Lack of Oncogenicity of Wood Creosote, the Principal Active Ingredient of Seirogan, an Herbal Antidiarrheal Medication, in Sprague-Dawley Rats

Tomoo Kuge,[1] Takashi Shibata,[1] Michael S. Willett,[2] Patricia Turck,[3] and Karl A. Traul[4]

[1]*Taiko Pharmaceutical Company, Ltd., Suita, Osaka, Japan*
[2]*Advanced Biomedical Research, Inc., Pennington, New Jersey, USA*
[3]*MPI Research, Inc., Mattawan, Michigan, USA*
[4]*Ingle and Traul Pharmaceutical Consulting, Inc., Princeton, New Jersey, USA*

Seirogan, an herbal medicine containing wood creosote (tablets, 10.0% *w/w*), has been developed and marketed for almost a century in various countries for the control of acute diarrhea and treatment of associated symptoms, such as abdominal cramping. Wood creosote (CAS no. 8021-39-4) is a mixture of simple phenolic compounds, including guaiacol and creosol and related compounds, and is chemically distinct from, and should not be confused with, coal tar creosote, a known carcinogen. In the current study, the oncogenic potential of wood creosote was assessed in a 96/103-week oral gavage study in Sprague-Dawley rats. Groups of 60 rats/sex received wood creosote at dose levels of 20, 50, or 200 mg/kg body weight [bw]/day. An additional group of rats received the vehicle, 0.5% carboxymethylcellulose in deionized, distilled water, at the same dose volume as the treatment groups (10 ml/kg) and served as the controls. Treatment-related decreases in survival, body weight, and food consumption, as well as increased incidences of clinical signs that included rales, decreased activity, and salivation, were noted at 200 mg/kg bw/day when compared with the control group. There was an increased incidence of reddened and edematous lungs in rats from the 200 mg/kg bw/day group that died during the study. The lung findings were suggestive of test article aspiration during dose administration or agonal aspiration preceding and possibly resulting in death, especially because these observations were not seen in animals that survived to scheduled sacrifice. Additionally, phenols are generally recognized as having corrosive properties. There were no changes in clinical pathology and no increases in neoplastic or non-neoplastic lesions, excluding the lung findings, related to treatment with wood creosote at any dose level. Although the results of this study indicate that the maximum tolerated dose of wood creosote was met or exceeded at 200 mg/kg bw/day, there was no evidence of oncogenicity at any dose level. The lack of any evidence of oncogenicity supports the safety profile of the active ingredient in Seirogan, wood creosote.

Keywords Antidiarrheal, Creosol, Guaiacol, Oncogenicity, Seirogan, Wood Creosote (CAS 8021-39-4)

Seirogan (wood creosote) tablets (10.0% *w/w*) have been developed and marketed for almost a century in various countries, particularly throughout Asia, as an herbal medicine for the control of acute diarrhea and the treatment of associated symptoms such as abdominal cramping. The active ingredient in Seirogan tablets is wood creosote, sometimes referred to as medicinal wood creosote or beechwood creosote (CAS 8021-39-4). In Japan, wood creosote is a compendial compound (Pharmacopoeia of Japan [JP]). Seirogan tablets marketed throughout multiple countries in Asia contain wood creosote as the principal active ingredient plus other herbal ingredients, including gambir, philodendron bark, glycyrrhiza, and citrus unshiu peel. Seirogan tablets being evaluated in clinical trials in North America contain only wood creosote without the other herbal ingredients. Wood creosote is a mixture of simple phenolic compounds, including guaiacol and creosol and related compounds, and is chemically distinct from and should not be confused with coal tar creosote (Ogata and Baba 1989). Another component of creosote, 4-ethylguaiacol, is also a major component of soy sauce.

Coal tar creosote (CAS no. 8001-58-9), which is used as a wood preservative, contains a mixture of compounds including polycyclic aromatic hydrocarbons, such as benzo(a)pyrene and benz(a)anthracene, which are known to be carcinogenic (Lenson 1956; Alben 1980; Malins et al. 1985; Rotard and Mailahn 1987). Creosote bush, which is chemically distinct from wood creosote and is also known as chaparral leaf, chico bush, or greasewood, is a plant indigenous to the southwestern United States, which is used as an herbal tea to treat a diverse group of ailments including ethanol withdrawal (Brody 1995). Chaparral leaf ingestion as an herbal tea has been linked

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Address correspondence to Tomoo Kuge, PhD, Taiko Pharmaceutical Co., Ltd., 3–34–14 Uchihonmachi, Suita, Osaka 564–0032, Japan. E-mail: kuge@seirogan.co.jp

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to several cases of hepatotoxicity (Alderman 1994; Brody 1995).

