

SC-19192: A 56 WEEK URINARY BLADDER TUMORIGENICITY  
STUDY IN THE MOUSE BY THE  
INTRAVESICAL PELLET IMPLANT TECHNIQUE

Pathology-Toxicology  
Project Nos. 1034ot73, 1036ot72, 1038ot72

And Addendum to:

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

Project No. 1032ot72

FINAL REPORT

Submitted to

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Division of G. D. Searle & Co.  
Chicago, Illinois 60680

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SC-19192: A 56 Week Urinary Bladder Tumorigenicity Study  
in the Mouse by the Intravesical Pellet Implant Technique

P-T No. 1034ot73

SC-19192: A 56 WEEK URINARY BLADDER TUMORIGENICITY  
STUDY IN THE MOUSE BY THE  
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FINAL REPORT

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SC-19192: A 56 WEEK URINARY BLADDER TUMORIGENICITY  
STUDY IN THE MOUSE BY THE  
INTRAVESICAL PELLET IMPLANT TECHNIQUE

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## SUMMARY

A 56 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 (XAE) (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. Each of the three treatment groups was composed of two sub-groups, A and B, each initially composed of 100 mice, a total of 200 mice being subjected to each treatment.

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 Chi square test and the exact method for 2 x 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 A - 7/77 (9.1%), Group B - 10/78 (12.8%,  $p = 0.5$ ), total - 17/155 (10.6%); and SC-19192-- Group A - 9/57 (15.8%), Group B - 8/68 (11.8%,  $p = 0.5$ ), total - 17/125 (13.6%). No statistical difference in duration of survival, growth rate, or incidence of vesical neoplasia was detected between the negative control group and SC-19192 (bladder neoplasia  $P$ -value = 0.5). The following urinary bladder neoplasia incidences were recorded for the XAE positive control group: Group A - 23/59 (39.0%), Group B - 17/52 (32.7%,  $p = 0.5$ ), total - 40/111 (36.0%). When compared with the appropriate sub-groups or total

group of the negative control mice these incidences of vesical neoplasia associated with the XAE treatment were statistically greater ( $p < 0.01$ ). 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 56-week period of observation.

## 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 56-week period of observation. The studies described were initiated June 15, 1972, and were terminated August 25, 1973. This report presents the data recorded for the entire experimental period.

## MATERIALS:

<u>Identification</u>	<ol style="list-style-type: none"><li>1. SC-19192</li><li>2. 8-Methyl Ether of Xanthurenic Acid (Positive Control Compound)</li></ol>
<u>Description</u>	<ol style="list-style-type: none"><li>1. A fine, white powder</li><li>2. A crystalline, yellow substance</li></ol>
<u>Received</u>	<ol style="list-style-type: none"><li>1. From Searle Laboratories February 25, 1972 designated as Lot #IR A6906</li><li>2. Synthesized in The Division of Clinical Oncology, University of Wisconsin School for Health Sciences</li></ol>
<u>Purity</u>	<ol style="list-style-type: none"><li>1. Specified by Searle Laboratories</li><li>2. No detectable impurities</li></ol>

## METHODS:

### Experimental Animals

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

Weight Range at Initiation of Study: 29 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 220 mice each from the supplier, and each lot was assigned as received to a study group. Mice were randomly allocated to treatment sub-groups, A or B. Each mouse was provided with a unique code number so that none of the investigators were aware of its treatment allocation until experimental termination.

### Groups and Dosage Levels

<u>Group No.</u>	<u>Sub-groups</u>	<u>No. of Mice</u>	<u>Dosage Level mgm/mouse</u>
1. (Negative Control)	A	100	0
	B	100	0
3. SC-19192	A	100	4.0 - 4.4
	B	100	4.0 - 4.4
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control)	A	100	4.0 - 4.4
	B	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 260-280 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  $\mu$  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 Chi square test and the exact method for 2 x 2 tables (9).

### RESULTS

#### Animal Survival

The number of mice surviving at various time periods in Groups A and B following surgical placement of pellets into urinary bladder lumina is presented in Table 1 (pg. 9) for cholesterol alone (Negative Control); Table 2 (pg. 10) for SC-19192; and Table 3 (pg. 11) for XAE (Positive Control). The survival of the



TABLE 1

Cholesterol alone, negative control groups for P-T 1034 of 73.  
 Mean Weights and Survival of Mice Subjected to a 56 Week Urinary Bladder  
 Tumorigenicity Study by the Intravesical Pellet Implant Technique.

Time (weeks)	Group A		Group B	
	No. Examined	Mean wt. $\pm$ SEM (gm)	No. Examined	Mean wt. $\pm$ SEM (gm)
0	100	$31.9 \pm 3.1$	100	$30.9 \pm 3.4$
1	95	$31.4 \pm 3.2$	95	$30.5 \pm 3.5$
2	93	$33.9 \pm 3.4$	92	$32.4 \pm 3.6$
3	90	$35.1 \pm 3.5$	91	$34.5 \pm 3.7$
4	90	$36.4 \pm 3.6$	91	$36.1 \pm 3.7$
6	89	$37.6 \pm 3.5$	91	$37.4 \pm 3.6$
8	89	$38.2 \pm 3.7$	90	$38.1 \pm 3.8$
10	88	$39.3 \pm 3.9$	89	$38.9 \pm 4.1$
12	86	$41.4 \pm 3.7$	87	$39.9 \pm 3.9$
16	85	$41.8 \pm 4.1$	86	$41.0 \pm 4.3$
20	82	$42.5 \pm 4.4$	82	$42.3 \pm 4.5$
24	81	$44.2 \pm 4.6$	82	$43.7 \pm 4.2$
28	78	$45.2 \pm 4.9$	79	$44.8 \pm 5.1$
32	73	$45.2 \pm 5.6$	73	$45.1 \pm 5.7$
36	70	$45.4 \pm 5.7$	71	$45.4 \pm 5.9$
40	66	$46.3 \pm 6.1$	67	$46.2 \pm 6.3$
44	65	$47.1 \pm 6.3$	65	$46.9 \pm 6.2$
48	63	$46.9 \pm 6.1$	62	$47.0 \pm 6.3$
52	59	$47.0 \pm 7.5$	53	$47.2 \pm 8.1$
56	52	$47.6 \pm 7.6$	48	$47.7 \pm 7.8$

TABLE 2

SC-19192 (20%), cholesterol (80%) groups for P-T 1034 to 73.  
Mean Weights and Survival of Mice Subjected to a 56 Week Urinary Bladder  
Tumorigenicity Study by the Intravesical Pellet Implant Technique.

Time (weeks)	Group A		Group B	
	No. Examined	Mean wt. ±SEM (gm)	No. Examined	Mean wt. ±SEM (gm)
0	100	33.5 ± 3.5	100	33.1 ± 3.2
1	90	32.7 ± 3.5	87	32.6 ± 3.4
2	84	35.1 ± 3.9	82	35.2 ± 3.7
3	81	36.9 ± 3.8	80	37.2 ± 3.9
4	80	37.5 ± 3.7	80	37.8 ± 3.8
6	77	38.7 ± 3.8	80	38.4 ± 3.6
8	76	40.4 ± 4.1	80	40.2 ± 3.9
10	73	42.3 ± 3.9	79	42.1 ± 4.1
12	70	42.7 ± 4.2	78	42.6 ± 4.3
16	68	43.4 ± 4.7	76	43.7 ± 4.8
20	67	43.9 ± 5.1	76	44.2 ± 5.3
24	66	45.7 ± 5.2	72	45.9 ± 5.4
28	62	45.3 ± 5.2	69	45.2 ± 5.5
32	61	45.4 ± 5.3	68	45.7 ± 5.2
36	57	45.9 ± 5.6	66	46.2 ± 5.5
40	54	46.2 ± 5.8	65	46.5 ± 5.7
44	51	46.7 ± 5.9	63	46.9 ± 5.6
48	47	47.2 ± 6.4	58	47.1 ± 6.1
52	47	48.1 ± 6.9	57	48.3 ± 7.0
56	39	49.6 ± 7.3	47	49.9 ± 7.5

TABLE 3

8-Methyl ether of xanthurenic acid (20%), cholesterol (80%) positive control groups for P-T 1034ot73.

Mean Weights and Survival of Mice Subjected to a 56 Week Urinary Bladder Tumorigenicity Study by the Intravesical Pellet Implant Technique.

