THE CARCINOGENIC ACTIVITY OF SOME PETROLEUM FRACTIONS AND EXTRACTS

COMPARATIVE RESULTS IN TESTS ON MICE REPEATED AFTER AN INTERVAL OF EIGHTEEN MONTHS

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WITH A STATISTICAL ANALYSIS OF THE RESULTS

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INTRODUCTION

During the years 1926–35 extensive researches were carried out on the carcinogenic properties of mineral oils by C. C. and J. M. Twort, who described the relative carcinogenic activity of petroleum and shale oils from many sources in a number of publications. A detailed statistical analysis of their results has been published by Irwin & Goodman (1945–6). As a consequence of the development of new industrial processes there has been an increased use of diverse types of lubricants and additives, and during the war period extended utilization of materials derived from mineral oils was anticipated. There is a possibility that frequent contact with such products over long periods might result in the occurrence of skin lesions among workers, some of a cancerous nature. The existence of such hazards in the engineering industry has in fact been demonstrated by Cruikshank & Squire (1950).

In 1944 an examination of a series of petroleum crudes, extracts and residues for possible carcinogenicity was undertaken at this centre at the request of the Petroleum Board. Since the chemical structure of the carcinogens in oil is still unknown, the estimation of the potency of such complex materials can only be made by tests on experimental animals. It was considered that some of the problems inherent in such experiments had not hitherto received adequate attention, especially those appertaining to the reliability and reproducibility of results. With this in view Prof. Garner (Department of Chemical Engineering, University of Birmingham) suggested that it would be of great value to compare the results on a series of selected fractions, in experiments repeated after an interval of one year or more, under conditions as constant as possible.

An outline of the technique and the results obtained on twenty-two petroleum fractions and on some other substances are given in this communication, together with the duplicate experiments on ten fractions. The data concerning these duplicate experiments have been examined by statistical methods.

FRACTIONS TESTED

During the period November 1944 to early in 1946, sixteen specimens, which had been selected by a committee representing the Petroleum Board and the Institute of petroleum, were utilized in the first experiment. These fractions varied in character from thin, amber or greenish oils to solid pitch-like residues, and were representative of crudes, extracts and residues from different sources and processes. At this time neither the origin nor the method of production was known to the writer, and the samples were designated by the letters A to P. A number of related materials were also tested, including specimens of creosote, anthracene oil, linseed oil and pine oil.

The second series of tests was conducted during the period July 1946 to November 1947. The specimens were selected from the above sixteen fractions in the light of the general effects previously observed, with the idea of utilizing representative types of the different grades of carcinogenicity as identified by the previous experiment. At the same time six spindle oils and three white oils (which had the appearance of medicinal liquid paraffin) were also subjected to the tests.

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 entirely miscible with this medium Benzene or toluene, however, yielded homogeneous solutions. It is known that both of these are somewhat toxic, but the latter has been shown to be less injurious to animals (Smith, 1931). The amount added to individual fractions is given in Table 1, which also indicates the general character of the fractions and their effects on the mice during the early part of the experiment.

GENERAL ARRANGEMENTS FOR THE TESTS

Animals

Each fraction was tested on fifty albino white 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 $10 \times 4\frac{1}{2} \times 4\frac{1}{2}$ in., two animals per box, bedded with sawdust and cellulose 'wool'. A varied diet of whole-meal bread and rusks with water and fresh milk daily was given, with the addition of crushed oats and a little bran two or three times a week.

