Jpn. J. Cancer Res. 83, 949–954, September 1992

Enhancing Effects of Harman and Norharman on Induction of Preneoplastic and Neoplastic Kidney Lesions in Rats Initiated with N-Ethyl-N-hydroxyethylnitrosamine

Akihiro Hagiwara,[1] Masashi Sano, Emiko Asakawa, Hikaru Tanaka, Ryohei Hasegawa and Nobuyuki Ito

First Department of Pathology, Nagoya City University Medical School, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467

The modifying potential of two nephrotoxic agents, harman and norharman, on N-ethyl-N-hydroxyethylnitrosamine (EHEN)-induced renal and hepatic carcinogenesis was investigated in male F344/DuCrj rats. Animals were given 0.1% EHEN in their drinking water for the first 2 weeks as an initiator. Subsequently, starting 3 weeks from the commencement, they were fed diet containing these compounds at concentrations of 1000, 500 or 0 ppm until week 26, and then killed for light microscopic examination. The mean numbers of renal tubular cell hyperplasias/cm^2 and those of tumors/cm^2 in rats given harman and norharman at 1000 ppm after initiation, but not at 500 ppm, were significantly increased as compared to the control values. However, neither compound modified liver carcinogenesis. It is concluded that harman and norharman show enhancing effects on rat kidney carcinogenesis, when ingested at dose levels which cause renal tubular damage.

Key words: Harman — Norharman — Renal tumor promotion — N-Ethyl-N-hydroxyethylnitrosamine — F344 rat

Harman and norharman, β-carboline derivatives, are present in some foodstuffs[1, 2] and in pyrolysis products of tobacco[3] and meat.[4, 5] It is reported that both chemicals, while lacking mutagenicity themselves, possess co-mutagenic potential in several *in vitro* assays.[6–13] No carcinogenicity was evident in an *in vivo* long-term assay,[14] and no liver tumor promotion[15] or skin tumor promotion[12] was observed. However, harman and norharman exerted renal tubular toxicity with obvious dose-dependence in our earlier oral administration studies.[16, 17] This prompted us to investigate the renal tumor-promoting effects of both chemicals, since there is ample evidence in the literature indicating a close relationship between renal tubular damage and enhancement of renal tumor development.[18–27]

The present report documents the positive influence of harman or norharman on renal carcinogenesis assayed using an EHEN initiation-promotion protocol in male F344 rats.

MATERIALS AND METHODS

Chemicals Harman (1-methyl-9*H*-pyrido[3,4-*b*]indole) and norharman (9*H*-pyrido[3,4-*b*]indole) were manufactured by Aldrich Chemical Company, Inc., Milwaukee, WI, and Sigma Chemical Company, St. Louis, MO, respectively. N-Ethyl-N-hydroxyethylnitrosamine (EHEN[2]) was obtained from Sakai Research Laboratories, Fukui.

Animals and maintenance A total of 90 male F344/DuCrj rats were purchased from Chales River Japan, Inc., Kanagawa. The rats were about 6 weeks old at the commencement of the experiment and were housed five to a plastic cage, with hardwood chips for bedding. The room temperature was kept at 22±2°C and relative humidity was 55±10% with a 12-h light/dark cycle. A positive air pressure was maintained with more than 15 air changes/hour.

Experimental Procedure Animals were randomized into five groups of 15 rats each and 3 groups of 5 rats each with equal group mean body weights and treated as shown in Fig. 1. Rats in groups 1–4 were given 0.1% EHEN in their drinking water for 2 weeks and then tap water until the termination. Starting one week from the end of the EHEN treatment, rats were fed on powdered diet (Oriental MF, Oriental Yeast Co., Tokyo) containing harman or norharman at 1000 or 500 ppm for up to 26 weeks. These doses were chosen based on the results of previous studies.[14–17] Animals in group 5 were given basal diet subsequent to carcinogen exposure. Rats of groups 6–8 were given 1000 ppm harman, 1000 ppm norharman, or basal diet without prior EHEN administration. Individual body weights were recorded weekly for the first 6 weeks and then biweekly. Food and water

[1] To whom correspondence should be addressed.

