3. Studies of Cancer in Experimental Animals

Previous evaluation

Various inorganic arsenic compounds were tested for carcinogenicity by oral administration, skin application, inhalation and/or intratracheal administration, subcutaneous and/or intramuscular administration, intravenous administration and other experimental systems in mice, rats, hamsters, dogs or rabbits. Arsenic trioxide produced lung adenomas in mice after perinatal treatment (Rudnay & Börzsönyi, 1981) and in hamsters after its intratracheal instillation (Ishinishi *et al.*, 1983; Pershagen *et al.*, 1984). It induced a low incidence of adenocarcinomas at the site of its implantation into the stomach of rats (Katsnelson *et al.*, 1986). A higher incidence of lung carcinomas was induced in rats following a single intratracheal instillation of a Bordeaux pesticide mixture (copper sulfate and calcium oxide in a concentration of 1–2%) containing calcium arsenate (IARC, 1987). Intratracheal instillations of calcium arsenate into hamsters resulted in a borderline increase in the incidence of lung adenomas, while no such effect was observed with arsenic trisulfide (Pershagen & Björklund, 1985). These studies provide *limited evidence* for carcinogenicity of inorganic arsenics (IARC, 1980, 1987).

No adequate data on the carcinogenicity of organic arsenic compounds were available to the previous working group (IARC, 1980, 1987).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 24 male A/J mice, 6 weeks of age, were given tap-water (control) or a solution of 50, 200 or 400 ppm [μg/mL] dimethylarsinic acid (DMA^V) as drinking-water for 25 (10 mice per group) or 50 weeks (14 mice per group). The incidences of lung tumours were 2/10 (20%), 3/10 (30%), 4/10 (40%) and 3/10 (30%) in control, 50-, 200- and 400-ppm groups, after 25 weeks, with average numbers of tumours/mouse of 0.2 ± 0.42, 0.3 ± 0.48, 0.5 ± 0.71 and 0.4 ± 0.70, respectively; no significant differences were apparent, nor did average tumour size vary significantly (0.9, 0.5, 1.4 and 1.1 mm, respectively). After 50 weeks, a non-significant increase in the incidence of lung tumours (50, 71.4, 64.3 and 78.6% in 14 animals per group, respectively), a significant increase in multiplicity (0.5 ± 0.52, 1.07 ± 1.0, 1.07 ± 1.07 and 1.36 ± 1.01, respectively; $p < 0.05$ for the 400-ppm group) and an increase in average diameter (1.0, 1.2, 1.4 and 1.5 mm, respectively) were observed. The numbers of mice with papillary lung adenoma and/or adenocarcinoma at 50 weeks were two, five, seven and 10 ($p = 0.002$ for the 200- and 400-ppm groups) and increased with increasing dose of DMA^V. In animals that received 0, 50, 200 or 400 ppm DMA^V, the number of alveolar adenomas ranged from 3/14 to 5/14 per treatment group (Hayashi *et al*., 1998).

Groups of 90 female C57BL/6J mice and 140 female metallothionein heterozygous mice ($MT^{-/-}$), aged 4–5 weeks, were given drinking-water containing sodium arsenate (500 μg/L arsenic) *ad libitum* for up to 26 months. Groups of 60 control mice were given tap-water. Preliminary findings indicate that tumours were observed in the lung (C57BL/6J, 17.8%; $MT^{-/-}$, 7.1%), gastrointestinal tract (14.4%; 12.9%), liver (7.8%; 5.0%), spleen (3.3%; 0.7%), reproductive organs (3.3%; 5.0%), skin (3.3%; 1.4%), bone (2.2%; 0%) and eye (1.1%; 0%) of treated animals. No tumours were observed in the control groups (Ng *et al.*, 1999). [The Working Group decided that this study was preliminary because no histopathological findings were reported.]

Groups of 20–30 K6/ODC transgenic mice, 7 weeks of age, were administered DMA^V at either 10 or 100 ppm [μg/mL] in their drinking water or sodium arsenite at 10 ppm for 5 months. The incidence of squamous skin tumours was 0% in the controls, 8 and 22% in the 10- and 100-ppm DMA groups, respectively, and 15% in the arsenite groups (Chen *et al.*, 2000).