Table 1

First Series

Fraction	Country of origin	Nature of fraction	Character of fraction	How applied	Toxicity by survival rates
A	Venezuela	Residue	Shiny black tar. Almost solid at 18° C.	Solution of 50 g. in 30 ml. toluene	Moderate
B	United Kingdom	Residue	Similar to A but slightly more liquid	Solution of 50 g. in 30 ml. toluene	Slight
C	Venezuela	Lub. distillate	Very viscous oil. Yellow-green fluorescence	With 10 % toluene added	Pronounced
D	Venezuela	Lub. distillate extract	Similar to C but slightly more liquid	With 10 % toluene added	Pronounced
E	Venezuela	Lub. distillate residue	Black pitch. Residue from furfural lub. oil distillate extract	Solution of 50 g. in 30 ml. toluene	Slight
F	U.S.A.	Lub. distillate	Fairly thin brownish oily liquid	Without diluent	Very toxic
G	U.S.A.	Lub. distillate	Very viscous dark brown tarry liquid	With 10 % toluene	Moderate
H	U.S.A.	Lub. distillate reprocessed	Amber oil. Green fluorescence	Without diluent	Moderate
I	United Kingdom	Lub. oil distillate	Thin, dark brown tar	Without diluent	Moderate
J	Iran	Lub. oil distillate	Viscous brown tar	Solution of 50 g. in 30 ml. toluene	Moderate
K	Columbia	Lub. oil distillate	Thick brown oil	With 10 % toluene	Very pronounced
L	Columbia	Lub. oil distillate residue	Very viscous brown tar	With 10 % toluene	Pronounced
M	Columbia	Lub. oil distillate-residue-sulphur hardened	Solid black pitch	50 % wt./vol. 'suspension' in toluene	Slight
N	Columbia	Residue	Solid black pitch	50 % wt./vol. 'suspension' in toluene	Very slight
O	Venezuela	Distillate	Yellow oil marked green fluorescence	Without diluent	Slight
P	Equador	Distillate	Very viscous brown tar	With 10 % toluene	Fairly great

Second Series

		Anthracene oil	Thin, yellow-brown oil	Without diluent	High
		Creosote oil	Thick dark brown liquid	Without diluent	Pronounced
		Linseed oil	Yellowish oily liquid	As supplied	None
		Pine tar	Black, tarry, viscous liquid	Without diluent	Fairly great
		Spindle oil: R	Fairly thin fluorescent oil	Without diluent	Moderate
		S	Fairly thin fluorescent oil	Without diluent	Moderate
		T	Fairly thin fluorescent oil	Without diluent	Moderate
		U	Fairly thin fluorescent oil	Without diluent	Moderate
		V	Fairly thin fluorescent oil	Without diluent	Fairly great
		W	Fairly thin fluorescent oil	Without diluent	Moderate

Every animal was identified with a label and number, and records of oil applications, deaths, etc., were kept throughout.

Experimental technique

The applications of the oils were made to the skin in the interscapular area after removing the hair with dilute sodium sulphide some days before commencing the paintings. The oils were applied from bottles by means of short glass rods. An area about $1\frac{1}{2}$ cm. in diameter was covered, and the amount used 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, and on the death of any animal the skin from the treated area was removed for microscopic examination from all those which had survived a period of at least 12 weeks.

RESULTS

The number of animals surviving for 25 weeks or more and the total tumours produced by the various fractions in the experiments are given in Tables 2 and 3. Fuller analysis of the results by statistical methods is given on pp. 128–132 of this

Table 2

First Series

	1st experiment				2nd experiment			
Fraction	Animals surviving 25 weeks*	Papillomas 50 weeks	Carcinomas period	Total tumours	Animals surviving 25 weeks	Papillomas 50 weeks	Carcinomas period	Total tumours
B	32	1	1	2	22	2	1	3
D	15	2	1	3	12	1	3	4
E	35	2	0	2	22	1	2	3
F	12–62	2	4	6	15	5	3	8
G	23	2	0	2	12	2	0	2
H	26	3	4	7	16	3	5	8
I	29	2	6	8	15	5	3	8
K	20–70	4	4	8	20–60	5	3	8
L	30–60	2	1	3	20	3	1	4
M	30	0	0	0	20	1	0	1

* Survivors out of 50 unless otherwise stated.

Table 3

Second Series

Fraction	Animals surviving 25 weeks	Papillomas	Carcinomas	Total tumours
Spindle oil: R	19	3	3	6
S	21	7	2	9
T	25	2	2	4
U	16	3	3	6
V	15	1	2	3
W	19	1	0	1
Anthracene oil	20	8	6	14
Creosote oil	19	10	9	19
Pine oil	18	0	0	0
Linseed oil	44	0	0	0

communication. Two illustrative samples of the data from which these statistical calculations were made are also set out in Tables 4 and 5 (Exp. 1, Fraction K, a moderately carcinogenic sample; and Exp. 1, Fraction L, a weak carcinogen).