[2] Abbreviations: EHEN, N-ethyl-N-hydroxyethylnitrosamine; BrdU, 5-bromo-2′-deoxyuridine; ALP, alkaline phosphatase; GOT, glutamic oxaloacetic transaminase, GPT, glutamic pyruvic transaminase; GGT, γ-glutamyl transpeptidase; BUN, blood urea nitrogen.

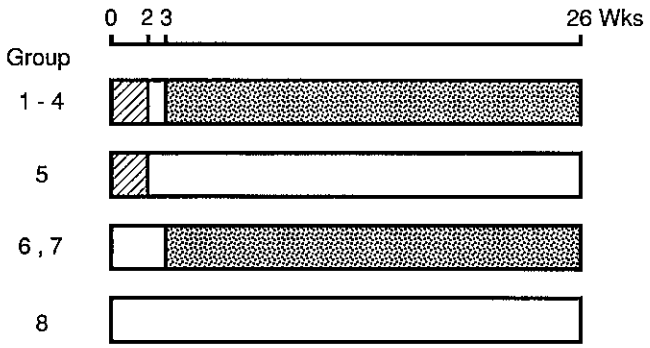

Fig. 1. Experimental design. Animals in groups 1–5 were initially given 0.1% EHEN (▨) for 2 weeks. From week 3, they were fed basal diet containing harman and norharman (□) at concentrations of 1000 and 500 ppm or basal diet (▩). Groups 6–8 were given 1000 ppm harman or norharman or basal diet without EHEN initiation. All surviving rats were killed at week 26.

consumption levels were measured over the 2-day period before each weighing. All survivors were killed under ether anesthesia, blood samples being taken from the abdominal aorta, at the end of week 26.

Blood biochemistry Blood biochemistry assessment was performed for all rats at termination using sera obtained from the aforementioned blood samples. The parameters examined were alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGT), blood urea nitrogen (BUN) and creatinine. These analyses were performed at the Chunichi Clinic Center, Ohgaki, Japan.

Histopathology and BrdU immunohistochemistry The final body weight, kidneys and liver weights were measured for all surviving animals, and organ-to-body weight ratios were determined. The kidneys, liver, and small intestine were quickly immersed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned, and then processed for routine hematoxylin and eosin staining. Five rats in each group (except group 6) were given an i.p. injection of 5-bromo-2′-deoxyuridine (BrdU; Sigma Chemical Co.) at a dose of 100 mg/kg 1 h before sacrifice. The numbers of labeled nuclei per 1000 renal tubular or hepatocyte nuclei were counted in sections stained immunohistochemically for BrdU.[28]

Statistical analysis Comparisons of quantitative data in this study were made by using the two-sided Student's *t* test. Insufficient homogeneity of variance was corrected with respect to the degree of freedom according to Welch.

RESULTS

Antemortem examination Marked retardation of body weight increase was found in rats exposed to EHEN (groups 1–5) when compared to rats maintained on tap water (groups 6–8) during the 2-week initiation period. In the promotion period, severe body weight retardation was observed in rats fed harman or norharman at 1000 ppm from week 4 to the termination, when compared to the corresponding controls. Body weights in rats fed 500 ppm harman and norharman were significantly lower from week 10 or 12 to the termination. Six out of 15 rats in group 1 were found dead during weeks 22 to 26, and 5/5 rats in group 6 (both groups being fed 1000 ppm harman) also died during weeks 18–22. No deaths were observed in any of the other groups. Water intake in the 1000 ppm harman or norharman groups was extremely high, and that of the 500 ppm harman group, but not the 500 ppm norharman group, was increased slightly. There was no adverse effect on food consumption in any group. The average test chemical intakes calculated from nomi-

Table I. Blood Biochemistry Data (mean±SD)

Group	Treatment (ppm)	No. of rats	BUN (mg/dl)	Creatinine (mg/dl)
1	EHEN; harman (1000)	9[a]	154.3±64.2**	3.30±1.71**
2	EHEN; harman (500)	15	21.9±3.7**	0.61±0.09**
3	EHEN; norharman (1000)	15	128.1±50.4**	2.73±1.00**
4	EHEN; norharman (500)	15	18.9±2.0**	0.53±0.06**
5	EHEN	15	16.7±1.4	0.43±0.06
6	harman (1000)	0[b]	—	—
7	norharman (1000)	5	223.0±99.7**	4.52±1.65**
8		5	17.4±0.5	0.44±0.05

a), *b*) Six and five rats respectively, died of severe toxic nephrosis.
** Significantly different from the corresponding control value, $P<0.01$.

