RAPID COMMUNICATION Jpn. J. Cancer Res. 81, 207–212, March 1990

Stomach Carcinogenicity of Caffeic Acid, Sesamol and Catechol in Rats and Mice

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The carcinogenic potential of caffeic acid, sesamol and catechol was examined in male and female F344 rats and B6C3F$_1$ mice, groups of 30 animals being treated with diets containing 2% caffeic acid, 2% sesamol or 0.8% catechol for 104 weeks (rats) or 96 weeks (mice). Histological examination revealed that caffeic acid induced forestomach squamous cell carcinoma in 57% ($P<0.001$ vs. controls) and 50% ($P<0.001$) of male and female rats, respectively, whereas sesamol was associated with squamous cell carcinoma at incidences of 31% ($P<0.001$) in male rats, and 38% ($P<0.001$) and 17% ($P<0.05$) in male and female mice, respectively. Catechol induced glandular stomach adenocarcinomas in 54% ($P<0.001$) and 43% ($P<0.001$) of male and female rats, respectively. The results thus clearly demonstrated that all three antioxidants are carcinogenic in rodent stomach epithelia.

Key words: Carcinogenicity — Antioxidants — Rat — Mouse

Of the many synthetic and naturally occurring antioxidants in our environment, butylated hydroxyanisole has attracted particular attention since it was the first to be shown to possess carcinogenic activity in rat and hamster forestomach epithelium.[1,2] However, more recently, the naturally occurring antioxidants caffeic acid and sesamol have also been shown to induce hyperplasias in rat[3] and hamster[4] forestomach epithelium in short-term experiments, as well as papillomas in this organ of rats in a 60-week experiment.[2] Catechol similarly induced hyperplasia in both forestomach and glandular stomach of hamsters,[4] and strongly enhanced forestomach and glandular stomach carcinogenesis of rats pretreated with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG).[5,6] Furthermore, treatment with catechol alone for 51 weeks induced adenomatous hyperplasia in all rats as well as a low incidence of adenocarcinomas.[5,6] These findings indicate potential carcinogenicity of these antioxidants in long-term experiments. The present report documents the effects of caffeic acid, sesamol and catechol administration to F344 rats and B6C3F$_1$ mice of both sexes for two years.

Groups of 30, 6-week-old F344 rats and B6C3F$_1$ mice of both sexes (Charles River Japan Inc., Kanagawa) were treated with 2% caffeic acid (Tokyo Kasei Kogyo Co., Tokyo, purity >98%), 2% sesamol (Fluka Chemie, AG, Switzerland, purity > 98%) or 0.8% catechol (Wako Pure Chemical Industries, Osaka, purity >99%) in Oriental MF powdered basal diet (Oriental Yeast Co., Tokyo), or basal diet alone for 104 weeks (rats) or 96 weeks (mice). Food and water were given *ad libitum*. Chemicals were incorporated into powdered diet using a mixer and the diets stored at 4°C until use. Animals which died during the experiment were necropsied and all surviving animals were killed under ether anesthesia and subjected to complete autopsy at the end of week 96 for mice and 104 for rats. Livers and kidneys were weighed before fixation in 10% buffered formalin solution. Formalin was injected into the stomach, which was then opened via an incision along the greater curvature. Three sections each were cut from the anterior and posterior walls of the forestomach and six sections from the glandular stomach. Tissues were processed in the usual way for histopathological examination. Animals which survived more than 77 weeks (caffeic acid, rat), 82 weeks (sesamol, rat), 26 weeks (catechol, male rats), 52 weeks (catechol, female rats), 74 weeks (caffeic acid, mice), 30 weeks (sesamol, mice) and 73 weeks (catechol, mice), when the first tumor appeared, were included in the effective numbers. Student's t test and Fisher's exact probability test were used for statistical evaluation of the data.

At the end of the experiment, body weights of animals treated with antioxidants were generally lower than in the controls, particularly for rats and mice of both sexes treated with catechol (17.1–41.1% reduction compared to controls). However, the relative liver and kidney weights were all higher in animals receiving the antioxidants.

