CUTTING OILS AND SQUAMOUS-CELL CARCINOMA

PART II: AN EXPERIMENTAL STUDY OF THE CARCINOGENICITY OF TWO TYPES OF CUTTING OILS*

BY

J. P. W. GILMAN and S. D. VESSELINOVITCH

From the Division of Biology, Ontario Veterinary College, Guelph, Ontario

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That mineral oils and their derivatives would induce squamous-cell carcinoma when painted on to the skin of mice was reported by Twort and Twort (1931), and in a series of other papers by these authors which have since been statistically analysed by Irwin and Goodman (1946). More recent work has demonstrated that both straight-run distillates (Woodhouse and Irwin, 1950) and high boiling point fractions of catalytically cracked petroleum oils (Smith, Sunderland, and Sugiura, 1951) may contain numerous carcinogenic hydrocarbons, although to date no pure carcinogens have been isolated from such compounds.

The literature which we have reviewed contains surprisingly few references to occupational cancers directly attributable to cutting oil exposure in the engineering industry. However, the relationship between excessive exposure to such products and a high incidence of other occupational skin diseases is well documented (Cruickshank, 1950; Shapiro, 1950).

Cruickshank and Squire (1950) have presented evidence, from both clinical observations and biological tests on mice and rabbits, implicating exposure to cutting oils as an occupational cancer hazard. A later publication by Cruickshank and Gourevitch (1952) supported this opinion. In the latter paper the authors suggested that it was quite possible that this cancer risk (exposure to cutting oils in the engineering industry) had not been long enough in existence for it to become apparent from mortality rate studies such as those of Henry (1947). It is interesting to note that in recent years the numbers and uses of the so-called "cutting fluids" have increased considerably. Such cutting fluids generally consist of a relatively stable aqueous emulsion of sulphurized petroleum oil distillates

with vegetable and mineral oils in relatively small amounts and various cutting compound additives, including emulsifying, wetting, and penetrating agents. Disinfectants are also frequently added.

A preliminary report by Gilman and Vesselinovitch (1955) demonstrated that certain fractions of soluble cutting oil were capable of inducing a high incidence of tumours in the mouse. The fractions that proved carcinogenic were cut at 630, 800, and 930° F., under vacuum distillation to avoid cracking.

The present contribution is concerned with an analysis of the tumour responses obtained in several experiments involving prolonged exposure of mice to samples and dilutions of a soluble cutting oil as well as preliminary results obtained from tests using a straight cutting oil. Both of these products were cutting oils in use at the metal working plant referred to in Part I.

Cutting oils are generally sprayed in a continuous stream over the point of contact between the cutting edge of the machine tool and the metal being processed. At this point, minute quantities of the coolant are subjected to extremely high temperatures which might quite possibly cause cracking of the oils. The oil spray is collected under each machine and generally filtered through a system of weirs before returning to a sump for recirculation. Hence, one lot of oil, subject to periodic replenishment, frequently remains in use for several weeks.

Materials Tested

One of the cutting oils under investigation is marketed in the form of a water-soluble emulsion and is used in dilutions of one part to eight or more of water. As dilutions of this order might tend to obscure the presence of a weak carcinogen, tests were run using both the undiluted emulsion and various dilutions of this fluid. Samples of used cutting oils were also tested because it has been shown that cracking may greatly enhance the carcino-

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genicity of petroleum oils (Smith and others, 1951) and it seemed probable that the used oil might contain minute amounts of such cracked materials.

The properties of the two types of cutting oil and the several samples tested may be summarized as follows :

Cutting Oil A.—This is a soluble cutting fluid in the form of a stable oil-in-water emulsion containing active additives, about 45% sulphurized mineral oil base, and 40% water when marketed. It is usually diluted with eight or more parts of water before use.

Cutting Oil B.—This is a straight cutting oil, a sulphurized mineral oil blended with fatty oils. It is usually cut back with a low viscosity straight paraffin oil before use.

Samples Tested

Soluble Oil A-1-51	Undiluted ; unused (market product)
Soluble Oil A-1-53	As above—separate sample obtained over 12 months later
Soluble Oil A-2	Dilute 1 : 4 water ; unused
Soluble Oil A-3	Dilute 1 : 8 water ; unused
Soluble Oil A-4	Dilute approx. 1 : 8 water ; used*
Straight Oil B-1	Dilute 6 : 4 parts paraffin oil ; unused
Straight Oil B-2	Dilute 6 : 4 parts paraffin oil ; used.†

Methods

Samples of oil A were tested against C₃H, C₅₇ black and R.F.‡ mice, while oil B was tested against C₃H mice only. Males and females were used in about equal numbers in all trials. Exposure consisted of painting the skin three times weekly with from 50 to 100 mg. of the test materials. The oils were applied with a camel-hair brush to a clipped area at the base of the neck.

