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Correlation between Medium-term Multi-organ Carcinogenesis Bioassay Data and Long-term Observation Results in Rats

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The effects of four test chemicals [2-acetylaminofluorene (2-AAF), D,L-ethionine (ethionine), butylated hydroxyanisole (BHA), and catechol] were compared in medium- and long-term *in vivo* systems. In the medium-term assay, animals were sequentially treated with N-diethylnitrosamine (100 mg/kg body weight, i.p., single injection), N-methylnitrosourea (20 mg/kg body weight, i.p., 4 times during weeks 1 and 2), N-butyl-N-(4-hydroxybutyl)nitrosamine (0.05% in the drinking water during weeks 1 and 2), 1,2-dimethylhydrazine (40 mg/kg body weight, s.c., 4 times during weeks 3 and 4) and dihydroxy-di-N-propylnitrosamine (0.1% in the drinking water during weeks 3 and 4) for multi-organ initiation, and then treated with one of the four test chemicals for 24 weeks, and killed at week 28 (group 1). In the long-term assay, animals were treated in the same manner and then given basal diet and tap water (group 3) or test chemical continuously (group 4) for the remainder of the lifespan. Animals receiving multi-organ initiation and then maintained on basal diet for 24 weeks (group 2) or their lifespan (group 5) served as controls. Detailed histopathological examinations were performed on all rats. Hepatocellular carcinoma incidences in the long-term assay were found to reflect closely the respective medium-term results. Induction of proliferative forestomach or glandular stomach lesions by BHA and/or catechol, and bladder lesions by 2-AAF and BHA in the medium-term assay also correlated with tumor development in the long-term. Furthermore, inhibition of thyroid proliferative lesions by all test chemicals corresponded with low thyroid tumor incidences in the long-term assay. The observed strong correlation between medium- and long-term results confirms the applicability of our medium-term multi-organ carcinogenesis bioassay system for detection of modifying effects of test chemicals in different organs.

Key words: Medium-term assay — Multi-organ carcinogenesis — Long-term assay — F344 rats

Two-year long-term *in vivo* carcinogenicity studies using small rodents have been considered to be most reliable for the prediction of carcinogenic potential in man.[1,2] Since these long-term tests are expensive and time-consuming, a large number of *in vitro* short-term assays, which are based on induction of different types of genetic alterations, have been developed with the aim of predicting the carcinogenicity of test chemicals.[3–7] However, the usefulness of these *in vitro* short-term tests is limited by substantial rates of false-positive or false-negative determinations and their inability to determine target organ-specific chemical carcinogenicity or promoting activity.[8–11] As a bridge to overcome the disadvantages of both *in vitro* short-term screening tests and the long-term *in vivo* bioassay, various medium-term *in vivo* single organ bioassay systems, based on the initiation-promotion protocol, have been developed for predicting carcinogenicity of test chemicals.[12] Since these assays primarily provide information on whether a test chemical is carcinogenic to one tissue depending on initiation with a specific carcinogen, we have extensively investigated several medium-term multi-organ bioassay systems, including newly developed DEN-MNU-BBN-DMH-DHPN[4] (DMBDD) models, named for the combined initiators, for systemic evaluation of carcinogenic, enhancing or inhibiting potentials of test compounds.[12–14] These models have proved to be of advantage for whole-body surveys of carcinogenic potential in a relatively short experimental period and at low cost, for rapid screening of large numbers of chemicals.[12–21]

The objectives of the present study were to investigate the relation between proliferative lesion induction in our newly developed multi-organ carcinogenesis bioassay and subsequent cancer development with or without further exposure to the test chemicals (2-AAF as a strong hepatocarcinogen; ethionine as a hepatotoxic and carcinogenic agent; BHA as a forestomach carcinogen and

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[4] Abbreviations: DEN, diethylnitrosamine; MNU, N-methylnitrosourea; BBN, N-butyl-N-(4-hydroxybutyl)nitrosamine; DMH, 1,2-dimethylhydrazine; DHPN, dihydroxy-di-N-propylnitrosamine, 2-AAF, 2-acetylaminofluorene; BHA, butylated hydroxyanisole.

bladder tumor promoter; catechol as a stomach carcinogen), and also to confirm the validity of this bioassay system.