Forestomach tumors were mostly observed in rats of both sexes treated with caffeic acid (Fig. 1) and sesamol (Fig. 2), and mice treated with sesamol. Tumors or

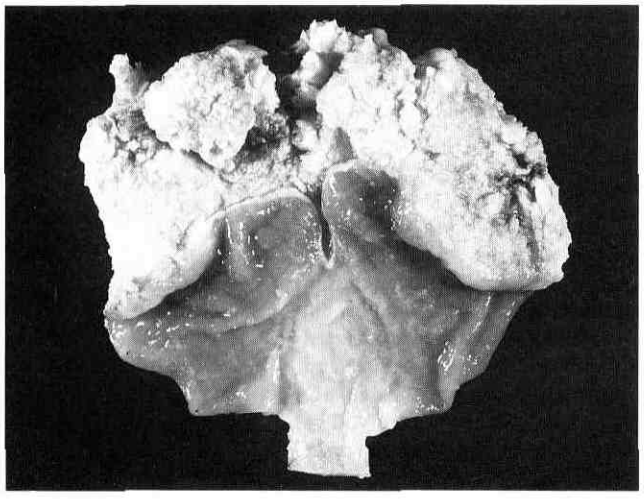

Fig. 1. Stomach of a male F344 rat treated with 2% caffeic acid for 104 weeks. The entire forestomach is occupied by large tumor masses.

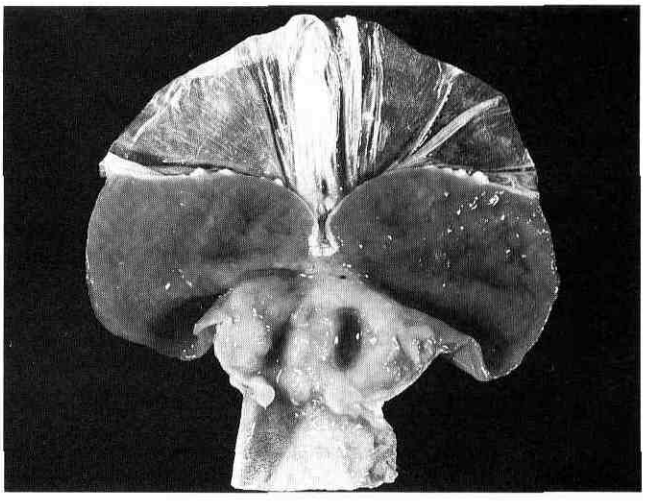

Fig. 3. Stomach of a male F344 rat treated with 0.8% catechol for 104 weeks. The pyloric region of the glandular stomach is thickened irregularly with ulcer formation.

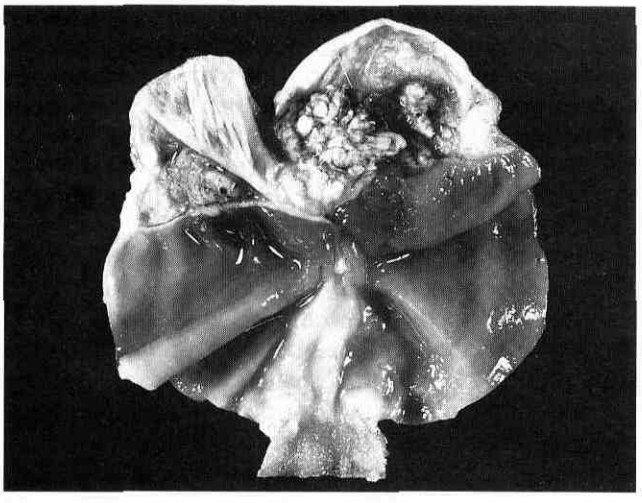

Fig. 2. Stomach of a male F344 rat treated with 2% sesamol for 104 weeks. Papillary or nodular tumors are distributed in the forestomach.

thickening of glandular stomach epithelium were found only in rats and mice of both sexes receiving catechol (Fig. 3).

