Dose Dependence of 1-*O*-Hexyl-2,3,5-trimethylhydroquinone Promotion of Forestomach Carcinogenesis in Rats Pretreated with *N*-Ethylnitrosourethane

Yasumoto Mizoguchi,[1] Masao Hirose,[1, 4] Tsuyoshi Yamaguchi,[1] Plemjit Boonyaphiphat,[1, 3] Tokutaro Miki[2] and Tomoyuki Shirai[1]

[1]*First Department of Pathology, Nagoya City University, Medical School, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601 and* [2]*Research and Development Division, Nippon Hypox Laboratories, Inc., 9420 Nanbu, Nanbu-cho, Minamikoma-gun, Yamanashi 409-2212*

Post-initiation dose-dependent effects of the chemopreventive antioxidant 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), a potent inhibitor of heterocyclic amine-induced mutagenesis and carcinogenesis, on the development of forestomach and tongue tumors were investigated in male F344 rats. Groups of 22 rats were treated with 0.01% ethylnitrosourethane (ENUR) as an initiator in the drinking water for 4 weeks, then placed on diet containing 1.0%, 0.5%, 0.25% or 0.125% HTHQ, or basal diet alone for 36 weeks. Further groups of 12 rats each were similarly treated with the different doses of HTHQ or given basal diet alone for 36 weeks without prior ENUR treatment. All animals were killed at week 40. Tongue papillary hyperplasia and papillomas tended to be increased in the groups treated with ENUR followed by 0.5–0.125% HTHQ, though there was no effect at the highest dose, in line with increased bromodeoxyuridine labeling indices. In the forestomach, the incidences of papillomas and carcinomas were also significantly elevated only in the group treated with ENUR followed by 0.125% HTHQ. Without ENUR pretreatment, papillary hyperplasia was found in the 1–0.125% HTHQ groups and the labeling index was also increased, though without clear dose dependence. The results indicate that HTHQ may have very weak or weak promotion potential for tongue and forestomach carcinogenesis, but that both minimum and maximum thresholds for active dose levels may exist.

Key words: Forestomach carcinogenesis — Antioxidant — Dose-dependence — Rat

1-*O*-Hexyl-2,3,5-trimethylhydroquinone (HTHQ) is a potent lipophilic phenolic antioxidant, which exhibits much stronger anti-lipid peroxidation activity than butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) or α-tocopherol in rat liver microsomes.[1] It also has strong antimutagenic activity against eight major carcinogenic heterocyclic amines (HCAs) in the Ames assay in the presence of an S9 mixture.[2] In *in vivo* animal experiments, it potently reduced both the multiplicity and the area of 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1)- or 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)-induced liver glutathione *S*-transferase placental form-positive foci in our *in vivo* medium term liver bioassay system,[3] indicating that HTHQ inhibits MeIQx- and Glu-P-1-induced hepatocarcinogenesis. HTHQ also inhibits 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary carcinogenesis when given together with PhIP,[4] and 7,12-dimethylbenz(*a*)anthracene (DMBA)-initiated mammary carcinogenesis in the post-initiation stage in female Sprague-Dawley rats.[5] Therefore, HTHQ has come to be regarded as a powerful cancer chemopreventive agent, particularly against HCAs. On the other hand, it has recently been shown to slightly enhance forestomach carcinogenesis and to induce equivocal tongue carcinogenesis in a medium term multi-organ carcinogenesis model.[6] It also weakly increases *N*-ethylnitrosourethane (ENUR)-initiated forestomach and tongue carcinogenesis when given at a dose of 1% (unpublished results). The present experiment was designed to evaluate the possibility that HTHQ might promote the development of forestomach and tongue tumors, and, if it does, to establish whether the effect is dose-dependent. For this purpose, ENUR, which is carcinogenic for the tongue, esophagus, forestomach and duodenum,[7, 8] was employed as an initiator.

MATERIALS AND METHODS

Animals and chemicals Five-week-old male F344 rats were obtained from Charles River Japan Inc., Atsugi. They were randomly housed at 3–4 animals per cage with hard wood chips as bedding in an air-conditioned room at

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22±2°C and 55±5% humidity with a 12 h light and dark cycle. ENUR was purchased from the Nard Institute, Osaka and bromodeoxyuridine (BrdU) from Sigma Chemical Co., St. Louis, MO. HTHQ was synthesized at Dainippon Ink Co., Tokyo. Food (Oriental MF basal diet, Oriental Yeast Co., Tokyo) and tap water were available ad libitum throughout the experiment.