Wood creosote appears to have potent antisecretory and antispasmodic properties. In vivo and in vitro studies with wood creosote suggest that its mechanism of action is multifaceted and includes electrolyte-mediated decreases in secretory activity and increases in absorption by the gastrointestinal mucosa as well as decreases in propulsive motility and gastrointestinal transit through a direct effect on smooth muscle (Ogata, Baba, and Shibata 1993; Ogata, Toyoda, and Shibata 1992; Greenwood-Van Meerveld et al. 2000; Kuge, Venkova, and Greenwood-Van Meerveld 2001).

Seirogan tablets, containing wood creosote (10.0% *w/w*) as the only active ingredient, are being evaluated as an antidiarrheal herbal medicine in placebo- and active ingredient–controlled clinical trials in North America. The most common side effects in these studies to date have included altered taste, somnolence, headache, and dizziness, most often mild in intensity. These effects are reported to be reversible and to occur in less than 10% of patients who use Seirogan at recommended dosages (up to 135 mg per day for 1 to 3 days). Uncontrolled studies in Japan and the United Kingdom and extensive postmarketing experience with Seirogan provide further support for the safety and tolerability of Seirogan. Previously published chronic toxicity studies, conducted prior to the implementation of Japanese Good Laboratory Practice (GLP) regulations, have demonstrated a lack of evidence of oncogenicity in mice and rats (Miyazato et al. 1984a, 1984b).

The present study was intended to supplement previous safety experience with wood creosote and was designed to assess the potential oncogenicity of wood creosote in rats in accordance with current, internationally accepted GLP regulations. The study was conducted at MPI Research, Mattawan, MI, USA.

MATERIALS AND METHODS

Test Article

The test article, wood creosote, Lot TA-03 (reference standard) was received directly from Taiko Pharmaceutical Co., Ltd., Suita, Osaka, Japan. Taiko Pharmaceutical Co., Ltd., provided documentation on the strength, purity, composition, stability, physical properties, and other pertinent information on the test article used in this study. This batch of material was made specifically for use in clinical trials and documentation regarding the manufacturing processes and analyses are on record with Taiko Pharmaceutical Co. The specifics of the composition are considered confidential information at this time, but these will be used as the guideline for the preparation of future batches. The carboxymethylcellulose (CMC) used to prepare the dosing suspensions was received from Sigma Chemical Company, St. Louis, MO, USA. The test article suspensions were analyzed for homogeneity, stability, and concentration. The analytical method used for these determinations is a gas chromatography–mass spectrometry (GC-MS) method developed by Taiko Pharmaceutical Co., Ltd. Results of these analyses indicated that the dosing formulations were homogeneous, stable for the duration of use, and were within 15% of the specified concentrations.

Animals

Four-week-old, male and female Sprague-Dawley Crl:CD VAF/Plus rats were received from Charles River Laboratories, Raleigh, NC, USA. The rats were observed daily for any clinical signs of disease during an approximate 2-week acclimation period and were given a detailed physical examination. A representative group of randomly selected male and female rats was also given a pretest health screen. Throughout the duration of the study, the rats were singly housed in stainless steel cages with wire mesh floors and maintained in environmentally controlled air-conditioned rooms with an ambient room temperature of 66°F to 77°F, relative humidity of 34% to 79%, and a 12-hour light/dark cycle. The rats were provided with Certified Rodent Chow #5002 (PMI International, Inc., St. Louis, MO) and tap water ad libitum. The manufacturer performed certification analysis of the diet and the water supply was analyzed on a quarterly basis for the presence of heavy metal, pesticides, and specified contaminants. There were no known contaminants in either the food or water that were considered to have impacted the outcome of the study. The care and use of animals conformed to Association for Assessment and Accreditation of Laboratory Care (AAALAC) standards and those published in the *Guide for Care and Use of Laboratory Animals* (NIH 1985). This study was conducted in accordance with the U.S. Food and Drug Administration (FDA) GLP Regulations 21 CRF Part 58, final rule effective October 5, 1987, and with Ordinance No. 21, GLP Regulations March 26, 1997, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare.

Dosing

Groups of 60 rats/sex received wood creosote orally by gavage at dose levels of 20, 50, or 200 mg/kg bw/day and a constant dose volume of 10 ml/kg bw for approximately 2 years, starting at about 6 weeks of age. The test article was prepared as a suspension in 0.5% *w/v* CMC in deionized, distilled water. An additional group (60/sex) of rats received an equivalent volume of the CMC vehicle and served as the control.