MATERIALS AND METHODS

Animals Male F344/DuCrj rats, 5 weeks of age, were obtained from Charles River Japan, Inc., Kanagawa. They were housed five to a plastic cage with hardwood chips for bedding, and fed powdered diet MF (Oriental Yeast, Co., Ltd., Tokyo) and water *ad libitum*. Animals were kept in an environmentally controlled room maintained at a temperature of 22 ± 2°C and a relative humidity of 55 ± 10% under a 12-h light/dark cycle. After a one-week acclimation period, they were used in the study.

Chemicals DEN and BBN (Tokyo Kasei Kogyo Co., Ltd., Tokyo), MNU (Iwai Chemical Co., Tokyo). DMH (Aldrich Chemical Co., Milwaukee, WI) and DHPN (Nacalai Tesque Inc., Kyoto) were used as carcinogens in the present DMBDD model. Four test chemicals, namely 2-AAF and ethionine (Tokyo Kasei Kogyo Co., Ltd.), BHA and catechol (Wako Pure Chemical Industries, Osaka) were selected for the investigation.

Experimental design A total of 255 rats were divided randomly into 5 groups, groups 1, 3 and 4 being subdivided into subgroups (15 rats each for groups 1a–1d, 20 rats each for groups 3a–3d and 4a–4d) for treatment with test chemicals as indicated in Fig. 1. All animals were treated sequentially with DEN (100 mg/kg body wt., i.p., single dose at commencement), MNU (20 mg/kg body wt., i.p., 4 times during weeks 1 and 2), BBN (0.05% in the drinking water during weeks 1 and 2), DMH (40 mg/kg body wt., s.c., 4 times during weeks 3 and 4), and DHPN (0.1% in the drinking water during weeks 3 and 4) as previously reported.[14] Starting 4 weeks later, the rats in groups 1a–1d were administered one of four test chemicals (a, 0.01% 2-AAF; b, 0.25% ethionine; c, 2.0% BHA; d, 0.8% catechol) for 24 weeks. Dose and administration route of test chemicals were based on previous reports.[22–39] Groups 3a–3d were treated in the same manner as described for groups 1a–1d and then received basal diet and tap water for their lifetime. Animals in groups 4a–4d were also treated as described for groups 1a–1d and then maintained on diet containing the respective test chemical for the remainder of their lifespan. Animals in groups 2 and 5 were given basal diet and tap water after the initiation procedure and served as controls. All animals were killed under ether anesthesia for examination of lesion development at week 28 (groups 1 and 2) or at the end of their lives (groups 3, 4 and 5).

Histopathological examination At autopsy (groups 1 and 2), the liver, kidneys and spleen were immediately excised and weighed, and organ-to-body weight ratios calculated. In addition, after fixation, urinary bladders were weighed and relative weights calculated. The following organs of each rat were preserved in 10% buffered formalin and processed routinely for histopathological examination of hematoxylin and eosin-stained slides: heart, lymph node, spleen, bone marrow, thymus, pituitary, thyroid, adrenal, trachea, lung, tongue, esophagus, stomach, small intestine, large intestine, pancreas, liver, kidney, urinary bladder, testis, prostate, seminal vesicle, brain, spinal cord, and any other tissues demonstrating an abnormal appearance.

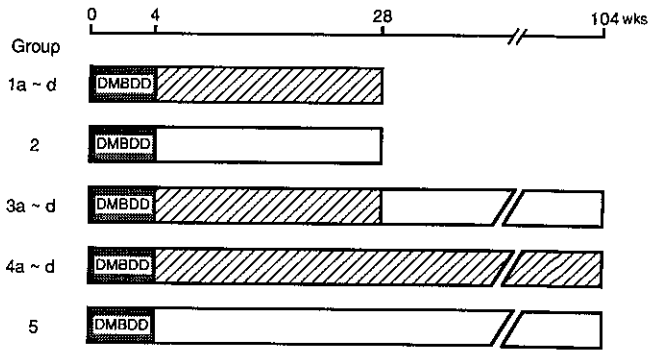

Fig. 1. Experimental design. DMBDD treatment: Animals were sequentially given DEN (100 mg/kg, i.p. single dose at the commencement), MNU (20 mg/kg, i.p. 4 times during weeks 1 and 2), BBN (0.05% in the drinking water during weeks 1 and 2), DMH (40 mg/kg, sc, 4 times during weeks 3 and 4) and DHPN (0.1% in the drinking water during weeks 3 and 4). Test chemicals (▨) were given at the following dietary concentrations: a, 2-AAF (0.01%); b, ethionine (0.25%); c, BHA (2.0%); and d, catechol (0.8%). Groups 2 and 5 were maintained on basal diet (□) after the DMBDD treatment, and served as controls.

