

FYI-0204-01286

VINYL ACETATE COUNCIL

1250 Connecticut Avenue, N.W. • Suite 700 • Washington, D.C. 20036  
Phone: 202-419-1500 • Fax: 202-659-8037

RECEIVED  
OPPT/NCIC

04 FEB 24 AM 6:03

February 13, 2004

Document Processing Center  
EPA East (Mail Code 7407M)  
Attn: TSCA Section 8(e)  
U.S. Environmental Protection Agency  
1201 Constitution Avenue, NW  
Washington, DC 20460-0001

CONTAINS NO CBI

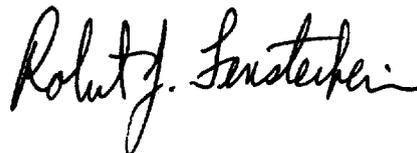
**Re: Supplemental Submission Related to Japan Bioassay Research Center Study; Follow-up Submission to Filing Doc # 99020000132 and FYI-OTS-0297-1286**

Dear TSCA Section 8(e) Coordinator:

On April 23, 2002, the Vinyl Acetate Council, formerly the Vinyl Acetate Toxicology Group, submitted to EPA an incomplete report relating to a two-year cancer bioassay on vinyl acetate conducted by the Japan Bioassay Research Center. That report presented supplemental information to an initial submission that EPA received in 1997 from the Japan Bioassay Research Center itself. As part of our continuing effort of keeping the Agency informed of new developments relating to vinyl acetate, we are hereby providing an advance copy of the results section from a publication on the two-year cancer bioassay that is scheduled to be published in the March 2004 issue of the *Journal of Occupational Health* (<http://joh.med.uoeh-u.ac.jp/>).

Please contact me if I can provide any further clarification.

Sincerely,



Robert J. Fensterheim  
Executive Director

2004 FEB 27 PM 2:36  
RECEIVED  
OPPT/NCIC



cc: Annie Jarabek, EPA



273302

**Excerpt from "In Press" Japan Bioassay Research Center (JBRC) manuscript  
scheduled to be published in the *Journal of Occupational Health***

## **Results**

### *Purity and stability of VA in drinking water*

The VA concentrations in drinking water measured 4 d after the preparation were found to decrease to 72-80% of the initial concentrations for rats and 86-96% for mice. The decrease in the VA concentration was attributed mainly to the loss due to vaporization of VA from the drinking bottle. The acetic acid concentration and pH measured 4 d after the preparation were 9.2 ppm and pH 4.0 for the 400 ppm VA-formulated drinking water, 47 ppm and pH 4.2 for the 2,000 ppm water and 263 ppm and pH 3.6 for the 10,000 ppm water at the end of the 4-day administration period.

### *Mice study*

*Survival, body weight, food and water consumptions, clinical signs:* In the 10,000 ppm group, 6 males and 4 females died of oral cavity tumor, 1 male died of esophagus tumor, 1 male and 2 females died of stomach tumor, and 1 male and 1 female died of larynx tumor. Those tumor deaths started to occur sporadically after a 1-yr elapse of the 2-yr administration period, but no significant difference in the survival rate was found between any VA-administered group of each sex (Table 1) and the respective control. The incidence of the oral cavity nodules (Fig. 2) significantly increased in the 10,000 ppm group of both sexes: three males after the 57th week and five females after the 95th week. Body weights of the 10,000 ppm males and females were significantly lower by 30 and 18% respectively, than those of controls at the end of the 2-yr administration period (Table 1). There was no significant difference in food consumption between any VA-administered group of each sex and the respective control (Table 1). Water consumption was lower in the 10,000 ppm group of each sex than in the respective control, especially in the later period of the 2-yr administration. Daily VA intake per body weight, which was estimated by the bottle concentration of VA and the amount of water consumed, was found to increase proportionally with the increase in the VA concentration in the drinking water except for the 10,000 ppm group (Table 1). The estimated VA intake per body weight was greater in female mice than in male mice at all the VA concentrations.

*Organ weights and macroscopic findings:* Statistically significant changes in the absolute and relative organ weights were observed in several organs of the 10,000 ppm-exposed males and females, but those changes were not associated with histopathological changes.