Table 4. *Fraction K—Exp.* 1

Weeks	Survivors (out of 50)	Survivors with tumours	% survivors with tumours	New tumours in previous week
12	45	—	—	—
13	44	—	—	—
14	44	—	—	—
15	42	—	—	—
16	39	—	—	—
17	38	—	—	—
18	36	—	—	—
19	36	—	—	—
20	34	2	5·9	—
21	31	3	9·7	2
22	29	4	13·8	1
23	29	4	13·8	1
24	28	5	17·8	—
25	26	6	23·1	1
26	26	6	23·1	1
27	25	7	28·0	—
28	25	7	28·0	1
29	25	7	28·0	—
30	25	7	28·0	—
31	25	7	28·0	—
32	25	7	28·0	—
33	24	7	29·2	—
34	23	7	30·4	—
35	23	7	30·4	—
36	23	7	30·4	—
37	23	7	30·4	—
38	23	7	30·4	—
39	23	7	30·4	—
40	23	6	26·1	—
41	22	6	27·3	—
42	21	5	23·8	—
43	20	5	25·0	—
44	18	5	27·8	—
45	17	4	23·5	—
46	15	4	26·6	—
47	14	3	21·4	—
48	13	3	23·1	—
49	12	2	16·6	—
50	11	2	18·2	—

A relatively small number of tumours was obtained in these tests, the highest incidence being eight in fractions F, H, I and K. Fraction M yielded only one papilloma in the second test. It is of interest that the white oils mentioned previously produced no tumours, and a similar negative result was obtained with the pine oil and the linseed oil. The creosote oil, anthracene oil and the spindle oils all had definite carcinogenic action, producing large keratinizing carcinomas.

Table 5. *Fraction L—Exp.* 1

Weeks	Survivors (out of 60)	Survivors with tumours	% survivors with tumours	New tumours in previous week
12	53	—	—	—
13	53	—	—	—
14	53	—	—	—
15	53	—	—	—
16	51	—	—	—
17	50	—	—	—
18	48	—	—	—
19	48	—	—	—
20	45	—	—	—
21	44	—	—	—
22	41	—	—	—
23	36	—	—	—
24	33	—	—	—
25	32	—	—	—
26	32	—	—	—
27	31	—	—	—
28	31	—	—	—
29	30	—	—	—
30	30	—	—	—
31	30	—	—	—
32	30	—	—	—
33	28	—	—	—
34	28	1	3·6	—
35	28	1	3·6	1
36	28	1	3·6	—
37	26	1	3·6	—
38	25	2	8·0	—
39	25	2	8·0	1
40	24	3	12·5	—
41	22	3	13·6	1
42	19	3	15·8	—
43	17	3	17·7	—
44	17	3	17·7	—
45	17	3	17·7	—
46	14	3	21·4	—
47	13	3	23·1	—
48	12	2	16·6	—
49	9	1	11·1	—
50	6	1	16·6	—

DISCUSSION

The question of a suitable and reliable method of interpreting the results of experiments employing external applications of either solutions of pure chemicals in a bland vehicle, or, as in these tests, of mixtures of ill-defined composition containing many unknown constituents, has presented difficulty to all workers in this field.

In view of the many factors involved, some of which can be controlled only partially, it is improbable that a grading of cancer-producing potency can be made to a fine degree of accuracy. There is no necessity to dwell upon these factors except to mention some of the more obvious, e.g. the strain, age, sex and general health of the animal, the site, area and frequency of application, the action of other substances in depressing the activity either mechanically or physiologically, or in 'enhancing' the normal activity as exemplified by the action of croton oil following benzpyrene applications.

For assessing carcinogenicity, the time intervals for the appearance of the first and subsequent macroscopic tumours and the total number of induced tumours, benign and malignant, are the chief data which must be considered. The effect of differential survival rates among tumourless animals should, however, always be considered.

A number of methods of computing 'carcinogenic activity' have been suggested. Twort & Twort (1931, 1933) discussed many of these problems and devised methods by which a relative index was calculated for oils of widely different activity, such as mineral oils producing only one benign tumour in 100 mice to those producing tumours in over 50 % of the animals with similar experimental treatment. An endeavour was made to allow for specially sensitive or resistant animals and for mortality among animals with or without tumours.

They outlined three methods, of which the simplest was based on the 'addition cumulative tumour frequency'. In this, animals which die without tumours are given a hypothetical tumour according to the ratio of tumour-bearing survivors at a particular week to those already dead without tumours.

A more statistical approach was made by Moseley (*A Scheme for Recording the Potency of Carcinogenic Agents*), in which the 'mean induction time', and the standard errors attached, were computed with the object of comparing the activity of different agents.