Statistical analysis The significance of intergroup differences in numerical data obtained for body and organ weights was assessed by using the two-sided Student's *t* test. Insufficient homogeneity of variance was corrected with respect to the degree of freedom according to the method of Welch. The significance of differences in the incidences of proliferative lesions between the treated and control groups was evaluated by using Fisher's exact probability test.

RESULTS

Medium-term multi-organ bioassay results Average final body and relative organ weights of animals from the present medium-term multi-organ carcinogenesis bioassay (DMBDD model) in groups 1a–1d and 2 are shown in Table I. Body weights of rats in groups 1a–1d were significantly reduced as compared to group 2 (con-

Table I. Final Body and Relative Organ Weights in the Medium-term Multi-organ Carcinogenesis Bioassay

Group[a]	No. of rats examined	Final body weights (g) (mean±SD)	Relative organ weights (%) (mean±SD)			
			Liver	Kidneys	Spleen	Urinary bladder
1a	14	288±18**	7.14±0.92**	0.71±0.04**	0.25±0.04**	0.038±0.010**
1b	13	230±15**	4.77±0.61**	0.77±0.08**	0.21±0.08	0.044±0.020*
1c	14	282±12**	3.07±0.16**	0.74±0.17*	0.19±0.02	0.044±0.018**
1d	13	277±14**	2.82±0.11**	0.75±0.05**	0.21±0.03*	0.032±0.010
2	15	344±13	2.38±0.07	0.61±0.04	0.18±0.02	0.029±0.008

a) For test chemicals and dietary levels, see Fig. 1.
*, ** Significantly different from group 2 at $P<0.05$ or 0.01, respectively.

Table II. Incidences of Proliferative Lesions Developing in the Medium-term Multi-organ Carcinogenesis Bioassay

Site and type of lesion	Group	1a	1b	1c	1d	2
	Test chemical	2-AAF	Ethionine	BHA	Catechol	None
	No. of rats	14	13	14	13	15
Thyroid						
Follicular cell hyperplasia		9 (64)	0**	3 (21)**	0**	12 (80)
Follicular cell adenoma		0**	0**	1 (7)**	0**	8 (53)
Follicular cell carcinoma		0	0	0	0	3 (20)
Forestomach						
Squamous cell hyperplasia		4 (29)	2 (15)	14 (100)**	13 (100)**	6 (40)
Squamous cell papilloma		0	1 (8)	6 (43)**	7 (54)**	0
Squamous cell carcinoma		0	0	13 (93)**	3 (23)	0
Glandular stomach						
Submucosal hyperplasia		0	0	0	11 (85)**	0
Adenoma		0	0	0	11 (85)**	0
Liver						
Hyperplastic focus		14 (100)**	13 (100)**	4 (29)	5 (38)	4 (27)
Neoplastic nodule		11 (79)**	2 (15)	0	0	1 (7)
Hepatocellular carcinoma		2 (14)	0	0	0	0
Urinary bladder						
PN hyperplasia[a]		6 (43)	1 (8)	7 (50)*	3 (23)	2 (13)
Transitional cell papilloma		0	1 (8)	4 (29)	0	1 (7)
Transitional cell carcinoma		0	1 (8)	2 (14)	0	0

a) Papillary or nodular hyperplasia.
*, ** Significantly different from group 2 at $P<0.05$ or 0.01, respectively.

trol), especially in rats fed ethionine (group 1b). Relative liver weights in rats given 2-AAF (group 1a) and ethionine were markedly increased, with concomitant appearance of macroscopic liver nodules. Other organ weight changes were considered to be related to the retardation of body weight gain.

Histopathological findings for the medium-term multi-organ carcinogenesis bioassay are summarized in Table II. Squamous cell hyperplasia and papilloma in the forestomach were clearly increased in rats fed BHA and catechol (groups 1c and 1d). Submucosal hyperplasias[37-39] and adenomas in the glandular stomach were observed in rats fed catechol (group 1d). The incidences of hyperplastic foci and/or neoplastic nodules in the liver were significantly increased in rats given 2-AAF and ethionine (groups 1a and 1b) as compared to control values. Increased incidences of proliferative transitional cell lesions of the urinary bladder were found in rats given BHA, and those in rats fed 2-AAF also tended to be increased. In contrast, the incidences of preneoplastic and neoplastic thyroid follicular cell lesions were significantly reduced in all treated groups, especially in rats exposed to ethionine and catechol. Although many other types of hyperplastic and/or neoplastic lesions were found in rats given test chemicals, the incidences were within the control ranges.

