

SC-19192: A 26 WEEK URINARY BLADDER TUMORIGENICITY
STUDY IN THE MOUSE BY THE
INTRAVESICAL PELLET IMPLANT TECHNIQUE

P-T NO. 1032ot72

FINAL REPORT

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by

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SUMMARY

A 26 week urinary bladder tumorigenicity study of SC-19192 was conducted in albino female 60-90 day old mice by the intravesical pellet implant technique. Pellets of 20-22 mgm mass composed of purified cholesterol (80%) and SC-19192 (20%; 4.0-4.4 mgm each) were prepared and surgically placed into the murine urinary bladder lumina. A negative control group of mice exposed only to pellets of purified cholesterol, and a positive control group of mice exposed to pellets composed of cholesterol (80%) and the 8-methyl ether of xanthurenic acid (20%; 4.0-4.4 mgm each) were established. The latter compound was selected as a positive control on the basis that in previous studies with the intravesical pellet implant technique it was associated with a statistically significantly augmented incidence of urinary bladder tumors when compared to an appropriate negative control group, and the characteristics of exposure of the murine urinary bladder mucosa in vivo of this compound and SC-19192 were found to be similar.

Criteria evaluated for compound effect were morbidity, mortality, motor and behavioral activity, growth, general external features, and digital palpation of protruding tissue masses. All animals dying, killed in extremis, or killed at experimental termination were subjected to thoracic, abdominal, and pelvic necropsy, as well as cutaneous inspection. All grossly abnormal tissues were recorded and preserved for subsequent histopathologic inspection.

The study was designed to specifically examine and compare the incidence of urinary bladder neoplasia present in the treated groups with that present in the

negative control group. All bladders were distended and fixed in Bouin's solution, injected per urethram, bisected in the midsagittal plane, inspected grossly under low magnification, and six intermittent sagittal sections of each bladder half were prepared for histopathologic inspection. The incidence of bladder neoplasia present in the treated groups was compared with that present in the negative control group by the exact method for 2×2 tables.

No bladder neoplasia were observed in animals dying or killed prior to 175 days of experimental observation, a finding compatible with previously published data employing the pellet implantation technique in mice. The incidence of bladder neoplasia recorded is based only on those mice surviving 175 days or more subjected to histopathologic inspection. The following urinary bladder neoplasia incidences recorded were: negative control group - 5/69 (7.2%); SC-19192 - 11/64 (17.2%; $p = 0.07$); and positive control group - 7/44 (15.9%; $p = 0.12$). Thus, these data provide no evidence for a statistically significantly augmented incidence of urinary bladder neoplasia associated with SC-19192 as assayed by the intravesical pellet implantation technique with a 26-week period of observation.

The utilization of the pellet implantation technique in a 26-week study of the type employed herein is unique. Previous published studies have generally employed a 40 to 56 week period of experimental observation. Thus, there is a paucity of historical data relative to the positive and negative control groups that are relevant in the present experimental context. Separate studies of conventional 56 week duration in the mouse are currently in progress with expected animal termination date of August, 1973.

INTRODUCTION:

The purpose of this study was to evaluate and assess the possible urinary bladder tumorigenicity of SC-19192 in the female albino mouse by the intravesical pellet implant technique during a 26-week period of observation. The studies described were initiated March 9, 1972, and were terminated September 28, 1972. This report presents the data recorded for the entire experimental period. A separate study of conventional 56 week duration is in progress, with anticipated termination date of August 1973.

MATERIAL:

<u>Identification</u>	1. SC-19192
	2. 8-Methyl Ether of Xanthurenic Acid (Positive Control Compound)
<u>Description</u>	1. A fine, white powder
	2. A crystalline, yellow substance
<u>Received</u>	1. From Searle Laboratories February 25, 1972 designated as Lot #IR A6 906
	2. Synthesized in The Division of Clinical Oncology, University of Wisconsin School for Health Sciences
<u>Purity</u>	1. Specified by Searle Laboratories
	2. No detectable impurities

METHODS

Experimental Animals

Three hundred and five female 60-90 day old Swiss albino mice obtained from Rolfsmeyer Company, Madison, Wisconsin.

Weight Range at Initiation of Study: 30 to 34.5 grams each.

Housing: 5 or less mice per raised, stainless steel, screen-bottomed cage.

Basal Diet: Wayne Lab-Blox (Allied Mills, Inc., Chicago, Illinois) and water available ad libitum.