Berenblum (1945) also described a system for grading carcinogenic potency in which the whole range of activity between such compounds as 9:10-dimethyl-1:2-benzanthracene, which will induce tumours when applied in 0·1 % solution for a few weeks, and feebly active types requiring 50 or more weeks for wart production is divided into twelve grades. The index is obtained by calculating the time to the nearest week at which 50 % of the animals have visible tumours taking into account the survival rate. An approximation, useful for experiments in which a total of 50 % tumour-bearing animals is never obtained, was also suggested on the basis that the time to reach the 50 % level is about twice that required for the appearance of the first tumour. While this assumption may be valid for pure substances applied to pure strains of animals, it is not so in our experience for oils, etc., and Twort & Twort (1933) gave data to illustrate 'the relatively slight value of the time of arrival of the first tumour'. Berenblum also states 'it is generally agreed that variations in the concentration of the carcinogen within the limits of 0·3–1·0 % have relatively little effect on the neoplastic response, and that difference in response due to frequency of application are not great provided the frequency is not less than once per week or more than three times'. This also is not in accord with the early workers (Twort & Twort, 1931), who abandoned twice-weekly paintings in favour of daily applications (five times weekly), but we consider that the latter routine is impractical when using substances of a toxic character, and that the twice-weekly routine should be utilized wherever possible in order to afford comparison between experiments in different laboratories.

Irwin & Goodman (1945–6) published a detailed analysis of Twort & Twort's data and stressed the importance of expectation of tumourless life as a measure of carcinogenic response.

CONCLUSIONS

From a general study of the data in Table 2 and the 'life sheets' as exemplified in Tables 3 and 4 it is possible to draw some general conclusions.

(1) There was a fair agreement in the results in the two series, though some distinct deviations occurred.

(2) On the whole the survival rate in Exp. 2 was consistently less than in Exp. 1.

(3) There appeared to be a slightly greater tumour response in many tests in Exp. 2. The reason for this is difficult to discover, but may be due, for instance, to heavier dosage applied in this period. This might also be correlated with the higher mortality.

(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.

These views are confirmed by the statistical analysis which, however, also affords some further information. A point of interest concerns whether early results can be relied upon to give a reasonably good indication of the final potency. The data of Table 10 of Dr Irwin's analysis support the belief that a preliminary idea of the final value can be obtained from the period at which 10 % of the surviving animals bear tumours.

(5) The investigation indicates that a general estimate of the carcinogenic properties can be obtained with the number of animals employed, but that fine degrees of difference cannot be detected in this type of material.

For reliable comparison of certain carcinogenic potencies, fractions should always be compared in a concurrent experiment carried out on randomized batches of mice, the paintings of the whole test to be carried out by a single individual on any one day. Under these conditions it appears possible to achieve reproducible results.

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A STATISTICAL ANALYSIS OF THE RESULTS

By J. O. IRWIN

Table 6 shows the numbers of survivors at 25 weeks per 50 animals tested. The average survival time was shorter in Exp. 2 than in Exp. 1, but there are no significant differences between fractions.

Tables 7, 8 and 9 show respectively the numbers of papillomas, carcinomas and total tumours per 50 animals tested.

Table 6. *Survivors at 25 weeks*

Fraction	Exp. 1	Exp. 2	Mean	Standard error*
B	32	22	27·0	
D	15	12	13·5	
E	35	22	28·5	
F	10	15	12·5	
G	23	10	16·5	
H	26	16	21·0	3·7
I	29	15	22·0	
K	14	20	17·0	
L	25	20	22·5	
M	30	20	25·0	
Mean	23·9	17·2		
Standard error	1·6	1·6		

Analysis of variance

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Experiments	224·45	1	224·45	8·4 sig. at 5 %
Fractions	548·45	9	60·94	2·3 n.s.
Error	240·05	9	26·67	
Total	1012·95	19		

* In this and the subsequent tables, the standard errors are derived from the error term in the analysis of variance, e.g. $1{\cdot}6 = \sqrt{(26{\cdot}67/10)}$.