Ages at the beginning of the experiments varied slightly but all groups fell within a range of from 90 to 130 days. Animals were housed in glass jars in groups of five and fed a standard commercial ration of fox cubes and rabbit pellets.

Calculations

Tumour response and carcinogenicity in this report are measured by means of the following simple statistics :—

$$(1) \text{ Mean Tumour Time (M.T.T.)} = \frac{\Sigma t}{TT}$$

Where Σt = sum of the times in days to the appearance of the first tumour on each tumour-bearing mouse.

Where TT = total number of animals with either squamous-cell papilloma or carcinoma.

$$(2) \text{ Percentage tumours} = \frac{TT}{N_{\text{corr}}} \times 100$$

Where N = actual number of animals set up for each experiment.

Where N_{corr} = N less those mice that died

without developing tumours before the M.T.T. minus two times its standard deviation.

$$(3) \text{ Percentage cancer} = \frac{C}{N_{\text{corr}}} \times 100$$

Where C = total number of mice developing histologically confirmed squamous-cell carcinoma.

$$(4) \text{ Mean Non-tumour Survival Time (M.S.T.)} = \frac{\Sigma t_s}{N_{\text{nt}}}$$

Where Σt_s = sum of survival times in days of all non-tumour mice, those still alive at the termination of the experiment being credited with survival times equal to the duration of the experiment in days.

Where N_{nt} = number of animals that failed to develop a tumour.

The M.S.T. is accompanied by a statement of the number of non-tumour survivors at the end of the experiment and its duration in days.

Results

Table 1 summarizes the results obtained from two separate tests of sample A-1 begun 18 months apart. In each of these trials a different batch of the soluble cutting oil A was used. Of the three strains of mice exposed, the C₃H and C₅₇ black were inbred stock from our own colony while the R.F. mice, obtained commercially, were not necessarily inbred.

It will be readily noticed that there was, in both trials, an appreciable difference in response between the two inbred strains. This is expressed both in the percentage of tumours and to a less marked degree in the induction period (M.T.T.).

TABLE 1

TUMOUR RESPONSE OF MICE TO SKIN PAINTING WITH A SOLUBLE CUTTING OIL AND WITH CORN OIL (CONTROL GROUP)

Sample	Strain	Number		Tumour Response			Non-tumour Mice	
				310 Days' Exposure			End of Experiment	
		N	N _{corr.}	% Tumour	MTT (days)	% Cancer	Survivors	Days
A-1-51	C ₃ H	20	18	61	204	22	2	401
	C ₅₇ Bl.	40	39	19	244	3	5	395
A-1-53	C ₃ H	30	26*	58	224	19	0	365
	C ₅₇ Bl.	30	30	27	255	3	1	406
	R.F.	30	30	33	144	7	0	310
Control	C ₃ H	20	—	0	—	0	9	401
	C ₅₇ Bl.	20	—	0	—	0	18	401

* Four deaths from injection occurred in a single cage between 161 and 175 days ; all animals showed similar necrotic liver and kidney lesions on necropsy. These four mice were discounted in calculating the response and MST.

Oil A proved highly toxic to the R.F. mice tested, with the result that this group had a non-tumour mean survival time (M.S.T.) of only 109 ± 35 days. Over 50% of the mice in this trial had died without

* Used for five weeks with daily replenishments with new emulsion
† Used for five weeks with daily replenishments with both new and reclaimed oils.

‡ Rockland Farm mice—a commercial strain of albino mice obtained from Rockland Farms, New City, N.Y., U.S.A.

having developed a tumour before reaching the M.T.T. for the group as a whole (144 days). All the R.F. mice were dead by 310 days, and for this reason length of exposure has been chosen for the comparison between samples and strains shown in Table 1. The control groups of C₃H and C₅₇ black were exposed to the same treatment as the test mice, except that they were painted with a domestic corn oil.

Regardless of the differences in response of the strains, it is quite evident that cutting oil A has, for a crude commercial product, quite a high tumour-inducing potency against mice and that this response shows a high level of repeatability.

In an initial investigation, the used oil (sample A-4) had shown only a very slight carcinogenic potency (Gilman, 1950-51, unpublished data). Consequently the tests summarized in Table 2 were undertaken, making use of similar dilutions of used (A-4) and unused (A-3) materials along with an unused intermediate dilution (A-2) of one part oil A to four parts of water. It was hoped that a comparison of the results obtained with these and with the undiluted product might help to clarify the role of dilution in reducing the tumour-producing activity of the used product.