Time (weeks)	Group A		Group B	
	No. Examined	Mean wt. ± SEM (gm)	No. Examined	Mean wt. ± SEM (gm)
0	100	32.6 ± 3.7	100	32.9 ± 3.5
1	88	33.8 ± 3.9	92	33.6 ± 4.0
2	85	34.9 ± 4.1	88	35.0 ± 4.2
3	84	36.8 ± 3.9	84	37.1 ± 4.1
4	84	37.9 ± 4.1	83	38.2 ± 4.3
6	82	39.4 ± 4.3	82	39.9 ± 4.2
8	80	41.1 ± 4.4	81	41.4 ± 4.3
10	78	41.9 ± 4.3	80	42.3 ± 4.4
12	77	43.5 ± 4.6	77	43.8 ± 4.5
16	75	44.3 ± 4.7	74	44.7 ± 4.3
20	72	44.9 ± 4.8	70	45.2 ± 4.8
24	69	45.2 ± 4.9	64	45.5 ± 5.1
28	64	45.8 ± 5.0	62	46.1 ± 5.2
32	61	46.4 ± 5.4	60	46.9 ± 5.5
36	60	46.1 ± 5.7	58	45.7 ± 5.8
40	53	47.5 ± 5.8	50	47.8 ± 5.9
44	53	46.9 ± 5.9	50	47.2 ± 6.0
48	50	47.6 ± 6.3	45	47.3 ± 6.4
52	49	48.7 ± 6.5	43	48.6 ± 6.7
56	36	49.5 ± 7.1	27	49.9 ± 7.8

mice in Table 2 approximated that of Table 1 and was somewhat better than that of Table 3. Previous studies conducted in these laboratories at different time periods (1959-1968) (4-8) show that the survival of mice in population groups similar to those of this study, and in treatment exposure comparable to those of Table 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 significant 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. 9) for cholesterol alone (Negative Control), Table 2 (pg. 10) for SC-19192, and Table 3 (pg. 11) for XAL (Positive Control). Growth of all treated groups was comparable to that observed for cholesterol alone (Table 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 (Tables 4-9, pgs. 14-19).

The survival and comparative incidence of vesical neoplasia for the duplicated sub-groups, A and B and the composite total for evaluated mice surviving more than 176 days was as follows: cholesterol alone, negative control (Table 4, pg. 14) -- Group A - 7/77 (9.1%), Group B - 10/78 (12.8%,  $p = 0.5$  compared to Group A), and Total - 17/155 (10.6%); SC-19192 (20%), cholesterol (80%) (Table 5, pg. 15) -- Group A - 9/57 (15.8%), Group B - 8/68 (11.8%,  $p = 0.5$  compared to Group A), and Total - 17/125 (13.6%); XAE (20%), cholesterol (80%), positive control (Table 6, pg. 16) -- Group A - 23/59 (39.0%), Group B - 17/52 (32.7%,  $p = 0.5$  compared to Group A), and Total - 40/111 (36.0%). Based on the incidence of vesical neoplasia respective treatment sub-groups appeared comparable within the total treatment group.

The survival and comparative incidence of vesical neoplasia for sub-groups A

TABLE 4

Summary of Survival and Comparison of Incidence of Neoplasia Observed in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique. P-T 1034t73.

Group	Cholesterol Alone									
	Survival (days)			Incidence of Bladder Changes						
				Metaplasia	Neoplasia Stage (all 176 - end days)			Total	%	P-value
	0-175	176 - Termination	Mean*		I	II	III			
A Assessed NA**	10 9	77 4	368	4	3	4	0	7/77	9.1	--
B Assessed NA**	10 9	78 3	362	4	8	2	0	10/78	12.8	0.556
Total Assessed NA**	20 18	155 7	365	8	11	6	0	17/155	10.6	--

\* Mean days survival of assessed animals surviving greater than 176 days.

\*\* NA--Animal cannibalized, too autolyzed or lost; or bladder tissue not available for microscopic inspection.

TABLE 5

Summary of Survival and Comparison of Incidence of Neoplasia Observed in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique. P-T 1034ot73.

SC-19192 (20%), cholesterol (80%)

Group	Survival (days)				Metaplasia	Neoplasia Stage (all 176 - end days)				Chi <sup>2</sup>	P-value
	0-175	176 - Termination	Mean*	%							
						I	II	III	Total		
A Assessed	17	57	372		3	6	3	0	9/57	15.8	--
NA**	17	9									
B Assessed	7	68	376		5	4	4	0	8/68	11.8	0.429
NA**	21	4									0.5
Total Assessed	24	125	374		8	10	7	0	17/125	13.6	--
NA**	38	13									

\* Mean days survival of assessed animals surviving greater than 176 days.

\*\* NA--Animal cannibalized, too autolyzed or lost; or bladder tissue not available for microscopic inspection.

TABLE 6

Summary of Survival and Comparison of Neoplasia Observed in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique. P-T 1034073.

8-Methyl ether of xanthurenic acid (20%), cholesterol (80%)

Group	Incidence of Bladder Changes										Chi <sup>2</sup>	P-value
	Survival (days)		Mean*	Metaplasia	Neoplasia Stage (all 176 - end days)							
					I	II	III	Total				
									%			
A Assessed	18	59	368	17	12	11	0	23/59	39.0	--	--	
A NA**	14	9										
B Assessed	26	52	366	15	6	10	1	17/52	32.7	0.471	0.5	
B NA**	10	12										
Total Assessed	44	111	367	32	18	21	1	40/111	36.0	--	--	
Total NA**	24	21										

\* Mean days survival of assessed animals surviving greater than 176 days.

\*\* NA--Animal cannibalized, too autolyzed or lost; or bladder tissue not available for microscopic inspection.



TABLE 7

Summary of Survival and Incidence of Neoplasia Observed in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Pellet Implant Technique. P-T 1034ot73.

Group A's

Group	Survival (days)			Metaplasia	Neoplasia Stage (all 176 - end days)				Chi <sup>2</sup>	P-value*	
	0-175	176 - Termination			I	II	III	Total			%
		176	Termination								
1. Cholesterol (Neg. Control) NA**	10	77	368	4	3	4	0	7/77	9.1	--	
3. SC-19192 NA**	17 17	57 9	372	3	6	3	0	9/57	15.8	1.4	
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control) NA**	18 14	59 9	368	17	12	11	0	23/59	39.0	17.8	
										<0.001	

\* P-value calculated by exact method for 2x2 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 and II, V-VIII).

TABLE 8

Summary of Survival and Incidence of Neoplasia Observed in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Pellet Implant Technique. P-T 10340173.

Group B's												
Group	Survival (days)				Incidence of Bladder Changes							
	0-175	176 - Termination	Mean	Metaplasia	Neoplasia Stage (all 176 - end days)					Chi <sup>2</sup>	P-value *	
					I	II	III	Total	%			
1. Cholesterol (Neg. Control) NA**	10	78	362	4	8	2	0	10/78	12.8	--	--	
	9	3										
3. SC-19192 NA**	7	68	376	5	4	4	0	8/68	11.8	0.0348	0.9	
	21	4										
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control) NA**	26	52	366	15	6	10	1	17/52	32.7	7.45	0.01>p>0.00	
	10	12										

\* P-value calculated by exact method for 2x2 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 and II, V-VIII).

TABLE 9

Summary of Survival and Incidence of Neoplasia Observed in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Pellet Implant Technique. P-T 1034of73.

## Pooled Groups A and B

Group	Survival (days)			Incidence of Bladder Changes						Chi <sup>2</sup>	P-value*
	0-175	176 - Termination	Mean	Metaplasia	Neoplasia Stage (all 176 - end days)				%		
					I	II	III	Total			
1. Cholesterol (Neg. Control) NA**	20 18	155 7	365	8	11	6	0	17/155	10.6	--	--
3. SC-19192 NA**	24 38	125 13	374	8	10	7	0	17/125	13.6	0.453	0.5
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control) NA**	44 24	111 21	367	32	18	21	1	40/111	36.0	24.8	<0.001

\* P-value calculated by exact method for 2x2 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 and II, V-VIII).

