

**NO SIGNIFICANT RISK LEVEL (NSRL)
FOR THE PROPOSITION 65 CARCINOGEN
POLYGEENAN**

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SUMMARY OF FINDINGS

The cancer potency of polygeenan was estimated from dose-response data of colorectal tumors among male and female rats exposed orally (Wakabayashi *et al.*, 1978). The cancer potency estimate corresponds to the upper 95 percent confidence bound on the linear term of the multistage model fit to cancer dose-response data in animals. The potency derivation takes into account body size differences between humans and experimental animals. The Proposition 65 “no significant risk level” (NSRL) is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The cancer potency estimate and the corresponding NSRL are given in Table 1.

Table 1. Cancer Potency and NSRL for Polygeenan

Chemical	Cancer Potency (mg/kg-day) ⁻¹	NSRL (µg/day)
<i>Polygeenan</i>	6.0×10^{-4}	1200

INTRODUCTION

This report describes the derivation of a cancer potency value and NSRL for polygeenan (CAS number 53973-98-1, commonly called degraded carrageenan, average molecular weight 20,000 to 40,000). Polygeenan was listed on January 1, 1988 as a chemical known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq.*). Polygeenan is formed by heated, strong-acid hydrolysis of native carrageenan (obtained from seaweed) (IARC, 1983; Kolbye *et al.*, 1987). Polygeenan is not in the U.S. food supply but is used to suspend barium sulfate in medical diagnostic procedures to aid x-ray visualization of the gastrointestinal tract (Kolbye *et al.*, 1987).

This document discusses the studies available for cancer dose-response assessment, and summarizes the derivations of the cancer potency estimate and NSRL. A description of the methodology used is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

The carcinogenic potential of polygeenan has been investigated in three series of studies in which degraded carrageenan was administered to rats by the oral route (Fabian *et al.*, 1973; Wakabayashi *et al.*, 1978; Oohashi *et al.*, 1981). The study by Fabian *et al.* (1973) was not utilized for cancer potency estimation due to small numbers of animals used for each dose group.

Wakabayashi *et al.* (1978) reported on three oral experiments in Sprague-Dawley rats in which polygeenan was administered in feed, drinking water or by gavage. In one experiment, Wakabayashi *et al.* (1978) administered degraded carrageenan in the diet to four groups of rats (30/sex/dose group) for up to 24 months at concentrations of 0, 1, 5 or 10 percent of the diet. In a second experiment, rats (20 males and 20 females) were administered a five percent aqueous solution of degraded carrageenan *ad libitum* as drinking water for 15 months. The control group (15 males and 15 females) was given distilled water for the length of the experiment. In a third experiment, three groups of rats (15 males and 15 females) were given an aqueous solution of degraded carrageenan by oral gavage, at concentrations of 0, 1 and 5 g/kg body weight, for 15 months. In the gavage study, the number of days per week was not specified, but is presumed to be seven days per week. For all three studies, Wakabayashi *et al.* (1978) reported the tumor incidences of males and females combined, but not separately. Both colorectal squamous

Table 2. Incidence of Colorectal Tumors in Male and Female Sprague-Dawley Rats Treated with Degraded Carrageenan in Diet, Drinking Water and Oral Gavage (Wakabayashi *et al.*, 1978)

Exposure Media (Study Duration)	Administered Dose	Average Dose ¹ (mg/kg-day)	Percent of Rats with Metaplasia	Tumor Incidence ²	Statistical Significance ³ of Tumors
Diet (24 months)	0 %	0	0	0/60	--
	1 %	441	0	0/60	--
	5 %	2206	88	12/60	p<0.001
	10 %	4412	98	19/60	p<0.0001
Drinking water (15 months)	0 %	0	0	0/30	--
	5 %	2647	100	11/40	p<0.01
Oral gavage (15 months)	0 g/kg	0	0	0/30	--
	1 g/kg	1000	37	0/30	--
	5 g/kg	5000	100	8/29	p<0.01

¹ Lifetime average dose was calculated by using reference values for chronic dosing of male and female rats (combined): average daily food intake of male and female rats (0.01875 kg/day), average daily drinking water intake of male and female rats (0.0225 L/day) and an average body weight of male and female rats (0.425 kg) (Gold and Zeiger, 1997).