Groups of 29 or 30 male $p53^{+/-}$ heterozygous or $p53^{+/+}$ mice (C57BL/6J background) were exposed to 0, 50 or 200 ppm [μg/mL] DMA^V in the drinking-water for 80 weeks. In $p53^{+/+}$ mice, a significant increase in the incidence (control, 10%; 50-ppm, 30% [$p < 0.05$]; and 200-ppm, 30% [$p < 0.05$] in 30 animals per group) and multiplicity (0.2, 0.6 [$p < 0.02$] and 0.6 [$p < 0.02$] tumours per mouse, respectively) of total tumours was

observed at the terminal killing, but with no dose dependence. In the heterozygotes, a non-significant increase in incidence was observed (control, 14/29 [48.3%]; 50-ppm, 18/29 [62.1%]; and 200-ppm, 19/30 [63.3%]), but the number of tumours per mouse was significantly increased at 200 ppm ($p < 0.05$) (control, 0.8; 50-ppm, 1.1; 200-ppm, 1.2). No effects were observed in either heterozygous or $p53^{+/+}$ mice regarding the number of tumours per tumour-bearing animal (control, 1.6; 50-ppm, 1.8; 200-ppm, 1.9 in heterozygous mice; control, 2; 50 ppm, 1.9; 200 ppm, 2 in $p53^{+/+}$ mice). No significant influence on tumour development was noted in any particular organ or tissue site. The tumours induced in the $p53^{+/-}$ heterozygous mice were mainly malignant lymphomas or leukaemia (control, 8/29 [28%]; 50-ppm, 13/29 [45%]; 200-ppm, 10/30 [33%]), fibrosarcomas (5/29 [17%], 8/29 [28%], 10/30 [33%]) and osteosarcomas (3/29 [10%], 2/29 [8%], 4/30 [13%]), with lower incidences of other types of tumours such as hepatocellular carcinomas, thyroid follicular carcinomas, squamous-cell carcinomas of the skin and lung adenomas. In $p53^{+/+}$ mice, tumours were generally malignant lymphomas or leukaemia (2/30 [7%], 9/30 [30%], 9/30 [30%]) with very low incidences of the other types of tumour. No fibrosarcomas or osteosarcomas were detected in $p53^{+/+}$ mice. Tumour latency curves in DMA^V-treated $p53^{+/-}$ heterozygous and $p53^{+/+}$ mice showed a dose-dependently significant shift towards early induction ($p < 0.03$) in comparison with untreated controls (Salim *et al*., 2003).

3.1.2 *Rat*

Groups of 36 male Fischer 344/DuCrj rats, 10 weeks of age, received 0, 12.5, 50 and 200 ppm DMA^V [μg/mL] (100% pure) in the drinking-water for 104 weeks. There was no significant difference in body weight or survival (25, 28, 28 and 24 animals) among the groups at week 104. At week 97, the first tumour in the urinary bladder was observed in one animal of the 200-ppm DMA^V group. Effective numbers were considered to be the numbers of animals alive at week 97. Incidences of urinary bladder tumours were 0/28, 0/33, 8/31 (26%; two papillomas and six carcinomas; $p < 0.01$, Fisher's exact probability test) and 12/31 (39%; two papillomas and 12 carcinomas; $p < 0.001$, Fisher's exact probability test), respectively, and were multiple in two animals given the highest dose. Histopathologically, the carcinomas were transitional-cell carcinomas. Urinary pH did not differ significantly between groups during the experiment. Bladder calculi were not observed in any of the rats (Wei *et al*., 1999, 2002). In a more exhaustive examination of the urinary bladder in the same animals, preneoplastic lesions (papillary or nodular hyperplasia) were observed in 0/28, 0/33, 12/31 (39%; $p < 0.01$) and 14/31 (45%, $p < 0.01$) animals in the 0-, 12.5-, 50- and 200-ppm groups, respectively. The incidences of tumours, other than those of the urinary bladder, in all DMA^V-treated groups were not different from those of controls (Wei *et al.*, 2002).