(Table 7, pg. 17), B (Table 8, pg. 18) and the combined total treatment groups (Table 9, pg. 19) are presented. For each comparison, the incidence of bladder neoplasia associated with treatment by SC-19192 was not statistically different from that displayed by the cholesterol alone, negative control groups. Conversely, the incidence of vesical neoplasia associated with treatment by XAE, positive control, was highly statistically significantly greater ( $p < 0.01$ ) than that for the cholesterol alone, negative control groups. The incidence with XAE found in this study was comparable to that reported previously (4). Thus, the mice employed in the present study appeared susceptible to the vesical tumorigenic effects associated with XAE treatment. Treatment with SC-19192 was not associated with an augmented bladder tumor incidence compared with the cholesterol alone, negative control group, and does not thusly appear to possess murine vesical carcinogenic potential.

The negative and positive control groups for this study and for P-T No. 1033ot73 were common between the two studies.

Other Tumors: Table 10 (pg. 21) presents a summary of the mice at risk and the incidence of other tumors detected. The total incidence associated with treatment by SC-19192 was not greater than that present in mice serving as the negative control. No other tumors were present in mice surviving less than 175 days of observation. The incidence of other tumors observed in each group is comparable to that recently reported as present in untreated Swiss female mice (12) surviving a comparable period of time.

TABLE 10

Summary of Tumors Observed in Organs Other than Urinary Bladder in a 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Pellet Implant Technique. P-T 1034ot73.

Treatment	Groups	No. Mice	Lesions in Evaluated Mice Surviving > 176 Days							
			Mammary Fibroadenoma	Leukemia	Pulmonary Adenoma	Stomach Papilloma	Intestinal Fibroma	Ovarian Cyst	Liver Cholangioma	Other
1. Cholesterol (Neg. Control)	A	77	4	2	4	1				
	B	78	2	2	5		1			
2. SC-19192	A	57	2	2	1			2		
	B	68	2		3			1	3	Submaxillary Gland-Adenoma (2)
3. 8-Methyl Ether of Xanthurenic Acid (Positive Control)	A	59	10	3	4					
	B	52	8	2	3		2			

## CONCLUSION

This study provides no evidence that SC-19192, administered by the intravesical pellet implant technique to mice, provides an augmented, statistically significant incidence of vesical neoplasia or extra vesical neoplasia of other organs.

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APPENDIX TO

SC-19192: A 56 WEEK URINARY BLADDER TUMORIGENICITY  
STUDY IN THE MOUSE BY THE INTRAVESICAL  
• PELLET IMPLANT TECHNIQUE

P-T No. 1034 of 73

## Key to Column Designations of Appendixes I - VIII

- 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

Key to Column Designations of Appendixes I - VIII (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.

Key to Column Designations of Appendixes I - VIII (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 groups for P-T 1034 and 73.

Tabulation of fate and assessment of individual mice in "A 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique".

## Group A Mice

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	1	1	1	0	0				
2	3	2	1	4	4				
3	3	0	1	0	1				
4	7	3	0	NA					
5	7	3	0	NA					
6	11	3	0	NA					
7	13	0	1	0	2				
8	15	0	1	1	2				
9	16	3	0	NA					
10	21	2	1	4	4				
11	33	0	1	1	2				
12	60	3	0	NA					
13	75	3	0	NA					
14	77	3	0	NA					
15	101	3	0	NA					
16	123	0	1	2	3				
17	125	3	0	NA					
18	135	0	1	1	2				
19	146	0	1	3	3				
20	183	0	1	0	0				
21	185	3	0	NA					
22	192	0	1	2	3				
23	210	1	1	0	2				
24	212	3	0	NA					
25	214	0	1	0	2				
26	222	0	1	0	1				
27	224	2	1	4	4				
28	230	0	1	0	2				
29	245	3	0	NA					
30	247	0	1	1	2				

APPENDIX I (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	272	0	1	2	2				
32	276	1	1	0	1				
33	276	3	0	NA					
34	278	1	1	0	3				
35	293	0	1	3	3	S-2	1		
36	309	0	1	1	2				
37	335	0	1	1	1				
38	339	0	1	0	0				
39	344	0	1	3	3				
40	345	0	1	3	2				
41	358	2	1	4	4				
42	382	0	1	3	3				
43	386	0	1	0	3				
44	390	0	1	2	2				
45	390	0	1	0	1				
46	392	0	1	2	1	S-1	1		
47	392	0	1	2	3				
48	392	0	1	1	3				
49	393	0	1	0	2				
50	394	0	1	1	0				
51	397	0	1	1	1				1
52	397	0	1	1	3				
53	397	0	1	2	1				
54	398	0	1	2	3				
55	398	0	1	1	0				
56	398	0	1	0	0				
57	398	0	1	1	1				
58	398	0	1	0	2				
59	398	0	1	2	2				
60	398	0	1	2	2				
61	398	0	1	0	2				
62	398	0	1	1	2				
63	398	0	1	0	2				
64	398	0	1	0	1				
65	398	0	1	3	3				1

APPENDIX I (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	398	0	1	2	3	S-1		1	
67	398	0	1	1	0				
68	398	0	1	2	1				
69	398	0	1	2	3			1	
70	398	0	1	2	1				
71	398	0	1	0	0				
72	398	0	1	1	0				
73	399	0	1	1	3				
74	399	0	1	3	3	S-2			
75	399	0	1	0	2				
76	399	0	1	1	0				
77	399	0	1	0	1				
78	399	0	1	2	3				
79	399	0	1	1	2				
80	399	0	1	1	0				
81	399	0	1	3	2				
82	399	0	1	2	1				
83	399	0	1	0	1				
84	399	0	1	1	1				
85	399	0	1	1	1				
86	399	0	1	1	0				
87	399	0	1	0	2				
88	399	0	1	2	2			1	
89	399	0	1	0	0				
90	399	0	1	1	0				
91	399	0	1	0	1				
92	400	0	1	1	2				
93	400	0	1	0	1				
94	400	0	1	1	1				
95	400	0	1	0	0				
96	400	0	1	1	3				
97	400	0	1	2	3				
98	401	0	1	3	3				
99	401	0	1	1	0				
100	401	0	1	0	1				

APPENDIX I (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	398	0	1	2	3	S-1		1	
67	398	0	1	1	0				
68	398	0	1	2	1				
69	398	0	1	2	3			1	
70	398	0	1	2	1				
71	398	0	1	0	0				
72	398	0	1	1	0				
73	399	0	1	1	3				
74	399	0	1	3	3	S-2			
75	399	0	1	0	2				
76	399	0	1	1	0				
77	399	0	1	0	1				
78	399	0	1	2	3				
79	399	0	1	1	2				
80	399	0	1	1	0				
81	399	0	1	3	2				
82	399	0	1	2	1				
83	399	0	1	0	1				
84	399	0	1	1	1				
85	399	0	1	1	1				
86	399	0	1	1	0				
87	399	0	1	0	2				
88	399	0	1	2	2			1	
89	399	0	1	0	0				
90	399	0	1	1	0				
91	399	0	1	0	1				
92	400	0	1	1	2				
93	400	0	1	0	1				
94	400	0	1	1	1				
95	400	0	1	0	0				
96	400	0	1	1	3				
97	400	0	1	2	3				
98	401	0	1	3	3				
99	401	0	1	1	0				
100	401	0	1	0	1				



APPENDIX II

Cholesterol alone, negative control groups for P-T 1034ot73.

Tabulation of fate and assessment of individual mice in "A 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique".