In-Life Evaluations

All rats were examined at least twice daily for mortality and general physical condition and detailed clinical examinations were performed on each rat weekly. Body weights for all rats were measured prior to initiation of dosing and at regular intervals throughout the duration of the study. Food consumption was measured for all rats throughout the study and additional calculations of food efficiency (food consumption divided by body weight gain) were made for the first 14 weeks of treatment. Ophthalmoscopic examinations were performed on all rats pretest and on all surviving rats prior to termination. Hematology evaluations were performed on a subset of 10 rats/sex

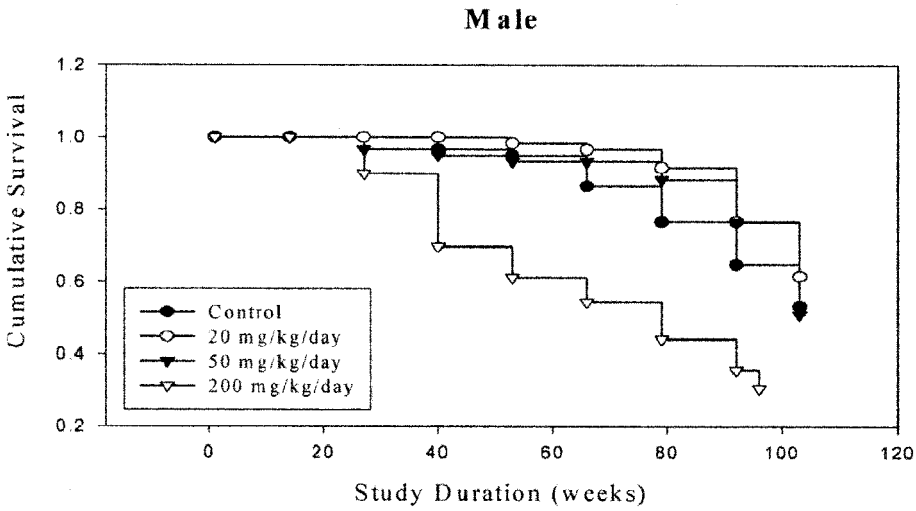

FIGURE 1

Summary of cumulative survival in male Sprague-Dawley rats receiving wood creosote 20, 50, or 200 mg/kg bw/day ($p \leq .01$ for overall survival between wood creosote 200 mg/kg bw/day versus control).

prior to study initiation and on all surviving rats prior to terminal necropsy. The rats had free access to food and drinking water prior to blood collection. The blood samples (approximately 1.5 to 2.0 ml) were collected by closed cardiac puncture after carbon dioxide inhalation. Any rat showing signs of severe debility or toxicity, particularly when death appeared imminent, was euthanized both for humane reasons and to prevent the loss of tissues through autolysis. Due to the increasing mortality of the 200-mg/kg bw/day group (after about week 40 for males and week 80 for females), this group and 10 rats/sex from the control group were sacrificed early during week 96 of the study. The other study groups (27 males and 26 females of the 20-mg/kg bw/day group; 21 males and 23 females of the 50-mg/kg bw/day group) and the remaining control animals (12 males, 9 females) were sacrificed during week 103.

Anatomic and Microscopic Pathology

A complete necropsy was performed on all rats under the supervision of a veterinary pathologist. The rats were examined for external abnormalities including palpable masses. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs were removed and examined. Selected

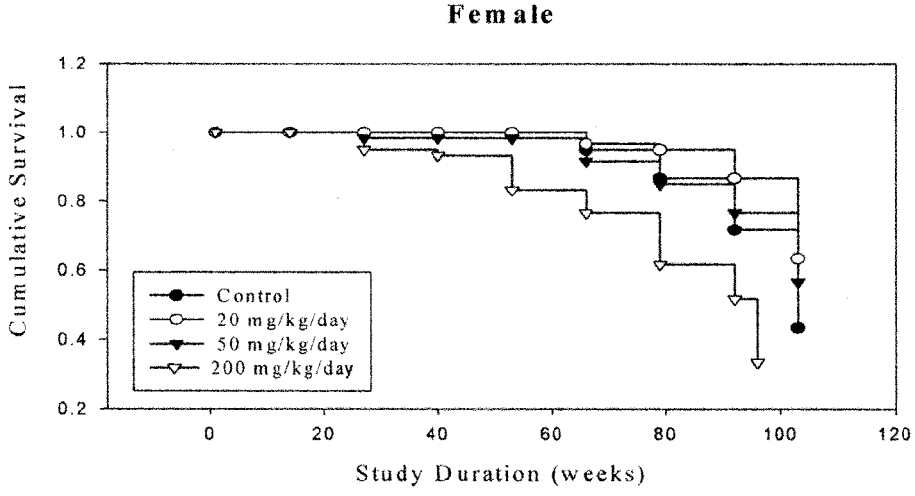

FIGURE 2

Summary of cumulative survival in female Sprague-Dawley rats receiving wood creosote 20, 50, or 200 mg/kg bw/day.