Selection for Groups: Mice were received in lots consisting of about 110 mice each from the supplier, and each lot was assigned as received to a study group.

Groups and Dosage Levels

<u>Group No.</u>	<u>No. of Mice</u>	<u>Dosage Level mgm/mouse</u>
1. (Negative Control)	100	0
3. SC-19192	105	4.0 - 4.4
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control)	100	4.0 - 4.4

Administration of Test Material

Pellets of 20-22 mgm mass and 0.4 cm diameter, composed of one part of powdered SC-19192 mixed with four parts of three times recrystallized, powdered cholesterol (obtained from Sigma Chemical Co., St. Louis, Mo.) were fashioned.

The cholesterol and each chemical selected to be tested for tumorigenic activity were separately ground to a fine powder in an agate mortar. The test compound was then carefully mixed with cholesterol by grinding thoroughly in a mortar. The mixture was compressed into spheroidal pellets with a standard, rounded cup die in a Colton pellet press. The dies were dusted frequently with fine magnesium stearate powder to prevent capping of the pellet. Pellets of comparable size were also prepared from pure cholesterol. The dies and the pellet press were thoroughly cleaned between preparation of different lots of pellets to avoid any chemical cross contamination. Lots of pellets numbering 130-140 were prepared for each group to encompass the needs of the study and to insure uniformity and reproducibility of the chemical composition of the pellets. All pellets were weighed following preparation, those exceeding the tolerance limits were discarded, and those retained were placed in individually labeled small glass vials for storage at room temperature (72°F) prior to animal administration. Storage in this manner was no more than 7 days prior to animal placement.

The mice were individually anesthetized with pentobarbital (Nembutal sodium, Abbott Laboratories, North Chicago, Illinois) and ether. Each study mouse had a pellet surgically inserted into the urinary bladder lumen by the technique of Jull (1) as modified by Allen et al (2). These techniques utilized have been amply described (3-8).

Observations and Records

The mice were inspected twice daily for morbidity, mortality, motor and

behavioral activity. Individual body weights were recorded weekly up to 4 weeks, biweekly for the next 8 weeks, once every 4 weeks thereafter, and at death. Pertinent observations, including general external features and digital palpation of protruding tissue masses were recorded.

Clinical Laboratory Studies - None

Terminal Studies

Animals Found Dead: Terminal body weights were recorded, necropsies performed under the supervision of a pathologist, and representative tissues preserved. All tissues in the thoracic, abdominal, and pelvic cavities were examined, as well as the skin.

Animals Killed in Extremis or by Design: Terminal body weights were recorded, animals were killed by ether anesthesia, necropsies performed under the supervision of a pathologist, tissues of the thoracic, abdominal, and pelvic cavities, as well as the skin, were examined, representative tissues were preserved, and all gross abnormalities were sampled for histologic preparation and inspection.

Postmortem Procedures:

Preservation of Tissues - All preserved tissues, except the urinary bladders, were fixed in 10% neutral buffered formalin. All urinary bladders were distended with Bouin's fixative inserted through the urethra. The bladders were bisected in the mid-sagittal line and inspected grossly.

Microscopic Examination - The bisected halves of each bladder were imbedded and six intermittent sagittal sections of each half were prepared at 5 μ thickness and stained with hematoxylin and eosin. All tissues preserved, imbedded tissues, and stained tissue sections are on file at the Division of Clinical Oncology.

Statistical Evaluation

Parameters Analyzed: Statistical evaluation was restricted to a comparison of the incidence of carcinoma observed in animals surviving more than 175 days. The comparison was made between the incidence of carcinomas related to the introduction of pellets of cholesterol containing a test chemical with the cholesterol alone (Negative Control) group, and probabilities of statistical significance were computed by the exact method for 2 x 2 tables (9).

RESULTS

Animal Survival

The number and percentage of mice surviving at various time periods following surgical placement of pellets into urinary bladder lumina is presented in Table 1 (pg. 8) for Groups 1 (Negative Control), 3 (SC-19192), and 4 (Positive Control). The survival of the mice in Group 3 approximated that of Group 1 and was somewhat better than that of Group 4. Previous studies conducted in these laboratories at different time periods (1959-1968) (4-8) show that the survival of

TABLE 1

Summary of Mean Weights of Mice Subjected to a 26 Week Urinary Bladder Tumorigenicity Study
by the Intravesical Pellet Implant Technique. P-T 1032of72.