Long-term assay results The survival rates of long-term assay animals, undergoing the multi-organ carcinogenesis protocol and then maintained with or without further chemical treatment, are shown in Fig. 2. The average weeks of survival in rats give 2-AAF and ethionine, with or without subsequent administration (groups 3a, 3b, 4a and 4b) were 42.7, 40.4, 41.8 and 39.9, respectively, all being clearly shorter than the control (group 5) value of 48.5. Mean survival of rats fed BHA and catechol (groups 3c, 3d, 4c and 4d) was essentially the same as in controls.

Histopathology results for the long-term assay without subsequent test chemical exposure after application of the multi-organ carcinogenesis bioassay protocol are summarized in Table III. Increased incidences of squamous cell papillomas and carcinomas in the forestomach were found in rats fed BHA (group 3c). All animals given 2-AAF and ethionine (groups 3a and 3b) developed hepatocellular carcinomas. Most liver carcinomas found in these groups gave rise to metastatic lung lesions. An intestinal and a urinary bladder metastasis were also observed in group 3a. Increased incidences of transitional cell papillomas in the urinary bladder were found in animals given 2-AAF (group 3a). Combined incidences of transitional cell papillomas and carcinomas tended to be increased in rats fed BHA (group 3c). Induction of thyroid follicular cell adenomas was reduced in groups 3a, 3b and 3d when compared to the controls. Incidences of squamous cell papillomas in the forestomach and nephroblastomas in the kidney were low in rats fed ethionine (group 3b).

Histopathology results for the long-term assay with continuous test chemical treatment after application of the multi-organ carcinogenesis bioassay protocol are summarized in Table IV. Increased incidences of squamous cell papillomas or carcinomas in the forestomach were found in rats fed BHA and catechol (groups 4c and 4d). Adenomas in the glandular stomach were significantly increased in rats fed catechol (group 4d). All animals given 2-AAF and ethionine (groups 4a