Mandibular nodules (Fig. 2) were observed in 3 males and 5 females of the 10,000 ppm group, whereas maxillary nodules were observed in 3 males and one female of the 10,000 ppm group.

*Hematology, blood chemistry and urinalysis:* Neither a consistent nor VA treatment-related effect on the parameters of hematology and blood chemistry was observed, although statistically significant changes occurred sporadically in several parameters.

**Excerpt from "In Press" Japan Bioassay Research Center (JBRC) manuscript  
scheduled to be published in the *Journal of Occupational Health***

*Histopathological examinations:* Incidences of squamous cell tumors in the oral cavity and forestomach of male and female mice and in the esophagus of male mice significantly increased in a VA treatment-related manner as indicated by Peto's test (Table 2). The 10,000 ppm VA administration significantly increased the incidence of the squamous cell carcinomas in those organs (Table 2). Besides, the squamous cell carcinomas were observed in the esophagus and larynx of the 10,000 ppm females, in the larynx of the 2,000 ppm females and in the larynx of the 10,000 ppm males. Although the incidences of squamous cell carcinomas in those organs were not statistically significant, they exceeded the range of the historical control data of the Japan Bioassay Research Center (Table 2). Squamous cell papillomas were also observed in the oral cavity and forestomach of both 10,000 ppm males and females and in the esophagus of a 2,000 ppm female.

The squamous cell papilloma was characterized by exophytic projection of the tumor tissue above the stratified squamous epithelium, which was composed of a central core of the tumor connective tissue covered with hyperplastic squamous cells having neither cellular nor structural atypia. In contrast, squamous cell tumors having either cellular or structural atypia were diagnosed as squamous cell carcinoma (Fig. 3). Of those squamous cell carcinomas, 20 oral cavity carcinomas, 4 esophagus carcinomas and all 10 forestomach carcinomas were characterized by endophytic growth in which the tumor cells were proliferated inward into the subepithelial tissue. The endophytic growth type of squamous cell carcinomas, 14 oral cavity, 4 esophagus and 8 forestomach, had invasion of the tumor tissue into the muscle layer that was indicative of severe malignancy. Moreover, 5 male mice were found to bear squamous cell carcinomas in multiple organs. Six oral cavity squamous cell carcinomas and 2 forestomach squamous cell carcinomas were metastasized into the lungs, the pancreas, the liver, the kidneys, and the lymph nodes (Fig. 4).

Pre-neoplastic lesions were observed in the stratified squamous epithelium of the upper digestive tract, and were further classified into basal cell hyperplasia, squamous cell hyperplasia and epithelial dysplasia (Table 3). In the oral cavity, basal cell hyperplasia and the squamous cell hyperplasia occurred not only in the 10,000 ppm but also in the 2,000 ppm groups of both sexes, and the epithelial dysplasia increased markedly in the 10,000 ppm group of both sexes. The 10,000 ppm administration increased those three pre-neoplastic lesions in the esophagus, the forestomach and the larynx. Basal cell hyperplasia was characterized by multiplication of the basal cell layer, often protruding into the subepithelial tissue. Squamous cell hyperplasia was diagnosed as proliferation of the entire cell components of normal squamous epithelium. Squamous epithelium having either cellular or structural atypia was diagnosed as the epithelial dysplasia which was characterized by abnormally differentiated squamous layers and usually accompanied by thickening of the epithelium.

As shown in Table 4, mapping of the neoplastic and pre-neoplastic lesions in the oral cavity of male and female mice administered 10,000 ppm VA revealed that the incidence of those lesions was higher at Level V of the mandibular region than at any other Levels. It was also striking that more than 60% of male mice and 35% of female

**Excerpt from "In Press" Japan Bioassay Research Center (JBRC) manuscript  
scheduled to be published in the *Journal of Occupational Health***

mice had two or more pre-neoplastic lesions at different sites, whereas there was only one male mouse bearing a carcinoma and basal cell hyperplasia at different sites.

