FINAL ANALYSIS

EXPERIMENT 1.1.1.9

THE EFFECT OF STOPPING PAINTING

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1. OBJECTIVES OF THE EXPERIMENT

1.01 In J1310 the objective of Experiment 1.1.1.9 were laid down as follows:-

1. To determine whether the effect of stopping painting is one of the following possibilities:-

(a) an immediate reduction of the incidence rate for the treated animals to a rate equal to that for the untreated animals.

(b) an immediate reduction of the incidence rate for the treated animals but the reduction is not as great as in 1 (a) .

(c) no change of the incidence rate from the point where painting was stopped.

(d) an increase in incidence rate but not as great as would have occurred if painting had continued.

(e) no effect of stopping, i.e. the incidence rate continues to rise exactly as if painting had continued.

(f) any, of the effects a to d but delayed by a latent period of magnitude to be determined.

2. To provide data to examine the hypothesis put forward in J1058 for a multi-stage mechanism for mouse skin carcinogenesis.

1.02 A further unstated objective was to try to determine the relationship between malignancy and tumour size and tumour growth rate.

2. DESCRIPTION OF THE EXPERIMENT

2.01 The experimental structure was as follows:

Treatment	Dose per week	Duration of painting	Number of mice
S.W.S.	180 mg	0-10 weeks	75
(stale whole smoke	180 mg	0-20 weeks	75
condensate from	180 mg	0-30 weeks	75
normal flue cured	180 mg	0-40 weeks	75
cigarette T57)	180 mg	0-50 weeks	75
	180 mg	Painted for life	75
G T57	600 mg	0-10 weeks	75
(Fraction G	600 mg	0-20 weeks	75
from T57)	600 mg	0-30 weeks	75
	600 mg	0-40 weeks	75
	600 mg	0-50 weeks	75
	600 mg	Painted for life	75
BP	36 µg	0-15 weeks	75
(Benzo(a)pyrene)	36 µg	0-25 weeks	75
	36 µg	0-35 weeks	75
	36 µg	Painted for life	75
	60 µg	0-15 weeks	75
	60 µg	0-25 weeks	75
	60 µg	0-35 weeks	75
	60 µg	Painted for life	75
G T57	600 mg	10-50 weeks	75
(Ageing groups)	600 mg	20-60 weeks	75
	600 mg	30-70 weeks	75
Untreated	-	-	102
Solvent	-	Painted for life	102

The animals were aged 10 weeks at the start of the experiment (week 0)

2.02 The mice were painted three times a week, on Monday, Wednesday, and Friday.

2.03 The solvent used alone and with S.W.S. and G was iso-propyl alcohol (I.P.A.).

2.04 The first four sets of groups were designed to measure the change in tumour rate with increasing duration of painting.

2.05 The fifth set of groups was designed to test how the tumour rate due to 40 weeks painting of Fraction G depended on the age at which treatment was started. For this purpose the G 0-40 weeks group from the second set can be used as an additional group in this set.

2.06 The experimental groups used 1929 mice, all kept in one room (the same room as for 1.1.3.29) with three mice in a cage, and the cages randomized over the batteries. The experiment ran until the last mouse died. (week 112).

3. TUMOUR INFORMATION RECORDED

3.01 In all previous experiments carried out in Harrogate the information recorded relevant to analysis of tumour rates has basically consisted of only four figures per mouse.

1. The time of appearance of the first tumour.
2. The number of tumours.
3. The tumour classification of the most malignant of the tumours - papilloma, carcinoma, infiltrating carcinoma or sarcoma.
4. Whether complete regression occurred.

Analysis normally ignored 2. and 4.

3.02 In this experiment a far more detailed recording system was used. There were four tumour sizes measured by calipers as follows:-

A. Tumour of at least 2 mm.

B. Tumour of at least 6 mm.

C. Tumour of 10 mm.

D. Tumour greater than 10 mm.

3.03 For each tumour on each mouse measurements were made weekly and the date at which each successive size was reached was recorded on the card.

3.04 If a tumour reached a given size but later regressed the date at which it no longer satisfied the criteria for that size was noted.

3.05 If two or more tumours became indistinguishable this was also noted and the combined tumour was considered to be one tumour subsequently.

4.1 UNSTANDARDISED AND STANDARDISED PERCENTAGES

4.1.1 Tables 1 to 4 give, for each treatment group by 16 week intervals, unstandardised and standardised percentages of tumour and infiltrating carcinoma bearing animals.

4.1.2 The standardised percentages are not used in any subsequent analysis and are presented solely to enable easy comparison to be made with previous Final Analyses.

4.1.3 The classification "tumour bearing animals" was based on those animals which ever had a recorded tumour greater than 2 mm. and is approximately comparable with the old standard classification.

4.1.4 The classification "infiltrating carcinoma bearing animals" is identical to that always used in the past and is independent of tumour size.

TABLE 1

UNSTANDARDISED PERCENTAGES

Tumour Bearing Animals

1.1.1.9.

	Week 32	48	64	80	96	FINAL
S.W.S. T57 180 mgs.						
0 - 10 weeks	0.0	2.7	5.3	9.3	10.7	12.0
0 - 20 weeks	0.0	4.0	8.0	10.7	12.0	12.0
0 - 30 weeks	1.3	9.3	10.7	14.7	17.3	18.7
0 - 40 weeks	1.3	10.7	16.0	17.3	18.7	18.7
0 - 50 weeks	1.3	13.3	22.7	34.7	37.3	37.3
Painted for Life	2.6	13.3	29.3	40.0	42.7	44.0
G T57 600 mgs.						
0 - 10 weeks	0.0	2.7	8.0	10.7	14.7	14.7
0 - 20 weeks	2.7	12.0	16.0	21.3	22.7	22.7
0 - 30 weeks	5.3	8.0	8.0	14.7	16.0	16.0
0 - 40 weeks	6.7	20.0	26.7	33.3	38.7	38.7
0 - 50 weeks	6.7	29.3	38.7	45.3	46.7	48.0
Painted for Life	4.0	29.3	49.3	57.3	61.3	61.3
B.P. 36 µgs.						
0 - 15 weeks	2.7	17.3	29.3	40.0	44.0	44.0
0 - 25 weeks	2.7	21.3	49.3	57.3	60.0	60.0
0 - 35 weeks	2.7	26.7	57.3	68.0	68.0	68.0
Painted for Life	5.3	44.0	76.0	77.3	77.3	77.3
B.P. 60 µgs.						
0 - 15 weeks	5.3	29.3	44.0	50.7	54.7	54.7
0 - 25 weeks	10.7	65.3	82.7	88.0	88.0	88.0
0 - 35 weeks	29.3	84.0	89.3	89.3	90.7	90.7
Painted for Life	24.0	82.7	86.7	86.7	86.7	86.7
G T57 600 mgs.						
10 - 50 weeks	0.0	8.0	20.0	26.7	29.3	29.3
20 - 60 weeks	0.0	1.3	5.3	9.3	13.3	14.7
30 - 70 weeks	0.0	1.3	4.0	10.7	12.0	13.3
UNTREATED	0.0	0.0	0.0	0.0	0.0	2.0
SOLVENT	0.0	0.0	1.0	1.0	2.0	2.0

TABLE 2

STANDARDISED PERCENTAGES

Tumour Bearing Animals

1.1.1.9.

	Week					
	32	48	64	80	96	FINAL
S.W.S. T57 180 mgs.						
0 - 10 weeks	0.0	2.5	4.9	9.1	10.9	12.9
0 - 20 weeks	0.0	4.0	8.0	10.5	12.0	12.0
0 - 30 weeks	1.3	9.3	10.7	14.9	17.7	19.5
0 - 40 weeks	1.3	10.5	15.9	18.1	20.4	20.4
0 - 50 weeks	1.3	12.7	20.9	31.2	33.7	33.7
Painted for Life	2.8	13.7	30.9	43.2	48.0	49.6
G T57 600 mgs.						
0 - 10 weeks	0.0	2.5	7.6	9.9	14.3	14.3
0 - 20 weeks	2.7	11.2	14.7	19.2	21.5	21.5
0 - 30 weeks	5.2	7.7	7.7	14.8	16.4	16.4
0 - 40 weeks	6.7	19.9	26.1	33.2	39.7	39.7
0 - 50 weeks	6.3	27.7	36.5	42.9	45.1	46.5
Painted for Life	3.9	28.3	50.0	60.0	64.7	64.7
B.P. 36 µgs.						
0 - 15 weeks	2.5	16.1	26.1	35.2	40.1	40.1
0 - 25 weeks	2.8	21.1	48.7	57.5	62.8	62.8
0 - 35 weeks	2.5	24.8	50.7	63.5	63.5	63.5
Painted for Life	5.3	42.0	72.1	76.0	76.0	76.0
B.P. 60 µgs.						
0 - 15 weeks	5.2	27.7	42.1	49.5	54.3	54.3
0 - 25 weeks	10.7	63.5	77.9	81.6	81.6	81.6
0 - 35 weeks	28.8	80.9	85.6	85.6	86.1	86.1
Painted for Life	24.0	84.1	88.1	88.1	88.1	88.1
G T57 600 mgs.						
10 - 50 weeks	0.0	7.3	18.0	23.3	25.5	25.5
20 - 60 weeks	0.0	1.2	4.7	8.4	12.9	14.5
30 - 70 weeks	0.0	1.2	3.9	10.9	12.4	16.3
UNTREATED	0.0	0.0	0.0	0.0	0.0	2.7
SOLVENT	0.0	0.0	0.8	0.8	1.8	1.8

TABLE 3

UNSTANDARDISED PERCENTAGES

INFILTRATING CARCINOMA BEARING ANIMALS

1.1.1.9.

	Week					
	32	48	64	80	96	FINAL
SWS. T57 180mgs.						
0 - 10 weeks	0.0	0.0	1.3	1.3	1.3	1.3
0 - 20 weeks	0.0	0.0	0.0	0.0	0.0	0.0
0 - 30 weeks	0.0	0.0	1.3	2.7	4.0	4.0
0 - 40 weeks	0.0	0.0	1.3	2.7	2.7	2.7
0 - 50 weeks	0.0	0.0	4.0	10.7	16.0	16.0
Painted for Life	0.0	2.7	5.3	10.7	13.3	16.0
G T57 600mgs.						
0 - 10 weeks	0.0	0.0	0.0	0.0	0.0	0.0
0 - 20 weeks	0.0	0.0	1.3	2.7	2.7	2.7
0 - 30 weeks	0.0	0.0	1.3	2.7	2.7	2.7
0 - 40 weeks	0.0	1.3	2.7	4.0	5.3	5.3
0 - 50 weeks	0.0	0.0	4.0	12.0	13.3	14.7
Painted for Life	0.0	1.3	5.3	21.3	25.3	25.3
B.P. 36 ugs.						
0 - 15 weeks	1.3	5.3	12.0	14.7	16.0	16.0
0 - 25 weeks	1.3	6.7	16.0	29.3	33.3	33.3
0 - 35 weeks	0.0	1.3	21.3	46.7	50.7	52.0
Painted for Life	1.3	8.0	46.7	64.0	68.0	68.0
B.P. 60 ugs.						
0 - 15 weeks	1.3	2.7	14.7	25.3	28.0	28.0
0 - 25 weeks	0.0	10.7	57.3	69.3	70.7	70.7
0 - 35 weeks	2.7	36.0	62.7	64.0	64.0	64.0
Painted for Life	1.3	37.3	73.3	76.0	76.0	76.0
GT57 600mgs.						
10 - 50 weeks	0.0	0.0	2.7	6.7	8.0	8.0
20 - 60 weeks	0.0	0.0	0.0	1.3	4.0	4.0
30 - 70 weeks	0.0	0.0	0.0	0.0	0.0	0.0
UNTREATED	0.0	0.0	0.0	0.0	0.0	0.0
SOLVENT	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 4

STANDARDISED PERCENTAGES

INFILTRATING CARCINOMA BEARING ANIMALS

1.1.1.9.