Group		Weeks				
		1	4	8	12	20
1. Cholesterol (Neg. Control)	(No. Examined)	97	85	80	78	74
	(Percent Surviving)	97	85	80	78	74
	(Mean Weights - gms)	30.8	37.4	38.9	42.2	43.1
3. SC-19192	(No. Examined)	97	75	72	70	68
	(Percent Surviving)	92.4	71.4	68.6	66.7	64.8
	(Mean Weights - gms)	32.2	38.6	41.6	42.9	44.6
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control)	(No. Examined)	87	68	59	55	50
	(Percent Surviving)	87	68	59	55	50
	(Mean Weights - gms)	34.5	40.2	42.0	44.9	45.2

mice in population groups similar to those of this study, and in treatment exposure comparable to those of Group 1 (Negative Control) ranged from 24-63% at 175 days after surgery. The data observed in the present experiment for control and treated groups is not at variance with previous experience (4-8).

Animal Weights

The mean weights of mice surviving at various time periods following surgical pellet placement is presented in Table 1 (pg. 8) for Groups 1 (Negative Control), 3 (SC-19192), and 4 (Positive Control). Growth of all treated groups was comparable to that observed for Group 1. Growth measurements have not been a part of any previously published data concerning the pellet implantation technique.

Tumor Incidence

Urinary Bladder: All available urinary bladders were inspected microscopically and the presence of hyperplasia, cystitis, metaplasia, and neoplasia was noted and scored. The scoring criteria employed as well as that attributed to each animal are submitted as Appendixes. The major emphasis was placed on the assessment, tabulation, and statistical relevance of bladder neoplasia. The histopathologic criteria of Bonser and Jull (10) and of Roe (11) were employed. Lesions possessing cellular characteristics compatible with epithelial-derived neoplasia and with extension into the bladder submucosa were classified as Stage I; those that were additionally seen to invade muscle as Stage II; those that additionally

presented evidence of serosal spread or local pelvic metastases as Stage III; and those that additionally demonstrated regional nodal or distant metastases as Stage III-M.

No urinary bladder neoplasms were found in mice subject to study by the pellet implantation technique dying or killed prior to 175 days following surgery in these or previous (4-8) studies. Thus only those mice surviving a minimum of 176 days were included for incidence tabulations and statistical analysis (Table 2, pg. 11).

The incidence of urinary bladder neoplasms associated with exposure to SC-19192 was about 2.4 times that present in Group 1 (Negative Control Group), but was not statistically significantly greater than that present in Group 1. Conversely, the incidence related to exposure to SC-19192 was slightly greater than that present in Group 4 (Positive Control Group). It is concluded that treatment with SC-19192 did not produce a statistically significant augmentation of urinary bladder neoplasia during the 26-week experimental period.

The incidence of urinary bladder neoplasms associated with exposure to the 8-methyl ether of xanthurenic acid (Group 4, Positive Control Group) was about 2.2 times that present in Group 1 (Negative Control Group), but was not statistically significantly greater than that present in Group 1. Thus, the incidence observed in the Positive Control Group (Group 4) was not significantly greater than that present in the Negative Control Group (Group 1).

The present study is unique in relationship to previously published studies

TABLE 2

Summary of Survival and Incidence of Neoplasia Observed in a 26 Week Urinary Bladder Tumorigenicity Study

in the Mouse by the Intravesical Pellet Implant Technique. P-T 1032ot72.

Group	Survival (days)						Incidence of Bladder Changes				P-Value*	
							Neoplasia Stage (all 176-200 days)					
	0-50	51-100	101-150	151-175	176-200	Unknown	Metaplasia	I	II	III	Total	
1. Cholesterol (Neg. Control) NA**	6	3	4	1	69		1	3	2	0	5/69	---
	3	0	1	0	2	11						
3. SC-19192 NA**	19	2	2	2	64		2	2	9	0	11/64	0.07
	1	0	0	0	2	13						
4. 3-Methyl Ether of Xanthurenic Acid (Positive Control) NA**	21	2	2	0	44		1	2	5	0	7/44	0.12
	8	1	1	1	2	18						

* P-value calculated by exact method for 2 x 2 table.