Group B Mice

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	2	2	1	4	4				
2	3	3	0	NA					
3	3	0	1	0	2				
4	7	3	0	NA					
5	7	0	1	0	0				
6	10	0	1	0	1				
7	11	2	1	4	4				
8	14	3	0	NA					
9	16	3	0	NA					
10	52	3	0	NA					
11	58	0	1	1	2				
12	74	3	0	NA					
13	78	3	0	NA					
14	107	3	0	NA					
15	119	3	0	NA					
16	123	0	1	2	3				
17	125	2	1	4	4				
18	140	0	1	2	3				
19	170	1	1	0	2				
20	183	0	1	1	2				
21	188	0	1	1	3				
22	197	3	0	NA					
23	211	1	1	0	3				
24	213	1	1	0	0				
25	221	0	1	3	3				
26	222	0	1	2	1				
27	223	0	1	2	3				
28	230	3	0	NA					
29	243	0	1	1	1				
30	266	1	1	0	3				

APPENDIX II (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	274	3	0	NA					
32	276	0	1	2	2				
33	276	0	1	0	1				
34	285	0	1	2	2				
35	291	0	1	1	1				
36	309	0	1	1	2				
37	321	0	1	0	2				
38	327	0	1	3	2	S-2	1		
39	337	0	1	0	2				
40	339	2	1	4	4				
41	347	0	1	4	1				
42	350	0	1	0	0				
43	353	0	1	4	3				
44	356	0	1	1	0				
45	359	0	1	0	1				
46	363	0	1	1	1				
47	364	0	1	1	2				
48	366	0	1	1	3				
49	379	0	1	0	0				
50	380	2	1	4	4				
51	380	0	1	1	2				
52	385	0	1	0	1				
53	397	0	1	2	3				1
54	398	0	1	0	2				
55	398	0	1	3	2		1		
56	398	0	1	2	3				
57	398	0	1	3	3	S-2	1		
58	398	0	1	2	2				
59	398	0	1	3	2				
60	398	0	1	2	0				
61	398	0	1	1	0				
62	398	0	1	1	2				
63	398	0	1	1	2				
64	398	0	1	1	0				
65	398	0	1	1	2				

APPENDIX II (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	398	0	1	3	3		1		
67	398	0	1	1	0				
68	398	0	1	3	2				
69	398	0	1	0	1				
70	398	0	1	2	3				
71	399	0	1	0	1				
72	399	0	1	1	1				
73	399	0	1	0	0				
74	399	0	1	0	2				
75	399	0	1	0	1				
76	399	0	1	1	3				
77	399	0	1	1	0				
78	399	0	1	0	2				
79	399	0	1	3	3	S-2			
80	399	0	1	2	3				
81	399	0	1	1	2				
82	399	0	1	0	2				
83	399	0	1	2	2				
84	399	0	1	1	0				
85	399	0	1	1	0				
86	399	0	1	3	3				
87	399	0	1	1	2				
88	399	0	1	1	0				
89	399	0	1	1	2				
90	399	0	1	1	3				
91	399	0	1	2	2				
92	399	0	1	2	1		1		
93	399	0	1	1	3		1		
94	400	0	1	2	1		1		
95	401	0	1	3	1		1		
96	401	0	1	2	2			1	
97	401	0	1	2	0				
98	401	0	1	2	1				
99	401	0	1	0	0				
100	401	0	1	1	0				

APPENDIX V (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	86	2	1	4	4				
32	102	3	0	NA					
33	138	3	0	NA					
34	147	0	1	1	0				
35	177	1	1	1	1				
36	183	3	0	NA					
37	184	0	1	3	2				
38	195	1	1	3	1				
39	203	3	0	NA					
40	235	1	1	1	2				
41	242	3	0	NA					
42	245	3	0	NA					
43	249	3	0	NA					
44	255	0	1	3	1				
45	275	0	1	1	0				
46	275	3	0	NA					
47	287	1	1	1	0				
48	293	3	0	NA					
49	297	3	0	NA					
50	315	3	0	NA					
51	318	0	1	1	0				
52	333	0	1	1	0				
53	335	0	1	1	0				
54	367	0	1	3	0				
55	378	1	1	1	2				
56	384	0	1	3	2				
57	391	0	1	1	2				
58	391	0	1	1	0				
59	391	0	1	2	2				
60	391	0	1	2	2				
61	391	0	1	1	2		1		
62	395	0	1	2	3				
63	395	0	1	1	0				
64	395	0	1	2	1		1		
65	396	0	1	3	3	S-2		1	

APPENDIX V (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	396	0	1	2	0				
67	396	0	1	2	3				
68	396	0	1	3	1				
69	396	0	1	2	3				
70	396	0	1	1	2				
71	396	0	1	3	3	S-2			
72	397	0	1	2	3			1	
73	397	0	1	0	0				
74	397	0	1	2	1				
75	397	0	1	1	0				
76	397	0	1	2	2				
77	397	0	1	1	2				
78	398	0	1	2	3		1		
79	398	0	1	1	0				
80	398	0	1	2	3		1		
81	398	0	1	3	2				
82	398	0	1	1	1				
83	398	0	1	1	1				
84	398	0	1	1	3				
85	398	0	1	3	3		1		
86	398	0	1	2	1				
87	398	0	1	0	0				
88	398	0	1	2	2				
89	398	0	1	2	3			1	
90	399	0	1	2	2				
91	399	0	1	1	1				
92	399	0	1	1	2				
93	399	0	1	3	3	S-2			
94	400	0	1	1	1				
95	400	0	1	3	3				
96	400	0	1	1	2				
97	400	0	1	1	0				
98	400	0	1	3	3				
99	400	0	1	2	3				
100	400	0	1	2	0		1		

APPENDIX VI

SC-19192 (20%), cholesterol (80%) groups for P-T 1034ot73.  
 Tabulation of fate and assessment of individual mice in "SC-19192: A 56 Week  
 Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet  
 Implant Technique".

Group B Mice

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	1	3	0	NA					
2	5	3	0	NA					
3	5	3	0	NA					
4	5	3	0	NA					
5	6	3	0	NA					
6	6	3	0	NA					
7	6	3	0	NA					
8	6	1	1	4	2				
9	7	2	1	4	4				
10	7	0	1	1	1				
11	7	3	0	NA					
12	7	3	0	NA					
13	7	3	0	NA					
14	9	3	0	NA					
15	10	3	0	NA					
16	12	1	1	3	2				
17	14	3	0	NA					
18	14	3	0	NA					
19	18	3	0	NA					
20	21	3	0	NA					
21	61	3	0	NA					
22	82	3	0	NA					
23	89	0	1	3	1				
24	112	3	0	NA					
25	144	3	0	NA					
26	144	3	0	NA					
27	151	0	1	2	1				
28	165	0	1	3	2				
29	180	1	1	1	3				
30	183	3	0	NA					

## APPENDIX VI (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	194	1	1	4	1				
32	207	0	1	3	1				
33	245	0	1	3	0				
34	247	3	0	NA					
35	273	1	1	2	3				
36	282	0	1	3	0				
37	297	0	1	3	1				
38	312	3	0	NA					
39	318	3	0	NA					
40	323	1	1	1	2				
41	327	0	1	0	1				
42	334	0	1	1	0				
43	337	0	1	3	2				
44	370	0	1	1	1	S-1			
45	372	1	1	1	0				
46	382	1	1	1	2				
47	384	1	1	2	1				
48	391	0	1	3	2	S-1		1	
49	391	0	1	3	3				
50	391	0	1	2	2			1	
51	391	0	1	3	2		1		
52	391	0	1	3	0				
53	391	0	1	2	2				
54	394	0	1	3	1				
55	395	0	1	0	0				
56	395	0	1	0	1				
57	395	0	1	3	3			1	
58	395	0	1	0	2				
59	396	0	1	1	0				
60	396	0	1	0	0				
61	396	0	1	3	3				
62	396	0	1	2	2				
63	396	0	1	1	1				
64	396	0	1	1	0		1		
65	396	0	1	3	2	S-1			

APPENDIX VI (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	396	0	1	1	1				
67	397	0	1	2	1	S-1	1		
68	397	0	1	1	2				
69	397	0	1	1	3				
70	397	0	1	3	1			1	
71	397	0	1	1	1				
72	397	0	1	3	0				
73	397	0	1	2	0				
74	398	0	1	0	1				
75	398	0	1	0	1				
76	398	0	1	1	0				
77	398	0	1	1	1				
78	398	0	1	2	1				
79	398	0	1	3	1				
80	398	0	1	0	0				
81	398	0	1	3	3	S-1			
82	398	0	1	3	3				
83	398	0	1	0	2				
84	398	0	1	2	3				
85	398	0	1	2	2				
86	398	0	1	1	0				
87	398	0	1	2	3				
88	398	0	1	2	2				
89	398	0	1	2	3				
90	398	0	1	3	2				
91	398	0	1	2	1				
92	399	0	1	0	2				
93	399	0	1	0	2				
94	399	0	1	2	1				
95	399	0	1	1	1		1		
96	399	0	1	2	0				
97	400	0	1	3	1				
98	400	0	1	0	1				
99	400	0	1	1	2				
100	400	0	1	2	3				



## APPENDIX VII

8-Methyl ether of xanthurenic acid (20%), cholesterol (80%), positive control groups for P-T 1034 to 73. Tabulation of fate and assessment of individual mice in "A 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique".