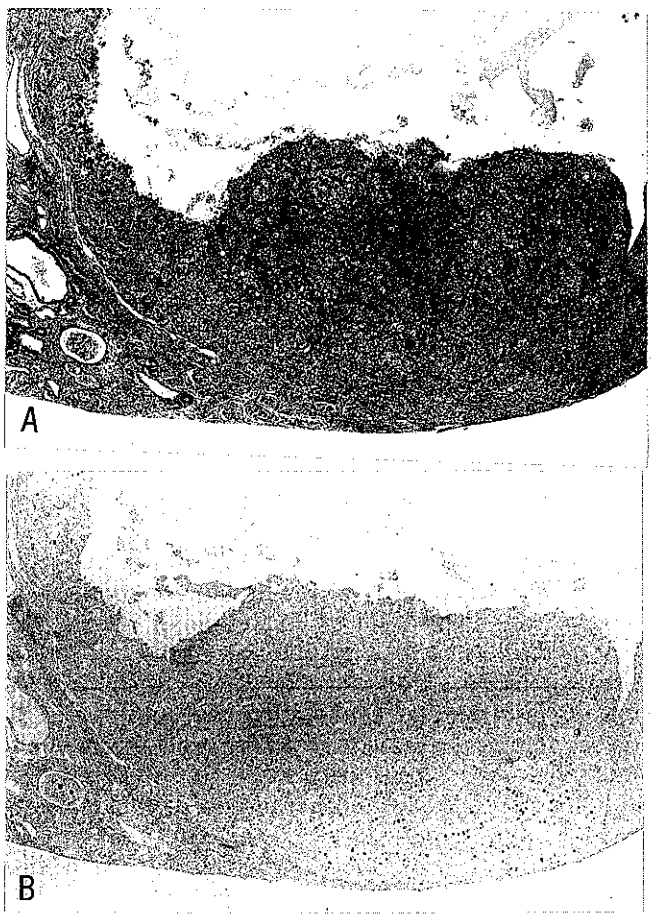

Fig. 2. (A) A renal cell carcinoma with cyst formation in a rat given EHEN + 1000 ppm norharman. H & E, ×40. (B) Specific BrdU-positive cell nuclei are evident in a semi-serial section to preparation (A). Immunohistochemical BrdU staining, ×40.

nal dietary levels, the mean food consumption, and the mean body weights for groups 1–4, 6 and 7 were 67, 27, 63, 26, 101 and 68 mg/kg/day, respectively.

Blood biochemistry analysis In the blood biochemistry analyses, marked elevations of BUN and creatinine were noted in rats exposed to 1000 ppm harman or norharman. Slight, but statistically significant, increases in both parameters were also observed in rats fed 500 ppm harman or norharman (Table I). Although ALP levels in groups 1, 3 and 7 were significantly higher than the corresponding control values, no test chemical-related changes were found for GOT, GPT or GGT levels (data not shown).

Gross findings and organ weights On macroscopic observation, the kidneys in rats given 1000 ppm harman and norharman were small and demonstrated roughened surfaces. Some of them bore white solid or cystic nodules. Kidney-to-body weight ratios in groups 1, 2, 3 and 7 were significantly decreased as compared to corresponding control values. The liver-to-body weight ratio in group 7 was significantly higher than that in group 8. However, no adverse effects were found in groups 1–4.

Kidney histopathology Proliferative renal tubular cell lesions were classified into hyperplasia, adenoma and adenocarcinoma (Fig. 2) categories as described previously.[23, 27] There were no intergroup differences in incidences of preneoplastic or neoplastic renal lesions. However, the average number/cm^2 of renal tubular cell hyperplasias and adenomas were markedly increased in rats given 1000 ppm harman. Density (mean no./cm^2) of hyperplasias was also significantly higher in rats fed 1000 ppm norharman (Table II). However, no equivalent significant increase was evident in rats exposed to either of the compounds at a dietary concentration of 500 ppm. Severe toxic renal degenerative/necrotic and regenerative tubular changes were obvious in rats exposed 1000 ppm harman or norharman, whereas these were only mild-to-moderate with 500 ppm harman, and not apparent in the 500 ppm norharman case. Under the light microscope BrdU immunohistochemistry of the kidneys and liver was evaluated for each group, and values of LI were determined (data not shown). The values for incorporation in renal tubular cells of rats fed 1000 ppm harman or norharman were significantly higher than those of controls, in line with findings for chronic nephrosis.