Table 7. *Papillomas (per 50 animals)*

Fraction	Exp. 1	Exp. 2	Mean	Standard error
B	1	2	1·5	
D	2	1	1·5	
E	2	1	1·5	
F	2	5	3·5	
G	2	2	2·0	
H	3	3	3·0	0·7
I	2	5	3·5	
K	3	5	4·0	
L	2	4	2·5	
M	0	1	0·5	
	19	29		
Mean	1·9	2·8		
Standard error	0·3	0·3		

Analysis of variance

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Experiments	4·05	1	4·05	3·86 n.s.
Fractions	23·05	9	2·56	2·44 n.s.
Error	9·45	9	1·05	
Total	36·55	19		

Apparent order of carcinogenicity: K I F H, L G B D E, M

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Table 8. *Carcinomas* (*per* 50 *animals*)

Fractions	Exp. 1	Exp. 2	Mean	Standard error
B	1	1	1·0	
D	1	3	2·0	
E	0	2	1·0	
F	3	3	3·0	
G	0	0	0·0	0·7
H	4	5	4·5	
I	6	3	4·5	
K	3	3	3·0	
L	1	1	1·0	
M	0	0	0·0	
Mean	1·9	2·1		
Standard error	0·3	0·3		

Analysis of variance

	Sum of squares	Degree of freedom	Mean square	Variance ratio
Experiments	0·2	1	0·20	n.s.
Fractions	51·0	9	5·67	5·7 sig. at 1 %
Error	8·8	9	0·98	
Total	60·0	19		

Apparent order of carcinogenicity: H I K F, D B E L, G M

Table 9. *Total tumours* (*per* 50 *animals*)

Fraction	Exp. 1	Exp. 2	Mean	Standard error
B	2	3	2·5	
D	3	4	3·5	
E	2	3	2·5	
F	5	8	6·5	
G	2	2	2·0	1·3
H	7	8	7·5	
I	8	8	8·0	
K	6	8	7·0	
L	3	4	3·5	
M	0	1	0·5	
Mean	3·8	4·9		
Standard error	0·2	0·2		

Analysis of variance

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Experiments	6·05	1	6·05	16 sig. at 1 %
Fractions	127·05	9	14·12	37 sig. at 0·1 %
Error	3·45	9	0·38	
Total	136·55	19		

Apparent order of carcinogenicity: I H K F, D L B E G, M

For *papillomas* there are no significant differences either between experiments or between fractions, but the apparent order of activity in producing them—K I F H, L G B D E, M—is almost the same as for carcinomas, and the mean number of papillomas for Exp. 2 is somewhat higher than for Exp. 1.

The *mean number of carcinomas* is also higher in Exp. 2, though not significantly; but in this case there *are* significant differences between fractions in carcinogenicity. The order is H I K F, D B E L G M, and the first four and the next six of these could perhaps be grouped together as of roughly equal carcinogenic power.

When *total tumours* are analysed collectively there are significant differences both between experiments and between fractions. The order of carcinogenicity is I H K F, D L B E G, M. The mean number of tumours is significantly higher in Exp. 2.

Table 10. *Date when* 10 % *of survivors had tumours*

Fraction	Exp. 1	Exp. 2	Mean	Standard error
B	30·6 (5·6)*	27·8 (1·4)	29·2	
D	30·2 (0·5)	23·6 (0·6)	26·9	
E	43·0 (6·4)	39·2 (1·0)	41·1	
F	20·2 (0·8)	21·9 (0·8)	21·1	
G	35·0 (6·7)	27·2 (1·0)	31·1	1·8
H	21·1 (5·3)	21·2 (0·6)	21·2	
I	23·0 (0·8)	22·4 (0·4)	22·7	
K	16·2 (4·4)	16·9 (4·9)	16·6	
L	39·2 (1·3)	31·4 (0·6)	35·3	
Mean	28·4	25·7		
Standard error	0·8	0·8		

* Figures in brackets are standard errors calculated from internal evidence.

Analysis of variance

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Experiments	40·20	1	40·20	6·5 sig. at 5 %
Fractions	972·43	8	121·55	20 sig. at 0·1 %
Error	55·44	8	6·16	
Total	1068·07	17		

Apparent order of carcinogenicity: K, F H I, D B G L E, M.

Table 10 shows *the dates when* 10 % *of survivors had tumours*. Fraction M gave only one papilloma, in Exp. 2. Consequently for this fraction the date was never reached, and the statistical analysis given is for the other nine fractions. Exp. 2 gave an earlier date than Exp. 1, and the differences between the different fractions are significant. The order of carcinogenic activity is K, F H I, D B G L E, M, four groups being distinguishable. The standard error of each determination was calculated from internal evidence, i.e. the square root of the average error variance is 3·1, while from the analysis of variance the corresponding value is 2·6.