Histopathologically, changes in the forestomach were classified into hyperplasia, papilloma, squamous cell carcinoma and sarcoma categories as previously reported,[6,7] the results being summarized in Table I. Caffeic acid induced high incidences of hyperplasia in rats and mice of both sexes. However, significant increases in the incidences of papillomas and squamous cell carcinomas were apparent only in male and female rats. Sesamol also induced hyperplasia in most animals but a striking difference between rats and mice was that whereas 34–47% incidences of papillomas were induced in rats no such lesions were found in mice. Significant increase in the development of squamous cell carcinoma was, however, observed in male rats ($P<0.001$), male mice ($P<0.001$) and female mice ($P<0.05$). Squamous cell carcinomas induced in rats and mice by these antioxidants were mostly of well to moderately differentiated type (Fig. 4) except for two of poorly differentiated type in male rats and also in mice given sesamol (Fig. 5). Although catechol also induced hyperplasia in both rats and mice, no significant increase in tumor incidence was found in either species. Hyperplasias induced in rats and mice by caffeic acid and catechol were mainly characterized by upward cell growth, basal cell proliferation being minimal. However, lesions induced in rats by sesamol were characterized by downward basal cell growth, while hyperplasias associated with sesamol administration to mice also showed downward growth, but the proliferating cells were not of basal but rather of spinous type. One sarcoma was found in a female mouse treated with sesamol.

Lesions of the glandular stomach were classified into submucosal growth, adenomatous hyperplasia, adenocarcinoma and sarcoma categories as previously reported,[6,7] the results being summarized in Table II. Catechol induced submucosal growth in most animals,

Table I. Histopathological Changes Observed in the Forestomach

Chemical	Species	Sex	Effective number	No. of animals with			
				Hyperplasia	Papilloma	Squamous cell carcinoma	Sarcoma
Caffeic acid	Rat	M	30	30 (100)***	23 (77)***	17 (57)***	0
		F	30	30 (100)***	24 (80)***	15 (50)***	0
	Mouse	M	30	27 (77)***	4 (13)	3 (10)	0
		F	29	29 (100)***	0	1 (3)	0
Sesamol	Rat	M	29	29 (100)***	10 (34)***	9 (31)***	0
		F	30	30 (100)***	14 (47)***	3 (10)	0
	Mouse	M	29	29 (100)***	0	11 (38)***	0
		F	30	28 (93)***	0	5 (17)*	1 (3)
Catechol	Rat	M	28	24 (86)***	2 (7)	0	0
		F	28	23 (82)***	0	0	0
	Mouse	M	30	16 (53)***	1 (3)	0	0
		F	29	25 (86)***	1 (3)	0	0
Basal diet	Rat	M	30	1 (3)	0	0	0
		F	30	5 (17)	0	0	0
	Mouse	M	27	1 (4)	0	0	0
		F	29	3 (10)	0	0	0

Significantly different from respective basal diet values at *** $P<0.001$ or * $P<0.05$.

Table II. Histopathological Changes Observed in the Glandular Stomach

Chemical	Species	Sex	Effective number	No. of animals with			
				Submucosal growth	Adenomatous hyperplasia	Adenocarcinoma	Sarcoma
Caffeic acid	Rat	M	30	0	1 (3)	0	0
		F	30	0	0	0	0
	Mouse	M	30	0	0	0	0
		F	29	0	0	0	0
Sesamol	Rat	M	29	0	1 (3)	0	0
		F	30	1 (3)	0	0	0
	Mouse	M	29	0	0	0	1 (3)
		F	30	5 (17)*	0	0	0
Catechol	Rat	M	28	28 (100)***	28 (100)***	15 (54)***	0
		F	28	28 (100)***	28 (100)***	12 (43)***	0
	Mouse	M	30	30 (100)***	29 (97)***	0	0
		F	29	26 (90)***	21 (72)***	0	0
Basal diet	Rat	M	30	0	0	0	0
		F	30	0	0	0	0
	Mouse	M	27	0	0	0	0
		F	29	0	0	0	0

Significantly different from respective basal diet values at *** $P<0.001$ or * $P<0.05$.

the incidences of adenomatous hyperplasias being 100% in male and female rats, but slightly lower in mice. Well differentiated adenocarcinomas (Fig. 6) were induced in 54 and 43% of male and female rats, respectively, but were not found in mice. One sarcoma was found in a male mouse treated with sesamol, and a significant incidence of submucosal growth in female mice was also associated with this chemical. No lesions were observed in the fundic region of the glandular stomach of rats and mice treated with these antioxidants.