Treatment At the age of 6 weeks, groups of 22 rats were treated with 0.01% ENUR in the drinking water for 4 weeks, then placed on powdered diet containing 1.0%, 0.5%, 0.25% or 0.125% HTHQ, or basal diet alone for 36 weeks. Further groups of 12 rats each were similarly treated with the different doses of HTHQ or given basal diet alone for 36 weeks without prior ENUR treatment. Body weights and food consumption were recorded once every 4 weeks. One rat given ENUR followed by 1.0% HTHQ became moribund and was killed and autopsied before the end of experiment. All surviving rats were killed under ether anesthesia at the end of week 40. Five animals each in the groups not receiving ENUR pretreatment were given a single i.p. injection of 100 mg/kg b.w. BrdU 1 h before being killed. The liver, kidneys, esophagus, stomach and intestines of each animal were excised, then the liver and kidneys were weighed and fixed in 10% buffered formalin solution. The esophagus, intestines and stomach were inflated with formalin and later opened via an incision along the greater curvature. One longitudinal section from the tongue, six from the forestomach, including tumors, and 2 from the esophagus and grossly abnormal lesions were made and routinely stained with HE. Tissues from the animals injected with BrdU were immunohistochemically stained with anti-BrdU antibody. For the analysis of BrdU labeling indices, 100 basal cells each from 6 slides (total 600 basal cells) in the mid region of the forestomach, and 1000 basal cells adjacent to the orifices of the salivary ducts in the squamous epithelium (where tongue tumors preferentially develop) in the tongue were counted, and the data were expressed as numbers of labeled cells per 100 cells. Fisher's exact test and Student's t test were used for statistical analyses.

RESULTS

The final body weights were significantly decreased in the groups given the 1% HTHQ diet with or without the ENUR pretreatment. On the other hand, the relative liver weights were increased in the groups fed 1–0.25% HTHQ with ENUR and in those fed 1–0.5% HTHQ without ENUR pretreatment, and the relative kidney weights were increased in those fed 1–0.25% HTHQ with ENUR and in those fed 1% HTHQ without ENUR pretreatment (Table I). Quantitative data for histopathological findings in the tongue are summarized in Table II. In the groups pretreated with ENUR, hyperplasias were observed in 48–76% of rats, with no significant inter-group differences. A slight increase in the incidence of papillomas was noted in the 0.5% HTHQ group and a carcinoma was found in a rat treated with 0.125% HTHQ. In the groups not given ENUR, no hyperplasias or neoplastic lesions were found in any groups, but the BrdU labeling indices were significantly increased in all groups given HTHQ and the greatest increase was found at the 0.5% dose.

Macroscopic data for multiplicity of forestomach tumors, which are divided into sizes of 2–3 mm and those larger than 3 mm in diameter, are shown in Table III. The total number of tumors was greatest at a dose of 0.25%. Multiplicity of tumors of 2–3 mm decreased in proportion to dose, while those larger than 3 mm increased in inverse proportion to dose up to 0.25%. Histopathological findings in the forestomach are summarized in Table IV. Hyperplasias were found in all rats treated with ENUR. The incidences of both papillomas and carcinomas were

Table I. Final Body and Relative Organ Weights

Treatment	No. of rats	Body weight (g)	Relative organ weights (g/100 g b.w.)	
			Liver	Kidney
ENUR→HTHQ (1%)	20	387±21***	3.29±0.15***	0.65±0.03***
ENUR→HTHQ (0.5%)	22	421±25	2.99±0.10***	0.58±0.03**
ENUR→HTHQ (0.25%)	22	425±33	2.83±0.17**	0.58±0.04*
ENUR→HTHQ (0.125%)	22	429±40	2.72±0.11	0.54±0.03
ENUR→Basal diet	22	431±21	2.69±0.12	0.55±0.04
HTHQ (1%)	12	394±21**	3.13±0.07***	0.62±0.04***
HTHQ (0.5%)	12	431±19	2.98±0.11***	0.57±0.03
HTHQ (0.25%)	12	432±22	2.79±0.12	0.56±0.04
HTHQ (0.125%)	12	439±30	2.71±0.09	0.54±0.03
Basal diet	12	425±29	2.73±0.08	0.56±0.03

* $P<0.05$, ** $P<0.01$ and *** $P<0.001$ vs. respective Basal diet group values.