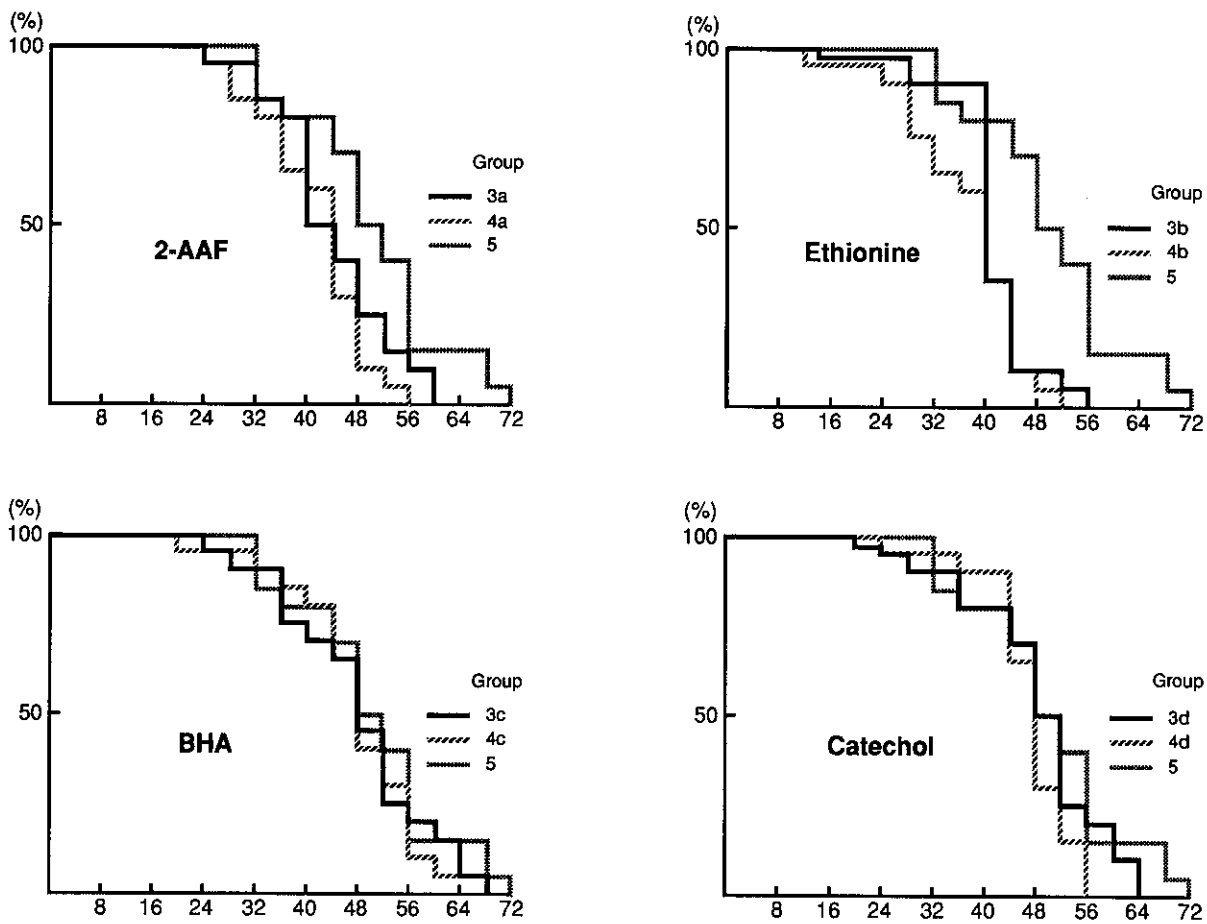

Fig. 2. Survival rates of rats given 2-AAF, ethionine, BHA, catechol or basal diet. For group details, see Fig. 1.

Table III. Incidences of Neoplastic Lesions Developing in the Long-term without Continuous Test Chemical Treatment after Application of the Multi-organ Carcinogenesis Bioassay Protocol

Site and type of tumor	Group Test chemical No. of rats	3a 2-AAF 20	3b Ethionine 19	3c BHA 18	3d Catechol 19	5 None 20
Thyroid						
Follicular cell adenoma		12 (60)*	2 (11)**	13 (72)	9 (47)**	18 (90)
Follicular cell carcinoma		0	0	2 (11)	0	1 (5)
[Adenoma or carcinoma		12 (60)*	2 (11)**	13 (72)	9 (47)**	18 (90)]
Forestomach						
Squamous cell papilloma		9 (45)	2 (11)*	14 (78)*	7 (37)	9 (45)
Squamous cell carcinoma		0	0	18 (100)**	1 (5)	0
[Papilloma or carcinoma		9 (45)	2 (11)*	18 (100)**	8 (42)	9 (45)]
Glandular stomach						
Adenoma		0	0	0	2 (11)	1 (5)
Adenocarcinoma		0	0	0	2 (11)	0
[Adenoma or adenocarcinoma		0	0	0	4 (21)	1 (5)]
Liver						
Neoplastic nodule		9 (45)	8 (42)	1 (6)	1 (5)	5 (25)
Hepatocellular carcinoma		20 (100)**	14 (74)**	4 (22)	3 (16)	4 (20)
[Neoplastic nodule or carcinoma		20 (100)**	16 (84)*	5 (28)	4 (21)	9 (45)]
Kidney						
Nephroblastoma		7 (35)	1 (5)**	4 (22)	8 (42)	9 (45)
Urinary bladder						
Transitional cell papilloma		14 (70)**	4 (21)	9 (50)	3 (16)	5 (25)
Transitional cell carcinoma		3 (15)	2 (11)	4 (22)	0	2 (10)
[Papilloma or carcinoma		15 (75)**	6 (32)	10 (56)	3 (16)	6 (30)]
Zymbal's gland						
Squamous/sebaceous carcinoma		9 (45)**	0	3 (17)	2 (11)	1 (5)

*, ** Significantly different from group 5 at $P<0.05$ or 0.01, respectively.

and 4b) developed hepatocellular carcinomas. Increased incidences of transitional cell papillomas in the urinary bladder were found in animals given 2-AAF (group 4a). Combined incidences of transitional cell papillomas and carcinomas were significantly increased in rats fed BHA (group 4c). Induction of thyroid follicular cell adenomas was reduced in groups 4b, 4c and 4d when compared to the controls. Incidences of neoplastic nodules/hepatocellular carcinomas of the liver and nephroblastoma of the kidney were low in rats fed BHA (group 4c).