	Week					
	32	48	64	80	96	FINAL
SWS. T57 180mgs						
0 - 10 weeks	0.0	0.0	1.2	1.2	1.2	1.2
0 - 20 weeks	0.0	0.0	0.0	0.0	0.0	0.0
0 - 30 weeks	0.0	0.0	1.3	2.7	4.1	4.1
0 - 40 weeks	0.0	0.0	1.3	2.8	2.8	2.8
0 - 50 weeks	0.0	0.0	3.6	9.7	15.2	15.2
Painted for Life	0.0	2.8	5.7	12.0	15.9	19.9
G T57 600 mgs.						
0 - 10 weeks	0.0	0.0	0.0	0.0	0.0	0.0
0 - 20 weeks	0.0	0.0	1.2	2.7	2.7	2.7
0 - 30 weeks	0.0	0.0	1.3	2.5	2.5	2.5
0 - 40 weeks	0.0	1.2	2.5	3.9	5.3	5.3
0 - 50 weeks	0.0	0.0	3.9	11.7	13.6	15.3
Painted for Life	0.0	1.3	5.1	23.7	28.1	28.1
B.P. 36 ugs.						
0 - 15 weeks	1.3	4.9	10.7	13.7	15.6	15.6
0 - 25 weeks	1.3	6.5	15.6	29.1	35.2	35.2
0 - 35 weeks	0.0	1.2	17.9	42.9	48.5	49.3
Painted for Life	1.3	7.6	42.8	58.5	60.9	60.9
B.P. 60 ugs.						
0 - 15 weeks	1.3	2.5	14.3	26.1	29.2	29.2
0 - 25 weeks	0.0	10.3	50.3	59.1	61.2	61.2
0 - 35 weeks	2.7	35.6	67.7	70.5	70.5	70.5
Painted for Life	1.3	38.9	75.3	77.6	77.6	77.6
G T57 600 mgs.						
10 - 50 weeks	0.0	0.0	2.4	5.6	6.7	6.7
20 - 60 weeks	0.0	0.0	0.0	1.3	3.9	3.9
30 - 70 weeks	0.0	0.0	0.0	0.0	0.0	0.0
UNTREATED	0.0	0.0	0.0	0.0	0.0	0.0
SOLVENT	0.0	0.0	0.0	0.0	0.0	0.0

4.2 A SIMPLE ANALYSIS OF THE EFFECT OF STOPPING PAINTING

4.2.1 One conceptually simple approach to the problem of assessing the effect of stopping painting is as follows.

Firstly, fit a distribution of time to tumour to the continuously painted time periods. Secondly, compare the incidence rates observed after stopping with those expected if the fitted distribution were extrapolated according to the various hypotheses between which one is trying to discriminate. Although this method of analysis is not fully efficient, it was carried out as a first step as it is reasonably simple to compute.

4.2.2 Analysis was restricted to tumour sizes A and D as it was felt that little extra would be gained by a separate consideration of the intermediate sizes.

4.2.3 Thus, for each of these tumour sizes, a Weibull distribution was fitted assuming that groups which had the same treatment but stopped at different times could be described by the same distribution as long as painting was continued. Three distributions were therefore fitted per tumour size, one to the 6 S.W.S. groups, one to the 6 G groups and one to the 8 BP groups. For the last distribution k and w but not b was assumed to be the same for the two dose levels. k was taken as 3 for S.W.S. and G, and as 4 for BP, since analysis in which k was unrestricted proved not to be any better fit to the data.

4.2.4. Three hypotheses were tested to describe the distribution of time to tumour after stopping painting. They were as follows:

4.2.5 1) No effect of stopping i.e. the distribution fitted to the continuously painted time period was assumed to hold for the whole experiment.

It is in fact clear from the relation between total percentage of tumour bearing animals and total length of time painted that stopping painting reduces the yield of tumours. However, this hypothesis was tested here to quantify the effect of stopping.

4.2.6 2) The tumour rate remains constant w weeks after stopping. This is an amended version of the Doll hypothesis. One interpretation of the fact that a Weibull distribution with parameter w fits is that tumours appearing at time t really represent an effect at time t - w. If this were so then clearly no effect would be seen until at least w weeks after stopping.

4.2.7 3) The tumour rate remains constant at the time of stopping painting. This is the pure Doll hypothesis. From the fact that the groups painted for only 10 weeks had zero tumour rate at 10 weeks and yet had an overall tumour yield far in excess of the untreated controls, this hypothesis is clearly not true. However, again, it serves as a baseline for comparison.

4.2.8 Tables 5 to 12 give details of the number of tumours observed in the periods after stopping by 10 week intervals and those expected under the three hypotheses, Exp.1, Exp.2 and Exp. 3. Tables 5 to 8, which are for tumour size A, relate to S.W.S., G., BP 36μg. and BP 60μg. respectively. Tables 9 to 12 are similar, for tumour size D. Details of the methods of calculations of the expected numbers of tumours are given in the Statistical Appendix (section 7(a)).

4.2.9 From an inspection of these Tables it is quite clear that none of these hypotheses fit the data at all adequately. However, a comparison of the observeds and expecteds affords a useful quantitative assessment of the

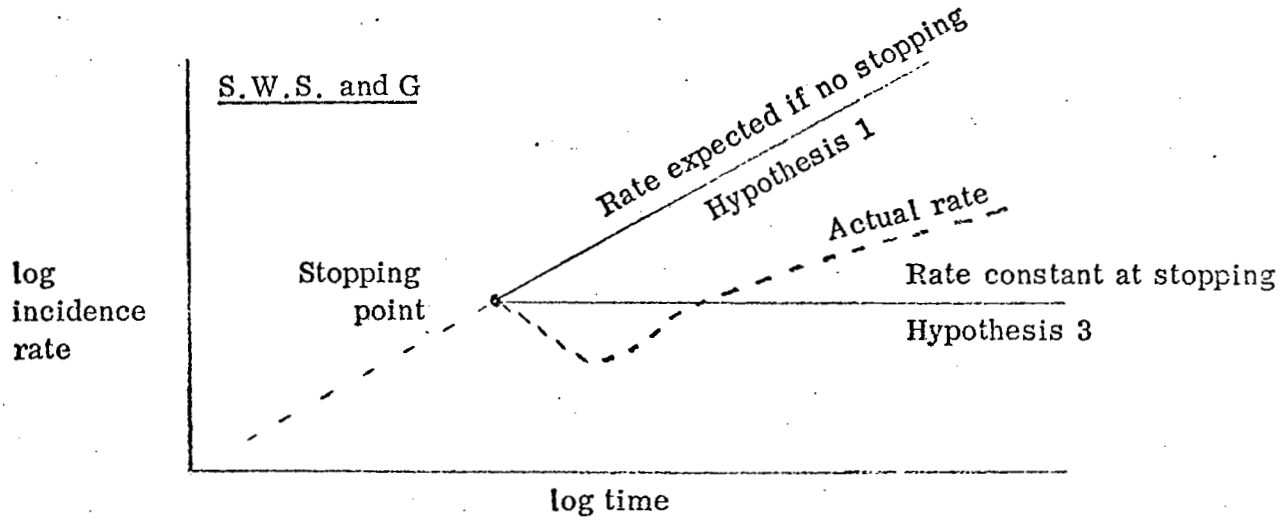
effect of stopping painting.

4.2.10 For tumours of size A the effect of stopping differs between the smoke-derived treatments and benzo(a)pyrene.

4.2.11 For S.W.S. and G there is a marked drop off in response evident in the first 10 weeks after stopping. In this period in both treatments taken together, 21 new animals with tumours were observed as against 59.4 expected if the rate had continued to rise as expected and 39.9 if it had stayed constant after stopping. Thus there is evidence of an immediate reduction in rate on stopping treatment.

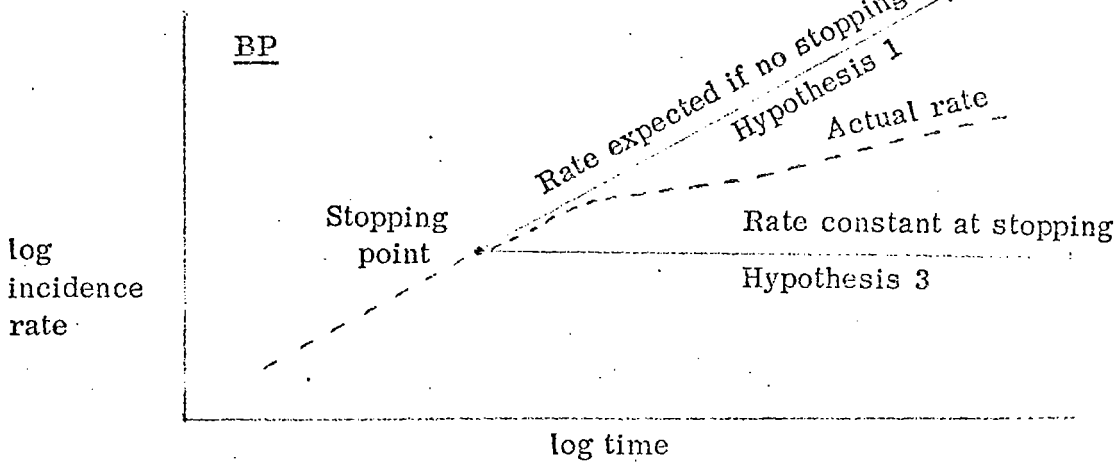
Later, the response, although always vastly less than it would have been had treatment not stopped, does in fact rise and towards the end of the experiment significantly exceeds that expected under hypotheses 2 or 3.

Graphically the picture can be represented approximately as follows:



4.2.12 For BP, on the other hand, the rate continues to rise for the first 10 weeks much as if painting had been continued (Obs. = 74 Exp. = 86.6). Only then is the effect of stopping seen. Although the rate continues to rise steadily it becomes of the order of a hundred times less than it would have been had painting been continued. Here the picture looks some-

thing like



4.2.13 The pattern for tumour size D is not so clear, especially for S.W.S. and G where numbers are small, but seems slightly different.

4.2.14 For S.W.S. there is no evidence of any drop off in rate after 20 weeks. (Period S+11 to S+20 Obs. = 5 Exp. = 5.9). For G on the other hand there may be a slightly earlier drop off (Obs. = 7, Exp. = 14.7) although this is of dubious significance. After that, rates drop off markedly and incidence is very sparse.

4.2.15 For BP the pattern is fairly much the same as for tumour size A . There is however some evidence of a drop off in the first 10 weeks after stopping for 60μg. (Obs. = 1 Exp. = 8.8), though none for 36μg. (Obs. = 24 Exp. = 25.8). After that, as for tumour size A, rates rise but far less than for continuous painting.

TABLE 5 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE A UNDER 3 HYPOTHESES

S.W.S. 180 mg. K = 3 W = 18.07 B = 5.527_{10}-06

Stopping Time (S) - Weeks.

Period			10		20		30		40		50		Continuous		Totals	
0 - S	Obs	Exp 1	0	0.00	0	0.00	1	0.65	6	3.86	13	11.08	33	37.40	53	52.99
	Exp 2	Exp 3	0.00	0.00	0.00	0.00	0.65	0.65	3.86	3.86	11.08	11.08			52.99	52.99
S+1-S+10	Obs	Exp 1	0	0.00	0	0.66	2	3.15	3	6.76	4	10.56			9	21.13
	Exp 2	Exp 3	0.00	0.00	0.66	0.04	3.15	1.54	6.76	4.48	10.56	7.94			21.13	14.00
S+11-S+20	Obs	Exp 1	0	0.66	3	10.29	4	6.93	2	10.45	4	12.98			13	41.31
	Exp 2	Exp 3	0.61	0.00	3.20	0.04	6.83	1.36	10.34	3.69	12.89	6.07			33.87	11.16
S+21-S+30	Obs	Exp 1	2	3.33	0	7.01	1	11.13	1	12.10	5	11.37			9	44.94
	Exp 2	Exp 3	1.14	0.00	3.85	0.04	7.36	1.17	8.91	2.68	8.91	3.64			30.17	7.53
S+31-S+40	Obs	Exp 1	2	7.85	3	11.43	2	13.68	1	10.50	2	8.05			10	51.51
	Exp 2	Exp 3	1.07	0.00	3.37	0.03	5.64	0.89	5.28	1.58	4.56	1.86			19.92	4.36
S+41-S+50	Obs	Exp 1	0	12.83	0	14.40	1	12.90	1	5.60	0	15.28			2	51.01
	Exp 2	Exp 3	0.94	0.00	2.63	0.02	3.61	0.57	2.05	0.61	2.28	0.93			11.51	2.13
S+51-S+60	Obs	Exp 1	0	15.98	2	15.85	2	11.01	0	3.36	0	1.93			4	48.13
	Exp 2	Exp 3	0.73	0.00	1.98	0.02	2.26	0.36	0.93	0.28	0.67	0.27			6.57	0.93
S+61-		Exp 1	3	14.59	1	11.96	1	6.06	0	0.46					5	33.07
		3	0.46	0.00	1.09	0.02	0.94	0.14	0.11	0.03					2.60	0.19
			2	17.04	0	6.56	0	3.39							2	26.99
				0.00	0.42	0.03	0.40	0.06							1.15	0.09
				28	9	78.16	14	58.90	14	53.09	28	61.25	33	37.40	107	361.08
					17.20	0.24	30.84	6.74	58.24	17.21	50.95	31.79	37.40	37.40	199.91	93.38