One tongue squamous cell papilloma and one carcinoma were observed in the 10,000 ppm female group, but the incidence of those tumors neither significantly increased over the respective control nor exceeded the range of historical control data. Five and two animals bearing squamous cell tumors in the 10,000 group had atrophy of the salivary gland at Level V and Level II, respectively. Two and one animals bearing pre-neoplastic lesions also had salivary gland atrophy in the mandible at Levels IV and V, respectively. Other types of tumors were observed in the spleen, lungs, uterus and nasal cavity of the VA-administered groups, but the incidences of those tumors were not statistically different from those in the respective controls (Table 2).

*Rats Study*

*Survival, body weight, food and water consumptions, clinical signs:* In the 10,000 ppm group, two male rats died of oral cavity tumors in the 97th and 100th wk, but there was no statistical difference in the survival rate at the end of the 2-yr administration period between any VA-administered group of each sex and the respective control (Table 1). As a clinical sign, an oral cavity nodule appeared in a 400 ppm female after the 87th wk. Body weight of the 10,000 ppm males and females measured at the end of the 2-yr administration period were significantly lower by 8 and 10%, respectively, than those of the respective controls (Table 1). No difference in food consumption was found between any VA-administered group of each sex and the respective control (Table 1). Water consumption of the 10,000 ppm males and females decreased by 15 and 18%, respectively, compared to the corresponding controls throughout the experimental period. The estimated daily VA intake per body weight was found to increase with an increase in the VA concentration in the drinking water except for the 10,000 ppm group which exhibited only a 4.0 to 4.5-fold increase in the intake whereas the bottle VA concentration was increased by 5.0-fold. The estimated daily VA intake per body weight was greater in female rats than in male rats at all the VA concentrations. The daily VA intake per body weight of the rats was smaller by 2- to 2.4-fold than that of the mice.

*Organ weights and macroscopic findings:* Statistically significant changes in the absolute and relative organ weights were observed in several organs of the 10,000 ppm-exposed males and females, but those changes were not associated with the histopathological changes. The mandibular nodule also occurred in 3 males in the 10,000 ppm group, and a female in the 400 ppm group. The maxillary nodule appeared in a female in the 10,000 ppm group.

*Hematology, blood chemistry and urinalysis:* Nether a consistent nor VA treatment-related effect on the parameters of hematology and blood chemistry was observed, although statistically significant changes occurred sporadically in several parameters.

**Excerpt from "In Press" Japan Bioassay Research Center (JBRC) manuscript  
scheduled to be published in the *Journal of Occupational Health***

*Histopathological examinations:* Squamous cell carcinomas and squamous cell papillomas were observed in the oral cavity and the esophagus, and their histological characteristics were the same as those observed in the mice. In males, squamous cell carcinomas and papillomas were seen in the oral cavity of five and two 10,000 ppm males, respectively. The incidence of oral cavity squamous cell carcinomas in the 10,000 ppm males was significantly higher than that in the control group, and increased in a VA treatment-related manner as indicated by Peto's test. In females, squamous cell carcinomas were observed in the oral cavity of 3 rats and in the esophagus of 1 rat in the 10,000 ppm group, and the incidence of oral cavity and esophagus squamous cell carcinomas exceeded the range of historical control data of our Institute (Table 5). One oral cavity squamous cell carcinoma was also seen in the 2,000 ppm group and one in the 400 ppm female group. All the squamous cell carcinomas except two has endophytic growth which was characterized by proliferative invasion of the tumor tissue inward into the subepithelial tissue. Of those endophytic carcinomas, 2 oral cavity carcinomas in the 10,000 ppm males and 1 oral cavity and esophagus carcinoma each in the 10,000 ppm females had invasion of the tumor tissues into the muscle layer, indicative of severe malignancy (Fig. 5).

Pre-neoplastic lesions were observed in the stratified squamous epithelium of the upper digestive tract (Table 6). All the pre-neoplastic lesions occurred in the 10,000 ppm group except for the forestomach squamous cell hyperplasia in both a control and a 2,000 ppm male. Mapping of those neoplastic and pre-neoplastic lesions in the rat oral cavity of the 10,000 ppm group of both sexes is shown in Table 7. It was striking that 4 squamous cell carcinomas out of 8 tumors tended to occur at Level VI, although neoplastic and pre-neoplastic lesions were distributed throughout the oral cavity. Focal basal cell hyperplasia was observed at two different sites in the oral cavity of a single animal (Table 7).