The present results thus clearly demonstrated that caffeic acid is carcinogenic for rat forestomach epithe-

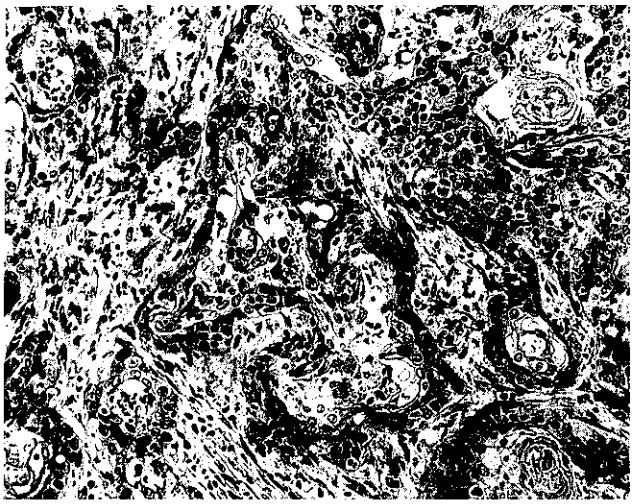

Fig. 4. Moderately differentiated squamous cell carcinoma in the Fig. 1 rat. Cancer cell nests invading into the submucosa show differentiation towards stratified squamous epithelium. Cornification is not prominent.

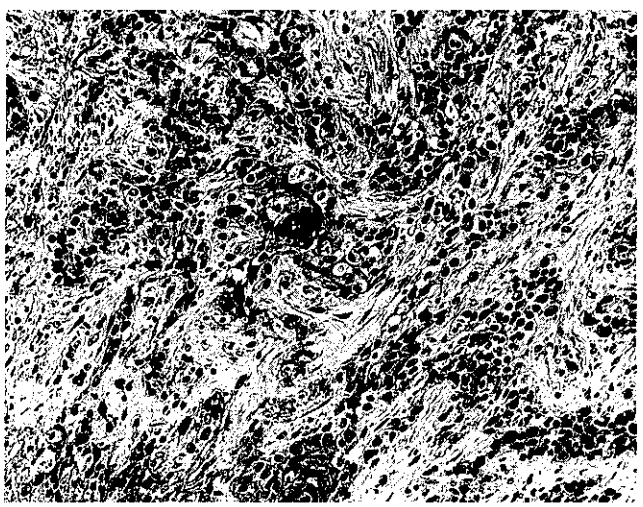

Fig. 5. Poorly differentiated squamous cell carcinoma in the Fig. 2 rat. Cancer cell nests are less differentiated than in Fig. 4, and lack cornification.

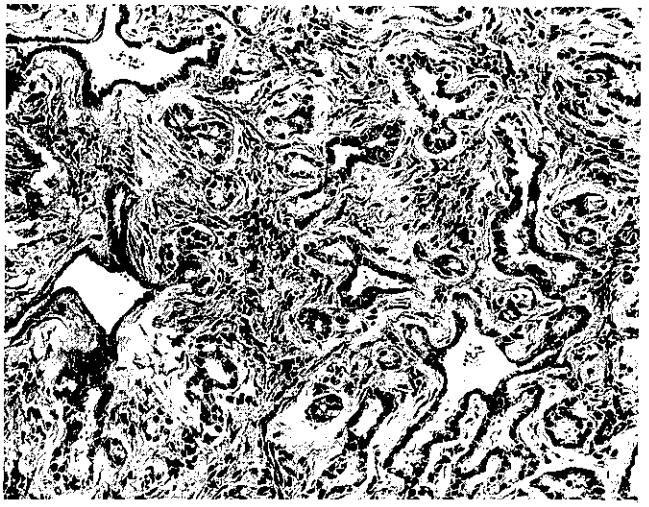

Fig. 6. Well differentiated glandular stomach adenocarcinoma in the Fig. 3 rat. Surface epithelium-like irregular glands are observed proliferating into the submucosa.

lium in both sexes, and that sesamol, though slightly less potent, can induce forestomach carcinomas in male rats, as well as male and female mice. Furthermore, catechol was confirmed to be carcinogenic for rat glandular stomach epithelium. With regard to mutagenicity, catechol is negative in the Ames test[8,9] and caffeic acid, in fact, inhibits mutagenicity induced by chemical carcinogens.[10,11] However, catechol has been reported to possess comutagenic activity with benzo[*a*]pyrene (B(a)P) in an *in vitro* transformation assay.[12] Genotoxicity of caffeic acid and catechol has also been described by some investigators.[8,10,13,14] *In vivo,* however, caffeic acid inhibits B(a)P-induced forestomach carcinogenesis,[15] and mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA)[16] but promotes rat forestomach carcinogenesis induced by 7,12-dimethylbenz-[*a*]anthracene (DMBA).[17] Catechol exerts cocarcinogenic activity in skin and esophageal carcinogenesis,[18,19] and promotes tongue, esophagus, forestomach and glandular stomach carcinogenesis of rats pretreated with methyl-N-amylnitrosamine[20] or MNNG.[6]