TABLE 6

OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE A UNDER 3 HYPOTHESES

G 600 mgs. K = 3 W = 13.69 B = 8.357_{10}-06

Stopping Time (S) Weeks

Period			10		20		30		40		50		Continuous		Total	
0 - S	Obs	Exp 1	0	0.00	0	0.15	2	2.59	13	9.53	23	22.09	46	49.65	84	84.01
	Exp 2	Exp 3	0.00	0.00	0.15	0.15	2.59	2.59	9.53	9.53	22.09	22.09	49.65	49.65	84.01	84.01
S+1-S+10	Obs	Exp 1	0	0.16	2	2.41	3	7.88	3	12.37	4	15.48			12	38.30
	Exp 2	Exp 3	0.16	0.00	2.41	0.71	7.88	4.54	12.37	8.69	15.48	12.00			38.30	25.94
S+11-S+20	Obs	Exp 1	0	2.53	3	7.84	1	14.84	4	18.32	6	17.17			14	60.70
	Exp 2	Exp 3	1.64	0.00	6.37	0.67	13.00	4.03	16.66	7.48	16.01	8.73			53.68	20.91
S+21-S+30	Obs	Exp 1	0	8.09	4	15.30	0	21.65	1	19.02	1	15.07			6	79.13
	Exp 2	Exp 3	1.76	0.00	6.23	0.62	11.44	3.38	11.71	5.07	10.09	5.33			41.23	14.40
S+31-S+40	Obs	Exp 1	2	15.45	2	22.66	0	28.12	4	18.10	0	11.31			8	95.64
	Exp 2	Exp 3	1.58	0.00	5.33	0.53	9.64	2.85	7.78	3.36	5.64	2.98			29.97	9.72
S+41-S+50	Obs	Exp 1	2	22.82	4	28.11	5	26.85	3	11.91	1	4.75			15	94.44
	Exp 2	Exp 3	1.34	0.00	4.32	0.43	6.55	1.93	3.85	1.66	1.81	0.96			17.87	4.98
S+51-S+60	Obs	Exp 1	3	29.76	1	24.44	1	15.88	1	3.06	0	1.86			6	75.00
	Exp 2	Exp 3	1.13	0.00	2.64	0.27	2.84	0.84	0.76	0.33	0.56	0.30			7.93	1.74
S+61-S+70	Obs	Exp 1	1	27.87	1	11.16	0	6.13	0	0.58					2	45.74
	Exp 2	Exp 3	0.75	0.00	0.90	0.09	0.88	0.26	0.12	0.05					2.65	0.40
S+71- End	Obs	Exp 1	3	36.09	0	7.18	0	1.20							3	44.47
	Exp 2	Exp 3	0.64	0.00	0.41	0.03	0.13	0.04							1.18	0.07
Totals	Obs	Exp 1	11	142.77	17	119.25	12	125.14	29	92.89	33	87.73	46	49.65	150	617.43
	Exp 2	Exp 3	9.00	0.00	28.76	3.50	54.95	20.46	62.78	36.17	71.68	52.39	49.65	49.65	276.82	162.17

TABLE 7 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE A UNDER 3 HYPOTHESES

B.P. 36 µg K = 4 W = 13.32 B = 3.538_{10}-07

Stopping Time (S) - Weeks

Period			15		25		35		Continuous		Totals	
0 - S	Obs	Exp 1	0	0.00	1	0.46	3	5.49	58	56.05	62	62.00
	Exp 2	Exp 3	0.00	0.00	0.46	0.46	5.49	5.49	56.05	56.05	62.00	62.00
S+1-S+10	Obs	Exp 1	1	0.48	5	4.70	15	16.45			21	21.63
	Exp 2	Exp 3	0.48	0.00	4.70	1.50	16.45	8.74			21.63	10.24
S+11-S+20	Obs	Exp 1	3	5.00	6	15.85	16	46.45			25	67.30
	Exp 2	Exp 3	3.04	0.00	11.96	1.30	25.44	6.37			40.44	7.67
S+21-S+30	Obs	Exp 1	7	17.21	11	31.01	9	36.16			27	84.38
	Exp 2	Exp 3	2.99	0.00	9.92	1.01	15.65	3.72			28.56	4.73
S+31-S+40	Obs	Exp 1	6	38.74	15	34.60	8	20.27			29	93.61
	Exp 2	Exp 3	2.64	0.00	5.48	0.56	5.21	1.24			13.33	1.80
S+41-S+50	Obs	Exp 1	6	62.17	3	29.07	0	0.70			9	91.94
	Exp 2	Exp 3	2.09	0.00	2.59	0.27	0.12	0.03			4.80	0.30
S+51-S+60	Obs	Exp 1	5	75.29	4	14.46					9	89.75
	Exp 2	Exp 3	1.41	0.00	0.82	0.08					2.23	0.08
S+61-S+70	Obs	Exp 1	5	48.40							5	48.40
	Exp 2	Exp 3	0.58	0.00							0.58	0.00
S+71- End	Obs	Exp 1	0	40.03							0	40.03
	Exp 2	Exp 3	0.28	0.00							0.28	0.00
Totals	Obs	Exp 1	33	287.32	45	130.15	51	125.52	58	56.05	187	599.04
	Exp 2	Exp 3	13.51	0.00	35.93	5.18	68.36	25.59	56.05	56.05	173.85	86.82

TABLE 8 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE A UNDER 3 HYPOTHESES

B.P. 60 µgs. K = 4 W = 13.32 B = 2.047_{10}-06

Stopping Time (S) - Weeks

Period			15		25		35		Continuous		Totals	
0 - S	Obs	Exp 1	0	0.00	1	2.70	29	24.91	65	67.37	95	94.98
	Exp 2	Exp 3	0.00	0.00	2.70	2.70	24.91	24.91	67.37	67.37	94.98	94.98
S+1-S+10	Obs	Exp 1	1	2.73	22	25.82	30	36.40			53	64.95
	Exp 2	Exp 3	2.73	0.03	25.82	8.42	36.40	21.10			64.95	29.55
S+11-S+20	Obs	Exp 1	8	27.36	21	54.63	6	24.21			35	106.20
	Exp 2	Exp 3	16.82	0.02	42.52	4.68	20.91	5.34			80.25	10.04
S+21-S+30	Obs	Exp 1	7	91.24	13	63.51	2	16.78			22	171.53
	Exp 2	Exp 3	15.81	0.02	20.98	2.14	7.73	1.84			44.52	4.00
S+31-S+40	Obs	Exp 1	12	166.28	5	53.87	0	15.02			17	235.17
	Exp 2	Exp 3	11.55	0.02	8.44	0.86	3.52	0.84			23.51	1.72
S+41-S+50	Obs	Exp 1	5	217.72	4	41.36	0	24.41			[illegible]	[illegible]
	Exp 2	Exp 3	7.30	0.01	3.71	0.38	3.52	0.84			14.53	1.23
S+51-S+60	Obs	Exp 1	5	206.91	0	4.03	1	28.47			6	239.41
	Exp 2	Exp 3	3.95	0.00	0.26	0.03	2.81	0.67			7.02	0.70
S+61-S+70	Obs	Exp 1	1	179.40							1	179.40
	Exp 2	Exp 3	207	0.00							2.07	0.00
S+71- End	Obs	Exp 1	2	90.41							2	90.41
	Exp 2	Exp 3	0.69	0.00							0.69	0.00
Totals	Obs	Exp 1	41	982.05	66	245.92	68	170.20	65	67.37	240	1465.54
	Exp 2	Exp 3	60.92	0.10	104.43	19.21	99.80	55.54	67.37	67.37	332.52	142.22

TABLE 9 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE D UNDER 3 HYPOTHESES

S.W.S. 180 mg. K = 3 W = 29.43 B = 1.817_{10}-06

Stopping Time (S) - Weeks

Period			10		20		30		40		50		Continuous		Totals	
0 - S	Obs	Exp 1	0	0.00	0	0.00	0	0.00	0	0.14	1	1.07	10	9.80	11	11.01
	Exp 2	Exp 3	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.14	1.07	1.07	9.80	9.80	11.01	11.01
S+1-S+10	Obs	Exp 1	0	0.00	0	0.00	0	0.14	1	0.84	5	2.08			6	3.06
	Exp 2	Exp 3	0.00	0.00	0.00	0.00	0.14	0.00	0.84	0.38	2.08	1.35			3.06	1.73
S+11-S+20	Obs	Exp 1	0	0.00	0	0.14	2	0.85	1	1.87	2	3.01			5	5.87
	Exp 2	Exp 3	0.00	0.00	0.14	0.00	0.85	0.00	1.87	0.32	3.01	1.01			5.87	1.33
S+21-S+30	Obs	Exp 1	0	0.15	0	0.82	0	1.96	1	2.69	1	3.36			2	8.98
	Exp 2	Exp 3	0.14	0.00	0.82	0.00	1.96	0.00	2.68	0.24	3.36	0.69			8.96	0.93
S+31-S+40	Obs	Exp 1	1	0.89	1	1.97	0	2.90	0	2.37	2	2.43			4	10.56
	Exp 2	Exp 3	0.36	0.00	1.20	0.00	2.08	0.00	1.87	0.13	2.01	0.34			7.52	0.47
S+41-S+50	Obs	Exp 1	0	2.14	0	2.98	0	3.26	0	1.58	0	1.62			0	11.58
	Exp 2	Exp 3	0.33	0.00	0.95	0.00	1.43	0.00	0.83	0.05	0.97	0.17			4.51	0.22
S+51-S+60	Obs	Exp 1	0	3.22	0	3.68	1	3.00	0	1.07	0	0.53			1	11.50
	Exp 2	Exp 3	0.26	0.00	0.71	0.00	0.89	0.00	0.41	0.03	0.24	0.04			2.51	0.07
S+61-S+70	Obs	Exp 1	0	3.39	0	3.27	0	2.07	0	0.11					0	8.84
	Exp 2	Exp 3	0.17	0.00	0.43	0.00	0.44	0.00	0.03	0.00					1.07	0.00
S+71- End	Obs	Exp 1	1	4.48	0	2.14	0	1.09							1	7.69
	Exp 2	Exp 3	0.11	0.00	0.17	0.50	0.18	0.00							0.46	0.00
Totals	Obs	Exp 1	2	14.27	1	15.00	3	15.25	3	10.67	11	14.10	10	9.80	30	79.09
	Exp 2	Exp 3	1.37	0.00	4.42	0.00	7.97	0.00	8.67	1.29	12.74	4.67	9.80	9.80	44.97	15.76

TABLE 10 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE D UNDER 3 HYPOTHESES

G 600 mg K = 3 W = 27.69 B = 4.146_{10}-06

Stopping Time (S) - Weeks

Period		10		20		30		40		50		Continuous		Totals	
0 - S	Obs Exp 1	0	0.00	0	0.00	0	0.00	0	0.52	4	2.94	22	22.53	26	25.99
	Exp 2 Exp 3	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.52	2.94	2.94	22.53	22.53	25.99	25.99
S+1-S+10	Obs Exp 1	0	0.00	0	0.00	0	0.55	4	2.39	2	4.76			6	7.70
	Exp 2 Exp 3	0.00	0.00	0.00	0.00	0.55	0.04	2.39	1.19	4.76	3.18			7.70	4.41
S+11-S+20	Obs Exp 1	0	0.00	0	0.54	1	2.43	1	5.04	5	6.64			7	14.65
	Exp 2 Exp 3	0.00	0.00	0.54	0.00	2.43	0.04	5.04	1.03	6.64	2.42			14.65	3.49
S+21-S+30	Obs Exp 1	0	0.52	0	2.62	0	5.08	3	6.79	2	7.14			5	22.15
	Exp 2 Exp 3	0.48	0.00	2.53	0.00	4.97	0.04	6.70	0.75	7.07	1.61			21.75	2.40
S+31-S+40	Obs Exp 1	0	2.43	1	5.62	1	7.96	0	7.42	0	5.31			2	28.74
	Exp 2 Exp 3	0.80	0.00	3.02	0.00	5.16	0.03	5.36	0.51	4.10	0.81			18.44	1.35
S+41-S+50	Obs Exp 1	0	5.29	0	8.34	0	9.14	0	6.78	0	3.82			0	33.37
	Exp 2 Exp 3	0.70	0.00	2.41	0.00	3.74	0.02	3.38	0.32	2.12	0.82			12.35	0.76
S+51-S+60	Obs Exp 1	0	8.76	1	8.73	0	7.88	0	2.79	1	1.41			2	29.57
	Exp 2 Exp 3	0.63	0.00	1.59	0.00	2.18	0.02	1.02	0.09	0.62	0.12			6.04	0.23
S+61-S+70	Obs Exp 1	0	9.42	0	5.14	0	4.01	0	0.34					0	18.91
	Exp 2 Exp 3	0.43	0.00	0.64	0.00	0.83	0.02	0.10	0.01					2.00	0.03
S+71- End	Obs Exp 1	0	15.46	0	3.36	0	1.64							0	20.46
	Exp 2 Exp 3	0.38	0.00	0.27	0.00	0.26	0.00							0.91	0.00
Totals	Obs Exp 1	0	41.88	2	34.35	2	38.69	8	32.07	14	32.02	22	22.53	48	201.54
	Exp 2 Exp 3	3.42	0.00	11.00	0.00	20.12	0.21	24.51	4.42	28.25	11.50	22.53	22.53	109.83	38.66