While many other types of tumors were found in rats given test compounds, the incidences were essentially similar to control values.

DISCUSSION

The present study demonstrated a clear correspondence between influence of medium-term exposure on preneoplastic lesions and subsequent neoplastic lesion development for all 4 test chemicals. Therefore, the observed correlation between medium- and long-term results strongly validated the present medium-term multi-organ carcinogenesis bioassay system for detection of target organ-specific modifying effects of test chemicals.

The results of the current study are summarized in Table V. 2-AAF enhanced the induction of altered liver cell foci and neoplastic nodules in the multi-organ carcinogenesis bioassay (DMBDD model), and was also associated with hepatocellular carcinomas in the long-term. A similar correlation for proliferative liver lesions was also found for medium-term liver bioassay system data using F344 rats[22,23] and results of subsequent long-term observation.[23] Furthermore, 2-AAF, which has been reported to target the urinary bladder epithelium,[24,25] also induced transitional cell hyperplasias and papillomas of the urinary bladder in the multi-organ bioassay, and caused development of transitional cell tumors in the long-term test. Furthermore, the reduced induction of follicular cell hyperplasia of the thyroid by 2-AAF in the multi-organ bioassay, corresponded with inhibition of development of thyroid follicular cell tumors in the long-term. While the Zymbal's gland carcinomas which developed in group 3a were not evident in groups 1a or 4a, this outcome could clearly have been related to the shorter exprimental period and might require recovery from toxicity.

Ethionine, an ethyl analog of the naturally occurring amino acid methionine, has been reported to induce

Table IV. Incidences of Neoplastic Lesions Developing in the Long-term with Continued Test Chemical Treatment after Application of the Multi-organ Carcinogenesis Bioassay Protocol

Site and type of tumor	Group	4a	4b	4c	4d	5
	Test chemical	2-AAF	Ethionine	BHA	Catechol	None
	No. of rats	17	15	19	19	20
Thyroid						
Follicular cell adenoma		11 (65)	1 (7)**	9 (47)**	7 (37)**	18 (90)
Follicular cell carcinoma		1 (6)	0	0	1 (5)	1 (5)
[Adenoma or carcinoma		11 (65)	1 (7)**	9 (47)**	7 (37)**	18 (90)]
Forestomach						
Squamous cell papilloma		5 (29)	5 (33)	4 (21)	17 (89)**	9 (45)
Squamous cell carcinoma		0	0	16 (84)**	3 (16)	0
[Papilloma or carcinoma		5 (29)	5 (33)	19 (100)**	18 (95)**	9 (45)]
Glandular stomach						
Adenoma		0	0	0	9 (47)**	1 (5)
Adenocarcinoma		0	0	0	0	0
[Adenoma or adenocarcinoma		0	0	0	9 (47)**	1 (5)]
Liver						
Neoplastic nodule		7 (41)	8 (53)	0*	2 (11)	5 (25)
Hepatocellular carcinoma		17 (100)**	15 (100)**	0	1 (5)	4 (20)
[Neoplastic nodule or carcinoma		17 (100)**	15 (100)*	0**	3 (16)	9 (45)]
Kidney						
Nephroblastoma		4 (24)	4 (27)	2 (11)*	5 (26)	9 (45)
Urinary bladder						
Transitional cell papilloma		10 (59)*	6 (40)	8 (42)	3 (16)	5 (25)
Transitional cell carcinoma		0	2 (13)	6 (32)	2 (11)	2 (10)
[Papilloma or carcinoma		10 (59)	8 (53)	12 (63)*	5 (26)	6 (30)]

*, ** Significantly different from group 5 at $P<0.05$ or 0.01, respectively.