Liver histopathology Multiple altered cell foci developed in rats treated with EHEN (groups 1–5), along with hyperplastic (neoplastic) nodules and hepatocellular carcinomas. While the incidences and numbers of tumors/tumor-bearing animals in the 1000 ppm harman or norharman cases tended to be low, no statistically significant effect was evident (data not shown).

DISCUSSION

The results of the present investigation clearly show that harman and norharman both exert promoting effects on renal carcinogenesis initiated by EHEN in male F344 rats when administered at the 1000 ppm level (highly toxic). While renal tubular tumors arising from the collecting tubules (oncocytoma) were not found in the current study, marked toxic renal lesions were observed in proximal, distal and collecting tubules. This suggests that promoting activity for the collecting tubules might also be demonstrated, if initiators of renal collecting tubule tumors, such as N-nitrosomorpholine,[29] were used.

Although rats given 500 ppm harman or norharman for 23 weeks did show very slight toxic renal changes

Table II. Quantitative Data for Renal Tubular Lesions in Male F344 Rats Initiated with EHEN and Receiving Harman or Norharman for up to 26 Weeks

Group[a]	Treatment (ppm)	No. of rats	Hyperplasia		Adenoma		Carcinoma
			Cases (%)	Density[b]	Cases (%)	Density	Cases (%)
1	EHEN; harman (1000)	9	9 (100)	6.70±3.78**	9 (100)	1.93±0.95**	2 (22)
2	EHEN; harman (500)	15	7 (47)	1.11±0.65	8 (53)	0.65±0.27	1 (7)
3	EHEN; norharman (1000)	15	12 (80)	1.51±1.10*	12 (80)	0.86±0.49	6 (40)
4	EHEN; norharman (500)	15	10 (67)	1.25±0.87	10 (67)	0.43±0.15	3 (20)
5	EHEN	15	11 (73)	0.73±0.83	8 (53)	0.65±0.36	2 (13)

a) Data for groups 6–8 were excluded from this table, since no proliferative renal tubular lesions were observed.
b) No. of lesions/cm^2. Data shown are mean±SD values.
*, ** Significantly different from the corresponding control value (group 5), $P<0.05$ or 0.01, respectively.

under the present conditions, no enhancing effects on renal carcinogenicity were evident. It was similarly found that the renal tumor promoters potassium bromate[21] and trisodium nitrilotriacetate monohydrate[20] possess threshold levels, which are closely related to their renal toxicity. Furthermore, this concept of a threshold level for tumor promoters is generally recognized to exist for other organs, for example, 12-O-tetradecanoylphorbol-13-acetate in mouse skin carcinogenesis,[30] phenobarbital and DDT in rat liver carcinogenesis,[31–33] saccharin in rat urinary bladder carcinogenesis,[34] and phenobarbital in rat thyroid carcinogenesis.[35]

Norharman has been reported to act as a co-mutagen, but is not itself a mutagen.[7–10] In our previous long-term toxicity study, rats fed 500 ppm (mild renal toxic dose in the present study) norharman for 2 years did not develop renal cell tumors.[14] The available evidence thus indicates that norharman is a complete renal tubular toxic and renal tumor-promoting agent, but not a carcinogen. The mechanisms underlying this type of renal tumor promotion by non-genotoxic chemicals are considered to be related to the persistent tubular damage and subsequent cellular regeneration (increasing level of replicative DNA synthesis).[36–42] However, it was reported that some established renal toxic agents lack promoting activity for renal carcinogenesis initiated by EHEN in rats.[18, 22] Further investigations are therefore required to elucidate the precise mechanisms of renal tumor-promoting activity.

Harman and norharman are nontoxic for the liver and lacked modifying potential for hepatocarcinogenesis in our previous study.[15] In the current investigation, neither of the chemicals tested acted as a promoter for the liver carcinogenesis initiated by EHEN in male F344 rats. In fact harman and norharman both tended to reduce the incidence of liver tumors when given at the 1000 ppm level (severe toxic dose). However this effect was not statistically significant and might be related to body weight retardation.