3.2 Transplacental exposure

Mouse: Groups of 10 pregnant C3H mice were given drinking-water containing 0, 42.5 and 85 ppm [μg/mL] *ad libitum* from day 8 to 18 of gestation. Offspring were weaned at 4 weeks and then divided into separate groups of 25 males and 25 females. The offspring received no additional treatment with arsenic for the next 74 (males) or 90 (females) weeks. Transplacental exposure to arsenic did not reduce body weight in any group of offspring over the course of the experiment. In male offspring, there was a marked increase in the incidence of hepatocellular carcinomas (control, 3/24 [12%]; 42.5-ppm, 8/21 [38%]; 85-ppm, 14/23 [61%]; p for trend = 0.00006, two-sided chi-square test) and multiplicity per mouse (control, 0.13 ± 0.07; 42.5-ppm, 0.42 ± 0.13; 85-ppm, 1.30 ± 0.28; p for trend = 0.003) in a dose-related fashion. There was also a dose-related increase in the incidence of adrenal cortical adenomas (control, 9/24 [37.5%]; 42.5-ppm, 14/21 [66.7%]; 85-ppm, 21/23 [91.3%]; p for trend = 0.001) and multiplicity (control, 0.71 ± 0.20; 42.5-ppm, 1.10 ± 0.22; 85-ppm, 1.57 ± 0.32; p for trend = 0.016). In female offspring, there was a strong, dose-related increase in the incidence of ovarian tumours. Total tumour (benign and malignant) incidence was control, 2/25 (8%); 42.5-ppm, 6/23 (26%); and 85-ppm, 9/24 (38%) (p for trend = 0.015). Controls had one adenoma and one benign granulosa-cell tumour. The 42.5-ppm treatment group had three adenomas, one adenocarcinoma, one benign granulosa-cell tumour and one malignant granulosa-cell tumour. The 85-ppm treatment group developed seven adenomas, one luteoma and one haemangiosarcoma. Lung carcinomas developed (control, 0/25 [0%]; 42.5-ppm, 1/23 [4%]; 85-ppm, 5/24 [21%]; p for trend = 0.0086) in a dose-dependent manner. There were significant increases in the number of mice bearing at least one tumour (control, 11/24; 42.5-ppm, 17/21; 85-ppm, 22/23; p for trend = 0.0006 in males; control, 12/25; 42.5-ppm, 17/23; 85-ppm, 16/24; $p < 0.172$ in females) and in mice bearing at least one malignant tumour (control, 3/24; 42.5-ppm, 9/21; 85-ppm, 14/23; $p < 0.0001$ in males; control, 2/25; 42.5-ppm, 9/23; 85-ppm, 8/24; $p < 0.042$ in females) with both doses of arsenic. Exposure to arsenic also increased the incidence of hyperplasia of the uterus and oviduct. In this experiment, four of the organs that developed tumours or hyperplasia were endocrine-responsive organs: adrenal gland, liver, ovary and uterus (Waalkes *et al*., 2003).

3.3 Intratracheal administration

Hamster: Groups of 30 (20 for a control) male Syrian golden hamsters, 8 weeks of age, were given arsenic trioxide, calcium arsenate or arsenic trisulfide by intratracheal instillation once a week for 15 weeks. Each compound contained 0.25 mg arsenic suspended in 0.1 mL phosphate buffer solution. The control group received buffer solution alone. All hamsters were kept during their entire lifespan. Numbers of survivors after 15 instillations were 18/30 (60%) in the arsenic trioxide-treated group, 27/30 (90%) in the calcium arsenate-treated group, 23/30 (77%) in the arsenic trisulfide-treated group and 22/22 (100%) in the control group, showing a similar tendency in survival rates of all

groups. All hamsters had died by day 794 (arsenic trioxide group), day 806 (calcium arsenate group), day 821 (arsenic trisulfide group) and day 847 (control group) after the initial instillation. Incidences of lung tumours were 1/17 (5.8%; adenocarcinoma) arsenic trioxide-treated, 7/25 (28.0%; one adenocarcinoma, six adenomas; *p* value, significant versus controls) calcium arsenate-treated, 1/22 (4.5%; adenoma) arsenic trisulfide-treated and 1/21 (4.8%; adenosquamous carcinoma) control animals. No tumours of the upper respiratory tract including the trachea were observed in any group. Besides lung tumours, one adrenal adenoma and one liver haemangiosarcoma in the arsenic trioxide-treated group, two adrenal adenocarcinomas and one leukaemia in the calcium arsenate-treated group, one nephroblastoma and one adrenal adenoma in the arsenic trisulfide-treated group and one adrenal adenocarcinoma and one adrenal adenoma in the control group were found (Yamamoto *et al.*, 1987).

3.4 Administration with known carcinogens

3.4.1 *Mouse*

Groups of 30–32 female Swiss mice, 21–24 days of age, received concentrations of 0, 10, 50 or 100 µg/L sodium arsenate or sodium arsenite in the drinking-water for 15 weeks. At week 3, the animals were administered a single intraperitoneal injection of 1.5 mg/kg bw urethane in saline. When killed at week 15, the numbers of lung adenoma per mouse were 29.0 ± 5.4, 21.4 ± 2.6, 15.7 ± 1.8 and 16.0 ± 2.1 (p for trend = 0.0185) in the 0-, 10-, 50- and 100-µg/mL arsenate-treated groups, respectively, and 20.1 ± 1.8, 25.7 ± 4.0, 19.5 ± 2.1 and 10.8 ± 1.6 (p for trend = 0.00082) in 0-, 10-, 50- and 100-µg/mL arsenite-treated groups, respectively [suggesting that both forms of arsenic exerted an inhibitory effect]. Both arsenate and arsenite at 100 µg/mL caused a significant reduction in tumour size (0.64 ± 0.01, 0.65 ± 0.02; $p < 0.05$) compared with control animals (0.74 ± 0.01, 0.71 ± 0.02) (Blakley, 1987).