The uterine polyp and tumors in females as well as thyroid tumors in both males and females were observed, but the incidences of those tumors were not significantly higher than those in the corresponding controls.

*Dose-response relationships for tumors in mice and rats*

The combined incidence of squamous cell carcinomas and papillomas in the oral cavity of mice and rats of both sexes was plotted against the estimated daily VA intake per body weight but not against the drinking water level of VA, as shown in Figure 6. The tumor incidence increased discernibly above the daily intake of 400 mg VA/kg/d. A BMDL<sub>10</sub> for the combined incidences of squamous cell carcinomas and papillomas in the oral cavity of mice and rats resulted in 477 mg/kg/d, based on multistage model with an AIC value of 232 and a P-value greater than 0.09. The dose-response curve was shown in a multistage model.

**Table 1.** Survival rate, body weight, food and water consumptions and daily VA intake in mice and rats orally administered VA for two years

Dose (ppm)	Survival rate	Body weight at the end of study (g)	Averaged food consumption (g/d)	Averaged water consumption (g/d)	Daily VA intake (mg/kg)
<b>Mice:Male</b>					
Control	35/50	51.4 ± 5.7	4.5	4.8	0
400	42/50	49.7 ± 7.1	4.6	4.8	42
2,000	38/50	50.7 ± 6.7	4.6	4.7	202
10,000	33/50	36.2 ± 7.3**	4.5	4.1	989
<b>Mice:Female</b>					
Control	26/50	35.7 ± 4.7	4.1	5.2	0
400	27/50	36.0 ± 3.4	4.1	5.1	63
2,000	25/50	37.2 ± 5.8	4.2	4.9	301
10,000	23/50	29.3 ± 4.8**	4.2	4.3	1418
<b>Rats:Male</b>					
Control	44/50	453 ± 53	16.7	20.5	0
400	40/50	453 ± 43	17.0	20.8	21
2,000	36/50	459 ± 37	16.5	19.6	98
10,000	39/50	420 ± 33**	16.2	16.9	442
<b>Rats:Female</b>					
Control	41/50	319 ± 38	12.5	18.1	0
400	40/50	319 ± 37	12.5	18.8	31
2,000	41/50	320 ± 35	12.5	17.9	146
10,000	37/50	301 ± 50	11.9	13.7	575

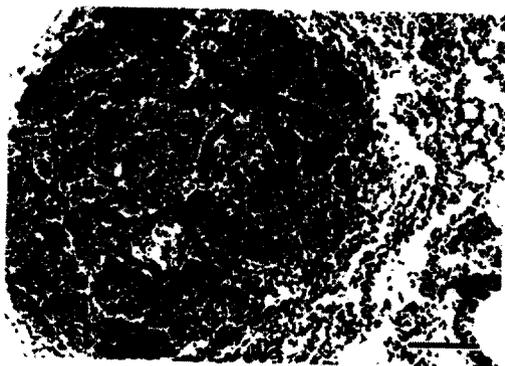
Data on food and water consumptions and VA intake are the mean values averaged over the 2-yr administration period.  
 \*\*: Significantly different at  $p < 0.01$  by Dunnett's test.



**Fig. 2.** Macroscopic finding of a mandibular nodule in a male mouse orally administered 10,000 ppm VA in drinking water for two years.



**Fig. 3.** The endophytic squamous cell carcinoma in the oral cavity of a male mouse administered 10,000 ppm VA. The malignant tumor cells were characterized by structural and cellular atypia, pleomorphism and loss of polarity, and invasion into the muscle layer (M). Bar=25  $\mu$ m.



**Fig. 4.** Metastasis to the lung from squamous cell carcinoma of the oral cavity in a male mouse administered 10,000 ppm VA. Bar=25  $\mu$ m.



**Fig. 5.** The endophytic squamous cell carcinoma in the oral cavity of a male rat administered 10,000 ppm VA. Tumor cells are protruding into the muscle layer (M) by nest of squamous epithelial cells including keratin pearls (arrows). Bar=30  $\mu$ m.