TABLE 11 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE D UNDER 3 HYPOTHESES

B.P. 36μg. K = 4 W = 16.96 B = 7.762_{10}-07

Stopping Time (S) - Weeks

Period			15		25		35		Continuous		Totals	
0 - S	Obs	Exp 1	0	0.00	0	0.23	5	5.64	58	57.12	63	62.99
	Exp 2	Exp 3	0.00	0.00	0.23	0.23	5.64	5.64	57.12	57.12	62.99	62.99
S+1-S+10	Obs	Exp 1	0	0.23	2	5.39	22	20.20			24	25.82
	Exp 2	Exp 3	0.23	0.00	5.39	1.11	20.20	9.74			25.82	10.85
S+11-S+20	Obs	Exp 1	1	5.52	8	24.07	16	27.89			25	57.48
	Exp 2	Exp 3	4.76	0.00	22.40	1.00	26.92	4.74			54.08	5.74
S+21-S+30	Obs	Exp 1	1	26.72	23	48.97	5	21.77			29	97.46
	Exp 2	Exp 3	7.06	0.00	21.59	0.72	12.52	1.71			41.17	2.43
S+31-S+40	Obs	Exp 1	5	66.28	11	46.89	0	7.36			16	120.53
	Exp 2	Exp 3	6.21	0.00	9.55	0.32	2.27	0.31			18.03	0.63
S+41-S+50	Obs	Exp 1	5	97.97	2	44.56	0	7.83			7	150.36
	Exp 2	Exp 3	4.21	0.00	4.70	0.16	1.34	0.18			10.25	0.34
S+51-S+60	Obs	Exp 1	2	104.56	2	26.35	0	12.16			4	143.07
	Exp 2	Exp 3	2.41	0.00	1.75	0.06	1.34	0.18			5.50	0.24
S+61-S+70	Obs	Exp 1	1	102.87	0	12.75	0	1.50			1	117.12
	Exp 2	Exp 3	1.41	0.00	0.53	0.02	0.13	0.02			2.07	0.04
S+71- End	Obs	Exp 1	0	102.64							0	102.64
	Exp 2	Exp 3	0.75	0.00							0.75	0.00
Totals	Obs	Exp 1	15	506.79	48	209.21	48	104.35	58	57.12	169	877.47
	Exp 2	Exp 3	27.04	0.00	66.14	3.62	70.36	22.52	57.12	57.12	220.66	83.26

TABLE 12 OBSERVED AND EXPECTED NUMBERS OF ANIMALS BEARING TUMOURS OF SIZE D UNDER 3 HYPOTHESES

B.P. 60μgs K = 4 W = 16.96 B = 2.041_{10}-07

Stopping Time (S) - Weeks

Period			15		25		35		Continuous		Totals	
0 - S	Obs	Exp 1	0	0.00	1	0.06	0	1.54	53	52.40	54	54.00
	Exp 2	Exp 3	0.00	0.00	0.06	0.06	1.54	1.54	52.40	52.40	54.00	54.00
S+1-S+10	Obs	Exp 1	0	0.06	0	0.39	1	7.31			1	8.76
	Exp 2	Exp 3	0.06	0.00	1.39	0.29	7.31	3.35			8.76	3.64
S+11-S+20	Obs	Exp 1	1	1.47	4	6.57	9	18.51			14	26.55
	Exp 2	Exp 3	1.27	0.00	6.10	0.27	17.68	3.00			25.05	3.27
S+21-S+30	Obs	Exp 1	3	7.05	2	16.59	11	28.96			16	52.60
	Exp 2	Exp 3	1.87	0.00	7.15	0.24	15.83	2.16			24.85	2.40
S+31-S+40	Obs	Exp 1	4	18.24	8	26.74	3	23.62			15	68.60
	Exp 2	Exp 3	1.68	0.00	5.28	0.18	7.08	0.97			14.04	1.15
S+41-S+50	Obs	Exp 1	1	32.82	6	28.65	4	7.35			11	68.82
	Exp 2	Exp 3	1.40	0.00	3.08	0.10	1.37	0.18			5.85	0.28
S+51-S+60	Obs	Exp 1	1	46.89	3	22.18	1	2.09			5	71.16
	Exp 2	Exp 3	1.07	0.00	1.42	0.05	0.25	0.03			2.74	0.08
S+61-S+70	Obs	Exp 1	0	33.33	1	5.22					1	38.55
	Exp 2	Exp 3	0.46	0.00	0.23	0.01					0.69	0.01
S+71- End	Obs	Exp 1	0	33.28							0	33.28
	Exp 2	Exp 3	0.28	0.00							0.28	0.00
Totals	Obs	Exp 1	10	173.14	25	107.40	29	89.38	53	52.40	117	422.32
	Exp 2	Exp 3	8.09	0.00	24.71	1.20	51.06	11.23	52.40	52.40	136.26	64.83

4.3 A MORE COMPLEX ANALYSIS BY MATHEMATICAL MODELS OF THE EFFECT OF STOPPING PAINTING

4.3.1 It can be shown that the Weibull distribution for a continuous painting experiment can be derived under the following assumptions:

1) That there are a constant number of cells, N, at risk.
2) That, for a tumour to appear, a cell must undergo k successive transformations.
3) That the instantaneous probability, b_r of a cell which has undergone r-1 transformations undergoing the rth transformation is small and constant.
4) That the time of appearance of the tumour is w weeks after the kth transformation of the cell.

4.3.2 The incidence rate, I, at time t will then be given by the formula

$$I = bk(t-w)^{k-1}$$

$$\text{where} \quad b = \frac{Nb_1b_2 \ldots b_k}{k!}$$

4.3.3. In untreated control animals tumours do occur so it is reasonable to assume that the parameters b_r are small and positive in the absence of treatment, and that the effect of treatment is to alter one or more of these parameters. Thus in the context of a stopping painting experiment the parameters can be taken as $c_1, c_2 \ldots . c_k$ during the treatment and $b_1, b_2 \ldots . b_k$ after stopping. The test of whether a treatment affects a particular stage, r, of carcinogenesis is whether the ratio $f_r = c_r/b_r$ is significantly greater than unity.

4.3.4 An expression for the incidence rate after stopping has been derived

and is a function of b, k, w, f_1, f_2, ...f_k. The formula and its derivation is given in the Statistical Appendix (section 7(b)).

4.3.5 An attempt was made to fit this formula to the data for tumours of size A only, due to the large amount of computing time involved.

4.3.6 A computer program was written which enabled maximum likelihood estimates of b and the f's to be calculated for a given treatment given "known values" of k and w with each of the f's being constrained, if required, to unity.

4.3.7 Lack of machine size and difficulties of programming made it impracticable to simultaneously estimate all the parameters, and thus, as k and w were required to be "known" for the program, it was necessary to try and choose reasonable pairs of values of k and w first.

4.3.8 To do this a preliminary analysis was carried out in which, for a number of selected values of the parameter f, the log likelihood of the data was calculated for each combination of k = 2, 3 and 4 and w = 0, 5, 10 and 15. It proved possible by this method to select one or two k, w pairs for use as "known" values in the computer program.

4.3.9 Tables 13(A), 14(A), 15(A) and 16(A) give for SWS, G, BP 36μg. and BP 60μg. respectively details of the relative likelihood of a number of models fitted of this type. In some cases, when the parameter f for two successive stages was allowed to vary in the program, a degenerate maximum was obtained in which one f was very large and one very small. However, it was in general true that where this occurred a solution with all f's less than 1 (as is physically sensible) fitted virtually as well.

4.3.10 Tables 13(B), 14(B), 15(B) and 16(B) correspondingly give details of the goodness of fit of a selected model from the Tables (A).

4.3.11 For S.W.S. 180 mg. it was not clear whether k = 3 or k = 4 was a better fit to the data. As was also true for G and BP k = 2 gave a far worse fit to the data whatever w was chosen. From a comparison of likelihoods for a number of k, w pairs, it was clear that the w values presented in Table 13(A) are near to the best available values for their respective k's.

4.3.12 It was clear that at least two of the f's had to be allowed to vary to get a sensible fit to the data. For k = 3 w = 15 the three models A5, A6 and A7 where only one f varied were at least 6.6 worse in log likelihood ($p < 0.001$) than model A1. The model A3 fitted virtually as well as A1 so could be taken as the "best" model as it involved less parameters. From the fact that A4 fitted far worse than A1 it was clear that the last stage was definitely affected but as A2 was not significantly worse than A3 it was not clear whether the first or second stage was affected.

4.3.13 Thus for S.W.S. 180 mg. we can postulate the best approximation to the data on a multistage hypothesis as "a three stage process in which the first stage transition probability is strongly affected (by a factor estimated as 11.9) and the third stage transition probability is less strongly, but also significantly, affected (by 3.6)".

4.3.14 The goodness of fit of this model is only fair, as can be seen from Table 13(B). The misfit lies in the assumption that the rate rises continuously until week 15 when in fact it drops in the first 10 weeks. (see section 4.2.11). However, the general fit is far better than any of the simpler hypotheses considered in section 4.2.

4.3.15 For G 600 mg. the drop off in the first 10 weeks after stopping was far more marked. (See Table 6. Observed Tumours of Size A in 10 week period = 12, Expected under assumption that rate continues to rise = 38.3). Thus the fit to a model with w much greater than 0 proved not to be a good fit to the data. Restricting w to 0 gave k = 4 as a better fit than k = 3.

4.3.16 Table 14(A) presents, therefore, only models fitted with k = 4 and w = 0. It was possible to show that at least two of the four stages must be affected by Fraction G. Model B3 in which the first, third and fourth stages are affected was as good a fit to the data as the fuller Model B1 in which all four f's were allowed to vary. The further restriction of fixing the f for stage 3 at 1 as in Model B5 gave a log likelihood 1.78 worse than Model B3. As there is some evidence that this gave a significant deterioration in fit (x^2 = 3.56 on 1d.f, p ≃ 0.06) Model B3 was taken as the best available.

4.3.17 Thus for G 600 mg. we can say that the best approximation to the data on a multistage hypothesis is "a four stage process in which the first stage transition probability is strongly affected (by 15.6), and the third and fourth stages are less strongly affected (by 2.4 and by 3.2 respectively)".

4.3.18 The goodness of fit to Model B3 is presented in Table 14(B). There is some indication of a misfit. During the first 20 weeks after stopping the Model under estimates the response (Obs. = 26 Exp. = 20.3) and in the next 20 weeks it overestimates it (Obs. = 14 Exp. = 23.7). The fit to the overall number of tumour bearing animals in each group is as good as can be expected, taking into account the fact that the observed rate

in the group painted for 30 weeks was less than that painted for 20 weeks. This observation could not be fitted by any plausible model, let alone a model of the type being used here. As before the general level of fit is far better than for any of the simpler hypotheses considered in section 4.2.

4.3.19 For both dose levels of BP, k = 4 w = 10 proved virtually as good a fit as was available to the data, k = 3 being clearly significantly worse for any w. Tables 15(A) and 16(A) give details of the models tested with k = 4 w = 10 and also with k = 3 w = 15. The results with the latter kw pair are presented to show more clearly the effect of the hypotheses on the number of stages affected.

4.3.20 From the Tables it is clear that it is necessary to postulate that at least two stages of the cancer process are involved. For the lower dose of BP Model B6, which postulates that only stages 2 and 4 are involved, is quite satisfactory. For the higher dose, it is statistically significantly worse than Model B1 ($x^2 = 7.42$ on 2d.f, $P < 0.05$) but for simplicity the results from this model have been considered in Table 16(B), as well as in Table 15(B).