In a two-stage protocol, groups of 9–13 male ddY mice, 6 weeks of age, were given a single subcutaneous injection of 10 mg/kg bw 4-nitroquinoline 1-oxide (4NQO) and then received tap-water, a 5% glycerol solution or a 200- or 400-ppm [µg/mL] DMA^V solution in drinking-water for 25 weeks. The incidences of lung tumour-bearing mice were 2/9 (22%), 5/10 (50%), 8/13 (62%) and 10/13 (77%), respectively, while the numbers of tumours per mouse were 0.22 ± 0.15, 1.40 ± 0.62, 3.92 ± 1.79 and 4.38 ± 1.07 ($p < 0.05$ Cochran-Cox t-test). Thus, DMA^V promoted lung tumorigenesis initiated by 4NQO (Yamanaka *et al.*, 1996). [The authors described a shift from papillary-type adenomas to adenosquamous carcinomas but no quantitative data were provided.]

Groups of 60 male and female C57BL6J outbred mice [sex distribution unspecified], 2 months of age, were fed a diet containing 10% lipids and were given either 0.01% arsenic trioxide in the drinking-water for 28 weeks or 3 mg per mouse benzo[*a*]pyrene in 0.2 mL corn oil by gavage once a week for 3 weeks or both arsenic trioxide and benzo[*a*]pyrene. No significant differences between groups given benzo[*a*]pyrene with or

without arsenic trioxide were observed at the end of the 28-week experimental period with regard to focal localized hyperplasia, ulcers, focal multiple hyperplasia, papillomatosis or papillomas of the forestomach (total number per mouse, 4.20 ± 0.39 in the benzo[*a*]pyrene-treated group and 5.40 ± 0.89 in the benzo[*a*]pyrene–arsenic-treated group) (Silva *et al*., 2000).

Groups of 10–11 female Hos:HR-1 hairless mice, 6 weeks of age, were administered 0, 400 or 1000 ppm [µg/mL] DMA^V in the drinking-water and irradiated twice weekly with 2 kJ/m^2 ultraviolet B (UVB) rays for 25 weeks. DMA^V had no effect on body weight gain. The number of skin tumours per mouse was significantly increased by 1000 ppm DMA^V compared with the 0-ppm value from weeks 13 to 19, and incidence of tumour-bearing mice was significantly increased at weeks 12 and 13 [exact data not clear as there were no tabulations]. No differences were noted at later time points up to week 25 (100% incidence in all groups by week 16), showing a shift towards early tumour induction following treatment with DMA^V. Malignant tumours were observed in only two animals in the 1000-ppm group (Yamanaka *et al*., 2000).

Groups of 15 female Crl: SK1-*hr*BR hairless mice, 21 days of age, received 0 or 10 mg/L sodium arsenite in the drinking-water and were irradiated with solar lamps at a dose of 1.7 J/m^2 (lamp output: 85% in the UVB range, < 1% UVC and 4% UVA, and the remainder visible) three times weekly. The UVR dose was chosen to be approximately half of the minimal erythemic dose. Two control groups of five mice received sodium arsenite only or no treatment. Sodium arsenite did not influence body weight gain. No skin tumours were observed with arsenite alone or in the untreated controls. The first tumours were noted after 8 weeks with arsenite and UVR but after 12 weeks with UVR alone (significantly earlier appearance). All UVR-treated animals had at least one tumour at 26 weeks; however, after 19 weeks of exposure to UVR, incidences were 100% for UVR and arsenite and 33% for UVR alone. The total number of tumours in the group treated with UVR alone (15 animals) was 53 and that in the group treated with UVR and arsenite (15 animals) was 127. In UVR and arsenite-treated animals, 64/127 (50.4%) tumours were highly invasive squamous-cell carcinomas, whereas in UVR alone-treated animals, 14/53 (26.4%) were highly invasive squamous-cell carcinoma ($p = 0.003$) (Rossman *et al*., 2001)