TABLE 1
Summary of body weights and body weight changes in rats treated with wood creosote

Mean body weights and body weight changes (g)				
	Males		Females	
Dose level (mg/kg bw/day)	Mean body weight (%)	Mean body weight change	Mean body weight (%)	Mean body weight change
0	706.3 (NA)	514.3	468.4 (NA)	322.6
0[a]	708.4 (NA)	529.9	473.0 (NA)	316.0
20	684.5 (−3.1)	498.6	442.2 (−5.6)	301.1
50	725.6 (+2.7)	536.2	448.5 (−4.2)	304.5
200[a]	659.7 (−6.9)	485.8	397.0[b] (−17.1)	241.4[b]

[a]Values through week 95, change: weeks 0–94; other values through week 102.
[b]Significantly different from comparative control group; $p < .05$.
NA = not applicable.
The numbers in parentheses are percent difference from control group values.

organs were weighed and representative samples were preserved for microscopic examination. A full complement of organs and tissues from all rats in the control and 200-mg/kg bw/day groups and for rats in the 20- and 50-mg/kg bw/day groups that died or were euthanized in extremis during the course of the study was examined. In addition, sections of all gross lesions and tissue masses with draining lymph nodes were prepared and examined microscopically for all groups.

Statistical Analyses

The data for each sex were analyzed separately in this study. For each specified end point and for all collection intervals, Levene's test (Milliken and Johnson 1992) was used to assess the homogeneity of group variances. If Levene's test was not significant ($p \geq .01$), Dunnett's test (Dunnett 1955) was used to compare each treatment group with the control group. If Levene's test was significant ($p < .01$), comparisons with the control group were made using Welch's t test (Welch 1937) with a Bonferroni correction. Results of all pair-wise comparisons were reported at the .05 and .01 significance levels. All end points were analyzed using two-tailed tests unless otherwise indicated.

Historical data indicate that leukocyte counts (total and differential) are not normally distributed; therefore, a log transformation was performed on these data. The transformed data were then analyzed as described above.

Survival data were analyzed using the Kaplan-Meier product-limit method. Each test article group was compared with the control group using the log rank test (Allison 1955).

Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test (Agresti 1990) was carried out and the Fisher's exact test (Zar 1999) was used to compare each treatment group with the control. The adjusted survival test was conducted according to prevalence/mortality methods (Peto et al. 1980).

RESULTS AND DISCUSSION

There was an increase in mortality in both males and females treated with 200 mg/kg bw/day wood creosote compared to the untreated controls. This decrease in survival was statistically significant ($p \leq .05$) in males and necessitated the early termination of this study group during week 96 of study along with 10 rats/sex of the control group for comparison purposes. Early termination was carried out to assure that there would be a reasonable minimum of control animals available for comparative analyses. The surviving rats from the remaining groups were terminated at week 103 due to the reduced survival of the remaining control animals. No statistically significant decreases in survival were noted in either the 20- or

TABLE 2
Summary of food consumption in rats treated with wood creosote

Average food consumption (g/day)				
	Males		Females	
Dose level (mg/kg bw/day)	Average consumption	(%)	Average consumption	(%)
0	26.7	(NA)	20.7	(NA)
20	27.0	(+1.1)	21.2	(+2.4)
50	26.9	(+0.7)	21.5	(+3.9)
200[a]	26.1	(−2.2)	20.4	(−1.4)

[a]Values through week 95, change: weeks 0–94; other values through week 102.
NA = not applicable.
(%) = percent difference from control group values.