TABLE 2
EFFECT OF DILUTION ON TUMOUR RESPONSE OF C₃H MICE TO SOLUBLE CUTTING OIL A

Sample	Dilution	Numbers		Tumour Response			Non-tumour Mice		MST (days)
				365 Days' Exposure			End of Experiment		
		N	N corr.	% Tumour	MTT (days)	% Cancer	Survivors	Days	
A-1	None	30	26*	61.5	224	23.1	0	365	226
A-2	1 : 4	20	20	15.0†	291	0	3	456	323
A-3	1 : 8	20	20	0	—	0	9	456	335
A-4	1 : 8 (used)	20	20	0	—	0	15	412	396

* See footnote to Table 1.

† One of the papillomas in the 1:4 group regressed by 310 days.

The percentage of C₃H mice developing tumours from the 1:4 solution is slightly less than a quarter of that observed for the pure product, while the induction period (M.T.T.) had increased on the average about 70 days. Neither the used nor the unused 1:8 dilutions had elicited any response after exposure for a full year.

Mice of the apparently less susceptible C₅₇ black strain were also exposed to the same dilution samples of the soluble oil as were used on the C₃H groups (Table 3). After 365 days' exposure the C₅₇ group showed similar but on the whole somewhat less marked responses to the treatments.

TABLE 3
RESPONSE OF C₅₇ BLACK MICE TO DILUTIONS OF SOLUBLE CUTTING OIL A

Sample	Dilution	Number		365 Days % Tumour	Response at End of Exposure Period			
		N	N corr.		% Tumour	MTT (days)	% Cancer	Duration (days)
A-1	None	30	29	41	45	255	17	406
A-2	1:4	20	20	5	20	408	0	456
A-3	1:8	20	20	0	0	—	0	456
A-4	1:8 (used)	40	35	3	3	—	3	395

It is interesting to note that after 456 days the 1:4 group showed 20% tumours, a figure only slightly in excess of the response registered by the C₃H mice after 90 days less exposure time to the same treatment.

The results recorded in Table 4 for the straight cutting oil (samples B-1 and B-2) are from a pilot test involving too few animals and too short an exposure time to warrant the making of comparisons between these and the other cutting oil samples tested. However, sample B-1 was apparently responsible for skin tumours in two of the 10 animals exposed. One of these developed into a typical squamous-cell carcinoma and eventually metastasized to the lung.

TABLE 4
TUMOUR RESPONSE OF C₃H MICE TO STRAIGHT CUTTING OIL (BLENDED 6:4 WITH PARAFFIN OIL)

Sample	Number		% Tumour	MTT (days)	% Cancer	Days on Experiment
	N	N corr.				
B-1	10	10	20	162	10	320
B-2	16	16	0	—	0	295

Thus it would appear that this cutting oil is also carcinogenic, although confirmation of this and detail as to potency will have to await the completion of experiments in progress.

Discussion

The oil base of the soluble cutting oil tested is a 50-50 blend of sulphurized and filtered straight-run distillates of a crude mineral oil. These base oil fractions are supposedly not cracked and contain no additives other than commercial flowers of sulphur. Thus it might be expected that oil A would give a tumour response comparable to those reported for similar straight-run distillates. However, it would appear to be considerably more potent to the mouse than the several mineral oils and fractions tested by Woodhouse (1951, 1952) and his colleagues. This discrepancy in intensity of response suggests the possibility that one or more of the additives used in compounding the cutting fluid may be acting

synergistically with the mineral oils. None of these additives have been recorded in the literature reviewed as being either carcinogens or cocarcinogens ; however, their possible role is being investigated at this laboratory.

The actual use of the cutting fluid A apparently does not either markedly reduce or enhance its tumour-producing potency. It had seemed possible that a reduction in carcinogenic activity might have been associated with oxidation of the carcinogenic components of the used oil, resulting possibly from extensive contact with metal particles. That oxidation may inhibit the potency of carcinogenic shale oil and synthetic tar has been shown by Twort and Twort (1930). However, in all probability the major part of those differences in response recorded in Table 2 is a simple function of the various levels of dilution. This contention is supported by evidence obtained from tests using C_{57}/C_3H hybrids. Such hybrids are hardy, long-lived and resistant to the toxicity of the oils, yet seemingly susceptible to their carcinogenic properties. Twenty such mice, out of C_{57} mothers and therefore free from spontaneous mammary tumours, were treated with sample A-3 (1 : 8 dilution) in exactly the same manner as the other mice reported in Tables 2 and 3 except that exposure was prolonged through a period of 525 days. At 365 and 450 days these hybrids showed no response. However, by 525 days, with all 20 hybrids still living, three had developed tumours, one of which had become cancerous, a response approximating that of the C_3H and C_{57} pure strain groups to the more concentrated 1 : 4 dilution after 365 and 456 days respectively. This suggests that, if exposure time to the various dilutions could be sufficiently extended, a tumour incidence comparable to that of the undiluted sample would ultimately be achieved. Apparently the induction period was the only variable appreciably affected by these dilutions. The advantage of using hybrid mice for testing substances that are highly toxic and where exposure may need to be continued over very long periods of time is also apparent.