4.3.21 Thus for BP we can say that a fair approximation to the data on a multistage hypothesis is "a four stage process in which the second stage transition probability is very strongly affected (36mg. by 190 and 60mg. by 460) and in which the fourth stage transition probability is affected far less strongly (36mg. by 1.7 and 60mg. by 3.5)".

4.3.22 From Tables 15(B) and 16(B) it can be seen that, in general, the fit to the data is quite satisfactory. The main misfit is in Table 16(B), for the period 11 to 20 weeks after stopping where the fitted tumour rate is

significantly less than the observed rate. This lack of fit is in fact mainly due to the contribution by the group painted for 25 weeks.

4.3.23 At this point we have shown:

a) that a multistage model fits the data reasonably well for each carcinogen.

b) that it is necessary, in each case, to postulate that at least one early and one late stage of the carcinogenic process is affected by the treatments.

c) that though S.W.S. and G both affect the early stage somewhat more than the late stage, BP affects the early stage very much more than the late stage. In fact at the doses applied its affect on the late stage was no more, probably less, than S.W.S. and G, but the total carcinogenic effect of BP was far greater.

4.3.24 We must now consider whether there is any serious deficiency in the model as defined in 4.3.1. It appears that there might be. As has been noted, the response for S.W.S. and especially G drops off in the first ten weeks after stopping. Yet experience has shown us in continuous painting experiments that it is normally necessary to postulate a w of at least 10 weeks* This suggests that 4.3.1. 4) is an over-simplification. A Weibull distribution for continuous painting would

* Footnote - It may be of interest to note here that in some recent work in Harrogate, M.C. Bibby painted mice with doses of BP 10 times higher than used here and did not observe tumours until the 11th week of painting. This is strong evidence that there is a true w of at least 10 weeks and that it is not an artefact of statistics.

equally well be observed if instead of a single waiting time of w after the kth transformation there was a waiting time w_i after each of the k transformations. In that case the w of the equation would be the sum of the k w_i's.

4.3.25 It is possible to derive the equation for the incidence rate pattern expected under this alternative hypothesis and this is given in Statistical Appendix (Section 7(c)). However, no work has been done to fit this to the data. This would involve maximizing a complex function of numerous variables (b, k, k f's and k w's) and would involve considerable computing time. Whether it would add anything much to the interpretation is a moot point. Perhaps it would be a job suitable for a postgraduate student.

TABLE 13(A)

COMPARATIVE LIKELIHOOD OF VARIOUS THEORETICAL MODELS FITTED TO S.W.S. 180 mg.DATA

k = 3 w = 15					k = 4 w = 0					
Model No.	F_1	F_2	F_3	log likelihood	Model No.	F_1	F_2	F_3	F_4	log likelihood
A1	0.118	0.628	0.350	- 633.4636	B1	0.072	L+	S+	0.711	- 633.5431
A2	1	0.117	0.480	- 635.2625	B2	1	0.106	0.666	0.508	- 635.8889
A3	0.084	1	0.279	- 633.7144	B3	0.116	1	0.278	0.596	- 633.9709
A4	0.557	0.079	1	- 640.0262	B4	0.164	0.329	1	0.362	- 635.2297
A5	0.032	1	1	- 654.0466	B5	0.060	1	1	0.256	- 636.9992
A6	1	0.049	1	- 640.0675	B6	1	0.079	1	0.410	- 636.0327
A7	1	1	0.140	- 644.3432	B7	1	1	0.076	0.804	- 637.6536
A8	1	1	1	- 694.3341						

+ L and S indicate very large and very small - Degenerate solution

TABLE J (iii)

GOODNESS OF FIT OF MODEL AS TO SWS 180mg. DATA

$k = 3$ $w = 15$ $B = 3.419_{10} - 6$

$f_1 = 0.084$ $f_2 = 1$ $f_3 = 0.279$

Stopping Time(s) - Weeks

Period		10	20	30	40	50	Continuous	Solvent	Totals
O - S	Obs	0	0	1	6	13	33	-	53
	Exp	0.00	0.03	0.80	3.56	9.10	27.51	-	41.02
S+1-S+10	Obs	0	0	2	3	4		0	9
	Exp	0.03	0.78	2.70	5.18	7.66		0.00	16.35
S+11-S+20	Obs	0	3	4	2	4		0	13
	Exp	0.36	1.56	3.13	4.64	5.82		0.00	15.51
S+21-S+30	Obs	2	0	1	1	5		0	9
	Exp	0.60	1.34	2.12	2.29	2.13		0.03	8.51
S+31-S+40	Obs	2	3	2	1	2		0	10
	Exp	0.99	1.78	2.29	1.81	1.41		0.09	8.37
S+41-S+50	Obs	0	0	1	1	0		0	2
	Exp	1.26	1.89	1.91	0.89	0.86		0.19	7.00
S+51-S+60	Obs	0	2	2	0	0		0	4
	Exp	1.30	1.81	1.47	0.49	0.29		0.32	5.68
S+61-S+70	Obs	3	1	1	0	0		1	6
	Exp	1.03	1.21	0.73	0.06	0.00		0.43	3.46
S+71 - End	Obs	2	0	0	-	-		1	3
	Exp	1.00	0.59	0.37	-	-		1.13	3.09
Totals	Obs	9	9	14	14	28	33	2	109
	Exp	6.57	10.99	15.52	18.92	27.29	27.51	2.19	108.99

COMPARATIVE LIKELIHOOD OF VARIOUS THEORETICAL MODELS FITTED TO G 600 mg. DATA

Model No.	F_1	F_2	F_3	F_4	log likelihood
B1	0.040	L^+	S^+	0.397	- 833.7695
B2	1	0.062	1.157	0.249	- 838.111
B3	0.064	1	0.413	0.316	- 834.3585
B4	0.073	0.537	1	0.212	- 835.3599
B5	0.045	1	1	0.167	- 836.1377
B6	1	0.069	1	0.272	- 838.1394
B7	1	1	0.084	0.500	- 842.9903

+ L and S indicate very large and very small - Degenerate solution

TABLE 14 (B)

GOODNESS OF FIT OF MODEL B3 TO G 600mg. DATA

Stopping Time(s) - Weeks

$k = 4 \quad w = 0 \quad B = 6.0413_{10}{}^{-6}$

$f_1 = 0.064 \quad f_2 = 1 \quad f_3 = 0.413 \quad f_4 = 0.316$

Period		10	20	30	40	50	Continuous	Solvent	Totals
O - S	Obs	0	0	2	13	23	46	-	84
	Exp	0.05	0.70	3.51	4.86	21.17	48.72	-	84.01
S+1-S+10	Obs	0	2	3	3	4	-	0	12
	Exp	0.12	0.63	1.76	2.89	3.94	-	0.00	9.34
S+11-S+20	Obs	0	3	1	4	6	-	0	14
	Exp	0.33	1.10	2.41	3.44	3.70	-	0.01	10.99
S+21-S+30	Obs	0	4	0	1	1	-	0	6
	Exp	0.66	1.72	3.04	3.20	2.96	-	0.03	11.61
S+31-S+40	Obs	2	2	0	4	0	-	0	8
	Exp	1.04	2.30	3.66	2.87	2.11	-	0.08	12.06
S+41-S+50	Obs	2	4	5	3	2	-	0	16
	Exp	1.39	2.70	3.37	1.83	0.86	-	0.16	10.31
S+51-S+60	Obs	3	1	1	1	0	-	0	6
	Exp	1.71	2.27	1.95	0.46	0.33	-	0.28	7.00
S+61-S+70	Obs	1	1	0	0	-	-	1	3
	Exp	1.55	1.02	0.75	0.09	-	-	0.40	3.81
S+71-End	Obs	3	0	0	-	-	-	1	4
	Exp	1.96	0.65	0.15	-	-	-	1.18	3.94
Totals	Obs	11	17	12	29	36	46	2	153
	Exp	8.80	13.07	20.59	24.63	35.06	48.72	2.15	153.07

TABLE 15(A)

COMPARATIVE LIKELIHOOD OF VARIOUS THEORETICAL MODELS FITTED TO BP 36 mg. DATA

k = 3 w = 15					k = 4 w = 10					
Model No.	F_1	F_2	F_3	log likelihood	Model No.	F_1	F_2	F_3	F_4	log likelihood
A1	0.144	0.550	0.654	- 865.222	B1	L^+	S^+	1.055	0.572	- 859.768*
A2	1	0.0063	1.125	- 871.820	B2	1	0.004	1.711	0.407	- 859.322
A3	0.011	1	0.485	- 865.948	B3	0.015	1	0.264	0.078	- 868.236
A4	0.022	0.265	1	- 866.796	B4	0.208	0.025	1	0.580	- 859.858
A5	0.0074	1	1	- 877.989	B5	1	1	0.006	1.498	- 882.506
A6	1	0.0071	1	- 872.132	B6	1	0.0053	1	0.589	- 859.874
A7	1	1	0.144	- 945.878	B7	0.009	1	1	0.323	- 868.541
A8	1	1	1	-1024.171						

+ L and S indicate very large and very small - Degenerate solution

* Not an absolute maximum (programming difficulties)

TABLE 15(B)

$k = 4 \quad w = 10 \quad B = 2.463_{10}{}^{-7}$

GOODNESS OF FIT OF MODEL B6 TO BP 36mg. DATA

$f_1 = f_3 = 1 \quad f_2 = 0.0053 \quad f_4 = 0.583$

Stopping Time(s) - Weeks

Period		15	25	35	Continuous	Solvent	Totals
O - S	Obs Exp	0 0.01	1 0.87	3 6.78	58 52.70	- -	62 60.36
S+1-S+10	Obs Exp	1 0.91	6 5.52	15 16.21		0 0.00	22 22.64
S+11-S+20	Obs Exp	3 2.76	6 8.32	16 15.36		0 0.00	25 26.44
S+21-S+30	Obs Exp	7 4.91	11 11.26	9 14.38		0 0.01	27 30.56
S+31-S+40	Obs Exp	6 6.52	15 8.87	8 6.43		0 0.05	29 21.87
S+41-S+50	Obs Exp	6 6.92	3 5.46	0 0.18		0 0.12	9 12.68
S+51-S+60	Obs Exp	5 5.91	4 2.10	- -		0 0.23	9 8.24
S+61-S+70	Obs Exp	5 2.88	0 0.00	- -		1 0.36	6 3.24
S+71-End	Obs Exp	0 1.74	- -	- -		0 1.23	0 2.97
Totals	Obs Exp	33 32.56	45 42.40	51 59.34	58 52.70	2 2	189 189.00

TABLE 16(A)

COMPARATIVE LIKELIHOOD OF VARIOUS THEORETICAL MODELS FITTED TO BP 60 mg. DATA

k = 3 w = 15					k = 4 w = 10					
Model No.	F_1	F_2	F_3	log likelihood	Model No.	F_1	F_2	F_3	F_4	log likelihood
A1	0.027	0.099	0.510	- 956.4368	B1	L	S	0.273	0.567	- 948.5023
A2	L	S	1	- 964.4362	B2	1	0.0043	0.271	0.568	- 948.5090
A3	0.0058	1	0.222	- 963.2709	B3	0.017	1	0.057	0.686	- 949.3050
A4	1	0.0024	0.592	- 957.0914	B4	L	S	1	0.287	- 952.1816
A5	0.0026	1	1	-1027.7086	B5	1	1	0.0009	0.892	- 954.1420
A6	1	0.0016	1	- 964.4391	B6	1	0.0022	1	0.285	- 952.2141
A7	1	1	0.038	-1067.6789	B7	0.0045	1	1	0.138	- 973.5930
A8	1	1	1	-1349.3047						

TABLE 16(B)

$k = 4 \quad w = 10 \quad B = 1.163_{10}-6$

GOODNESS OF FIT OF MODEL B6 TO BP 60mg. DATA

$f_1 = 1 \quad f_3 = 0.0022 \quad f_4 = 0.285$

Stopping Time(s) - Weeks

Period		15	25	35	Continuous	Solvent	Totals
O - S	Obs	0	1	29	65	-	95
	Exp	0.05	4.19	25.67	59.57	-	89.48
S+1-S+10	Obs	1	22	30	-	0	53
	Exp	4.17	24.79	29.56	-	0.00	58.52
S+11-S+20	Obs	8	21	6	-	0	35
	Exp	5.98	11.47	4.89	-	0.00	22.34
S+21-S+30	Obs	7	13	2	-	0	22
	Exp	10.23	9.23	2.70	-	0.01	22.17
S+31-S+40	Obs	12	5	0	-	0	17
	Exp	11.13	5.41	1.79	-	0.04	18.37
S+41-S+50	Obs	5	4	0	-	0	9
	Exp	9.46	3.07	2.28	-	0.11	14.92
S+51-S+60	Obs	5	0	1	-	0	6
	Exp	6.39	0.25	2.18	-	0.22	9.04
S+61-S+70	Obs	1	-	-	-	1	2
	Exp	4.07	-	-	-	0.35	4.42
S+71-End	Obs	2	-	-	-	1	3
	Exp	1.59	-	-	-	1.16	2.75
Totals	Obs	41	66	68	65	2	242
	Exp	53.06	58.40	69.06	59.57	1.90	241.99

4.4 THE EFFECT OF AGEING

4.4.1 As described in section 2.05 four groups of animals were tested with fraction G at 600 mg. for 40 weeks starting at ages which differed successively by 10 weeks from group to group.