These carcinogenic antioxidants are of particular importance since they are commonly present in our environment. For example, caffeic acid is a major phenolic compound in potatoes (0.028%), lettuce (up to 0.09%), apples (up to 0.13%), coffee beans (up to 0.14%), soybeans (0.002%) and cereals.[10,21] Sesamol is a minor component of sesame seed oil, being found at levels of 0.004 to 0.05% depending on the processing method. Catechol, a synthetic as well as naturally occurring antioxidant, is found in some plants, roast coffee, wood smoke, cigarette smoke, hair dyes and film developers.[22] While average intake is not known, human do ingest these antioxidants through foodstuffs. Although the carcinogenic activity of caffeic acid appears to be stronger than that of BHA and human exposure to caffeic acid may be much higher, considering its wider

distribution in our environment, the relevance of a forestomach carcinogen to man is questionable, mainly because humans do not possess this organ. On the other hand, the demonstrated carcinogenicity of catechol in glandular stomach is of unequivocal importance because the rat gastric epithelium is morphologically and functionally similar to that in man. The presence of catechol in the enviroment is reflected in the fact that catechol and its conjugates are actually excreted in human urine at levels up to 30 mg/24 h.[23] Although the carcinogenic dose of catechol in rats is much higher than the supposed human intake of this carcinogen, promotion of glandular stomach carcinogenesis by catechol has been found for a concentration of only 0.2% in the diet (unpublished data). Recently a related antioxidant, *p*-methylcatechol, was also shown to have strong promotion activity for glandular stomach carcinogenesis in rats pretreated with MNNG, and could itself induce glandular stomach tumors.[24] In this context, the finding that mixtures of low-dose promoters can strongly enhance carcinogenesis even if individually they have no effect, is of great interest.[25] Further work on the risk assessment of these chemicals in humans is urgently required.

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and from the Ministry of Health and Welfare, by a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, Japan, and a grant from the Experimental Pathological Research Association, Japan.

(Received November 10, 1989/Accepted January 22, 1990)