## Group A Mice

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically				
				Benign			Malignant Stag	
				Hyperplasia	Cystitis	Metaplasia	I	II III
1	2	2	1	4	4			
2	4	2	1	4	4			
3	4	0	1	0	1			
4	4	0	1	0	2			
5	5	0	1	0	2			
6	5	0	1	1	0			
7	7	3	0	NA				
8	7	3	0	NA				
9	7	0	1	1	3			
10	7	0	1	1	2			
11	7	0	1	1	2			
12	7	3	0	NA				
13	8	3	0	NA				
14	8	3	0	NA				
15	14	0	1	1	2			
16	21	0	1	1	3			
17	33	3	0	NA				
18	40	3	0	NA				
19	50	0	1	2	3			
20	53	3	0	NA				
21	64	0	1	3	2			
22	65	3	0	NA				
23	79	3	0	NA				
24	90	2	1	4	4			
25	92	3	0	NA				
26	116	0	1	3	3	S-1		
27	119	3	0	NA				
28	140	0	1	3	3	S-1		
29	155	3	0	NA				
30	162	0	1	3	3	S-1		

## APPENDIX VII (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	165	3	0	NA					
32	173	0	1	2	3				
33	182	3	0	NA					
34	185	2	1	4	4				
35	188	0	1	2	2	S-1			
36	189	3	0	NA					
37	203	3	0	NA					
38	216	1	1	2	1				
39	220	0	1	1	0	S-1	1		
40	242	0	1	1	3				
41	258	3	0	NA					
42	269	0	1	1	2				
43	269	3	0	NA					
44	269	1	1	3	3	S-2			
45	275	1	1	4	2				
46	275	0	1	3	3	S-1			
47	276	3	0	NA					
48	310	3	0	NA					
49	316	0	1	3	3	S-1			1
50	331	0	1	2	1	S-2			
51	349	1	1	2	2				
52	369	3	0	NA					
53	369	1	1	4	2				
54	373	0	1	1	1				
55	374	3	0	NA					
56	378	0	1	2	2				1
57	380	0	1	2	2		1		
58	385	0	1	1	1				
59	389	0	1	2	3				
60	392	0	1	2	3	S-1			
61	392	0	1	1	1		1		
62	392	0	1	2	2				
63	392	0	1	2	1				
64	392	0	1	3	1	G-1			1
65	394	0	1	1	2				

## APPENDIX VII (con't)

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stag		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
66	394	0	1	0	0				
67	394	0	1	0	0				
68	394	0	1	2	2		1		
69	395	0	1	1	1				1
70	395	0	1	1	2	S-1			1
71	395	0	1	2	2				
72	395	0	1	2	2				
73	396	0	1	2	1				
74	397	1	1	3	2				
75	398	0	1	1	1				1
76	398	0	1	1	1				
77	398	0	1	2	2				
78	398	0	1	1	0				
79	398	0	1	1	2				
80	398	0	1	1	1				
81	399	0	1	2	3				
82	399	0	1	1	2		1		
83	399	0	1	3	1		1		
84	399	0	1	3	3	S-2	1		
85	399	0	1	3	2	S-1			
86	399	0	1	3	2				
87	399	0	1	1	2				1
88	399	0	1	2	1				
89	399	0	1	1	3		1		
90	399	0	1	1	1		1		
91	399	0	1	0	3				
92	399	0	1	0	2				
93	399	0	1	2	1				
94	399	0	1	1	0				
95	399	0	1	2	3		1		
96	399	0	1	1	3	S-1			1
97	400	0	1	1	1				1
98	400	0	1	2	2		1		
99	400	0	1	3	3	S-1	1		
100	400	0	1	2	3	S-1			1

## APPENDIX VIII

8-Methyl ether of xanthurenic acid (20%), cholesterol (80%), positive control groups for P-T 1034ot73. Tabulation of fate and assessment of individual mice in "A 56 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Intravesical Pellet Implant Technique".

## Group B Mice

Mouse Number	Life Length (Days)	Animal Status Death	Tissue Available	Bladder Status Microscopically					
				Benign			Malignant Stage		
				Hyperplasia	Cystitis	Metaplasia	I	II	III
1	2	2	1	4	4				
2	3	1	1	0	1				
3	4	0	1	3	2				
4	4	3	0	NA					
5	5	1	1	1	2				
6	5	0	1	1	3				
7	6	0	1	1	2				
8	6	1	1	0	1				
9	8	0	1	1	1				
10	8	2	1	4	4				
11	8	3	0	NA					
12	12	0	1	2	2				
13	15	3	0	NA					
14	15	3	0	NA					
15	15	3	0	NA					
16	17	0	1	1	0				
17	22	1	1	0	3				
18	41	1	1	0	2				
19	54	3	0	NA					
20	62	0	1	1	3				
21	72	3	0	NA					
22	80	0	1	1	0				
23	81	3	0	NA					
24	92	0	1	3	3				
25	101	0	1	3	3	S-1			
26	112	0	1	2	1	S-1			
27	115	3	0	NA					
28	119	1	1	1	0				
29	120	3	0	NA					
30	135	0	1	2	3				

## APPENDIX VIII (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	146	0	1	3	2				
32	152	0	1	2	2				
33	154	0	1	2	2				
34	155	0	1	0	0				
35	162	0	1	1	2				
36	163	0	1	3	3				
37	185	0	1	3	1				
38	185	3	0	NA					
39	205	3	0	NA					
40	217	3	0	NA					
41	237	1	1	0	2				
42	244	0	1	1	0				
43	263	3	0	NA					
44	268	1	1	0	0				
45	269	1	1	0	2				
46	267	0	1	3	3	S-1			
47	273	3	0	NA					
48	273	3	0	NA					
49	276	1	1	0	2				
50	276	0	1	2	3		1		
51	315	0	1	3	3	S-1			
52	325	0	1	1	2				
53	328	3	0	NA					
54	332	1	1	4	2				
55	333	0	1	3	3	S-1			
56	349	3	0	NA					
57	360	0	1	2	3		1		
58	366	0	1	3	0	S-1			
59	371	0	1	2	2				
60	372	3	0	NA					
61	373	0	1	1	1				
62	373	3	0	NA					
63	376	3	0	NA					
64	378	0	1	2	2	S-1			
65	378	3	0	NA					

## APPENDIX VIII (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	379	1	1	4	3				
67	382	1	1	4	2				
68	392	0	1	1	0				
69	392	0	1	1	3				
70	392	0	1	2	1		1		
71	392	0	1	0	1				
72	392	0	1	2	3			1	
73	392	0	1	1	2	S-1			
74	394	0	1	1	1			1	
75	394	0	1	3	3	S-2		1	
76	394	0	1	3	2	S-1		1	
77	394	0	1	3	3			1	
78	394	0	1	3	3	S-1			
79	394	0	1	2	3				
80	398	0	1	2	2				
81	398	0	1	2	1				
82	398	0	1	1	3				
83	398	0	1	1	2				
84	398	0	1	1	3		1		
85	398	0	1	1	3	S-1	1		
86	398	0	1	2	2				
87	399	0	1	1	2				
88	399	0	1	2	3			1	
89	399	0	1	1	2			1	
90	399	0	1	2	3	G-1 S-1		1	
91	399	0	1	1	1				
92	399	0	1	2	2		1		
93	399	0	1	2	1	S-2		1	
94	399	0	1	1	3				
95	399	0	1	3	3	S-1			
96	400	0	1	2	3				
97	400	0	1	2	3			1	
98	400	0	1	3	2				1
99	400	0	1	2	0				
100	400	0	1	1	1				

## II

SC-19192: Assessment of Stability and Disappearance Rate  
of SC-19192 From the Intravesical Cholesterol Pellet

P-T No. 1036ot72

*Submitted 11/6/74*

SC-19192: ASSESSMENT OF STABILITY AND DISAPPEARANCE  
RATE OF SC-19192 FROM  
THE INTRAVESICAL CHOLESTEROL PELLET

P-T NO. 10360t72

FINAL REPORT

Submitted to

Searle Laboratories, Division of  
G. D. Searle and Company  
Chicago, Illinois

by

George T. Bryan, M.D., Ph.D.  
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January 29, 1974



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## SUMMARY

The in vivo rate of disappearance of C<sup>14</sup>-SC-19192 from cholesterol pellets surgically implanted into the urinary bladder lumina of Swiss albino female 60-90 day old mice was studied. The study was conducted to evaluate the potential of bladder exposure to SC-19192 under conditions of carcinogenicity testing by the Intravesical Pellet Implant Technique. The time required for 50 percent of SC-19192 to diffuse out of the pellet was determined to be 4.8 hours. The rate of elution was observed to follow first-order kinetics, and the specific elution-rate constant,  $K$ , was calculated to be 0.12/hour. This estimate provided a measure of the probable extent and duration of exposure of the mouse bladder mucosa to SC-19192.