² Tumor incidence reported here is the number of tumor-bearing animals/total number of animals. This includes squamous cell carcinomas, adenocarcinomas, adenomas and a myosarcoma, as reported in the study.

³ Results of pairwise comparison with controls using the Fisher exact test.

metaplasia and tumors were observed in the treated animals receiving the highest exposures. Increases in colorectal tumors, including squamous cell carcinomas, adenocarcinomas, adenomas

and a myosarcoma, were observed in treated animals relative to controls. Tumor incidences are presented in Table 2.

Oohashi *et al.* (1981) conducted a feeding study in eight-week old male F344 rats. During the 18-month experiment, a diet of 10 percent degraded carrageenan was administered for 2, 6, or 9 months. Following treatment, all animals were maintained on a basal diet and were sacrificed 18 months after the initial administration. The control group was given the basal diet for 18 months and then sacrificed. The results of the study demonstrate that even with short-term (two month) administration, high doses of degraded carrageenan induce tumors of the colorectum in these animals. The dose administered in this study is in the high dose range, where the dose rate-response relationships appear non-linear. Increases in the incidence of colorectal tumors, including squamous cell carcinomas, adenocarcinomas, adenomas and anaplastic carcinomas, were observed (Table 3). In contrast, no colorectal tumors or inflammatory changes were present in the control group.

Table 3. Incidence of Colorectal Tumors in Male F344 Rats Treated with Degraded Carrageenan by Dietary Administration (Oohashi *et al.*, 1981)

Administered Dose ¹ (% diet)	Months Dosed	Average Dose ² (mg/kg-day)	Percent Metaplasia	Tumor Incidence ³	Statistical Significance ⁴
0	--	0	0	0/46	--
10	2	502.3	100	5/39	p<0.02
10	6	1188	100	8/42	p<0.01
10	9	1477	100	17/42	p<0.001

¹ The treated groups were administered a diet containing 10% degraded carrageenan for 2, 6, or 9 months, followed by a basal diet for the remainder of the 18 month experiment. The control group was given the basal diet for the length of the study.

² Because the dosing periods are very short, weighted average dose is calculated by applying an approach based on the Armitage and Doll (1954) model of carcinogenesis, as described by Crouch (1983) and Crump and Howe (1984) (see Appendix). For this case, the dose (d) is calculated by multiplying the average dose during treatment (d_{avg}) by the weighting factor, as specified by values given below. Reference values for chronic dosing of male rats were: average daily food intake of 0.02 kg/day, and an average body weight of 0.5 kg (Gold and Zeiger, 1997).

$$d = d_{avg} \times 1/T^m \times [(T_e - a)^m - (T_e - b)^m]$$

where

T = lifespan	= 24 months
T _e = duration of the experiment	= 20 months
a = age at beginning of treatment	= 2 months
b = age at end of treatment	= 4, 8 or 11 months
m = constant that describes the degree of increase in cancer incidence with age	= 3

³ Tumor incidence reported here includes the occurrence of squamous cell carcinomas, adenocarcinomas, adenomas and anaplastic carcinomas.

⁴ Results of pairwise comparison with controls using the Fisher exact test.

APPROACH TO DOSE-RESPONSE ANALYSIS

Degraded carrageenan was not mutagenic in *Salmonella typhimurium* TA 89 and TA 100 or in Chinese hamster V79 cells (IARC, 1983). IARC (1983) concluded that inadequate data existed to evaluate the genotoxicity of degraded carrageenan. OEHHA located only one other study on the genotoxicity of degraded carrageenan published since 1983. Mori *et al.* (1984) reported that degraded carrageenan did not exhibit genotoxic activity in DNA repair tests employing cultured rat hepatocytes.

Degraded carrageenan and other sulfated polysaccharides, namely amylopectin sulfate and dextran sulfate sodium, all induce colorectal tumors in rats (reviewed in Ishioka *et al.*, 1987). All three compounds induce first ulcerative colitis, secondly irreversible squamous metaplasia, and finally colorectal adenomatous polyps and tumors in rats (reviewed in Oohashi *et al.*, 1981; Ishioka *et al.*, 1987). Degraded carrageenan-induced colorectal squamous cell metaplasia (Ishioka *et al.*, 1987) and colonic epithelial cell proliferation (Wilcox *et al.*, 1992) persist after termination of exposure. The affected colorectal mucosa progressed irreversibly toward colorectal cancer (reviewed in Ishioka *et al.*, 1987). Thus, it appears that metaplasia is likely an important step in polygeenan-induced tumorigenesis. In the cancer studies described above (Tables 2 and 3), high incidences of metaplasia were observed among animals receiving high doses of degraded carrageenan, whereas no metaplasia was observed among animals receiving the lowest dose. The induction of metaplasia following short-term exposure to high doses of degraded carrageenan has been observed in short-term studies of rats fed diets containing 10 percent degraded carrageenan after two or more weeks of dosing (as reviewed in Ishioka *et al.*, 1987).