** NA -- Animal cannibalized, too autolyzed or lost; or bladder tissue not available for microscopic inspection.
Please see detailed summary of each individual animal (Appendixes I, III, and IV).

employing the pellet implantation technique in that the present study was terminated after 26 weeks of observation. Thus, there is a paucity of historical data concerning the negative and positive control groups that might be cited as relevant to the present studies. It is hoped that conventional studies involving the compounds under present consideration designed to encompass 56 weeks of experimental observation and scheduled to terminate about August 1973 will clarify the data reported herein.

The negative and positive control groups for this study and for P-T No. 1031ot72 were common between the two studies.

Other Tumors: An occasional mammary tumor and thymic neoplasm was observed in all groups in low incidence. Histopathologic analyses of these tissues are incomplete, and relevant data are not available at this time. Supplementary data will be provided when they are available.

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APPENDIX TO

SC-19192: A 26 WEEK URINARY BLADDER TUMORIGENICITY
STUDY IN THE MOUSE BY THE INTRAVESICAL
PELLET IMPLANT TECHNIQUE

P-T No. 1032 of 72

TABLE 1

Key to Column Designations of Appendixes I-IV.

NA - Animal or tissue not available for data inclusion due to cannibalization, severe post mortem autolysis, animal disappearance from cage, or tissue unavailability for microscopic inspection.

Animal Status Death

- 0 - Animal killed and tissues fresh.
- 1 - Animal died with moderate post mortem autolysis. Urinary bladder epithelial surface is shed 30-60 minutes following death.
- 2 - Animal died with severe post mortem autolysis.
- 3 - Animal died and partially or wholly cannibalized by cage mates.

Tissue availability

- 0 - None saved due to severe autolysis or cannibalization.
- 1 - Tissue available for histologic processing.
- 2 - Tissue available for histologic processing, but blocks or slides misplaced and not available at this time for study.

Hyperplasia

- 0 - None
- 1 - Slight or focal
- 2 - Moderate or more extensive
- 3 - Severe, generally involving most of bladder epithelial surface
- 4 - Unable to determine, generally due to post mortem autolytic change

Cystitis

- 0 - None
- 1 - Slight or focal
- 2 - Moderate or more severe
- 3 - Severe, generally involving submucosa, muscularis, and serosa of bladder, and the majority of the bladder structures.
- 4 - Unable to determine, generally due to post mortem autolytic change

Metaplasia

- S - Squamous metaplasia
- G - Glandular metaplasia
- O - None (if not designated is specifically this)
- 1 - Slight or focal
- 2 - Moderate, involving 1/3 of bladder epithelial surface
- 3 - Severe, involving more than 1/3 of bladder epithelial surface

TABLE 1 (con't)

Malignant Stage

- I - Cellular characteristics compatible with epithelial-derived neoplasia, with extension into bladder submucosa.
- II - Cellular characteristics compatible with epithelial-derived neoplasia, with extension into or through bladder musculature.
- III - Cellular characteristics compatible with epithelial-derived neoplasia, with extension through bladder serosa, or into adjacent pelvic structures.
- III-M - Like III but with regional nodal or distant metastases.

APPENDIX I

Cholesterol alone, negative control group for P-T 1032ot72.

Tabulation of fate and assessment of individual mice in "A 26 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique."

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	10	1	1	2	2				
2	10	3	0	NA					
3	12	1	1	4	1				
4	20	2	1	4	4				
5	26	1	2	NA					
6	27	1	1	3	0				
7	33	3	0	NA					
8	45	1	1	1	0				
9	47	1	1	1	0				
10	60	1	1	0	1				
11	64	1	1	0	1				
12	86	1	1	1	1				
13	108	2	1	4	4				
14	120	1	1	0	1				
15	133	1	1	2	0				
16	144	1	1	1	2				
17	147	1	2	NA					
18	174	1	1	4	0				
19	190	0	1	1	2				
20	190	0	1	2	2				
21	190	0	1	0	0				
22	190	0	1	1	2				
23	189	0	1	0	0				
24	189	0	1	2	0		1		
25	189	0	1	2	2		1		
26	189	0	1	0	0				
27	189	0	1	0	1				
28	189	0	1	0	1				
29	189	0	1	2	2				
30	189	0	1	1	0				