We conclude from the current investigation that harman and norharman do exert renal tumor-promoting activity at dose levels which cause renal tubular damage. However, the actual renal carcinogenic hazard of these agents to humans may be negligible, because our intake levels are estimated to be very low.[1–5]

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare of Japan, and by a grant from the Society for Promotion of Pathology of Nagoya.

(Received April 3, 1992/Accepted June 9, 1992)

REFERENCES

1) Takase, S. and Murakami, H. Studies on the fluorescence of sake. I. Fluorescence spectrum of sake and identification of harman. *Agric. Biol. Chem.*, **30**, 869–876 (1966).
2) Takeuchi, T., Ogawa, K., Iinuma, H., Suda, H., Ukita, K., Nagatsu, T., Kato, M., Umezawa, H. and Tanabe, O. Monoamine oxidase inhibitors isolated from fermented broths. *J. Antibiot.*, **26**, 162–167 (1973).
3) Poindexter, E. H., Jr. and Carpenter, R. D. The isolation of harman and norharman from tobacco and cigarette smoke. *Phytochemistry*, **1**, 215–221 (1962).
4) Sugimura, T., Sato, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M. and Kawachi, T. Overlapping of carcinogens and mutagens. *In* "Fundamentals in Cancer Prevention," ed. P. N. Magee, S. Takayama,