The mechanism by which polygeenan induces cell proliferation, metaplasia and colorectal tumors is unknown (Wilcox *et al.*, 1992). However, there is evidence to suggest that the gastrointestinal flora play an important role (reviewed in Ishioka *et al.*, 1987). As discussed above, there is also strong evidence to suggest that metaplasia is likely to be an important step in the development of polygeenan-induced cancers. The dose-response curve for polygeenan-induced metaplasia appears to be highly non-linear (that is, metaplasia was present in all animals at the highest concentration of polygeenan and none at a concentration ten-fold lower) (see Table 2). Thus, it might be predicted that the dose-response relationship for polygeenan-induced colorectal tumors likewise would be non-linear. The observed dose-response data for polygeenan-induced tumors is not inconsistent with this, but it is also consistent with a linear relationship. Since the mode of action is unknown, the genotoxicity database is insufficient, insufficient data exist to support dose adjustments based on pharmacokinetic models, and the polygeenan-induced metaplasia is irreversible, deviation from the default approach (i.e., a linearized multistage model and interspecies scaling) is not warranted at this time. However, should data be forthcoming, the approach taken here should be revisited.

DOSE-RESPONSE ASSESSMENT

Cancer potency estimates for polygeenan-induced colorectal tumors were derived using methods described in the Appendix for male and female Sprague-Dawley rats (combined) from the dietary, drinking water and oral gavage studies reported by Wakabayashi *et al.* (1978) and for

male F344 rats from the dietary study reported by Oohashi *et al.* (1981). Additionally, a cancer potency was estimated from a combined dataset comprising the data from the three oral studies reported by Wakabayashi *et al.* (1978). The potency estimates are provided in Table 4. As discussed above, the dose-response relationships from these studies show indications of non-linearity. The Wakabayashi *et al.* dietary study was selected as the basis for the cancer potency estimate for two reasons. First, the study duration was 24 months, the standard lifespan. Secondly, the study utilized three treated dose groups, covering a wide range of dose rates, including the lowest dose rate given in the three experiments. The drinking water study (Table 2) used only one dose level and was of relatively short duration (15 months), and the gavage study, which used two dose levels, had only one responding group and was also of short duration. The Oohashi *et al.* diet study (Table 3) utilized a single high dietary concentration (causing metaplasia in 100 percent of the treated rats) but varied the duration of exposure (2, 6 or 9 months), which introduces uncertainty from the dose estimates calculated from these shorter-term exposures. The Wakabayashi *et al.* dietary study is the most data-rich, has the most power in the low-dose region, and was a full-length study. Thus, this study was selected to model the dose-response relationship.

Table 4. Human Cancer Potency Estimates for Polygeenan-induced Colorectal Tumors

Study	Human Cancer Potency Estimate (mg/kg-d) ⁻¹
Wakabayashi <i>et al.</i> (1978)	
Sprague-Dawley rat -- diet study	6.0 x 10⁻⁴
Sprague-Dawley rat -- drinking water study	4.3 x 10 ⁻³
Sprague-Dawley rat --oral gavage study	1.3 x 10 ⁻³
Oohashi <i>et al.</i> , 1981	
F344 rat – diet study ¹	1.8 x 10 ⁻³

Bolding indicates value selected as the basis of the NSRL.

¹ The cancer potency was corrected for less than lifetime dosing using the Doll-Armitage approach (see footnote 2 of Table 3 and Appendix).