Table 11 shows *the expectation of tumourless life* limited to 50 weeks. Exp. 2 gave a lower expectation than Exp. 1, and the differences between the different fractions are significant. The order of carcinogenic activity is F H I K, D L G, B E, M, four

Table 11. *Expectation of tumourless life* (*limited to* 50 *weeks*)

Fraction	Exp. 1	Exp. 2	Mean	Standard error
B	48·15 (1·29)*	47·89 (1·20)	48·02	
D	45·71 (2·28)	46·09 (1·91)	45·90	
E	49·06 (0·67)	48·09 (1·08)	48·58	
F	40·35 (3·46)	40·60 (3·18)	40·48	
G	48·42 (1·10)	46·17 (2·52)	47·30	
H	43·49 (2·21)	39·59 (2·99)	41·54	0·66
I	42·63 (2·27)	41·18 (2·41)	41·90	
K	44·69 (0·86)	43·66 (1·85)	44·18	
L	48·49 (2·05)	45·90 (2·27)	47·20	
M	50·00 —	48·87 (1·12)	49·44	
Mean	46·10	44·80		
Standard error	0·29	0·29		

* Figures in brackets are standard errors calculated from internal evidence.

Analysis of variance

	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Experiments	8·385	1	8·39	9·5 sig. at 5 %
Fractions	186·267	9	20·70	24 sig. at 0·1 %
Error	7·933	9	0·88	
Total	202·585	19		

Apparent order of carcinogenicity: F H I K, D L G, B E, M.

groups being distinguishable. The standard error of each determination was again calculated from internal evidence. The average value is 2·09, while from the analysis of variance the corresponding figure is 0·94. The latter value, if standard tables are used, is significantly lower than the former at the 5 % level; this may be due to the fact that, the expectations being limited, the error distribution is truncated at the upper end. If the larger standard error is used, B and E must be grouped together with D L G.

The types of data used in Tables 10 and 11, which take account of mortality, are for that reason preferable to those used in Tables 7, 8 and 9.

SUMMARY

Woodhouse's experiments on the carcinogenicity of ten 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 2nd series of experiments.

(2) There were significant differences in carcinogenicity. The fractions can be divided into three groups within which the carcinogenicities were about the same, viz. F H I K, B D G L E, M.

APPENDIX

Calculation of the standard error of the estimate of the date when a given percentage (say 10 %) of surviving animals have tumours

Suppose we have the percentage of survivors with tumours worked out at the end of weeks 0, 1, 2, 3, ..., etc. We can take the first date after this passes 10 % and determine the 10 % date from that value and previous weekly values. The most practical method is to fit a straight line to as many values as appear by eye to be linear.

There will often be only 2 and rarely more than 4. Fitting by a standard regression technique may then be used. If the fitted line is

$$Y = \bar{y} + b(t - \bar{t}), \tag{1}$$

when $y = \%$ of surviving animals with tumours, then the 10 % date is

$$M = \bar{t} + \frac{10 - \bar{y}}{b}. \tag{2}$$

But it is clearly wrong to apply the ordinary formulae to obtain the standard error of (4) because the times t are not being kept fixed in sampling. The y's are being kept fixed in the neighbourhood of 10 %, apart from sampling errors. We may take

$$V(\bar{y}) = \frac{\bar{y}(100 - \bar{y})}{n},$$

where n is the mean number of surviving animals.

Approximations to the standard error of b can be obtained in various ways, but it has been found that it is good enough to estimate $V(b)$ in the usual manner from deviations from regression, e.g. to take

$$V(b) = \frac{s^2}{S(t - \bar{t})^2},$$

and where k is the number of points used

$$(k-2)\, s^2 = S(y - Y)^2 = S(y - \bar{y})^2 - \frac{S^2\{y(t - \bar{t})\}}{S(t - \bar{t})^2} \quad \text{for} \quad k > 2.$$

When $k = 2$ we may take

$$V(b) = \frac{P(100 - P)}{n},$$

where P is the percentage of surviving animals which get tumours in the week in question.

The standard errors of the 10 % date were calculated in this way for Woodhouse's experiments, using

$$V(M) = \frac{V(\bar{y})}{b^2} + \frac{(10 - y)^2\, V(b)}{b^4}. \tag{3}$$

That this method is substantially correct is shown by the agreement between the average standard errors finally obtained and the result given by an analysis of variance.

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