Table II. Incidences of Tongue Lesions and BrdU Labeling Indices

Treatment	No. of rats	Hyperplasia (%)				Papilloma (%)	Carcinoma (%)	Labeling index[a] (%)
		Total	Simple	Papillary	Atypical			
ENUR→HTHQ (1%)	21	16 (76)	15 (71)	0	1 (5)	0	0	—
ENUR→HTHQ (0.5%)	22	11 (50)	8 (36)	3 (14)	1 (5)	3 (14)	0	—
ENUR→HTHQ (0.25%)	22	13 (59)	11 (50)	1 (5)	1 (5)	1 (5)	0	—
ENUR→HTHQ (0.125%)	21	16 (76)	16 (76)	2 (10)	1 (5)	0	1 (5)	—
ENUR→Basal diet	21	10 (48)	10 (48)	0	0	0	0	—
HTHQ (1%)	12	0	0	0	0	0	0	21.8±2.0***
HTHQ (0.5%)	12	0	0	0	0	0	0	30.9±3.2***
HTHQ (0.25%)	12	0	0	0	0	0	0	18.2±2.1***
HTHQ (0.125%)	12	0	0	0	0	0	0	16.6±1.6***
Basal diet	12	0	0	0	0	0	0	10.6±1.6

a) Average±SD for 5 rats.
*** $P<0.001$ vs. Basal diet group value.

Table III. Multiplicity of Macroscopic Forestomach Tumors in Rats Pretreated with ENUR

Treatment	No. of rats	Total No. of tumors ≥2 mm	Size of tumors (diameter)	
			2–3 mm (No./rat)	>3 mm (No./rat)
ENUR→HTHQ (1.0%)	21	40	1.52±1.50**	0.38±0.74
ENUR→HTHQ (0.5%)	22	45	1.41±1.76*	0.64±0.73
ENUR→HTHQ (0.25%)	22	55	1.32±1.46**	0.73±0.98
ENUR→HTHQ (0.125%)	22	27	0.59±0.73	0.64±1.09
ENUR→Basal diet	22	21	0.32±0.65	0.64±0.79

* $P<0.05$ and ** $P<0.01$ vs. Basal diet group value.

Table IV. Incidences of Forestomach Lesions and BrdU Labeling Indices

Treatment	No. of rats	Hyperplasia (%)			Papilloma (%)	Carcinoma (%)	Labeling index[a] (%)
		Total	Simple	Papillary			
ENUR→HTHQ (1%)	21	21 (100)	21 (100)	21 (100)	15 (68)	4 (19)	—
ENUR→HTHQ (0.5%)	22	22 (100)	22 (100)	22 (100)	16 (73)	6 (27)	—
ENUR→HTHQ (0.25%)	22	22 (100)	22 (100)	22 (100)	21 (95)**	8 (36)*	—
ENUR→HTHQ (0.125%)	22	22 (100)	22 (100)	22 (100)	18 (82)	5 (23)	—
ENUR→Basal diet	22	22 (100)	22 (100)	22 (100)	13 (59)	2 (9)	—
HTHQ (1%)	12	12 (100)	12 (100)	3 (25)	0	0	27.5±9.5*
HTHQ (0.5%)	12	12 (100)	12 (100)	4 (33)	0	0	35.5±11.5**
HTHQ (0.25%)	12	12 (100)	12 (100)	4 (33)	0	0	18.6±3.8***
HTHQ (0.125%)	12	12 (100)	12 (100)	0	0	0	17.4±8.5
Basal diet	12	0	0	0	0	0	7.8±2.5

a) Average±SD for 5 rats.
* $P<0.05$, ** $P<0.01$ and *** $P<0.001$ vs. respective Basal diet group values.