**Table 2.** Incidences of neoplastic lesions in B6D<sub>F</sub> mice orally administered VA in drinking water for two years

Dose (ppm)	Male						Female					
	0	400	2,000	10,000	Peto test	Historical control data	0	400	2,000	10,000	Peto test	Historical control data <sup>c</sup>
Number of mice examined	50	50	50	50			50	50	50	50		
<b>Oral cavity</b>												
Squamous cell papilloma <sup>1)</sup>	0	0	0	4	↑↑	0/996	0	0	0	3	↑↑	1/998
Squamous cell carcinoma <sup>2)</sup>	0	0	0	13**	↑↑	0/996	0	0	0	15**	↑↑	0/998
1)+2)	0	0	0	16***	↑↑		0	0	0	18**	↑↑	
<b>Esophagus</b>												
Squamous cell papilloma	0	0	0	0		0/996	0	0	1	0		1/998
Squamous cell carcinoma	0	0	0	7**	↑↑	0/996	0	0	0	1		0/998
<b>Forestomach</b>												
Squamous cell papilloma <sup>3)</sup>	0	0	0	2		2/996	0	0	0	1		4/998
Squamous cell carcinoma <sup>4)</sup>	1	0	0	7*	↑↑	0/996	0	0	0	3	↑↑	2/998
3)+4)	1	0	0	9**	↑↑		0	0	0	4	↑↑	
<b>Tongue</b>												
Squamous cell papilloma	0	0	0	0		0/996	0	0	0	1		1/998
Squamous cell carcinoma	0	0	0	0		0/996	2	0	0	1		2/998
<b>Larynx</b>												
Squamous cell carcinoma	0	0	0	2		0/996	0	0	1	1		0/998
<b>Liver</b>												
Hepatocellular adenoma	2	6	4	3			3	1	4	0		
Hemangiosarcoma	4	5	5	4			3	3	0	1		
Hepatocellular carcinoma	13	10	9	4*			1	0	0	0		
<b>Spleen</b>												
Malignant lymphoma	1	1	2	0			0	5*	1	1		
Hemangiosarcoma	2	1	4	0			1	1	1	0		
<b>Nasal cavity</b>												
Histiocytosarcoma	0	0	0	0			0	1	0	0		
<b>Lung</b>												
Bronchiolar-alveolar adenoma	3	3	4	3			1	3	1	2		
Bronchiolar-alveolar carcinoma	7	3	5	2			2	3	1	1		
<b>Uterus</b>												
Endometrial stromal polyp	--	--	--	--			0	1	0	0 <sup>b</sup>		
Histiocytosarcoma	--	--	--	--			10	11	8	10 <sup>b</sup>		

a: The value indicates the number of animals bearing both squamous cell papilloma and squamous cell carcinoma. b: Number of mice examined was 48. c: The data were obtained from the 2-yr carcinogenicity and chronic toxicity studies in mice used as a control group, conducted in the Japan Bioassay Research Center from 1987 to 2002. \* and \*\*: Significantly different at  $p < 0.05$  and  $p < 0.01$ , respectively, by Fisher's exact test. ↑ and ↑↑: Significantly different at  $p < 0.05$  and  $p < 0.01$ , respectively, by Peto's test.

**Table 3.** Incidences of pre-neoplastic lesions of digestive tract and larynx in B6D<sub>F</sub> mice orally administered VA in drinking water for two years

Dose (ppm)	Male				Female			
	0	400	2,000	10,000	0	400	2,000	10,000
Number of mice examined	50	50	50	50	50	50	50	50
<b>Oral cavity</b>								
Basal cell hyperplasia	0	0	1	18**	0	0	1	17**
Squamous cell hyperplasia	0	0	2	13**	0	0	1	6*
Epithelial dysplasia	0	0	0	24**	0	0	0	17**
<b>Esophagus</b>								
Basal cell hyperplasia	0	0	0	9**	0	0	0	15**
Squamous cell hyperplasia	0	0	0	2	0	0	0	2
Epithelial dysplasia	0	0	0	2	0	0	0	7**
<b>Forestomach</b>								
Basal cell hyperplasia	0	0	0	1	0	0	0	1
Squamous cell hyperplasia	0	0	0	3	0	2	0	4*
Epithelial dysplasia	0	0	0	1	0	0	0	0
<b>Larynx</b>								
Basal cell hyperplasia	0	0	0	3	0	0	0	6*
Squamous cell hyperplasia	0	0	0	1	0	0	0	0
Epithelial dysplasia	0	0	0	2	0	0	0	3

\* and \*\*: Significantly different at  $p < 0.05$  and  $p < 0.01$ , respectively, by Chi-square test.