TABLE 3
Summary of absolute organ weights in rats treated with wood creosote

	Dose level (mg/kg bw/day)				
	0[a]	0[b]	20[a]	50[a]	200[b]
Male					
Terminal body weight (g)	693	699	677	726	655
Brain (g)	2.17	2.21	2.11	2.14	2.16
Adrenal (mg)	87	79	99	80	72
Epididymis (g)	1.24	1.40	1.25	1.27	1.36
Heart (g)	2.04	2.10	2.07	2.08	1.86*
Kidney (g)	5.43	5.32	5.65	5.54	5.08
Liver (g)	21.8	22.6	21.9	23.6	22.3
Lung (g)	2.33	2.40	2.46	2.36	2.30
Pituitary (mg)	88 49	48	31*	33	
Prostate (g)	0.51	0.69	0.65	0.64	0.67
Spleen (g)	1.07	1.32	1.20	1.18	0.99**
Testis (g)	3.15	3.23	3.28	3.45	3.59*
Thymus (g)	0.19	0.31	0.21	0.21	0.32
Thyroid/parathyroid (mg)	45	36	41	44	32
Female					
Terminal body weight (g)	452	465	433	440	387**
Brain (g)	1.92	1.94	1.88	1.95	1.95
Adrenal (mg)	111	121	115	129	94
Heart (g)	1.52	1.53	1.47	1.44	1.45
Kidney (g)	3.34	3.38	3.34	3.35	3.44
Liver (g)	16.3	15.7	16.0	15.9	15.4
Lung (g)	1.73	1.89	1.83	1.70	1.84
Ovary (mg)	142	108	125	99	106
Pituitary (mg)	112	103	140	121	103
Spleen (g)	0.82	0.82	0.71	0.69	0.69
Thymus (g)	0.19	0.25	0.18	0.18	0.21
Thyroid/parathyroid (mg)	34	40	34	38	29**
Uterus/cervix	1.10	0.96	0.87	0.94	1.61

[a] Values are for control, low- and mid-dose group animals that were necropsied during week 102.
[b] Values are for control and high-dose group animals that were necropsied during week 95.
*Significantly different from control group; $p < .05$.
**Significantly different from control group; $p < .01$.

50-mg/kg bw/day groups compared to the untreated controls. In fact, a statistically significant trend toward increased survival was detected in the 20-mg/kg bw/day group females when compared to the controls. The survival rates at termination were 53%, 62%, 51%, and 30% for males and 43%, 63%, 57%, and 33% for females in the control, 20-, 50-, and 200-mg/kg bw/day groups, respectively. Survival curves are presented in Figure 1 for males and Figure 2 for females. Wood creosote–related clinical signs were limited to the 200-mg/kg bw/day dose group and consisted of rales, salivation, decreased activity, whole body tremors, and signs of apparent abdominal discomfort (stretching and pressing abdomens against the cage or feeder). Statistically significant reductions ($p \leq .05$) in body weight parameters, including a reduction in mean body weight of approximately 7% in males and 15% in females, and concomitant, but sporadic, statistically significant reductions ($p \leq .05$) in food consumption and food efficiency were also observed in the 200-mg/kg bw/day group. There were no clinical signs or effects on body weight, food consumption, or food efficiency attributed to wood creosote at dose levels of 20 or 50 mg/kg bw/day. There were no ophthalmoscopic findings, alterations in hematology parameters, organ weight changes, or effects on

TABLE 4
Summary of macroscopic findings in rats treated with wood creosote

Selected macroscopic observations at necropsy for male rats

	Dose level (mg/kg bw/day)							
	0		20		50		200	
	DOS[a] $n = 38$	SAC $n = 22$	DOS $n = 33$	SAC $n = 27$	DOS $n = 39$	SAC $n = 21$	DOS $n = 46$	SAC $n = 14$
Lung, red discoloration	7	0	2	0	8	0	21	0
Pituitary, enlarged	15	8	19	8	19	5	9	2
Skin, mass subcutis	3	3	5	0	0	1	3	0
Skin, mass	2	0	2	1	4	1	0	0
Foot, plantar ulcer	5	9	8	17	5	8	6	7

Selected macroscopic observations at necropsy for female rats

	Dose level (mg/kg bw/day)							
	0		20		50		200	
	DOS $n = 41$	SAC $n = 19$	DOS $n = 34$	SAC $n = 26$	DOS $n = 37$	SAC $n = 23$	DOS $n = 44$	SAC $n = 16$
Lung, red discoloration	6	0	2	0	3	0	17	0
Ovary, cyst	3	7	1	8	2	3	1	3
Pituitary, enlarged	31	19	26	20	27	19	24	14
Skin, mass subcutis	28	10	39	21	43	21	15	9
Skin, mass	0	0	0	1	1	0	0	0
Foot, plantar ulcer	3	2	0	5	4	5	2	3

[a]DOS = died on study; SAC = scheduled sacrifice.
n = total number examined.

the incidence of palpable masses attributed to wood creosote at any dose level in this study. Table 1 presents body weights and body weight changes and Table 2 presents data on food consumption during the study. Table 3 presents a summary of the organ weights for male and female rats. Although there were effects noted for organ weights, for example, thyroid, spleen, heart, and testis, no accompanying histopathologic changes were reported and no other observations provided insight into these changes.