T. Sugimura, and T. Matsushima, pp. 191–215 (1976). University of Tokyo Press, Tokyo.
5) Gross, G. A. Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. *Carcinogenesis,* **11**, 1597–1603 (1990).
6) Levitt, R. C., Legraverand, C., Nebert, D. W. and Pelkonen, O. Effects of harman and norharman on the mutagenicity and binding to DNA of benzo[a]pyrene metabolites *in vitro* and on aryl hydrocarbon hydroxylase induction in cell culture. *Biochem. Biophys. Res. Commun.,* **79**, 1167–1175 (1977).
7) Nagao, M., Yahagi, T., Honda, M., Seino, Y., Kawachi, T. and Sugimura, T. Comutagenic actions of norharman derivatives with 4-dimethylaminoazobenzene and related compounds. *Cancer Lett.,* **3**, 339–346 (1977).
8) Nagao, M., Yahagi, T., Honda, M., Seino, Y., Matsushima, T. and Sugimura, T. Demonstration of mutagenicity of aniline and *o*-toluidine by norharman. *Proc. Jpn. Acad.,* **53**, Ser. B, 34–37 (1977).
9) Nagao, M., Yahagi, T., Kawachi, T., Sugimura, T., Kosuge, T., Tsuji, K., Wakabayashi, K., Mizusaki, S. and Matsumoto, T. Comutagenic action of norharman and harman. *Proc. Jpn. Acad.,* **53**, 95–98 (1977).
10) Umezawa, K., Shirai, A., Matsushima, T. and Sugimura, T. Comutagenic effect of norharman and harman with 2-acetylaminofluorene derivatives. *Proc. Natl. Acad. Sci. USA,* **75**, 928–930 (1978).
11) Mita, S., Kamataki, T. and Kato, R. Effect of norharman on DNA strand breaks and mutation of Chinese hamster V79 cells by chemical carcinogens. *Carcinogenesis,* **5**, 715–718 (1984).
12) Zajdela, F., Perin-Roussel, O. and Saguem, S. Marked differences between mutagenicity in *Salmonella* and tumour-initiating activities of dibenzo[a,e]fluoranthene proximate metabolites; initiation inhibiting activity of norharman. *Carcinogenesis,* **8**, 461–464 (1987).
13) Lecoq, S., Perin, F., Plessis, M. J., Strapelias, H. and Duquesne, M. Comparison of *in vitro* metabolisms and mutagenicities of dibenzo[a,c]anthracene, dibenzo[a,h]-anthracene and dibenzo[a,j]anthracene: influence of norharman. *Carcinogenesis,* **10**, 461–469 (1989).
14) Hagiwara, A., Arai, M., Hirose, M., Nakanowatari, J., Tsuda, H. and Ito, N. Chronic effects of norharman in rats treated with aniline. *Toxicol. Lett.,* **6**, 71–75 (1980).
15) Hagiwara, A., Tanaka, H., Kurata, Y., Kato, T., Tsuda, H. and Ito, N. Lack of hepatotoxicity or promotion of enzyme-altered liver foci development in rats treated with harman or norharman. *J. Toxicol. Environ. Health,* **29**, 211–218 (1990).
16) Murasaki, G., Miyata, Y., Shirai, T., Arai, M., Kawachi, T. and Ito, N. Effects of the co-mutagen norharman in rats. *Toxicol. Lett.,* **3**, 157–161 (1979).
17) Hagiwara, A., Asakawa, E., Kurata, Y., Sano, M., Hirose, M. and Ito, N. Dose-dependent renal tubular toxicity of harman and norharman in male F344 rats. *Toxicol. Pathol.,* in press.
18) Shirai, T., Ohshima, M., Masuda, A., Tamano, S. and Ito, N. Promotion of 2-(ethylnitrosamine)ethanol-induced renal carcinogenesis in rats by nephrotoxic compounds: positive responses with folic acid, basic lead acetate, and N-(3,5-dichlorophenyl)succinimide but not with 2,3-dibromo-1-propanol phosphate. *J. Natl. Cancer Inst.,* **72**, 477–482 (1984).
19) Diwan, B. A., Rice, J. M., Ohshima, M., Ward, J. M. and Dove, L. F. Comparative tumor-promoting activities of phenobarbital, amobarbital, barbital sodium, and barbituric acid on liver and other organs of male F344/NCr rats following initiation with N-nitrosodiethylamine. *J. Natl. Cancer Inst.,* **74**, 509–516 (1985).
20) Hiasa, Y., Kitahori, Y., Konishi, N. and Shimoyama, T. Dose-related effect of trisodium nitrilotriacetate monohydrate on renal tumorigenesis initiated with N-ethyl-N-hydroxyethyl-nitrosamine in rats. *Carcinogenesis,* **6**, 907–910 (1985).
21) Kurokawa, Y., Aoki, S., Imazawa, T., Hayashi, Y., Matsushima, Y. and Takamura, N. Dose-related enhancing effect of potassium bromate on renal tumorigenesis in rats initiated with N-ethyl-N-hydroxyethylnitrosamine. *Jpn. J. Cancer Res.,* **76**, 583–589 (1985).
22) Kurokawa, Y., Matsushima, M., Imazawa, T., Takamura, N., Takahashi, M. and Hayashi, Y. Promoting effect of metal compounds on rat renal tumorigenesis. *J. Am. Coll. Toxicol.,* **4**, 321–330 (1985).
23) Hiasa, Y. and Ito, N. Experimental induction of renal tumors. *CRC Crit. Rev. Toxicol.,* **17**, 279–343 (1987).
24) Kurata, Y., Tsuda, H., Sakata, T., Yamashita, T. and Ito, N. Reciprocal modifying effects of isomeric forms of aminophenol on induction of neoplastic lesions in rat liver and kidney initiated by N-ethyl-N-hydroxyethylnitrosamine. *Carcinogenesis,* **8**, 1281–1285 (1987).
25) Hirose, M., Shirai, T., Kurata, Y., Yamaguchi, S. and Asakawa, E. Effects of nephrotoxic compounds on renal- and hepato-carcinogenesis. *J. Toxicol. Pathol.,* **1**, 193–198 (1988) (in Japanese).
26) Kurokawa, Y., Takamura, N., Matsushima, Y., Imazawa, T. and Hayashi, Y. Promoting effect of peroxisome proliferators in two-stage rat renal tumorigenesis. *Cancer Lett.,* **43**, 145–149 (1988).
27) Diwan, B. A., Ohshima, M. and Rice, J. M. Promotion by sodium barbital of renal cortical and transitional cell tumors, but not intestinal tumors, in F344 rats given methyl(acetoxymethyl)nitrosamine, and lack of effect of phenobarbital, amobarbital, or barbituric acid on development of either renal or intestinal tumors. *Carcinogenesis,* **10**, 183–188 (1989).
28) Hagiwara, A., Tamano, S., Ogiso, T., Asakawa, E. and Fukushima, S. Promoting effect of the peroxisome proliferator, clofibrate, but not di(2-ethylhexyl)phthalate, on urinary bladder carcinogenesis in F344 rats initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine. *Jpn. J. Cancer Res.,* **81**, 1232–1238 (1990).
29) Nogueira, E. and Bannasch, P. Cellular origin of renal