**Table 4.** Locations and incidences of neoplastic and pre-neoplastic lesions in the oral cavity of mice orally administered 10,000 ppm VA in drinking water for two years

Level Locations	I	II		III		IV		V	VI	Total*
		Hard palate	Buccal mucosa	Hard palate	Buccal mucosa	Lip mucosa	Gingiva			
<b>Male</b>										
Number of mice examined	50	50	50	50	50	44	44	43	43	
Squamous cell carcinoma			3		2	1		8		13 (1)
Squamous cell papilloma	1							3		4
Epithelial dysplasia		2	3		5	3		19	8	24 (14)
Squamous cell hyperplasia			2		3	4	1	10	5	13 (10)
Basal cell hyperplasia	1		3		1	4	4	18	6	18 (11)
<b>Female</b>										
Number of mice examined	49	49	49	49	49	35	35	35	35	
Squamous cell carcinoma			2			2		11		15
Squamous cell papilloma							1	2		3
Epithelial dysplasia			4		1	3		13	3	17 (7)
Squamous cell hyperplasia								3	3	6
Basal cell hyperplasia		1	6		1	8	1	12		17 (7)

\*: The value indicates the total number of animals bearing the lesions. The parenthesized value indicates number of animals having two or more lesions at different sites. The locations and levels are indicated in Fig. 1.

**Table 5.** Incidences of neoplastic lesions in F344 rats orally administered VA in drinking water for two years

Dose (ppm)	Male						Female					
	0	400	2,000	10,000	Peto test	Historical control data <sup>b</sup>	0	400	2,000	10,000	Peto test	Historical control data <sup>b</sup>
Number of rats examined	50	50	50	50			50	50	50	50		
<b>Oral cavity</b>												
Squamous cell papilloma	0	0	0	2		3/1199	0	0	0	0		1/1147
Squamous cell carcinoma	0	0	0	5*	↑↑	0/1199	0	1	1	3	↑	1/1147
<b>Esophagus</b>												
Squamous cell carcinoma	0	0	0	0		0/1199	0	0	0	1		0/1147
<b>Pituitary</b>												
Adenoma	19	16	14	11			8	11	9	14		
<b>Liver</b>												
Histiocytic sarcoma	0	0	2	0			0	1	0	1		
Hepatocellular carcinoma	0	0	0	1			0	0	0	0		
<b>Uterus</b>												
Endometrial stromal polyp	-	-	-	-			5	5	10	4		
Adenocarcinoma	-	-	-	-			1	0	1	0		
Endometrial stromal sarcoma	-	-	-	-			1	0	1	0		
<b>Thyroid</b>												
C-cell adenoma	7	9*	3*	7*			2	7	8*	5		
C-cell carcinoma	1	2*	2*	0*			0	0	1	2		

a: Number of rats examined was 49. b: The data were obtained from the 2-yr carcinogenicity and chronic toxicity studies in rats used as a control group, conducted in the Japan Bioassay Research Center from 1987 to 2002. \* and \*\*: Significantly different at  $p < 0.05$  and  $p < 0.01$ , respectively, by Fisher's exact test. ↑ and ↑↑: Significantly different at  $p < 0.05$  and  $p < 0.01$ , respectively, by Peto's test.