## INTRODUCTION

The purpose of this study was to provide an estimation of the rate of disappearance of SC-19192 from cholesterol pellets implanted intravesically into the urinary bladder lumina of female Swiss mice. These data were used to select an appropriate positive control compound with comparable bladder exposure characteristics for bladder tumorigenicity studies. Additionally, thin-layer chromatography of prepared pellets and pellets removed after varying periods in vivo were monitored by radiochromatographic scanning to assess the stability of SC-19192.

## MATERIALS

<u>Identification</u>	SC-19192-phe-C <sup>14</sup> , Specific Activity 1.765 $\mu$ Ci/mgm
<u>Description</u>	A fine, white powder
<u>Received</u>	From Searle Laboratories March 13, 1972 designated as SC-19192-phe-C <sup>14</sup>
<u>Purity</u>	Specified by Searle Laboratories

## METHODS

### Experimental Animals

Forty 60-90 day old Swiss albino female mice obtained from Rolfsmeyer Company, Madison, Wisconsin.

Weight Range at Initiation of Study: 30 to 33 grams each.

Housing: Individually in raised, stainless steel, screen-bottomed cages.

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

### Dosage Levels

4.0 - 4.4 mgm/mouse;  $0.94 \pm 0.08$   $\mu$ Ci/mouse of SC-19192.

### Administration of Test Material

Pellets of 20-22 mgm mass and 0.4 cm diameter, composed of one part of powdered C<sup>14</sup>-SC-19192 mixed with four parts of three times recrystallized,

powdered cholesterol (Sigma Chemical Co., St. Louis, Mo.) were fashioned. The cholesterol and the C<sup>14</sup>-SC-19192 were separately ground to a fine powder in an agate mortar. C<sup>14</sup>-SC-19192 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. Lots of pellets numbering 80 were prepared to insure uniformity and reproducibility of 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).

#### Analyses of the Pellets

The C<sup>14</sup> content of pellets was assayed by dissolving the pellets in 10 ml of chloroform followed by quantitative dilution to a final volume of 100 ml with absolute ethanol. Following mixing, aliquots were quantitatively transferred to vials, 18 ml of scintillation fluid (naphthalene, 259 gm.; diphenyloxazole,

18.4 gm; alpha-naphthylphenyloxazole, 0.184 gm; xylene, 1400 ml; dioxane, 1400 ml; and absolute ethanol, 840 ml) were added, and the samples were counted (68-74% efficiency) in a Nuclear Chicago Mark I scintillation counter. Efficiency corrections were made using automatic external standardization with the channel ratio method.

Aliquots of the chloroform-ethanol solutions containing C<sup>14</sup>-SC-19192 were concentrated under a stream of air and chromatographed on 0.25 mm Silica gel thin-layer plates with fluorescent indicator (Brinkmann Inst. Inc., Sil G-25 UV 254) employing a saturated atmospheres solvent system consisting of:

- 1) n-butanol:glacial acetic acid:water (8:2:2 v/v) Rf SC-19192 of 0.89, or
- 2) ethanol:water (7:3 v/v) Rf SC-19192 of 0.55. Following development, radioactivity was monitored with a Varian Series 6000 radiochromatogram scanner.

#### In Vivo Elution of SC-19192 from the Pellets

The pellets were randomly selected from those prepared, weighed, and the content of C<sup>14</sup>-SC-19192 calculated. Following surgical introduction into the mouse bladder lumina, the mice were housed as described. At intervals of 1, 2, 4, 6 or 8 hours following surgery, 2 to 4 mice were killed and the pellets recovered. The quantity of C<sup>14</sup>-SC-19192 remaining in the pellet was determined, and from this and the calculated content of test chemical present prior to insertion of the pellet into the mouse bladder, an estimate of the amount of compound that had disappeared from the pellet during the time that it was in the bladder in vivo could be made.

### Calculations and Statistical Evaluation

The percent of  $C^{14}$ -SC-19192 remaining in a pellet after x hours in the bladder was converted to its common logarithm. When the analytical results, representing several pellets remaining in the mouse bladders for varying periods of time, were plotted graphically versus the number of hours each pellet had been exposed to urine, a straight-line relationship was observed. The slope, b, of the regression equation best representing this linear relationship was computed by the method of least squares, tested for statistical significance by the F test, and a correlation coefficient calculated (9).

A specific elution-rate constant, K, was calculated for  $C^{14}$ -SC-19192 by multiplying the slope, b, by  $(-2.303)$ . The time required for 50% of  $C^{14}$ -SC-19192 to disappear from a pellet,  $T_{1/2}$ , was computed from K and was used as an index of the first-order elution time. Coordinates (x, y) lying on the regression line were computed from the regression equation, and a graphic representation of this line was drawn for  $C^{14}$ -SC-19192 (Chart 1, pg. 7).

### RESULTS

Analyses of 5 pellets not inserted into mouse bladders demonstrated a mean radioactivity content ( $C^{14}$ -SC-19192) of  $0.94 \pm 0.08$  (SD)  $\mu\text{Ci}$ . Thin-layer radiochromatograms of these pellets demonstrated only one spot corresponding to the chemical known to be present in the pellets.

The loci of the experimentally determined coordinates; the linear elution

curves, computed by the method of least squares from these values; the slope,  $b$ ; the correlation coefficient; and the 50% elution time,  $T_{1/2}$ , for SC-19192 are shown in Chart 1 (pg. 7). The elution rate constant,  $K$ , was calculated to be 0.12/hour. The slope of the computed regression line was highly significant ( $P < 0.01$ ). Thin-layer radiochromatograms of these pellets revealed only one spot corresponding to SC-19192 known to be present in the pellets.