oncocytoma. *Lab. Invest.*, **59**, 337–343 (1988).
30) Verma, A. K. and Boutwell, R. K. Effect of dose and duration of treatment with the tumor-promoting agent, 12-O-tetradecanoylphorbol-13-acetate on mouse skin carcinogenesis. *Carcinogenesis*, **1**, 271–276 (1980).
31) Takano, T., Tatematsu, M., Hasegawa, R., Imaida, K. and Ito, N. Dose-response relationship for the promoting effect of phenobarbital on the induction of liver hyperplastic nodules in rats exposed to 2-fluorenylacetamide and carbon tetrachloride. *Gann*, **71**, 580–581 (1980).
32) Peraino, C., Staffeldt, E. F., Haugen, D. A., Lombard, L. S., Stevens, F. J. and Fry, R. J. M. Effects of varying the dietary concentration of phenobarbital on its enhancement of 2-acetylaminofluorene-induced hepatic tumorigenesis. *Cancer Res.*, **40**, 3268–3273 (1980).
33) Kitagawa, T., Hino, O., Nomura, K. and Sugano, H. Dose-response studies on promoting and anticarcinogenic effects of phenobarbital and DDT in the rat hepatocarcinogenesis. *Carcinogenesis*, **12**, 1653–1656 (1984).
34) Nakanishi, K., Hagiwara, A., Shibata, M., Imaida, K., Tatematsu, M. and Ito, N. Dose response of saccharin in induction of urinary bladder hyperplasias in Fischer 344 rats pretreated with N-butyl-N-(4-hydroxybutyl)nitrosamine. *J. Natl. Cancer Inst.*, **65**, 1005–1010 (1980).
35) Hiasa, Y., Kitahori, Y., Konishi, N., Shimoyama, T. and Lin, J-C. Sex differential and dose dependence of phenobarbital-promoting activity in N-bis(2-hydroxypropyl)-nitrosamine-initiated thyroid tumorigenesis in rats. *Cancer Res.*, **45**, 4087–4090 (1985).
36) Choie, D. D. and Richter, G. W. Cell proliferation in rat kidney induced by lead acetate and effects of uninephrectomy on the proliferation. *Am. J. Pathol.*, **66**, 265–276 (1972).
37) Choie, D. D. and Richter, G. W. Cell proliferation in rat kidneys after prolonged treatment with lead. *Am. J. Pathol.*, **68**, 359–370 (1972).
38) Short, B. G., Burnett, V. L. and Swenberg, J. A. Histopathology and cell proliferation induced by 2,2,4-trimethylpentane in male rat kidney. *Toxicol. Pathol.*, **14**, 194–203 (1986).
39) Loury, D. J., Goldworthy, T. L. and Butterworth, B. E. The value of measuring cell replication as a predictive index of tissue-specific tumorigenic potential. *In* "Nongenotoxic Mechanisms in Carcinogenesis," ed. B. E. Butterworth and T. L. Slaga, Banbury Report 25, pp. 119–134 (1987). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
40) Loury, D. J., Smith-Oliver, T. and Butterworth, B. E. Assessment of unscheduled and replicative DNA synthesis in rat kidney cells exposed *in vitro* or *in vivo* to unleaded gasoline. *Toxicol. Appl. Pharmacol.*, **87**, 127–140 (1987).
41) Short, B. G., Burnett, V. L., Cox, M. G., Bus, J. S. and Swenberg, J. A. Site-specific renal cytotoxicity and cell proliferation in male rats exposed to petroleum hydrocarbons. *Lab. Invest.*, **57**, 564–577 (1987).
42) Hagiwara, A., Diwan, B. A. and Ward, J. M. Barbital sodium, a tumor promoter for kidney tubules, urinary bladder, and liver of the F344 rat, induces persistent increases in levels on DNA synthesis in renal tubules but not in urinary bladder epithelium or hepatocytes. *Fundam. Appl. Toxicol.*, **13**, 332–340 (1989).