**Table 6.** Incidences of pre-neoplastic lesions of the upper digestive system in F344 rats orally administered VA in drinking water for two years

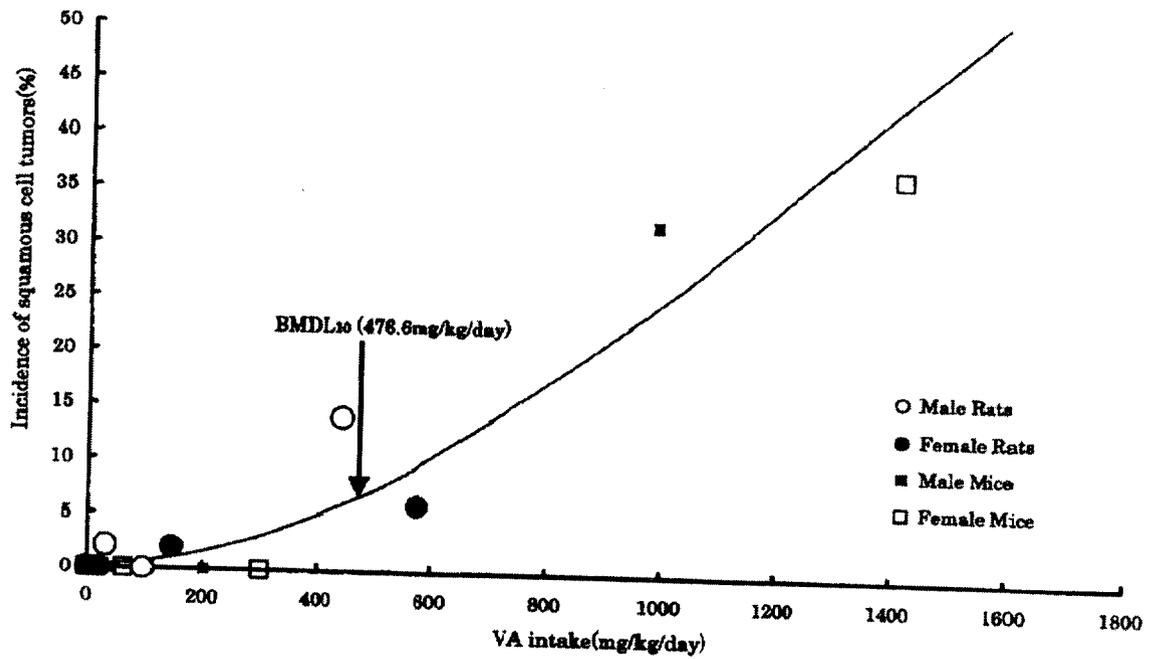
Dose (ppm)	Male				Female			
	0	400	2,000	10,000	0	400	2,000	10,000
Number of rats examined	50	50	50	50	50	50	50	50
Oral cavity								
Basal cell hyperplasia	0	0	0	2	0	0	0	1
Epithelial dysplasia	0	0	0	0	0	0	0	2
Esophagus								
Basal cell hyperplasia	0	0	0	0	0	0	0	4*
Squamous cell hyperplasia	0	0	0	1	0	0	0	1
Stomach								
Basal cell hyperplasia	0	0	0	2	0	0	0	5*
Squamous cell hyperplasia	2	0	1	0	0	0	0	0

\* and \*\*: Significantly different at  $p < 0.05$  and  $p < 0.01$ , respectively, by Chi-square test

**Table 7.** Locations and incidence of neoplastic and pre-neoplastic lesions in the oral cavity of rats orally administered 10,000 ppm VA in drinking water for two years

Level Locations	I	II		III		IV		V	VI	Total*
		Hard palate	Buccal mucosa	Hard palate	Buccal mucosa	Lip mucosa	Gingiva			
Male										
Number of rats examined	50	50	50	50	50	44	44	44	45	
Squamous cell carcinoma		1						1	3	5
Squamous cell papilloma							2			2
Basal cell hyperplasia						2		1		2 (1)
Female										
Number of rats examined	50	50	50	50	50	43	43	43	43	
Squamous cell carcinoma			1	1						3
Epithelial dysplasia							1	1		2
Basal cell hyperplasia			1							1

\*: The value indicates the total number of animals bearing lesions. The parenthesized value indicates number of animals having two or more lesions at different sites. The locations and levels are indicated in Fig. 1.



**Fig. 6.** Dose-response relationship between estimated daily VA intake (mg/kg/d) and incidence of oral cavity squamous cell tumors of mice and rats of both sexes orally administered VA in drinking water for two years. The dose-response curve was fit on Multistage model.