### CONCLUSION

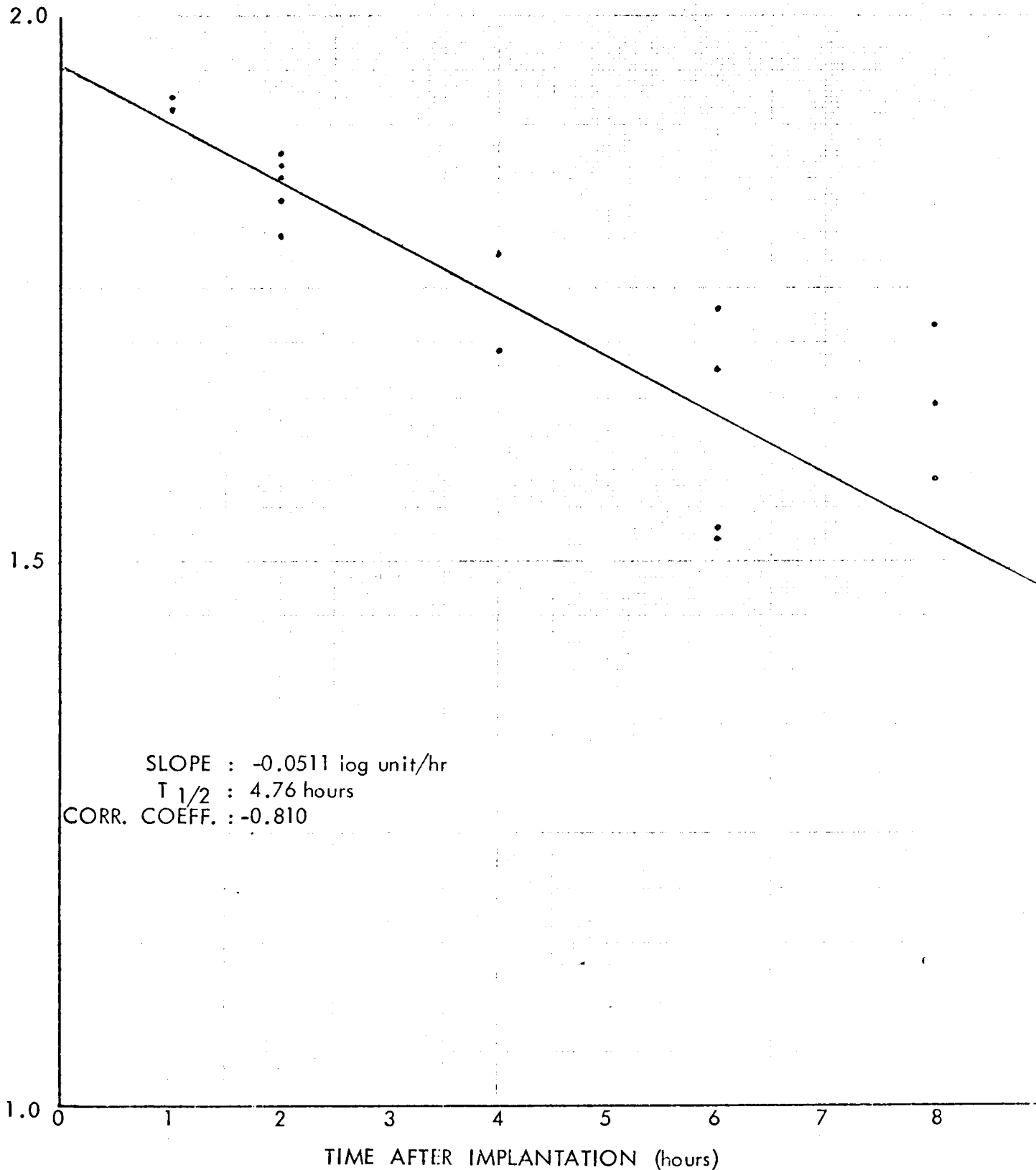
SC-19192 is very rapidly eluted by urine from cholesterol pellets placed into mouse bladder lumina, with a  $T_{1/2}$  of 4.8 hours. No decomposition of SC-19192 during the process of pellet formulation or in vivo exposure was ascertained. An appropriate Positive Control chemical for bladder tumorigenicity studies of SC-19192 by the intravesical pellet implant technique appears to be the 8-methyl ether of xanthurenic acid, which also is rapidly eluted from cholesterol pellets (3).

FORM C1

# CHART 1

## IN VIVO ELUTION OF DIKETOPIPERAZINE (SC-19192) FROM CHOLESTEROL PELLETS

LOG PER CENT DIKETOPIPERAZINE (SC-19192) REMAINING IN CHOLESTEROL PELLETS





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### III

SC-19192: Absorption By and Interaction Of SC-19192  
With the Mouse Urinary Bladder

P-T No. 1038ot72

*Submitted 11/6/74*

SC-19192: ABSORPTION BY AND INTERACTION OF SC-19192  
WITH THE MOUSE URINARY BLADDER

P-T NO. 10380t72

FINAL REPORT

Submitted to

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## SUMMARY

The distribution of SC-19192-phe- $C^{14}$  was investigated in 60-90 day old Swiss female mice by: (1) surgical insertion into the bladder lumina of cholesterol pellets containing 20% SC-19192; and (2) direct intraurethral instillation of an aqueous solution of SC-19192. Following surgical implantation of pellets, mice were killed at 4.8 ( $T_{1/2}$ ), 9.6 ( $2T_{1/2}$ ), and 14.4 ( $3T_{1/2}$ ) hours; 32-51% of SC-19192 was eluted from the pellets. Most of the  $C^{14}$  was retained in several tissues, or was present in the gastrointestinal contents and feces, urine, or expired  $CO_2$ . The urinary bladder retained much greater quantities of  $C^{14}$  than did other tissues. When an aqueous solution of SC-19192-phe- $C^{14}$  was directly instilled into the urinary bladder, in 4.8 hours ( $T_{1/2}$ ) about 25% of the  $C^{14}$  had disappeared from the bladder lumen and was retained in several tissues, or was present in the gastrointestinal contents and feces or expired  $CO_2$ . These data indicate that the urinary bladder was permeable to SC-19192, and had ample opportunity for intimate contact with this compound during the acute period of exposure of the bladder while assessing potential murine vesical tumorigenicity by the Intravesical Pellet Implant Technique.

## INTRODUCTION

It has been proposed that carcinogenic chemicals induce neoplasia through interactions with cellular or tissue components. In assessing the potential tumorigenicity of a chemical by the Intravesical Pellet Implant Technique a

negative result must be regarded as inconclusive unless it is shown that the test chemical disappears from the pellet (investigated in P-T 1036t72) and reaches the bladder epithelium; and that the test compound has had an opportunity to be metabolized (1). The purpose of this study was to investigate the absorption by and the interaction of SC-19192 with the murine urinary bladder under tumorigenicity test conditions.

### MATERIALS

<u>Identification</u>	SC-19192-phe-C <sup>14</sup> , Specific Activity 1.765 $\mu$ Ci/mgm
<u>Description</u>	A fine, white powder
<u>Received</u>	From Searle Laboratories March 13, 1972 designated as SC-19192-phe-C <sup>14</sup>
<u>Purity</u>	Specified by Searle Laboratories

### METHODS

#### Experimental Animals

Forty 60-90 day old Swiss albino female mice obtained from Rolfsmeyer Company, Madison, Wisconsin.

Weight Range at Initiation of Study: 30 to 33 grams each.

Housing: Individually in all-glass metabolism cages which permitted the separate collection of urine, feces and CO<sub>2</sub> (ethanolamine:methanol--20:80 v/v).

Basal Diet: Mice were not fed but were permitted distilled water ad libitum.

#### Dosage Levels

1. Administered in pellets: 4.0 - 4.4 mgm/mouse;  $0.94 \pm 0.08$   $\mu$ Ci/mouse of SC-19192.
2. Administered in aqueous solution by direct intraurethral instillation: 0.57 mgm/mouse; 1.0  $\mu$ Ci/mouse of SC-19192-phe-C<sup>14</sup>.

#### Administration of Test Material

Pellets of 20-22 mgm mass and 0.4 cm diameter, composed of one part of powdered C<sup>14</sup>-SC-19192 mixed with four parts of three times recrystallized, powdered cholesterol (Sigma Chemical Co., St. Louis, Mo.) were fashioned. The cholesterol and the C<sup>14</sup>-SC-19192 were separately ground to a fine powder in an agate mortar. C<sup>14</sup>-SC-19192 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. Lots of pellets numbering 80 were prepared to insure uniformity and reproducibility of 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. For the Intravesical Pellet Implant Technique study, each study mouse had a pellet surgically inserted into the urinary bladder lumen by the technique of Jull (2) as modified by Allen et al (3). For the direct intraurethral vesical instillation experiment the following procedures were employed. After anesthetization, a low abdominal midline incision was made, the bladder and urethra were exposed, and the urethra was carefully dissected away from the dorsal-lying vagina. Two small, round, wooden sticks (3-mm diameter) were inserted between the urethra and vagina to facilitate the entry of the needle (syringe, 0.05 ml, with needle, no. 705, Hamilton Co. Inc., Whittier, Calif.) into the urethra about 0.2 - 0.3 mm distal to the bladder. Two ligatures were placed around the urethra, but were not tightened until the needle had been passed into the urethra and inserted into the bladder lumen. Next the ureters were identified and ligated bilaterally about midway between the bladder and kidneys. After the injection of 0.2 ml of the sterile saline solution containing the test chemical, the needle was carefully drawn out past each ligature, and the ligatures were successively tightened to prevent leakage of the solution as the needle was removed. The methods employed were described (4). The abdomen was then closed and the animal placed into an all-glass metabolism chamber, and respiratory CO<sub>2</sub>, urine, and feces were collected until the animals were killed.

#### Analyses of Pellets

The C<sup>14</sup> content of pellets was assayed by dissolving the pellets in 10 ml of chloroform followed by quantitative dilution to a final volume of 100 ml with absolute ethanol. Following mixing, aliquots were quantitatively transferred

to vials, 18 ml of scintillation fluid (naphthalene, 259 gm; diphenyloxazole, 18.4 gm; alpha-naphthylphenyloxazole, 0.184 gm; xylene, 1400 ml; dioxane, 1400 ml; and absolute ethanol, 840 ml) were added, and the samples were counted (68-74% efficiency) in a Nuclear Chicago Mark I scintillation counter. Efficiency corrections were made using automatic external standardization with the channel ratio method.