Table V. Summary: Comparison of Multi-organ Bioassay and Long-term Assay Results

Test chemical	Results of the multi-organ bioassay	Long-term multi-organ observation results	
		Cessation of treatment	Continuous treatment
2-AAF	Thyroid, A⇩	Thyroid, A⇩	Thyroid, A↓
	Liver, F and A⬆	Liver, C⬆	Liver, C⬆
	Urinary bladder, H↑	Urinary bladder, P⬆	Urinary bladder, P⬆
Ethionine	Thyroid, H and A⇩	Thyroid, A⇩	Thyroid, A⇩
	Forestomach, H↓	Forestomach, P⇩	Forestomach, P↓
	Liver, F⬆	Liver, C⬆	Liver, C⬆
BHA	Thyroid, H and A⇩	Thyroid, A↓	Thyroid, A⇩
	Forestomach, H, P and C⬆	Forestomach, P and C⬆	Forestomach, C⬆
	Liver, ⇨	Liver, A and C↓	Liver, A and C⇩
	U. bladder, H⬆	Urinary bladder, P and C↑	Urinary bladder, P and C⬆
Catechol	Thyroid, H and A⇩	Thyroid, A⇩	Thyroid, A⇩
	Forestomach, H and P⬆	Forestomach, ⇨	Forestomach, P and C⬆
	Gl. stomach, H and A⬆	Gl. stomach, A and C↑	Gl. stomach, A⬆
	Liver, ⇨	Liver, A and C↓	Liver, A and C↓

Key: F, hyperplastic focus; H, hyperplasia; A, adenoma; P, papilloma; C, carcinoma; Gl., glandular ⬆, significant enhancement; ↑, tendency for enhancement; ⇩, significant inhibition; ↓, tendency for inhibition; ⇨, no change.

hepatocellular carcinomas in rats.[28,29] In the present investigation, it induced altered liver cell foci and neoplastic nodules in the multi-organ carcinogenesis bioassay, and also caused development of hepatocellular carcinomas within the animal's lifetime. These findings are again in line with our previous comparison of medium-term liver bioassay and long-term results.[22,30] Induction of follicular cell hyperplasias and adenomas of the thyroid was apparently reduced in rats fed ethionine in the current multi-organ carcinogenesis bioassay,

and in the long-term test. Thyroid tumor inhibition by ethionine was also noted in our previous multi-organ carcinogenesis bioassay.[18]

The phenolic antioxidant, BHA, is a well established forestomach carcinogen in rodents,[31–33] and in the present medium-term bioassay, it markedly enhanced the development of forestomach proliferative lesions, in line with early results with several types of initiation-promotion protocol.[33–36] Although proliferative squamous cell lesions of the forestomach induced by nongenotoxic antioxidants are generally reversible in nature,[33] regression was clearly not total under the current experimental conditions. In both medium-term and long-term assays, BHA showed enhancing effects on urinary bladder carcinogenesis, which coincided with our previous initiation-promotion study results.[33–35] In addition, the present study clearly demonstrated that BHA exerts inhibitory effects on liver carcinogenesis, especially after prolonged continuous treatment, in line with our previous investigations using medium-term liver bioassay and long-term tests.[22, 23] Although an inhibitory effect of BHA on liver carcinogenesis was not detected in the medium-term assay, we did not employ quantitative analysis of immunohistochemical glutathione *S*-transferase placental form (GST-P)-positive hepatocyte foci[12–23] in the present study. BHA did, however, show a slight inhibitory effect on induction of thyroid follicular cell tumors in the present case, although no effect was observed in previous studies.[18, 21, 35] The reason for this discrepancy might be related to the relatively low carcinogen dose or dietary level of BHA (1.0%).[18]

Catechol, which is a natural occurring phenolic antioxidant, induces adenomas and carcinomas of the glandular stomach in rats.[37, 38] In agreement with previous findings,[17] it exerted clear enhancing effects on forestomach and glandular stomach carcinogenesis in the present multi-organ carcinogenesis bioassay, apparently correlated with tumor induction in these sites after continuous exposure. Although an enhancing effect of forestomach carcinogenesis was not found in rats when the catechol treatment was discontinued, this was considered to be related to the reversibility of antioxidant-induced forestomach proliferative lesions.[33] In the current long-term study, catechol also showed inhibitory effects on liver carcinogenesis as demonstrated in our earlier investigations.[18, 22] Again the fact that the medium-term assay results relied only on histological assessment might explain the lack of significant influence.

In conclusion, the present findings indicate that statistical analysis of proliferative lesions developing in multi-organ carcinogenesis bioassays can reveal enhancing and inhibitory activity of test chemicals without the necessity of long-term *in vivo* studies. The results further confirm the advantages of this bioassay system for detection of target-organ-specific carcinogenic potential or promoting/inhibitory activity, especially in large-scale surveys of carcinogenic hazard.

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