4.4.2 One of the hypotheses of the Weibull distribution (section 4.3.1 3)) is that the risk of cellular transformation is constant, i.e. ageing per se has no effect on the cancer process. Under this assumption therefore one would predict that there would be no difference in the relation between tumour incidence rate and time from first treatment between the four groups.

4.4.3 This null hypothesis can be most easily tested by the logrank test of Peto and Peto (1972). Table 17 gives the observed numbers of animals bearing tumours of each size and those expected under hypothesis.

4.4.4 No difference significant at the $p < 0.05$ level was found between the groups for any tumour size. This agrees with the published result of Lee and Peto (1970) and also with that from Dr. F. J. C. Roe's large ageing experiment at Pollard's Wood.

TABLE 17

NUMBERS OF TUMOUR BEARING ANIMALS OBSERVED AND EXPECTED UNDER THE ASSUMPTION OF NO EFFECT OF AGEING.

Tumour Size A	Observed	Expected	
0 - 40 weeks	29	23.2	
10 - 50 "	22	22.9	$\chi^2 = 3.41$ on 3 d.f.
20 - 60 "	11	16.6	Not significant
30 - 70 "	10	19.4	
Tumour Size B			
0 - 40 weeks	18	13.8	
10 - 50 "	16	12.5	$\chi^2 = 6.72$ on 3 d.f.
20 - 60 "	3	8.6	$p < 0.1$
30 - 70 "	3	5.1	
Tumour Size C			
0 - 40 weeks	10	8.2	
10 - 50 "	8	7.5	$\chi^2 = 1.36$ on 3 d.f.
20 - 60 "	3	5.2	Not significant
30 - 70 "	3	3.0	
Tumour Size D			
0 - 40 weeks	7	5.3	
10 - 50 "	3	4.8	$\chi^2 = 1.34$ on 3 d.f.
20 - 60 "	3	3.3	Not significant
30 - 70 "	2	1.7	

4.5 THE RELATION BETWEEN TUMOUR SIZE AND PATHOLOGY

4.5.1 One of the reasons why the times to the different tumour sizes A, B, C and D were measured was to try and determine whether there was a critical size above which virtually all tumours proved to be malignant. If such a size were found it may prove feasible to carry out a carcinogenicity experiment in which animals were killed when a tumour reached a given size and no histopathology was performed.

4.5.2 In order to find this critical size it would have been better to kill animals at predetermined tumour sizes so as to directly answer the question "is an x mm. tumour likely to be malignant?" In this experiment this strategy was not followed so that the only question one can answer is "is a tumour of size y mm. or greater on a dead animal likely to be malignant?". Although the information that can be derived is not designed to answer the most relevant question it seems worthwhile to carry out an analysis.

4.5.3 For each tumour found on each animal for which pathology was possible classification was made into papilloma (P), carcinoma (C), infiltrating carcinoma (I) or sarcoma (S) in the normal way.

4.5.4 Table 18 gives the relationship between tumour classification and maximum tumour size. Sarcomas have been omitted from the table as there were only 4 (3 tumour size D, 1 B).

4.5.5 The first conclusion that can be made is that there is a clear relationship of tumour size to pathology. Only 5.6% of tumours less than 6 mm. proved to be infiltrating carcinomas whereas 86.8% of those greater than 10 mm. were.

4.5.6 However, there are differences in this respect between smoke derived materials and BP. In SWS and G taken together 32.4% of tumours greater than 10 mm. proved non-malignant whereas only 9.4% did with BP. There is also a smaller, but still highly significant difference in the proportion of 2 mm. tumours that proved to be carcinomas or infiltrating carcinomas (SWS + G 9.1%, BP 19.1%).

4.5.7 It is of interest also that there was a difference in the proportion of tumours of less than 6 mm. that were carcinomas or infiltrating carcinomas between the continuously painted groups (27.5%) and the stopping groups (11.2%). A plausible explanation for this is that in the continuously painted groups there is more chance that an animal with a small but malignant tumour, that would be expected to grow larger, dies of other causes before this growth can occur.

TABLE 18 RELATIONSHIP BETWEEN TUMOUR SIZE AND PATHOLOGY

Treatment		Tumour Size: 2 - 6 mm N	%	6 - 10 mm N	%	≤ 10 mm N	%	> 10 mm N	%
SWS 180 mg 0 - 10 weeks	P	4	100.0			0	0.0	1	33.3
	C	0	0.0	—		1	100.0	1	33.3
	I	0	0.0			0	0.0	1	33.3
SWS 180 mg 0 - 20 weeks	P	7	100.0			0	0.0	0	0.0
	C	0	0.0	—		1	100.0	1	100.0
	I	0	0.0			0	0.0	0	0.0
SWS 180 mg 0 - 30 weeks	P	1	100.0	1	100.0	2	100.0	0	0.0
	C	0	0.0	0	0.0	0	0.0	0	0.0
	I	0	0.0	0	0.0	0	0.0	3	100.0
SWS 180 mg 0 - 40 weeks	P	11	91.7	1	100.0			0	0.0
	C	1	8.3	0	0.0	—		0	0.0
	I	0	0.0	0	0.0			2	100.0
SWS 180 mg 0 - 50 weeks	P	16	94.1	4	50.0	0	0.0	1	12.5
	C	0	0.0	1	12.5	1	50.0	0	0.0
	I	1	5.9	3	37.5	1	50.0	7	87.5
SWS 180 mg Painted for Life	P	17	68.0	3	37.5	1	33.3	2	25.0
	C	6	24.0	2	25.0	0	0.0	0	0.0
	I	2	8.0	3	37.5	2	66.7	6	75.0
SWS 180 mg Total	P	56	84.8	9	50.0	3	33.3	4	16.0
	C	7	10.6	3	16.7	3	33.3	2	8.0
	I	3	4.5	6	33.3	3	33.3	19	76.0

TABLE 18 RELATIONSHIP BETWEEN TUMOUR SIZE AND PATHOLOGY

(continued - 1)

Treatment		Tumour Size: 2 - 6 mm		6 - 10 mm		≏10 mm		>10 mm	
		N	%	N	%	N	%	N	%
G 600 mg 0 - 10 weeks	P	8	100.0	2	100.0	1	100.0		
	C	0	0.0	0	0.0	0	0.0	—	
	I	0	0.0	0	0.0	0	0.0		
G 600 mg 0 - 20 weeks	P	12	100.0	4	66.7	0	0.0	0	0.0
	C	0	0.0	2	33.3	1	100.0	0	0.0
	I	0	0.0	0	0.0	0	0.0	1	100.0
G 600 mg 0 - 30 weeks	P	10	100.0	2	66.7			0	0.0
	C	0	0.0	1	33.3		—	0	0.0
	I	0	0.0	0	0.0			2	100.0
G 600 mg 0 - 40 weeks	P	13	100.0	2	50.0	1	100.0	3	42.9
	C	0	0.0	1	25.0	0	0.0	1	14.3
	I	0	0.0	1	25.0	0	0.0	3	42.9
G 600 mg 0 - 50 weeks	P	22	100.0	5	56.6	1	33.3	2	16.7
	C	0	0.0	3	33.3	1	33.3	0	0.0
	I	0	0.0	1	11.1	1	33.3	10	83.3
G 600 mg Painted for life	P	39	86.7	19	65.5	9	52.9	8	38.1
	C	6	13.3	4	13.8	2	11.8	2	9.5
	I	0	0.0	6	20.7	6	35.3	11	52.4
G 600 mg Total	P	104	94.5	34	64.2	12	52.2	13	30.2
	C	6	5.5	11	20.8	4	17.4	3	7.0
	I	0	0.0	8	15.1	7	30.4	27	62.8

TABLE 18 RELATIONSHIP BETWEEN TUMOUR SIZE AND PATHOLOGY

(continued - 2)

Treatment		Tumour Size: 2 - 6 mm. N	%	6 - 10 mm. N	%	≤ 10 mm. N	%	> 10 mm. N	%
BP 36µg 0 - 15 weeks	P	20	87.0	5	71.4	2	28.6	0	0.0
	C	2	8.7	0	0.0	2	28.6	1	14.3
	I	1	4.3	2	28.6	3	42.9	6	85.7
BP 36µg 0 - 25 weeks	P	31	88.6	12	63.2	0	0.0	1	3.9
	C	2	5.7	3	15.8	2	25.0	4	15.4
	I	2	5.7	4	21.1	6	75.0	21	80.8
BP 36µg 0 - 35 weeks	P	28	90.3	7	33.3	2	20.0	0	0.0
	C	1	3.2	4	19.0	1	10.0	2	6.1
	I	2	6.5	10	47.6	7	70.0	31	93.9
BP 36µg Painted for Life	P	53	67.9	5	23.8	1	4.5	0	0.0
	C	15	19.2	7	33.3	3	13.6	4	5.3
	I	10	12.8	9	42.9	18	81.8	71	94.7
BP 36µg Total	P	132	79.0	29	42.6	5	10.6	1	0.7
	C	20	12.0	14	20.6	8	17.0	11	7.8
	I	15	9.0	25	36.8	34	72.3	129	91.5

TABLE 18 RELATIONSHIP BETWEEN TUMOUR SIZE AND PATHOLOGY

(continued - 3)

Treatment		Tumour Size: 2 - 6 mm. N	%	6 - 10 mm. N	%	≤10 mm. N	%	>10 mm. N	%
BP 60µg 0 - 15 weeks	P	39	92.9	5	38.5	1	16.7	1	5.3
	C	3	7.1	4	30.8	0	0.0	0	0.0
	I	0	0.0	4	30.8	5	83.3	18	94.7
BP 60µg 0 - 25 weeks	P	48	81.4	10	30.3	1	7.7	2	3.6
	C	6	10.2	10	30.3	1	7.7	2	3.6
	I	5	8.5	13	39.4	11	84.6	51	92.7
BP 60µg 0 - 35 weeks	P	41	91.1	17	36.2	5	19.2	3	5.4
	C	2	4.4	16	34.0	4	15.4	5	8.9
	I	2	4.4	14	30.0	17	65.4	48	85.7
BP 60µg Painted for Life	P	44	69.8	32	50.0	5	18.5	3	3.8
	C	12	19.0	8	12.5	5	18.5	4	5.1
	I	7	11.1	24	37.5	17	63.0	72	91.1
BP 60µg Total	P	172	82.3	64	40.8	12	16.7	9	4.3
	C	23	11.0	38	24.2	10	13.9	11	5.3
	I	14	6.7	55	35.0	50	69.4	189	90.4
BP 36+60µg Total	P	304	80.9	93	41.3	17	14.3	10	2.9
	C	43	11.4	52	23.1	18	15.1	22	6.5
	I	29	7.7	80	35.6	84	70.6	318	90.9

TABLE 18 RELATIONSHIP BETWEEN TUMOUR SIZE AND PATHOLOGY

(Continued - 4)

Treatment		Tumour Size: 2 - 6 mm. N	%	6 - 10 mm. N	%	≤10 mm. N	%	>10 mm. N	%
G 600 mg 10 - 50 weeks	P	8	100.0	4	57.1	2	50.0	0	0.0
	C	0	0.0	1	14.3	0	0.0	0	0.0
	I	0	0.0	2	28.6	2	50.0	2	50.0
G 600 mg 20 - 60 weeks	P	10	100.0					0	0.0
	C	0	0.0	—		—		0	0.0
	I	0	0.0					3	100.0
G 600 mg 30 - 70 weeks	P	4	100.0			0	0.0	2	100.0
	C	0	0.0	—		1	100.0	0	0.0
	I	0	0.0					0	0.0
Untreated + Solvent Controls	P	1	50.0						
	C	1	50.0	—		—		—	
	I	0	0.0						
All Groups	P	487	84.5	140	46.2	34	21.8	29	6.9
	C	57	9.9	67	22.1	26	16.7	27	6.4
	I	32	5.6	96	31.7	96	61.5	367	86.8
All Continuous Groups	P	153	72.5	59	48.4	17	24.3	13	7.1
	C	39	18.5	21	17.2	10	14.3	10	5.5
	I	19	9.0	42	34.4	43	61.4	160	87.4

4.6 THE EFFECT OF AGE ON TUMOUR GROWTH RATES

4.6.1 In considering the effect of stopping painting on tumour growth rates (section 4.7) the analysis is considerably facilitated if the probability of a tumour of size x progressing to size y by time t can be estimated ignoring the age of the animal. This presupposes that age has no large effect on tumour growth rate, and the purpose of this section is to test this hypothesis.