These observations are of particular interest when it is remembered that it is with just such highly diluted oil-in-water emulsions that the machine operator is in contact. It is only to be expected that, if cutting oils are an aetiological factor in the occurrence of squamous-cell carcinoma amongst exposed workers, such cases will probably be preceded by long periods of exposure. It would seem then that the length of the exposure period should be considered as a very important factor in the evaluation of results of experiments, such as

those reported by Smith and others (1951), concerning the effect of dilution with an inactive material on the carcinogenicity of active fractions of catalytically cracked oils. These data seemingly influenced the interim recommendation by Holt, Hendricks, Eckardt, Stanton, and Page (1951) that blends of oil containing more than 10% of catalytically cracked oil boiling above 700° F. be considered carcinogenic.

Smith and Sunderland (1951) have reported differences in the response of various strains of mice to the same carcinogen. The evidence presented in this communication clearly supports their observations (Table 1). It appears advisable, therefore, to test against more than one strain of mice when attempting to determine whether or not a substance is carcinogenic. This would seem to be particularly important if the rabbit is not available for duplicate testing, in accordance with the suggestion of Hieger and Woodhouse (1952).

The inclusion of the mean survival time of those animals that failed to develop tumours along with the number of non-tumour survivors and the duration of each trial gives some indication of the reliability of the results in terms of the toxicity of the substances tested and response of the treated mice (other than tumour development) to the conditions of the particular experiment.

Summary

A soluble cutting fluid (oil-in-water emulsion) used in industry has been shown to be carcinogenic to three different strains of mice.

The response of test groups to a standard skin painting technique varied with the strain used from 19 to 61% tumours after 310 days' exposure.

Results proved reproducible and comparatively constant in two trials repeated after an interval of 18 months.

The responses in mice to 1 : 4 and 1 : 8 aqueous dilutions of this soluble oil were compared with the undiluted product and with a 1 : 8 used dilution after 365 days' exposure. A marked reduction in the percentage of mice bearing tumours and an increase in induction periods accompanied dilution.

Evidence was presented which suggests that the chief effect of dilution is the lengthening of the induction period. Thus when duration of treatment with a dilute oil was lengthened sufficiently the absolute tumour response approached that of mice exposed to the more concentrated emulsions.

Results of tests using a straight cutting oil (blend) are reported and suggest that this type may also be carcinogenic to the mouse.

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THE APRIL (1955) ISSUE

The April (1955) issue contains the following papers :—

Mortality from Lung Cancer in Asbestos Workers. By Richard Doll.

Primary Lung Cancer in South Wales Coal-workers with Pneumoconiosis. By W. R. L. James.

Pulmonary Fibrosis in Non-ferrous Foundry Workers. By H. E. Harding and A. I. G. McLaughlin.

Leptospiral Serology in Scottish Coal-miners. By R. S. F. Adam and P. N. Edmunds.

Diurnal Variation in Mental Performance. By Bo Bjerner, Åke Holm, and Åke Swensson.

Physiological Observations on Men Working in Supposedly Limiting Environments in a West African Gold-mine. By W. S. S. Ladell.

The Influence of Inspired Air Temperature on Tolerance to Work in the Heat. By A. R. Lind.

Investigations into the Effects of Exposure to Trichlorethylene in Mechanical Engineering. By E. Grandjean, R. Münchinger, V. Turrian, P. A. Haas, H.-K. Knoepfel, and H. Rosenmund.

Conference of the British Occupational Hygiene Society on Radiation Hazards in Industry :

The Health of Workers Exposed to Ionizing Radiations. By A. S. McLean.

Radiation Safety at an Atomic Energy Factory. By D. R. R. Fair.

Protection against X rays and Gamma rays in the Industrial Field. By W. Binks.

Safety Criteria in Atomic Energy. By F. R. Farmer.

Miscellanea :

Occupational Dermatitis Caused by Ethylene Oxide. By A. Royce and W. K. S. Moore.

Blood Examinations in Industry : A Comparison of Blood Cell Counts from Ear Lobe and Thumb Pulp. By A. Butterworth and N. Goodman.

Raynaud's Phenomenon. By Andrew Meiklejohn.

First Ordinary Meeting of the British Occupational Hygiene Society

Obituary : Cecil Gordon Roberts.

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