There is some uncertainty about selecting a cancer potency for polygeenan based on low-dose extrapolation. First, humans are potentially exposed at high dose rates. Polygeenan is used to suspend barium sulfate for medical imaging and may result in high, short-term exposures. As discussed above, there is clearly a dose-rate effect for polygeenan-induced metaplasia and likely to be one for cancer. As observed in the Oohashi *et al.* (1981) study, a short (two month) exposure to a high dose (ten percent polygeenan) resulted in 100 percent metaplasia which progressed irreversibly in some rats to cancer (Table 3). Studies in animals given polygeenan and other sulfated polysaccharides observed squamous metaplasia "as early as 2 weeks after the start of administration" (Ishioka *et al.*, 1987). It is unknown if current use of polygeenan in humans induces metaplasia. The number of potential medical procedures for affected individuals was not evaluated here. It is noteworthy that medical conditions precipitating the use of medical imaging may include hyperplastic and metaplastic lesions. It is unknown whether the extent to which exposures to polygeenan through the use of barium sulfate medical imaging may interact or otherwise increase risks to these individuals with pre-existing medical conditions.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5} . The cancer potency estimate of $6.0 \times 10^{-4} \text{ (mg/kg-d)}^{-1}$ was used to calculate the NSRL for polygeenan (1200 $\mu\text{g/day}$).

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR POLYGEENAN

Procedures for the development of Proposition 65 NSRLs are described in regulation (California Code of Regulations, Title 22, Sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRL for polygeenan are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

"Multistage" polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (CDHS, 1985; U.S. EPA, 1996; Anderson *et al.*, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \dots + q_id^i)] \quad (1)$$

with constraints,

$$q_i \geq 0 \text{ for all } i.$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter q_0 represents the background lifetime incidence of the tumor. The parameter q_1 , or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1^* , the upper 95% confidence bound on q_1 (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units of mg/kg-day, the parameters q_1 and q_1^* are given in units of $(\text{mg/kg-day})^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977). To estimate potency in animals (q_{animal}) from experiments of duration T_e , rather than the natural life span of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$q_{\text{animal}} = q_1^* \cdot (T/T_e)^3 \quad (2)$$

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural life span of mice and rats is assumed to be two years, so that for experiments lasting T_e weeks in these rodents:

$$q_{\text{animal}} = q_1^* \cdot (104/T_e)^3 \quad (3)$$

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson *et al.*, 1983) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

Calculation of the lifetime average dose

The lifetime average dose in units of mg/kg-day was calculated for each of the relevant dose groups, based on the dose level, duration and regimen described in the experiments above. When actual body weight information was not provided by the study authors, default values were utilized as described by Gold and Zeiger (1997). In this case, default body weights for male and female rats (0.5 kg and 0.35 kg, respectively), daily food intake for male and female rats (0.02 kg/day and 0.017 kg/day, respectively), and daily water intake for male and female rats (25 ml/day and 20 ml/day) were taken from Gold and Zeiger (1997).

For studies which employ short dosing periods, another technique is to estimate a weighted average dose using an approach based on the Armitage and Doll (1954) model of multistage carcinogenesis, as described by Crouch (1983) and Crump and Howe (1984) (see footnote 2 of Table 3 above). In the application here, exposures early in life are weighted more heavily than exposures later in life, and cumulative cancer incidence is assumed to increase as a power function of age, (i.e., cumulative incidence \propto age^m). A common default in cancer potency and statistical calculations is to assume that cumulative cancer incidence increases with the third power of age (i.e., the instantaneous incidence increases with the second power) (Anderson et al., 1983; Gold and Zeiger, 1997; NTP, 2000). Typical observations of the power function parameter “m” from human and animal epithelial tumors are between three and six (Armitage and Doll, 1954; Portier *et al.*, 1986). Thus, the value of three may, in general, undercorrect the cancer potency risk.

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California risk assessment guidelines (CDHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-third power when animal potency is expressed in units (mg/kg-day)⁻¹:

$$q_{\text{human}} = q_{\text{animal}} \cdot (bw_h / bw_a)^{1/3} \quad (4)$$

A.3 Risk-Specific Intake Level Calculation

The intake level (I, in mg/day) associated with a cancer risk R, from exposure is:

$$I = \frac{R \cdot bw_h}{q_{\text{human}}} \quad (5)$$

where bw_h is the body weight, and q_{human} the theoretical cancer potency estimate for humans.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the no significant risk level for cancer under Proposition 65 (Title 22 California Code of Regulations, Section 12703). Thus for a 70 kg person, the NSRL is given by:

$$\text{NSRL} = \frac{10^{-5} \cdot 70\text{kg}}{q_{\text{human}}} \quad (6)$$

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