significantly increased only in the 0.25% HTHQ group. In the non ENUR-initiated groups, simple hyperplasia was found in all rats given HTHQ, and papillary hyperplasia, which is a precursor lesion of papilloma, was found in 33–35% of the 1–0.125% HTHQ groups, although the incidences were not significant. BrdU labeling was significantly increased in the 1–0.25% HTHQ groups, the value being greatest at the 0.5% dose level. In the esophagus,

simple hyperplasia was found in all rats treated with ENUR. In addition, papillary hyperplasia was found in one rat treated with 0.125% HTHQ and two rats maintained on basal diet. Papilloma was found in only one rat treated with ENUR followed by 0.25% HTHQ. Erosion, ulceration or inflammation was not evident in any of the treatment groups. In the duodenum, one rat in each of the groups treated with ENUR followed by 1% HTHQ and 0.25% HTHQ demonstrated adenocarcinomas.

DISCUSSION

Previously, we showed that HTHQ at a dose of 1% weakly enhanced forestomach carcinogenesis and exerted equivocal effects on tongue carcinogenesis in a medium-term multi-organ model.[6] Another synthetic phenolic antioxidant, BHA, a known forestomach carcinogen, promotes development of forestomach squamous cell carcinomas in rats pretreated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) when given at 1%,[9] whereas catechol at 0.8% and *tert*-butylhydroquinone (TBHQ) at 1% only affected papillomas in multi-organ[10, 11] models. In line with the present study, the same dose of HTHQ also increased the incidence and numbers of tongue papillary or nodular (PN) hyperplasias ($P<0.001$), and forestomach PN hyperplasias ($P<0.01$) in rats after initiation with ENUR (unpublished results). In the present examination of the dose-dependence of HTHQ promotion, BrdU labeling of the tongue epithelium was increased in all the HTHQ-treated groups, but no significant effects on the incidences of any lesions were found. Therefore, from the results of the present and previous experiments, it can be concluded that if HTHQ possesses promotion activity, it is very weak. On the other hand, an unequivocal enhancing influence on forestomach carcinogenesis was confirmed in the present experiment.

It is of interest that the strongest promotion activity was seen at the dose of 0.25%, and the greatest BrdU labeling index at 0.5% in the forestomach. In the tongue, the labeling index was also greatest at the 0.5% level. In forestomach carcinogenesis by phenolic compounds, toxic high dose levels are more effective for the induction of cell proliferation and promotion of carcinogenesis, but the present experiment indicates that there may be some limitation in this regard. Non-genotoxic phenolic forestomach carcinogens such as BHA, caffeic acid, sesamol and 4-methoxyphenol induce both direct hyperplasia and strong secondary regenerative hyperplasia due to inflammation, erosion or ulceration.[12, 13] A toxic dose of BHA (2% in diet) has been shown to strongly enhance rat forestomach carcinogenesis.[11] Non-carcinogens that are promoters for the forestomach, such as catechol and TBHQ, induce only weak hyperplasia[10, 14] which may be due to lack of inflammation, erosion or ulceration. Induction of fore-

stomach hyperplasias by HTHQ appears to be as weak as was observed with catechol and TBHQ, and HTHQ showed no toxicity, such as inflammation, erosion or ulceration, at any dose level in the present experiment. The reason for the lower level of DNA synthesis with non-toxic 1% HTHQ and the lower levels of tumor incidence and multiplicity with 1 and 0.5% HTHQ is not clear. Recently, free radicals produced during prostaglandin H synthase-mediated metabolism have been regarded as an important factor in the cell proliferation and carcinogenesis caused by the phenolic antioxidant BHA.[15, 16] Reactive oxygen species generation has also been demonstrated during oxidation of hydroquinone.[17, 18] Although this is speculative, at quite high dose, excess amounts of free HTHQ may exert a self-protective action, namely free HTHQ could trap free radicals or electrophilic metabolites produced in the forestomach epithelium which are responsible for the generation of hyperplasia. Alternatively, it is possible that high dose levels of HTHQ could cause phenomena such as metabolic switching due to the saturation or exhaustion of the normal metabolic pathways or the induction of enzymes, which can alter the normal biotransformation processes, without apparent toxicity in the forestomach epithelium. The present results also indicate that more than two dose levels should be used to assess the modifying effects of chemicals on carcinogenesis.