APPENDIX 1 (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
31	NA								
32	189	0	1	2	1				
33	189	0	1	1	1				
34	189	0	2	NA					
35	NA								
36	NA								
37	189	0	1	0	0				
38	NA								
39	189	0	1	1	1				
40	189	0	1	0	1				
41	189	0	1	1	1				
42	189	0	1	1	0				
43	189	0	1	1	3				
44	189	0	1	0	0				
45	189	0	1	2	1				
46	189	0	1	0	0				
47	189	0	1	1	0				
48	189	0	1	0	0				
49	189	0	1	2	1				
50	186	0	1	1	1				
51	186	0	1	1	0				
52	186	0	1	1	2				
53	186	0	1	0	0				
54	186	0	1	1	1				
55	186	0	2	NA					
56	186	0	1	1	1				
57	NA								
58	186	0	1	2	2				
59	185	0	1	0	0				
60	185	0	1	1	0				
61	NA								
62	185	0	1	1	0				
63	185	0	1	1	1				
64	185	0	1	0	3				
65	185	0	1	2	1				

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APPENDIX I (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metoplasia	I	II	III
66	NA								
67	185	0	1	2	2				
68	184	0	1	0	0				
69	184	0	1	0	0				
70	184	0	1	1	2				
71	184	0	1	0	0				
72	184	0	1	3	3				
73	184	0	1	1	3				
74	NA								
75	184	0	1	0	1				
76	184	0	1	1	3	5-3		1	
77	184	0	1	1	2				
78	184	0	1	0	0				
79	183	0	1	0	0				
80	183	0	1	1	2				
81	183	0	1	0	0				
82	184	0	1	0	0				
83	184	0	1	0	1				
84	184	0	1	0	0				
85	184	0	1	1	3				
86	184	0	1	0	0				
87	184	0	1	0	0				
88	NA								
89	NA								
90	184	0	1	1	0				
91	184	0	1	3	2				
92	184	0	1	0	0				
93	184	0	1	1	1				
94	184	0	1	2	3			1	
95	184	0	1	0	0				
96	184	0	1	2	1				
97	184	0	1	0	0				
98	184	0	1	2	0				
99	184	0	1	2	3		1		
100	NA	3	0						

-1-
APPENDIX III

SC-19192 (20%), cholesterol (80%) group for P-T 1032 at 72.

Tabulation of fate and assessment of individual mice in "SC-19192: A 26 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique."

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Status		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	8	0	1	1	0				
2	8	2	1	4	0				
3	9	1	1	4	0				
4	9	0	1	0	0				
5	10	3	0	NA					
6	10	0	1	1	0				
7	11	2	1	4	0				
8	12	0	1	2	1				
9	14	2	1	4	0				
10	14	2	1	4	0				
11	17	1	1	0	0				
12	17	0	1	1	0				
13	18	1	1	4	0				
14	18	1	1	4	1				
15	25	2	1	4	0				
16	25	1	1	4	0				
17	26	0	1	0	2				
18	35	1	1	1	1				
19	49	0	1	0	0				
20	50	2	1	4	0				
21	59	0	1	2	1				
22	63	0	1	1	0				
23	114	2	1	4	3				
24	125	2	1	4	0				
25	170	2	1	4	0				
26	171	1	1	4	3				
27	179	2	1	4	3				
28	187	0	1	0	0				
29	187	0	1	1	0				
30	187	3	0	NA					

APPENDIX III (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
31	187	0	1	2	1				
32	187	0	1	2	1				
33	187	0	1	1	1				
34	NA								
35	NA								
36	187	0	1	2	1				
37	187	0	1	2	1				
38	187	0	1	2	1				
39	187	0	1	0	2				
40	187	0	1	0	0				
41	NA								
42	NA								
43	187	0	1	1	1				
44	187	0	1	1	2				
45	187	0	1	1	1				
46	187	0	1	1	1				
47	188	1	1	1	1				
48	188	0	1	0	0				
49	188	3	0	NA					
50	188	0	1	3	1				
51	188	0	1	3	3				1
52	188	0	1	1	1		1		
53	NA								
54	188	0	1	2	0				
55	NA								
56	188	0	1	0	0				
57	188	0	1	1	1				
58	188	0	1	2	1				
59	NA								
60	188	0	1	1	1				
61	188	0	1	2	1				
62	188	0	1	0	0				
63	188	0	1	0	0				
64	188	0	1	3	2				1
65	NA								

