difference in the percentage number which shows the condition. It can thus be said that in the distillation of oil 138 the first 25% which comes over contains little, if any, material which will cause a fatty infiltrative condition of the liver, but apparently contains substances capable of causing some degree of hyaline changes in the spleen. The next 25% will be more likely to cause fatty infiltrative changes in the liver and hyaline changes in the spleen than either the residue or the first 25%.

It was of interest to note that the incidence of lung tumours was rather higher than is usual among our mice painted with mineral oils for this length of time. The lungs of all the survivors were examined macroscopically and possible adenomas were clinically diagnosed in thirty of them. These thirty organs were examined microscopically and eleven of them were found to have definite adenomas, three of which were obviously malignant, and several others suspicious. There was only one questionable adenoma in the first three groups and this was not confirmed by the microscope. It was rather curious to note that the incidence of lung adenoma sometimes seemed to be higher in particular boxes of mice than in others. For example in one box where there were only four survivors the lungs of three were diagnosed clinically as bearing tumours. One of these was confirmed microscopically as an adenocarcinoma. In another box with four survivors three had questionable tumours of the lungs and two were confirmed microscopically one being a malignant bronchial carcinoma. In still another box where the survivors numbered five, two showed adenomas both confirmed by the microscope, one of which was probably malignant. There were ten boxes containing three or more animals with no apparent lung tumours. In Table X is set out the particulars as regards the incidence of lung tumours in the various experiments. From left to right is given (1) the number diagnosed macroscopically as possible adenomas, (2) the number confirmed microscopically, (3) the number which were obviously malignant, and (4) the number of mice in each group.

Table X

Cut no.	(1)	(2)	(3)	(4)
1, 2, 3	1	0	0	22
4, 5, 6	7	2	1	45
7, 8, 9	1	0	0	18
10	6	4	1	29
11	8	3	0	36
12	7	2	1	45

Scrutiny of the table reveals that the incidence of primary lung tumours in these experiments was somewhat higher in the group of mice painted with the more viscous distillates, but that the incidence did not appear to be related to any differences in their carcinogenicity for the skin.

DISCUSSION

The main object of these experiments was to ascertain the distillation range of the carcinogenic constituents of a given crude, with the intention of subsequently selecting the commercial cuts showing the greatest activity (cuts 7 and 8 in this case), and subjecting them to high vacuum distillation. In the early stages of the latter process the low viscosity, high density, and index, biologically (mostly dermatitic) very active constituents should be removed, leaving a residuum or intermediate fraction of relatively high viscosity and carcinogenicity, showing but a relatively small change in refractive index after 7 days' sojourn in the peritoneal cavity of the animal (Lyth & Twort, 1938). Solvent extraction of this intermediate fraction should result in a relatively inert residuum, with consequently an increased activity of the portion extracted.

SUMMARY

1. A range of commercial distillates of a topped crude Borneo, mineral oil were tested for carcinogenic activity and it was found that the peak was around a viscosity at 25° C. of 200 to 250 Centipoises.

2. The whole range of oils was biologically active. This activity had been foreseen from an examination of the physical characteristics, alone sufficient to condemn the utilization of any single representative as a lubricant when likely to come into contact with the user.

3. A greater concentration of the carcinogenic material was obtained by molecular distillation of oils than by ordinary distillation at from 3 to 10 mm.

4. The middle fractions of a Borneo crude oil were mostly responsible for pathological changes in the organs—fatty infiltration of the liver and hyaline degeneration of the spleen. These results again confirm previous findings.

5. Lung tumours were, according to our experience with mineral oils more prevalent than is usual when utilizing this type of agent for testing carcinogenicity for the skin. Their incidence was somewhat higher in the more viscous fractions but was not apparently related to their carcinogenicity for the skin.

In conclusion we should like to thank Dr C. C. Twort, former Director of this Department and now of the Portslade Laboratories, Sussex, for his helpful suggestions and advice in the compilation of this paper, and wish also to acknowledge their indebtedness to Dr J. R. Bowman of the Mellon Institute of Industrial Research, University of Pittsburgh, for a micro method of viscosity determination.

REFERENCES

LYTH, R. (1937). *Nature, Lond.*, **139**, 374.
LYTH, R. & TWORT, C. C. (1938). *J. Hyg., Camb.*, **38**, 269.
TWORT, C. C. & TWORT, J. M. (1933*a*). *J. Hyg., Camb.*, **33**, 396.
TWORT, C. C. & LYTH, R. (1933*b*). *J. Hyg., Camb.*, **33**, 464.
—— —— (1935). *J. Hyg., Camb.*, **35**, 404.
TWORT, C. C. & TWORT, J. M. (1933). *Amer. J. Cancer*, **17**, 293.
—— —— (1938). *Oil Col. Tr. J.* 22 and 29 July.

(*MS. received for publication* 17. XII. 38—Ed.)