We have earlier recommended HTHQ as an antioxidant with chemopreventive activity, particularly against HCA-induced carcinogenesis. The major synthetic antioxidants used in foods or cosmetics are now well established to possess promotion activity, i.e., BHA for the forestomach and urinary bladder,[9, 19, 20] BHT for the esophagus, thyroid glands and urinary bladder,[20–22] and TBHQ for the esophagus, forestomach and urinary bladder.[10, 23] The only exception appears to be propyl gallate.[10] The promoting activity of HTHQ on forestomach carcinogenesis is much weaker than that of BHA and the promoting action on tongue carcinogenesis is very weak, if present. HTHQ has the highest anti-mutagenic activity against Glu-P-1-induced mutagenesis[2] and chemopreventive effect against MeIQx-induced hepatocarcinogenesis among HTHQ, BHA, BHT, TBHQ, propyl gallate and α-tocopherol (unpublished results). In addition HTHQ inhibits colon[6] and mammary carcinogenesis[5] when given in the post-initiation stage in multi-organ and DMBA models, respectively. Thus, HTHQ still deserves consideration as a candidate chemopreventive agent suitable for human use.

REFERENCES

1) Nihro, Y., Furukawa, H., Sogawa, S., Wang, T. C., Miyataka, H., Natsumoto, H., Miki, T. and Satoh, T. Synthesis and anti lipid-peroxidation activity of hydroquinone monoalkyl ethers. *Chem. Pharm. Bull.*, **42**, 576–579 (1994).

2) Hirose, M., Iwata, S., Ito, E., Nihro, Y., Takahashi, S., Mizoguchi, Y., Miki, T., Satoh, T., Ito, N. and Shirai, T. Strong anti-mutagenic activity of the novel lipophilic antioxidant 1-*O*-hexyl-2,3,5-trimethylhydroquinone against heterocyclic amine-induced mutagenesis in the Ames assay and its effect on metabolic activation of 2-amino-6-methyl-dipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1). *Carcinogenesis*, **16**, 2227–2232 (1995).

3) Hirose, M., Hasegawa, R., Kimura, J., Akagi, K., Yoshida, Y., Tanaka, H., Miki, T., Satoh, T., Wakabayashi, K., Ito, N. and Shirai, T. Inhibitory effects of 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), green tea catechins and other antioxidants on 2-amino-6-methyldipyrido[1,2 *a*:3′,2′-*d*]imidazole (Glu-P-1)-induced rat hepatocarcinogenesis and dose dependent inhibition by HTHQ of lesion induction by Glu-P-1 or 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx). *Carcinogenesis*, **16**, 3049–3055 (1995).

4) Hirose, M., Akagi, K., Hasegawa, R., Yaono, M., Satoh, T., Hara, Y., Wakabayashi, K. and Ito, N. Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) induced mammary gland carcinogenesis by antioxidants in F344 female rats. *Carcinogenesis*, **16**, 217–221 (1995).

5) Futakuchi, M., Hirose, M., Miki, T., Tanaka, H., Ozaki, M. and Shirai, T. Inhibition of DMBA-initiated rat mammary tumor development by the novel synthetic ascorbic acid derivatives, synthetic phenolic antioxidant 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ) and phenylethyl isothiocyanate. *Eur. J. Cancer Prev.*, **7** (1998), in press.

6) Ogawa, K., Hirose, M., Futakuchi, M., Boonyaphiphat, P., Mizoguchi, Y., Miki, T. and Shirai, T. Stage and organ dependent effects of 1-*O*-hexyl-2,3,5-trimethylhydroquinone, ascorbic acid derivatives and n-heptadecane-8,10-dione, and phenylethyl isothiocyanate in a rat multi-organ carcinogenesis model. *Int. J. Cancer*, **73** (1998), in press.

7) Hirose, M., Maekawa, A., Kamiya, S. and Odashima, S. Carcinogenic effect of N-ethyl- and N-amylnitrosourethanes on female Donryu rats. *Gann*, **70**, 653–662 (1979).