The most consistent macroscopic finding at necropsy was an increased incidence of reddened lungs associated microscopically with evidence of edema in rats in the 200-mg/kg bw/day group that died. These findings were noted at a much lower incidence in rats from all other groups, including the controls, that died during the study. There was no evidence of gavage injury or perforated esophagus or trachea in these rats; however, some of these rats did have rales, suggesting that the necropsy and microscopic findings may be indicative of aspiration of the test article/vehicle. Phenols, as a class of compounds, are generally known to be corrosive and it is quite possible that the respiratory effects, seen in the high-dose group, may be due to secondary irritation of the lungs, possibly associated with aspiration, following dosing. Aspiration of this test material may have contributed to the premature demise of test and control group animals and would explain the observed effects only in animals that died prematurely on study. There is no evidence, from this or prior studies, that such irritation would result from the systemic exposure following oral administration. Table 4 presents the major findings observed at necropsy for animals that either died on study or were evaluated at scheduled sacrifice times. Histopathology examinations were conducted on a full set of tissues from the control and 200 mg/kg bw/day group as well as on any animal that died during the course of the study. The histopathology examinations showed an apparently high level of mammary adenomas, mammary adenocarcinomas, and pituitary adenomas across all groups. The results of the histopathology examinations showed, however, that there was no difference in the incidence of any neoplasm between the control and any wood creosote–treated group in this study. A review of the historical data prepared

TABLE 5
Summary of neoplastic findings in rats treated with wood creosote

Summary of neoplastic lesions in female rats—total incidence [%]				
	Dose level (mg/kg bw/day)			
Tissue and lesion	0	20	50	200
Adrenal gland, cortex				
Adenoma	1/60 [1.7%]	1/42 [2.4%]	0/40 [0.0%]	1/60 [1.7%]
Carcinoma	0/60 [0.0%]	0/42 [0.0%]	1/40 [2.5%]	0/60 [0.0%]
Adrenal gland, medulla				
Pheochromocytoma, benign	1/60 [1.7%]	0/34 [0.0%]	1/39 [2.6%]	0/58 [0.0%]
Brain				
Ependymoma, benign	0/60 [0.0%]	0/34 [0.0%]	0/37 [0.0%]	1/59 [1.7%]
Granular cell tumor, benign	0/60 [0.0%]	1/34 [2.9%]	0/37 [0.0%]	0/59 [0.0%]
Eye				
Carcinoma, squamous cell	1/60 [1.7%]	0/36 [0.0%]	0/37 [0.0%]	0/60 [0.0%]
Hemolymphoreticular system				
Hemangiosarcoma	1/60 [1.7%]	0/60 [0.0%]	0/60 [0.0%]	0/60 [0.0%]
Leukemia, granulocytic	1/60 [1.7%]	0/60 [0.0%]	0/60 [0.0%]	0/60 [0.0%]
Lymphoma	1/60 [1.7%]	0/60 [0.0%]	0/60 [0.0%]	0/60 [0.0%]
Sarcoma, histiocytic	0/60 [0.0%]	0/60 [0.0%]	1/60 [1.7%]	0/60 [0.0%]
Kidney				
Carcinoma, renal cell	0/60 [0.0%]	0/35 [0.0%]	0/40 [0.0%]	1/60 [1.7%]
Large intestine, cecum				
Lipoma	1/60 [1.7%]	0/34 [0.0%]	0/37 [0.0%]	0/60 [0.0%]
Liver				
Adenoma, hepatocellular	0/60 [0.0%]	1/47 [2.1%]	0/46 [0.0%]	3/60 [5.0%]
Cholangioma	0/60 [0.0%]	0/47 [0.0%]	1/46 [2.2%]	0/60 [0.0%]
Mammary gland				
Adenocarcinoma	7/60 [11.7%]	5/48 [10.4%]	15/51 [29.4%]	4/60 [6.7%]
Adenoma	8/60 [13.3%]	15/48 [31.3%]	12/51 [23.5%]	9/60 [15.0%]
Fibroadenoma	16/60 [26.7%]	23/48 [47.9%]	22/51 [43.1%]	15/60 [25.0%]
Ovary				
Granulosa cell tumor, malignant	0/59 [0.0%]	2/42 [4.7%]	0/40 [0.0%]	0/60 [0.0%]
Lipoma	1/59 [1.7%]	0/42 [0.0%]	0/40 [0.0%]	0/60 [0.0%]
Thecoma	0/59 [0.0%]	1/42 [2.4%]	0/40 [0.0%]	0/60 [0.0%]
Pancreas				
Adenoma islet cell	1/60 [1.7%]	0/34 [0.0%]	0/37 [0.0%]	1/60 [1.7%]
Pituitary gland				
Adenocarcinoma	0/57 [0.0%]	0/54 [0.0%]	0/54 [0.0%]	1/58 [1.7%]
Adenoma	48/57 [84.2%]	47/54 [87.0%]	44/54 [81.5%]	31/58 [53.5%]
Skin				
Keratoacanthoma	0/60 [0.0%]	0/35 [0.0%]	1/37 [2.7%]	1/60 [1.7%]
Papilloma	0/60 [0.0%]	0/35 [0.0%]	0/37 [0.0%]	1/60 [1.7%]
Skin, subcutis				
Fibrosarcoma	1/3 [33.3%]	2/2 [100.0%]	0/3 [0.0%]	0/0 [0.0%]
Hibernoma	0/3 [0.0%]	0/2 [0.0%]	1/3 [33.3%]	0/0 [0.0%]
Lipoma	2/3 [66.7%]	0/2 [0.0%]	1/3 [33.3%]	0/0 [0.0%]
Thymus gland				
Carcinoma, squamous cell	0/54 [0.0%]	0/29 [0.0%]	1/31 [3.2%]	0/55 [0.0%]
Fibrosarcoma	0/54 [0.0%]	0/29 [0.0%]	0/31 [0.0%]	1/55 [1.8%]
Thyroid gland				
Adenoma, C-cell	5/58 [8.6%]	4/32 [12.5%]	2/38 [5.3%]	0/60 [0.0%]
Adenoma, follicular	2/58 [3.4%]	0/32 [0.0%]	1/38 [2.6%]	0/60 [0.0%]
Uterus				
Adenoma	1/60 [1.7%]	1/40 [2.5%]	1/38 [2.6%]	1/60 [1.7%]
Polyp	1/60 [1.7%]	2/40 [5.0%]	2/38 [5.3%]	1/60 [1.7%]
Uterus, cervix				
Fibrosarcoma	1/60 [1.7%]	0/34 [0.0%]	0/37 [0.0%]	0/60 [0.0%]
Sarcoma, stromal cell	0/60 [0.0%]	0/34 [0.0%]	0/37 [0.0%]	1/60 [1.7%]
Vagina				
Carcinoma, squamous cell	1/60 [1.7%]	0/34 [0.0%]	0/36 [0.0%]	0/59 [0.0%]