4.6.2 Table 19 gives, summed over all treatments, and for each of a number of different ranges of treatment weeks of first appearance of tumour size A, the average time of growth to size D of those tumours that reached that size.

4.6.3 The results demonstrate that tumour growth rates are not strongly dependent on age or length of treatment.

TABLE 19

EFFECT OF AGE ON TUMOUR GROWTH RATE

Age at which tumour first reached size A (weeks)	Number of tumours progressing to size D	Average time of progression to size D
0 - 30	38	11.5
31 - 40	151	12.9
41 - 50	100	12.0
51 - 60	53	9.4
61 - 70	20	9.6
71 - 80	9	13.0
91 - 112	2	16.0

4.7 THE EFFECT OF STOPPING PAINTING ON TUMOUR GROWTH RATES

4.7.1 In order to ascertain the effect of stopping painting on tumour growth rates two life tables were constructed for each of the main four sets of treatment groups (S.W.S., G, BP 30 μg and BP 60 μg) and for each of four tumour growths (A to B, B to C, C to D, A to D) as follows.

Firstly, considering only those animals first getting a tumour of the smaller size after cessation of treatment, the probability of surviving a given number of weeks without reaching a tumour of the larger size in the absence of death was calculated. Secondly, a similar calculation was made considering only those animals first getting a tumour of the smaller size while treatment was being continued. In this case, if treatment was stopped before the tumour had reached the larger size then for the purposes of the construction of the life-table the animal was treated as "dying" at the week of stopping.

4.7.2 Table 20 gives details of the median growth times estimated from these life-tables. This is the first week when the estimated probability of survival from the appearance of the tumour of larger size dropped below 0.5. Also given in the Table are the number of animals with tumours of the smaller size, and the number of those animals who later had tumours of the larger size (ignoring those in the pre-stopping group who reached the larger size post-stopping).

4.7.3 From Table 20 two major facts emerge. One is that the speed of growth is very much faster in the BP treated groups than in the smoke material treated groups. The second is that the tumour growth rate is markedly greater in the groups still being painted than in those groups no longer being painted. This is most marked, and highly statistically

significant, for the growth from A to D.

TABLE 20 MEDIAN TIME BEFORE AN ANIMAL WITH A TUMOUR OF SIZE X HAS A TUMOUR OF SIZE Y (X→Y)

		A → B			B → C			C → D			A → D		
		N_A	N_B	Med.	N_B	N_C	Med.	N_C	N_D	Med.	N_A	N_D	Med.
S.W.S.	Post-stopping	54	22	18	30	21	8	24	17	2	54	11	43
	Pre-stopping	53	30	7	30	18	7	18	11	4	53	11	21
G	Post-stopping	67	32	19	44	19	15	25	19	6	67	7	> 50
	Pre-stopping	84	56	7	56	41	9	41	26	6	82	26	27
BP 30µg	Post-stopping	125	95	5	95	83	3	84	63	4	125	63	18
	Pre-stopping	62	59	3	59	58	4	58	54	2	62	53	10
BP 60µg	Post-stopping	145	123	5	135	111	4	115	100	4	145	86	15
	Pre-stopping	95	81	5	81	73	3	73	63	3	95	63	10

4.8 PREDOMINANT PATHOLOGY AT DEATH

4.8.1 Table 21 gives details of the percentage of animals in each treatment group, and in all groups together, of each major predominant pathology at death. Each animal is assigned to one, and only one, of the cause of death categories.

4.8.2 The percentages as presented are not directly comparable between groups partly due to survival differences and partly due to the fact that, e.g. in the BP 60μg groups, such a high proportion of the animals died with carcinoma of the painted area due to the treatment, that one would expect a lower percentage of other, treatment independent, pathologies in these groups than in e.g. the untreated controls.

4.8.3 No formal analysis has been done to validly compare rates of all these pathologies but it is visually apparent that, apart from the relationship between treatment and dermatis, skin sepsis and carcinoma of the painted area, there is little reason to suspect any effect of treatment on other pathologies. The main effects of skin painting are restricted to the painted area.

TABLE 21

PREDOMINANT PATHOLOGIES - PERCENTAGES

CAUSE OF DEATH	TOTAL	S.W.S. T57 180mgs						G T57 600mgs					
		0-10 weeks	0-20 weeks	0-30 weeks	0-40 weeks	0-50 weeks	Painted For life	0-10 weeks	0-20 weeks	0-30 weeks	0-40 weeks	0-50 weeks	Painted for life
Lymphoma, Splenomegally	14.46	9.3	18.7	18.7	21.3	12.0	14.7	21.3	21.3	16.0	13.3	14.7	9.3
Dermatitis or Skin Sepsis	13.37	6.7	9.3	5.3	16.0	17.3	17.3	4.0	14.7	9.3	5.3	14.7	20.0
Diagnosis Impossible-Autolysed	13.06	20.0	16.0	12.0	16.0	2.7	8.0	20.0	8.0	9.3	9.3	13.3	13.3
Diagnosis Not Determined	12.86	17.3	16.0	14.7	9.3	16.0	16.0	16.0	18.7	14.7	17.3	9.3	14.7
Carcinoma Infil. Muscle (P.A.)	11.61	0.0	0.0	2.7	1.3	13.3	13.3	0.0	1.3	1.3	5.3	10.7	12.0
Adenoma of Lung	8.97	8.0	13.3	14.7	10.7	10.7	8.0	9.3	9.3	13.3	16.0	13.3	5.3
Inflamations	5.24	8.0	10.7	10.7	6.7	2.7	8.0	2.7	6.7	9.3	6.7	2.7	6.7
Nephropathy	4.30	4.0	5.3	4.0	2.7	6.7	2.7	4.0	2.7	6.7	9.3	1.3	1.3
Haemorrhage	3.53	4.0	4.0	2.7	5.3	8.0	2.7	4.0	5.3	2.7	4.0	5.3	2.7
Keratopathy	2.59	5.3	1.3	1.3	1.3	4.0	1.3	1.3	4.0	4.0	1.3	2.7	1.3
Abscess	1.61	2.7	0.0	8.0	2.7	2.7	0.0	2.7	2.7	1.3	4.0	1.3	0.0
Fibro-Sarcoma of Subcutaneous Tissue (Not P.A.)	1.30	6.7	0.0	2.7	1.3	0.0	1.3	2.7	0.0	1.3	1.3	0.0	1.3
Adeno-Carc of Mammary Gland	1.14	1.3	0.0	0.0	0.0	0.0	1.3	0.0	2.7	2.7	1.3	1.3	2.0
Other	5.96	6.7	5.4	3.2	5.4	3.0	5.4	12.0	2.6	8.1	9.6	9.4	6.1
TOTAL MICE = 100%	1929	75	75	75	75	75	75	75	75	75	75	75	75

TABLE 21 (Continued)

PREDOMINANT PATHOLOGIES - PERCENTAGES.

CAUSE OF DEATH	TOTAL	B.P. 36 µg				B.P. 60 µg				GT37 600mg			Untreated	Solvent
		0-15 weeks	0-25 weeks	0-35 weeks	Painted for life	0-15 weeks	0-25 weeks	0-35 weeks	Painted for life	10-50 weeks	20-60 weeks	30-70 weeks		
Lymphoma Splenomegally	14.46	25.3	13.3	13.3	5.3	13.3	6.7	5.3	10.7	13.3	8.0	13.3	24.5	14.7
Dermatitis or Skin Sepsis	13.37	16.0	6.7	18.7	21.3	12.0	25.3	34.7	29.3	4.0	9.3	14.7	3.9	4.9
Diagnosis Impossible - Autolysed.	13.06	13.3	10.7	8.0	12.0	12.0	8.0	12.0	8.0	9.3	22.7	17.3	19.6	21.7
Diagnosis Not Determined	12.86	12.0	5.3	4.0	0.0	17.3	6.7	5.3	1.3	26.7	16.0	20.0	14.7	11.8
Carcinoma Infil.Muscle (P.A.)	11.61	4.0	29.3	36.0	36.0	17.3	40.0	26.7	32.0	6.7	2.7	0.0	0.0	0.0
Adenoma of Lung	8.97	10.7	8.0	5.3	4.0	6.7	1.3	6.7	6.7	13.3	8.0	5.3	5.9	10.8
Inflammations	5.24	6.7	5.3	2.7	4.0	4.0	4.0	0.0	0.0	2.7	5.3	4.0	4.9	5.9
Nephropathy	4.30	4.0	2.7	2.7	1.3	9.3	0.0	1.3	4.0	8.0	4.0	5.3	6.9	5.9
Haemorrhage	3.53	2.7	4.0	1.3	0.0	2.7	0.0	0.0	0.0	2.7	4.0	1.3	9.8	5.9
Nephropathy	2.59	2.7	0.0	4.0	2.7	0.0	2.7	0.0	2.7	4.0	2.7	5.3	1.0	6.9
Abscess	1.61	1.3	0.0	1.3	0.0	2.7	1.3	0.0	0.0	1.3	2.7	1.3	0.0	1.0
Fibro-Sarcoma of Subcutaneous Tissue (Not P.A.)	1.30	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	2.7	2.7	2.7	1.0	2.0
Adeno-Carc.of Mammary Gland	1.14	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	1.3	4.0	2.7	2.0	3.9
Others	5.96	1.3	9.3	2.7	13.4	2.7	4.0	8.0	5.3	4.0	7.9	6.8	5.8	4.8
Total Mice = 100%	1929	75	75	75	75	75	75	75	75	75	75	75	102	102

5. DISCUSSION

5.1 It is well-known that the rise in incidence rates of most human cancers (Nordling, 1953) and of skin tumours in mice subject to continuous painting (Lee and O'Neill, 1971) is related to age by a power law. What is not so clear is whether in fact this increased incidence is due to an increased susceptibility to carcinogen with increasing age or to a cumulative effect of the carcinogen. The ageing experiment described by Lee and Peto (1970), that of Dr. F.J.C. Roe (unpublished) and the ageing groups in this experiment all indicate, by showing approximate equality of rates after comparable lengths of treatment in groups starting at different ages, that rate depends on length of treatment and not age.

5.2 It could be argued, perhaps, that this result could be explained if the effect of the carcinogen were to "start" a tumour within the first, 10 weeks say, in all young rats and within the first, 5 weeks say, in all old rats and that differences in observed times to tumour were purely a result of an age and treatment independent growth process. If this were so, then it would probably not be possible to observe any differences in the relationship between rate and weeks of treatment between young and old rats, although in one sense the old rats are twice as susceptible. It was difficult to believe such a theory in any case, as it would not predict dose-response relationships of the type found by Lee and O'Neill (1971), but it is now completely refuted by the observation that length of exposure to the carcinogen is of crucial importance and that the growth rate of the visible tumour is treatment dependent.

5.3 It seems absolutely clear that the major reason these incidence

rates rise with age is that the effect of the carcinogen is cumulative and not that the animal is inherently weaker at old age. It may be weaker in one sense, but this must be due to the previous effect of carcinogen.

5.4 Having established that cumulative effect is the important factor, how does one measure it and what happens if the carcinogen is stopped? As Armitage and Doll (1954) pointed out, a power law relationship of rate to time is what would be expected if, before a tumour were to occur, a cell had to pass through a number of independent stages. This model has been examined mathematically in this document, making the assumption that there is a background risk of transformation for each stage of the process and that the effect of the carcinogen is to increase one or more of these risks. It has been shown that if one assumes that the carcinogens affect one early and one late stage of the process, (BP having relatively more effect on the early stage than SWS or G), then a fit to the observed data is obtained that is, though by no means perfect, good enough to fit, not only far better than any other plausible model tried so far, but also well enough not to cast serious doubt on any major hypothesis.