APPENDIX III (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
66	NA								
67	NA								
68	188	1	1	4	0				
69	189	0	1	2	3	S-1		1	
70	189	0	1	1	1		1		
71	189	0	1	0	0			1	
72	187	0	1	1	2				
73	189	0	1	1	2			1	
74	189	0	1	2	2			1	
75	189	0	1	0	0				
76	187	0	1	2	1				
77	187	0	1	0	0				
78	189	0	1	0	0				
79	187	0	1	2	2				
80	187	0	1	0	0				
81	187	0	1	2	2				
82	NA								
83	189	0	1	0	0				
84	189	0	1	2	2				
85	187	0	1	2	2			1	
86	189	1	1	1	0				
87	189	0	1	2	0				
88	189	0	1	0	1				
89	189	0	1	0	1				
90	189	0	1	0	0				
91	189	0	1	1	0				
92	189	0	1	0	0				
93	189	0	1	1	0				
94	NA								
95	187	1	1	1	2				
96	189	0	1	1	1				
97	187	0	1	2	1				
98	187	0	1	1	1				
99	187	0	1	2	1				
100	187	1	1	4	0				
101	189	0	1	2	1			1	
102	189	0	1	2	2	S-2		1	
103	189	0	1	2	2				
104	NA								
105	189	0	1	0	0				

APPENDIX IV

XAE (20%), cholesterol (80%), positive control group for P-T 1032ot72.

Tabulation of fate and assessment of individual mice in
 "A 26 Week Urinary Bladder Tumorigenicity Study in the Mouse by the
 Intravesical Pellet Implantation Technique."

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	2	1	1	4	0				
2	3	3	0	NA					
3	3	3	0	NA					
4	4	3	0	NA					
5	4	3	0	NA					
6	5	1	1	4	0				
7	7	1	1	4	0				
8	8	1	1	4	3				
9	9	0	1	2	1				
10	10	0	1	1	3				
11	10	2	1	4	0				
12	10	1	1	4	3				
13	10	1	1	4	3				
14	11	2	1	4	4				
15	11	2	1	4	2				
16	12	0	1	0	0				
17	12	0	1	1	1				
18	13	1	1	1	1				
19	13	0	1	1	3				
20	13	1	1	4	3				
21	13	1	1	4	2				
22	16	1	1	1	0				
23	17	3	0	NA					
24	17	1	1	NA					
25	29	1	0	NA					
26	34	1	1	4	0				
27	34	1	1	4	0				
28	37	0	1	1	0				
29	50	3	0	NA					
30	64	3	0	NA					

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APPENDIX IV (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
31	65	1	1	4	2				
32	74	1	1	0	1				
33	131	1	1	4	1				
34	137	1	1	0	0				
35	141	3	0	NA					
36	167	1	1	NA					
37	188	0	1	0	0				
38	188	0	1	0	0				
39	NA								
40	188	0	1	1	2				
41	188	0	1	1	0				
42	188	0	1	1	1				
43	188	0	1	3	2		1		
44	188	0	1	2	1				
45	NA								
46	188	0	1	1	3				
47	188	0	1	1	0				
48	188	0	1	1	0				
49	188	0	1	2	0				
50	NA								
51	188	0	1	1	0				
52	188	NA							
53	188	0	1	1	1			1	
54	188	0	1	1	1				
55	188	0	1	0	0				
56	188	0	1	3	2				
57	188	0	1	2	2				
58	188	0	1	1	0				
59	188	0	1	0	0				
60	188	0	1	0	0				
61	188	0	1	2	1				
62	188	0	1	2	2			1	
63	185	0	1	0	0				
64	185	0	1	2	3			1	
65	185	0	1	0	0				

APPENDIX IV (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
66	185	0	1	0	0				
67	185	0	1	NA					
68	185	0	1	0	0				
69	NA								
70	185	0	1	1	2				
71	NA								
72	185	0	1	1	1				
73	NA								
74	NA								
75	NA								
76	185	0	1	0	0				
77	NA								
78	NA								
79	NA								
80	185	0	1	0	0				
81	NA								
82	NA								
83	NA								
84	185	0	1	3	2				
85	185	0	1	1	2			1	
86	NA								
87	184	0	1	2	1				
88	184	0	1	1	2	S-3			
89	184	0	1	2	0				
90	184	0	1	0	0				
91	184	0	1	1	1		1		
92	184	0	1	1	2				
93	NA								
94	183	0	1	0	0				
95	183	0	1	1	0				
96	183	0	1	2	1				
97	183	0	1	2	1			1	
98	183	0	1	2	0				
99	NA								
100	NA								