REFERENCES

1) Ito, N. and Hirose, M. The role of antioxidants in chemical carcinogenesis. *Jpn. J. Cancer Res.*, **78**, 1011–1026 (1987).
2) Ito, N. and Hirose, M. Antioxidants — carcinogenic and chemopreventive properties. *Adv. Cancer Res.*, **53**, 247–302 (1989).
3) Hirose, M., Masuda, A., Imaida, K., Kagawa, M., Tsuda, H. and Ito, N. Induction of forestomach lesions in rats by oral administrations of naturally occurring antioxidants for 4 weeks. *Jpn. J. Cancer Res.*, **78**, 317–321 (1987).
4) Hirose, M., Inoue, T., Asamoto, M., Tagawa, Y. and Ito, N. Comparison of the effects of 13 phenolic compounds in induction of proliferative lesions of the forestomach and increase in the labelling indices of the glandular stomach and urinary bladder epithelium of Syrian golden hamsters. *Carcinogenesis*, **7**, 1285–1289 (1986).
5) Hirose, M., Kurata, Y., Tsuda, H., Fukushima, S. and Ito, N. Catechol strongly enhances rat stomach carcinogenesis: a possible new environmental stomach carcinogen. *Jpn. J. Cancer Res.*, **78**, 1144–1149 (1987).
6) Hirose, M., Fukushima, S., Kurata, Y., Tsuda, H., Tatematsu, M. and Ito, N. Modification of N-methyl-N′-nitro-N-nitrosoguanidine-induced forestomach and glandular stomach carcinogenesis by phenolic antioxidants in rats. *Cancer Res.*, **48**, 5310–5315 (1988).
7) Hirose, M., Kagawa, M., Ogawa, K., Yamamoto, A. and Ito, N. Antagonistic effect of diethylmaleate on the promotion of forestomach carcinogenesis by butylated hydroxyanisole (BHA) in rats pretreated with N-methyl-N′-nitro-N-nitrosoguanidine *Carcinogenesis*, **10**, 2223–2226 (1989).
8) Brandt, K. Final report on the safety assessment of hydroquinone and pyrocatechol. *J. Am. Coll. Toxicol.*, **5**, 123–165 (1986).
9) Haworth, S., Lawlor, T., Mortelmas, K., Spech, W. and Seiger, E. *Salmonella* mutagenicity test results for 250 chemicals. *Eviron. Mutag. (Suppl. 1)*, 3–42 (1983).
10) Stich, H. F. and Rosin, M. P. Naturally occurring phenolics as antimutagenic and anticarcinogenic agents. *Adv. Exp. Biol.*, **177**, 1–29 (1984).
11) San, R. H. C. and Chan, R. I. M. Inhibitory effect of phenolic compounds on aflatoxin B_1 metabolism and induced mutagenesis. *Mutat. Res.*, **177**, 229–239 (1987).
12) Atchison, H., Chu, C-S., Kakunaga, T. and Van Durren, B. L. Chemical cocarcinogenesis with the use of a subclone derived from BALB/3T3 cells with catechol as cocarcinogen. *J. Natl. Cancer Inst.*, **69**, 503–508 (1982).
13) Stich, H. F., Rosin, M. P., Wu, C. H. and Powrie, W. D. The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. *Cancer Lett.* **14**, 251–260 (1981).
14) Yamada, K., Shirahata, S., Murakami, H., Nishiyama, K., Shinohara, K. and Omura, H. DNA breakage by phenyl compounds. *Agric. Biol. Chem.*, **49**, 1423–1428 (1985).
15) Wattenberg, L. W., Coccia, J. B. and Lam, L. K. Inhibitory effects of phenolic compounds on benzo[*a*]-pyrene-induced neoplasia. *Cancer Res.*, **40**, 2820–2823 (1980).
16) Huang, M. T., Smart, R. C., Wong, C. Q. and Conney, A. H. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol-13-acetate. *Cancer Res.*, **48**, 5941–5946 (1988).
17) Hirose, M., Masuda, A., Fukushima, S. and Ito, N. Effects of subsequent antioxidant treatment on 7,12-dimethylbenz[*a*]anthracene-initiated carcinogenesis of the mammary gland, ear duct and forestomach in Sprague-Dawley rats. *Carcinogenesis*, **9**, 101–104 (1988).
18) Mirvish, S. S., Salmasi, S., Lawson, T. A., Pour, P. and Sutherland, D. Test of catechol, tannic acid, bidens pilosa,

croton oil, and phorbol for cocarcinogenesis of esophageal tumors induced in rats by methyl-*N*-amylnitrosamine. *J. Natl. Cancer Inst.*, **74**, 1283–1290 (1985).
19) Van Duuren, B. L. and Goldschmidt, B. M. Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J. Natl. Cancer Inst.*, **56**, 1237–1242 (1976).
20) Yamaguchi, S., Hirose, M., Fukushima, S., Hasegawa, R. and Ito, N. Modification by catechol and resorcinol of upper digestive tract carcinogenesis in rats treated with methyl-*N*-amylnitrosamine. *Cancer Res.*, **49**, 6015–6018 (1989).
21) Pratt, D. E. and Birac, P. M. Source of antioxidant activity of soybeans and soy products. *J. Food Sci.*, **44**, 1720–1722 (1979).
22) IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 15. "Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals," pp. 155–175 (1977), IARC, Lyon.
23) Carmella, S. G., LaVoie, E. J. and Hecht, S. S. Quantitative analysis of catechol and 4-methylcatechol in human urine. *Food Chem. Toxicol.*, **20**, 587–590 (1988).
24) Hirose, M., Yamaguchi, S., Hasegawa, R., Takahashi, S. and Ito, N. Promotion by dihydroxybenzene derivatives of N-methyl-N′-nitro-N-nitrosoguanidine-induced F344 rat forestomach and glandular stomach carcinogenesis. *Cancer Res.*, **49**, 5143–5147 (1989).
25) Yamada, M., Hirose, M., Takahashi, S., Mutai, M., Shirai, T. and Ito, N. Effects of combined low dose phenolic antioxidant administration on forestomach carcinogenesis in rats pretreated with MNNG. *Proc. Jpn. Cancer Assoc., 48th Annu. Meet.*, 74 (1989).