5.5. One would have expected the treatments to have a marked effect on the early stage of the process. If they had not, then one would not have observed the apparent lack of association of age per se with incidence rate. If only an early effect had been involved, say the first stage in a four stage process, then incidence rates would rise only slightly less fast after cessation of treatment than if treatment had been continued. In this case the measure of cumulative effect can be calculated by assuming that a dose, d, applied t weeks ago would contribute dt^3 to the

total. Thus after 60 weeks painting the first 10 weeks of painting would contribute approximately $6^3 = 216$ more to the observed incidence than the most recent 10 weeks. Thus, if painting had stopped at 50 weeks, the effect would not have been noticed.

5.6 It is clear from inspection that the treatments, especially the smoke derived treatments, have an effect on a later stage. If they had not, then one would not have observed the fairly immediate relative (absolute, in the case of the smoke derived treatments) drop off of rate. Nor would the observed growth rate of tumours have depended on treatment. If only a last stage effect had been seen then the drop off in rate would have been far more dramatic.

5.7 It has been shown therefore that the effect of treatment is quite complex and has at least two major effects. What effect would one expect stopping smoking to have on human lung cancer, assuming that the mechanisms relating cigarette smoking to lung cancer and smoke condensate to mouse skin cancer are comparable? The results of this experiment suggest that, though an immediate benefit may be expected compared to continuing smoking, there is little reason to suggest a complete reversion to non-smoker's lung cancer rates. Cigarette smoking, if the model were correct, would have caused a permanently increased risk of lung cancer. Stopping smoking could, however, reduce, and possibly very substantially, future risk. Accurate qualitative assessments of the benefits of stopping smoking obviously await human prospective surveys. It is of interest, however, that Richard Peto (personal communication) tells me that in the Doll and Hill study, the rates after stopping smoking show an initial decrease, followed by a rise much as in our SWS and G groups.

5.8 The other main purpose of the experiment, which was to show the relationship between tumour size and pathology, has demonstrated that, though 2 mm tumours are rarely infiltrating carcinomas and 10 mm usually are, the proportions vary between benzpyrene and smoke material. It would not be safe, therefore, unless perhaps very similar types of product were being tested for carcinogenicity, to use tumours of a given size only as the index of activity and to dispense with microscopic observations.

5.9 The analyses in this experiment, though perhaps sufficient to answer the main questions of interest, by no means extract all the possible useful information from the mass of data available. I would be happy to extract data relevant to any other questions readers may think important.

P.N.L.

30.4.74

6. SUMMARY OF CONCLUSIONS

1. The incidence rate of tumours due to smoke-derived material drops soon after stopping painting but later rises though to levels considerably less than had painting been continued.

2. The incidence rate of tumours due to benzpyrene continues to rise after stopping painting but very much less steeply than it would have done had painting been continued.

3. A multistage model in which the carcinogens affect at least two stages of the cancer process, one early and one late, fits the observed results quite well.

4. Though the estimated relative effect of all treatments to the background effect is greater for the early stage than the later stage this is far more marked for benzpyrene than for smoke-derived material.

5. There is no apparent effect of age per se on tumour incidence rate or on tumour growth rate.

6. The treatments affect the growth rate of the tumours; stopping painting reduces the growth rate.

7. There is a marked relationship between tumour size and pathology. 10 mm tumours are very commonly infiltrating carcinomas; 2 mm tumours rarely are. This relationship does depend, however, on treatment.

7. STATISTICAL APPENDICES

a) Calculation of expected number of animals with tumours for section 4.2

Let the incidence rate of tumours be given by

$I = bk\,(t - w)^{k-1}$ (Weibull distribution)

up to time Q and after that let it remain constant at the value

$I = bk\,(Q - w)^{k-1}$

For hypothesis 1 Q can be taken as infinite

2 $Q = S + w$

3 $Q = S$

where S is the actual time of stopping.

Consider the time period (t_1, t_2) and let

$X(t) = b\,(t - w)^{k}$

$Y(t) = bkt\,(Q - w)^{k-1}$

n_1 = the number of animals alive and tumourless at time t_1

n_2 = the number of animals alive and tumourless at time t_2

nq = the number of animals alive and tumourless at time Q

and all summations be over the times of tumour or tumourless death in the interval.

The expected number of animals with tumours E is then given by:

1) If $Q < w$ then $E = 0$

2) If $Q > w$ then either: (i) $t_2 < w$

$E = 0$

(ii) $t_1 < w$, $t_2 > w$, $t_2 < Q$

$E = n_2 X(t_2) + \sum_{t_2 \geq t > w} X(t)$

(iii) $t_1 > w,\ t_2 > w,\ t_2 < Q$

$$E = n_2 X(t_2) + \sum_{t_2 > t > t_1} X(t) - n_1 X(t_1)$$

(iv) $t_1 < Q < t_2$

$$E = n_2 Y(t_2) + \sum_{t_2 > t > Q} Y(t) - nq\, Y(Q) + nq\, X(Q) + \sum_{Q > t > t_1} X(t) - n_1 X(t_1)$$

(v) $t_1 > Q$

$$E = n_2 Y(t_2) + \sum_{t_2 > t > t_1} Y(t) - n_1 Y(t_1)$$

These formulae are derived by maximising the log likelihood function over the interval (t_1, t_2).

b) Derivation of the Weibull distribution for continuous and stopping painting experiments under the hypotheses of section 4.3.1

We shall consider the three stage model only in the derivation. Generalisation to a multi-stage model presents no new problems.

Continuous Experiment

The number of cells in the second stage, N_2, by time T is given by:

$$N_2 = N \int_0^T b_2 \int_0^V b_1 \, du \, dv$$

$$= \frac{N b_1 b_2 T^2}{2}$$

Thus the incidence rate at time T (which is the product of the number of cells at risk w weeks before, and the third stage transition probability) is given by:

$$I = \frac{N b_1 b_2 b_3 (T - w)^2}{2}$$

and the cumulative density function G by

$$G = 1 - \exp\left(-\frac{Nb_1b_2b_3}{6}(T - w)^3\right)$$

(derived from the fact that $I = dG/(1 - G)$ and that $G = 0$ at time $T = w$)

Stopping Painting Experiment

The number of cells in the second stage at time T is given here by

$$N_2 = N\left[\int_0^S c_2\int_0^V c_1\,du\,dv + \int_S^T b_2\int_0^S c_1\,du\,dv + \int_S^T b_2\int_S^V b_1\,du\,dv\right]$$

$$= N\left[\frac{c_1c_2S^2}{2} + c_1b_2S(T - S) + \frac{b_1b_2(T - S)^2}{2}\right]$$

The incidence rate is given by

$$I = N\left[\frac{c_1c_2b_3S^2}{2} + c_1b_2b_3S(T - S - w) + \frac{b_1b_2b_3(T - S - w)^2}{2}\right]$$

and the cumulative density function G by

$$G = 1 - \exp\left(-N\left[\frac{c_1c_2c_3S^3}{6} + \frac{c_1c_2b_3S^2(T - S - w)}{2} + \frac{c_1b_2b_3S(T - S - w)^2}{2} + \frac{b_1b_2b_3(T-S-w)^3}{6}\right]\right)$$

(derived from the fact that $I = dG/(1 - G)$ and that $G = 1 - \exp\left(-\frac{Nc_1c_2c_3S^3}{6}\right)$ at time $T = S + w$)

The latter two formulae clearly only apply for $T > S + w$. If $T < S + w$ the effect of stopping is not yet seen and the formulae for continuous painting apply.

c) Derivation of the Weibull distribution for continuous and stopping painting experiments under the alternative hypothesis of section 4.3.24

As in section 7(b) we shall consider only the three stage model and use similar notation. The only difference in the hypotheses from those used in section 7(b) is that instead of a single waiting time of w

after the last transformation there are waiting times w_i after each transformation.

Continuous Experiments

The number of cells ready for the third transformation, N_2, by time T is given by

$$N_2 = N \int_{w_1}^{T-w_2} b_2 \int_{0}^{V-w_1} b_1 \, du \, dv$$

$$= \frac{Nb_1b_2(T - w_1 - w_2)^2}{2}$$

thus

$$I = \frac{Nb_1b_2b_3(T - w_1 - w_2 - w_3)^2}{2}$$

which since $w = w_1 + w_2 + w_3$, is the same formula as in section 7(a).

Stopping Painting Experiments

Here the change of hypothesis does affect the formula. We will consider firstly the incidence rate at a time $T > S + w_1 + w_2 + w_3$ and assume that $S > w_1 + w_2$. Subsequently, we will consider how other assumptions affect the formula.

The number of cells ready for the third transformation, N_2, by time $T\ (> S + w_1 + w_2)$ is given by the sum of the following six expressions:

a) The number of cells ready for the third transformation by time S

$$X_a(T) = N \int_{w_1}^{S-w_2} c_2 \int_{0}^{V-w_1} c_1 \, du \, dv = \frac{Nc_1c_2(S-w_1-w_2)^2}{2}$$

b) The number of cells in the second waiting-time at time S

$$X_b(T) = N\int_{S-w_2}^{S} c_2 \int_{0}^{V-w_1} c_1\, du\, dv = \frac{Nc_1c_2}{2}\left[(S-w_1)^2 - (S-w_1-w_2)^2\right]$$

c) The number of cells ready for the second transformation at time S, undergoing the second transformation after S and ready for the third transformation at time T.

$$X_c(T) = N\int_{S}^{T-w_2} b_2 \int_{0}^{S-w_1} c_1\, du\, dv = Nc_1b_2(S-w_1)(T-S-w_2).$$

d) The number of cells in the first waiting time at time S undergoing the second transformation after time S+w, and ready for the third transformation at time T

$$X_d(T) = N\int_{S+w_1}^{T-w_2} b_2 \int_{S-w_1}^{S} c_1\, du\, dv = Nc_1b_2w_1(T-S-w_1-w_2)$$

e) The number of cells in the first waiting time at time S, undergoing the second transformation before time $S+w_1$, and ready for the third transformation at time T

$$X_e(T) = N\int_{S}^{S+w_1} b_2 \int_{S-w_1}^{V-w_1} c_1\, du\, dv = \frac{Nc_1b_2w_1^2}{2}$$

f) The number of cells undergoing the first two transformations after S, and ready for the third transformation at time T.

$$X_f(T) = N\int_{S+w_1}^{T-w_2} b_2 \int_{S}^{V-w_1} b_1\, du\, dv = \frac{Nb_1b_2(T-S-w_1-w_2)^2}{2}$$

The incidence rate at time T is therefore given by

$$I = \frac{Nc_1c_2b_3(S-w_1)^2}{2} - \frac{Nc_1b_2b_3w_1^2}{2} + Nc_1b_2b_3S(T-S-w_2-w_3) + \frac{Nb_1b_2b_3(T-S-w_1-w_2-w_3)^2}{2}$$

The cumulative density function can also be obtained, but requires a knowledge of the formulae for $T < S+w_1+w_2+w_3$ considered later.

We now consider how the expressions $X_a(T) \ldots\ldots X_f(T)$ alter, under different assumptions about S and T.

Firstly, we consider S

If $S < w_1$ then

$$\begin{cases} X_a(T) = X_b(T) = X_c(T) = 0 \\ X_d(T) = Nc_1b_2S(T-S-w_1-w_2) \\ X_e(T) = \dfrac{Nc_1b_2S^2}{2} \\ X_f(T) \text{ is unchanged} \end{cases}$$

If $S > w_1$ but $< w_1+w_2$ then

$$\begin{cases} X_a(T) = 0 \\ X_b(T) = \dfrac{Nc_1c_2(S-w_1)^2}{2} \\ X_c(T), X_d(T), X_e(T) \text{ and } X_f(T) \text{ are unchang} \end{cases}$$

Secondly, we consider T

If $T < w_1 + w_2$ then all $X(T) = 0$

If $T < S$ then the continuous formula applies

If $T > S$ but $< S + w_2$ then

$$\begin{cases} X_a(T) \text{ is unchanged} \\ X_b(T) = \dfrac{Nc_1c_2}{2}\left[(T-w_1-w_2)^2 - (S-w_1-w_2)^2\right] \\ X_c(T) = X_d(T) = X_e(T) = X_f(T) = 0 \end{cases}$$

If $T > S+w_2$ but $< S+w_1+w_2$ then

$$\begin{cases} X_a(T), X_b(T) \text{ and } X_c(T) \text{ are unchanged} \\ X_d(T) = X_f(T) = 0 \\ X_e(T) = Nc_1b_2\dfrac{(T-S-w_2)^2}{2} \end{cases}$$

There are in fact some other possibilities but as the equations for these are easily derivable from those given we shall not present them here. Nor shall we give the values of the incidence rate or cumulative density function in all these cases.

8. REFERENCES

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