#### Analyses of Tissues

Radioactivity of aliquots of the ethanolamine carbonate, urine, or homogenized feces was counted utilizing liquid scintillation techniques (5, 6). The mice were killed by cervical fracture. Liver, lung, kidney and urinary bladder were dissected, weighed, and digested individually in 2 N KOH:ethanol:toluene (10:5:1 v/v) for 24 hours, and then brought to volume with ethanol. The bladder was washed and processed separately from the bladder contents. Stomach and intestinal contents were pooled with feces prior to homogenization. The carcass was digested as the other tissues and then filtered to remove bone. Duplicate 0.5-ml samples of the different tissues, urine, feces, or CO<sub>2</sub> were counted in vials containing 20 ml of scintillation fluid for 10 minutes each. Background counts for each tissue were determined by testing 2 to 4 mice of the same age processed individually through the above procedures using "cold" SC-19192 instead of C<sup>14</sup>-SC-19192.

#### Calculations

The mean  $\pm$  standard deviation percentage of recovered C<sup>14</sup> was calculated

utilizing 4 mice for each time period studied. The mean tissue specific activity  $\pm$  standard deviation, expressed as disintegrations per minute (DPM)/mgm wet weight was computed.

## RESULTS

The percentage of total DPM recovered and the tissue specific activity measured at 4.8 (T  $\frac{1}{2}$ ), 9.6 (2T  $\frac{1}{2}$ ), and 14.4 (3T  $\frac{1}{2}$ ) hours following implantation of cholesterol pellets containing SC-19192-phe-C<sup>14</sup> into the vesical lumen of mice is presented in Table 1 (pg. 8). Radioactivity apparently disappeared and was detected in all tissues and biological samples measured. At all time periods, urinary bladder tissue specific activity was the highest of all tissues measured, indicating that SC-19192 penetrated into the bladder. Following bladder absorption, radioactivity was widely distributed.

The percentage of total DPM recovered and the tissue specific activity measured at 4.8 hours (T  $\frac{1}{2}$ ) following intraurethral instillation of an aqueous solution containing SC-19192-phe-C<sup>14</sup> into the isolated urinary bladder lumen of mice is displayed in Table 2 (pg. 9). Within the brief observation period of 4.8 hours, about 25% of the C<sup>14</sup> had passed into or through the bladder and was distributed into other tissues or CO<sub>2</sub>. These data strongly suggest that the intact bladder is permeable to SC-19192, and that the distribution observed in Table 1 (pg. 8) was not due simply to C<sup>14</sup> "leaking" through the surgical incision in the bladder dome.

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## CONCLUSION

These data indicate that under the conditions utilized in the Intravesical Pellet Implant Technique, SC-19192 is readily eluted from the cholesterol pellet, has an opportunity to interact with the urinary bladder epithelium, is absorbed through the vesical wall, and gains access to other organs where it is subjected to host metabolism and excretory processes. The absence of a vesical neoplastic response to exposure of the bladder to SC-19192 in the presence of demonstrable incorporation of SC-19192-phe-C<sup>14</sup> into bladder tissue utilizing the Intravesical Pellet Implantation Technique substantially strengthens the conclusion that SC-19192 is probably not a murine vesical carcinogen.

TABLE 1

Percentage of Total DPM Recovered and Tissue Specific Activity After Implantation of Cholesterol Pellets Containing SC-19192-phe-C<sup>14</sup> into the Urinary Bladder of Mice. P-T 10380172.

Sample	Animal Hours of Survival					
	4.8 (T 1/2)		9.6 (2T 1/2)		14.4 (3T 1/2)	
	% $\pm$ S.D.*	DPM/mg $\pm$ S.D.**	% $\pm$ S.D.*	DPM/mg $\pm$ S.D.**	% $\pm$ S.D.*	DPM/mg $\pm$ S.D.**
<sup>14</sup> CO <sub>2</sub>	0.2 $\pm$ 0.07	--	0.6 $\pm$ 0.4	--	0.9 $\pm$ 0.9	--
Urine	10.8 $\pm$ 8.4	--	19.9 $\pm$ 4.8	--	29.0 $\pm$ 7.3	--
Feces***	1.4 $\pm$ 1.6	--	16.9 $\pm$ 9.6	--	13.3 $\pm$ 8.3	--
Bladder	0.4 $\pm$ 0.06	124 $\pm$ 28	0.5 $\pm$ 0.2	335 $\pm$ 153	1.8 $\pm$ 0.5	635 $\pm$ 152
Carcass	19.7 $\pm$ 7.7	8.5 $\pm$ 3.1	10.4 $\pm$ 1.6	5.5 $\pm$ 0.8	6.9 $\pm$ 6.7	3.5 $\pm$ 25
Liver	0.2 $\pm$ 0.06	1.6 $\pm$ 0.4	0.3 $\pm$ 0.07	2.8 $\pm$ 1.3	0.2 $\pm$ 0.2	2.7 $\pm$ 1.1
Lung	0.04 $\pm$ 0.01	1.3 $\pm$ 0.4	0.08 $\pm$ 0.03	3.5 $\pm$ 2.0	0.04 $\pm$ 0.03	1.1 $\pm$ 0.7
Kidney	0.3 $\pm$ 0.09	7.6 $\pm$ 3.3	0.9 $\pm$ 0.4	27.5 $\pm$ 14.5	0.1 $\pm$ 0.1	3.2 $\pm$ 2.2
Pellet	67.8 $\pm$ 3.8	--	50.5 $\pm$ 8.8	--	49.3 $\pm$ 11.1	--

\* Percentage  $\pm$  Standard Deviation of 4 mice at each time period.

\*\* Tissue specific activity (disintegrations per mgm wet weight)  $\pm$  Standard Deviation.

\*\*\* Including intestinal contents.

TABLE 2

Percentage of Total DPM Recovered and Tissue Specific Activity After Intraurethral Instillation of Aqueous Solution Containing SC-19192-phe-C<sup>14</sup> into the Urinary Bladder Lumen of Mice.  
P-T 10386772.

Sample	4.8 Hours (T 1/2) After Instillation	
	% $\pm$ S.D.*	DPM/mg $\pm$ S.D.**
<sup>14</sup> CO <sub>2</sub>	0.03 $\pm$ 0.01	--
Bladder Contents	75.9 $\pm$ 11.9	--
Intestinal Contents and Feces	0.07 $\pm$ 0.11	--
Bladder	0.3 $\pm$ 0.08	56.6 $\pm$ 32.6
Carcass	13.3 $\pm$ 6.6	14.2 $\pm$ 5.1
Liver	2.7 $\pm$ 1.9	24.4 $\pm$ 14.7
Lung	0.6 $\pm$ 0.4	29.3 $\pm$ 13.8
Kidney	7.2 $\pm$ 3.5	151.2 $\pm$ 64.4

\* Percentage  $\pm$  Standard Deviation of 4 mice.

\*\* Tissue specific activity (disintegrations per mgm wet weight)  $\pm$  Standard Deviation.

## REFERENCES

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#### IV

Addendum To:

SC-19192: A 26 Week Urinary Bladder Tumorigenicity Study  
in the Mouse by the Intravesical Pellet Implant Technique

P-T No. 1032ot72

ADDENDUM TO:

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

P-T NO. 1032of72

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Other Tumors:

Table 3 presents the number of neoplastic lesions observed in each treatment group. No statistically significant augmented incidence of a single tumor type, or of the aggregated tumor incidence, compared with the Negative Control group, was detected for either chemical treatment group.

TABLE 3

Summary of Tumors Observed in Organs Other than Urinary Bladder in a 26 Week Urinary Bladder Tumorigenicity Study in the Mouse by the Pellet Implant Technique. P-T 1032ot72.

Group	No. Mice	Lesions in Evaluated Mice Surviving > 176 Days				
		Leukemia	Mammary Adenofibroma	Pulmonary Adenoma	Stomach Papilloma	
1. Cholesterol (Neg. Control)	69	4	2	1		
3. SC-19192	64	3	1		1	
4. 8-Methyl Ether of Xanthurenic Acid (Positive Control)	44	2		2		