8) Maekawa, A., Onodera, H., Matsushima, Y., Nagaoka, T., Todate, A., Shibutani, M., Kodama, Y. and Hayashi, Y. Dose-response carcinogenicity in rats on low-dose levels of N-ethyl-N-nitrosourethane. *Jpn. J. Cancer Res.*, **80**, 632–636 (1989).

9) Hirose, M., Kagawa, M., Ogawa, K., Yamamoto, A. and Ito, N. Antagonistic effect of diethylmaleate on the promotion of forestomach carcinogenesis by butylated hydroxy-anisole (BHA) in rats pretreated with N-methyl-N′-nitro-N-nitrosoguanidine. *Carcinogenesis*, **10**, 2223–2226 (1989).

10) Hirose, M., Yada, H., Hakoi, K., Takahashi, S. and Ito, N. Modification of carcinogenesis by α-tocopherol, *t*-butylhydroquinone, propyl gallate and butylated hydroxytoluene in a rat multi-organ carcinogenesis model. *Carcinogenesis*, **14**, 2359–2364 (1993).

11) Hagiwara, A., Tanaka, H., Imaida, K., Tamano, S., Fukushima, S. and Ito, N. Correlation between medium-term multi-organ carcinogenesis bioassay data and long-term observation results in rats. *Jpn. J. Cancer Res.*, **84**, 237–245 (1993).

12) Ito, N., Hirose, M. and Takahashi, S. Cell proliferation and forestomach carcinogenesis. *Environ. Health Perspect.*, **101** (Suppl. 5), 107–110 (1993).

13) Hirose, M., Takahashi, S. and Shirai, T. Characteristics of forestomach carcinogenesis by non-genotoxic phenolic compounds. *J. Toxicol. Pathol.*, **8**, 277–284 (1995).

14) 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).

15) Schilderman, P. A. E. L., van Maanen, J. M. S., Smeets, E. J., ten Hoor, F. and Kleinjans, J. C. S. Oxygen radical formation during prostaglandin H synthase-mediated biotransformation of butylated hydroxyanisole. *Carcinogenesis*, **14**, 347–353 (1993).

16) Schilderman, P. A. E. L., van Maanen, J. M. S., ten Vaarwerk, F. J., Lafleur M. V. M., Westmijze, E. J., ten Hoor, F. and Kleinjans, J. C. S. The role of prostaglandin H synthase-mediated metabolism in the induction of oxidative DNA damage by BHA metabolites. *Carcinogenesis*, **14**, 1297–1302 (1993).

17) Li, Y. and Trush, M. A. DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation. *Carcinogenesis*, **14**, 1303–1311 (1993).

18) Hiraku, Y. and Kawanishi, S. Oxidative DNA damage and apoptosis induced by benzene metabolites. *Cancer Res.*, **56**, 5172–5178 (1996).

19) Williams, G. M. Epigenetic promoting effects of butylated hydroxyanisole. *Food Chem. Toxicol.*, **24**, 1163–1166 (1986).

20) Imaida, K., Fukushima, S., Shirai, T., Ohtani, M., Nakanishi, K. and Ito, N. Promoting activities of butylated hydroxyanisole and butylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis*, **4**, 895–899 (1983).

21) Fukushima, S., Sakata, T., Tagawa, Y., Shibata, M. A.,

Hirose, M. and Ito, N. Different modifying response of butylated hydroxyanisole, butylated hydroxytoluene, and other antioxidants in N,N-dibutylnitrosamine esophagus and forestomach carcinogenesis of rats. *Cancer Res.*, **47**, 2113–2116 (1987).

22) Imaida, K., Fukushima, S., Shirai, T., Masui, T., Ogiso, T. and Ito, N. Promoting activities of butylated hydroxyanisole, butylated hydroxytoluene and sodium L-ascorbate on forestomach and urinary bladder carcinogenesis initiated with methylnitrosourea in F344 male rats. *Gann*, **75**, 769–775 (1984).

23) Tamano, S., Fukushima, S., Shirai, T., Hirose, M. and Ito, N. Modification by α-tocopherol, propyl gallate and tertiary butylhydroquinone of urinary bladder carcinogenesis in Fischer F344 rats pretreated with N-butyl-N-(4-hydroxybutyl)nitrosamine. *Cancer Lett.*, **35**, 39–46 (1987).