(Continued on next page)

TABLE 5
Summary of neoplastic findings in rats treated with wood creosote *(Continued)*

Summary of neoplastic lesions in male rats—total incidence [%]				
	Dose level (mg/kg bw/day)			
Tissue and lesion	0	20	50	200
Adrenal gland, medulla				
Pheochromocytoma, benign	2/59 [3.4%]	3/34 [8.8%]	2/37 [5.4%]	1/60 [1.7%]
Pheochromocytoma, malignant	0/59 [0.0%]	1/34 [2.9%]	0/37 [0.0%]	0/60 [0.0%]
Brain				
Glioma, malignant	0/60 [0.0%]	1/33 [3.0%]	0/39 [0.0%]	1/60 [1.7%]
Epididymis				
Lipoma	0/60 [0.0%]	0/33 [0.0%]	1/40 [2.5%]	0/60 [0.0%]
Heart				
Fibrosarcoma	0/60 [0.0%]	1/34 [2.9%]	0/39 [0.0%]	0/60 [0.0%]
Hemolymphoreticular system				
Hemangiosarcoma	0/60 [0.0%]	0/60 [0.0%]	1/60 [1.7%]	0/60 [0.0%]
Leukemia, granulocytic	0/60 [0.0%]	0/60 [0.0%]	1/60 [1.7%]	1/60 [1.7%]
Liposarcoma	0/60 [0.0%]	0/60 [0.0%]	1/60 [1.7%]	0/60 [0.0%]
Lymphoma	1/60 [1.7%]	0/60 [0.0%]	0/60 [0.0%]	2/60 [3.3%]
Liver				
Adenoma, hepatocellular	4/60 [6.7%]	5/46 [10.9%]	3/51 [5.9%]	2/60 [3.3%]
Cholangioma	0/60 [0.0%]	0/46 [0.0%]	1/51 [2.0%]	0/60 [0.0%]
Fibrosarcoma	0/60 [0.0%]	1/46 [2.2%]	0/51 [0.0%]	0/60 [0.0%]
Lung				
Fibrosarcoma	0/60 [0.0%]	1/36 [2.8%]	0/42 [0.0%]	0/60 [0.0%]
Lymph node, mediastinal				
Fibrosarcoma	0/55 [0.0%]	1/32 [3.1%]	0/37 [0.0%]	0/57 [0.0%]
Pancreas				
Adenoma	1/60 [1.7%]	0/33 [0.0%]	0/39 [0.0%]	0/60 [0.0%]
Adenoma, islet cell	2/60 [3.3%]	1/33 [3.0%]	1/39 [2.6%]	0/60 [0.0%]
Pituitary gland				
Adenocarcinoma	0/58 [0.0%]	0/41 [0.0%]	0/43 [0.0%]	1/58 [1.7%]
Adenoma	27/58 [46.6%]	29/41 [70.7%]	26/43 [60.5%]	16/58 [27.6%]
Skin				
Basal cell tumor, benign	0/59 [0.0%]	1/36 [2.8%]	0/40 [0.0%]	0/60 [0.0%]
Fibrosarcoma	0/59 [0.0%]	1/36 [2.8%]	0/40 [0.0%]	0/60 [0.0%]
Keratoacanthoma	1/59 [1.7%]	2/36 [5.6%]	1/40 [2.5%]	2/60 [3.3%]
Papilloma	2/59 [3.4%]	1/36 [2.8%]	2/40 [5.0%]	0/60 [0.0%]
Skin, subcutis				
Adenoma, sebaceous	0/6 [0.0%]	0/5 [0.0%]	1/2 [50.0%]	0/3 [0.0%]
Carcinoma, squamous cell	0/6 [0.0%]	2/5 [40.0%]	0/2 [0.0%]	0/3 [0.0%]
Fibroadenoma	0/6 [0.0%]	0/5 [0.0%]	1/20 [5.0%]	0/3 [0.0%]
Fibroma	2/6 [33.3%]	1/5 [20.0%]	0/2 [0.0%]	2/3 [66.7%]
Fibrosarcoma	1/6 [16.7%]	2/5 [40.0%]	0/2 [0.0%]	0/3 [0.0%]
Keratoacanthoma	2/6 [33.3%]	0/5 [0.0%]	0/2 [0.0%]	0/3 [0.0%]
Lipoma	1/6 [16.7%]	0/5 [0.0%]	0/2 [0.0%]	0/3 [0.0%]
Spinal cord, thoracic				
Glioma, benign	0/60 [0.0%]	0/33 [0.0%]	1/39 [2.6%]	0/59 [0.0%]
Testis				
Adenoma	0/60 [0.0%]	0/39 [0.0%]	1/43 [2.3%]	0/60 [0.0%]
Thymus				
Fibrosarcoma	0/48 [0.0%]	0/30 [0.0%]	1/32 [3.1%]	0/56 [0.0%]
Thymoma	1/48 [2.1%]	0/30 [0.0%]	0/32 [0.0%]	0/56 [0.0%]
Thyroid gland				
Adenoma, C-cell	7/60 [11.7%]	3/36 [8.3%]	1/39 [2.6%]	3/60 [5.0%]
Adenoma, follicular	3/60 [5.0%]	1/36 [2.8%]	1/39 [2.6%]	1/60 [1.7%]
Carcinoma, C-cell	0/60 [0.0%]	0/36 [0.0%]	0/39 [0.0%]	1/60 [1.7%]

by Giknis and Clifford (2001) shows also that there is a normally high incidence of pituitary adenomas, mammary adenomas, and mammary adenocarcinomas in this strain of rat. Table 5 presents a summary of neoplastic findings recorded during the histopathologic evaluations. There was no evidence of oncogenicity associated with wood creosote under the conditions of this study.

SUMMARY AND CONCLUSION

Wood creosote was administered to groups of Sprague-Dawley Crl:CDVAF/Plus rats (60 rats/sex/group) as a suspension in 0.5% *w/v* CMC with deionized, distilled water (vehicle) at doses levels of 20, 50, or 200 mg/kg bw/day. An additional group of rats (60/sex) received 10 ml/kg of vehicle, an equivalent dose volume to the treatment groups, and served as the control. Wood creosote-related decreases in survival, body weight, and food consumption, as well as increased incidences of clinical signs that included rales, decreased activity, and salivation, were noted in the group dosed at 200 mg/kg bw/day when compared with the control group. There was an increased incidence of reddened and edematous lungs in rats from the 200-mg/kg bw/day group that died during the study. The lung findings were suggestive of aspiration of the test article during dose administration or agonal aspiration preceding and possibly resulting in death. There were no changes in clinical pathology and no increases in neoplastic or other non-neoplastic lesions related to wood creosote at any dose level. It is considered possible that the observed high incidences of mammary adenomas/adenocarcinomas and pituitary adenomas in all groups, although not treatment related, contributed to the decreased survival across all groups. Although the results of this study indicate that the maximum tolerated dose of wood creosote was met or exceeded at 200 mg/kg bw/day, there was no evidence of oncogenicity at any dose level. The lack of any evidence of oncogenicity supports the safety profile of the active, and primary, ingredient and principal component in Seirogan, wood creosote.

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