Groups of 10 female Hos:HR-1 hairless mice, 6 weeks of age, were treated with a single topical application of 200 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA) dissolved in acetone and were then administered 0, 400 or 1000 ppm [µg/mL] DMA^V in the drinking-water and/or irradiated twice weekly with 0.3 kJ/m^2 UVB. All mice were killed after 50 weeks. Skin tumours occurred faster in the DMA^V-treated group. DMA^V without UVB increased the incidence of skin tumours ($p < 0.05$ at 20–22 weeks) but not dose-dependently. Greater effects were seen in combination with UVB, particularly at 1000 ppm [exact data not clear as there was no tabulation]. Incidences of papillomas in animals treated with DMA^V without UVB were 1/10, 9/10 and 7/10 in the 0-, 400- and 1000-ppm groups, respectively, and those of squamous-cell carcinomas were 2/10, 0/10 and 0/10, respectively. Incidences of papillomas in animals treated with DMA^V and UVB

were 0/10, 7/10 and 7/10, and those of squamous-cell carcinomas were 0/10, 3/10 and 1/10 in the 0-, 400- and 1000-ppm groups, respectively (Yamanaka *et al.*, 2001).

3.4.2 *Transgenic mouse*

Groups of 7–8 female *keratin (K6)/ODC* transgenic mice, 10–14 weeks of age, received two weekly applications of 3.6 mg DMA^V in neutral cream or 5 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 200 μL acetone 1 week after initiation with 50 μg DMBA in 200 μL acetone. A significantly accelerated development of skin tumours (first tumour after 8 weeks in DMA^V-treated animals and after 11 weeks in controls) was observed following treatment with DMA^V; 20 weeks after initiation, the numbers of tumours (average, 19.4 ± 10.2 per mouse compared with 9.7 ± 3.5 in controls) were increased. Promoting activity was similar to that achieved with application of 5 μg TPA (20.7 ± 8.4) twice weekly. Microscopically, most of the tumours were squamous papillomas, although squamous carcinomas occurred in some DMA^V- and some TPA-treated animals (Morikawa *et al*., 2000).

3.4.3 *Rat*

Sodium arsenite has been reported to enhance the incidence of renal tumours induced in rats by intraperitoneal injection of *N*-nitrosodiethylamine (NDEA) (Shirachi *et al*., 1983). A subsequent re-evaluation of the study indicated that not only sodium arsenite but also sodium arsenate enhanced NDEA-induced kidney tumours (Smith *et al*., 1992).

Groups of 20 male Fischer 344/DuCrj rats, 6 weeks of age, received a single intraperitoneal injection of 100 mg/kg bw NDEA, followed by intraperitoneal injections of 20 mg/kg bw *N*-methyl-*N*-nitrosourea on days 5, 8, 11 and 14 and subcutaneous injections of 50 mg/kg bw 1,2-dimethylhydrazine chloride on days 18, 22, 26 and 30. At the same time, animals received 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) in the drinking-water for the first 2 weeks then 0.1% *N*-bis(2-hydroxypropyl)nitrosamine for the next 2 weeks (so-called DMBDD model treatment). After a 2-week interval, the animals received 0, 50, 100, 200 or 400 ppm [μg/mL] DMA^V in the drinking-water from weeks 6 to 30, at which time they were killed. DMA^V significantly enhanced tumour induction in the urinary bladder (both papillomas and transitional-cell carcinomas), kidney (both adenomas and adenocarcinomas), liver (hepatocellular carcinomas) and thyroid (adenomas) (see Table 27). Values for preneoplastic lesions such as papillary or nodular hyperplasia in the urinary bladder, atypical tubules in the kidney and altered hepatocyte foci in the liver were also significantly increased. No promoting effects were noted in the lungs or the nasal cavity (Yamamoto *et al*., 1995).

To confirm the above-mentioned results and to evaluate low-dose effects of DMA in urinary bladder and liver carcinogenesis, the following two studies were conducted. Groups of 20 male Fischer 344 rats, 6 weeks of age, received 0.05% BBN in drinking-water for 4 weeks followed by 0, 2, 10, 25, 50 or 100 ppm [μg/mL] DMA^V for 32 weeks.

Table 27. Incidence of preneoplastic and neoplastic lesions in various organs of Fischer 344/Du Crj rats treated with DMA^V after initiation with DMBDD treatment

Organ and finding	DMA^V					Two-tailed Cochran-Armitage analysis[a]
	0 ppm *n* = 20 (%)	50 ppm *n* = 20 (%)	100 ppm *n* = 19 (%)	200 ppm *n* = 20 (%)	400 ppm *n* = 20 (%)	
Urinary bladder						
Papillary or nodular hyperplasia	4 (20)	13 (65)[c]	14 (73.7)[d]	11 (55)[b]	11 (55)[b]	NE
Papilloma	1 (5)	12 (60)[d]	12 (63.2)[d]	11 (55)[d]	7 (35)[b]	NE
Transitional-cell carcinoma	1 (5)	10 (50)[c]	11 (57.9)[d]	12 (60)[d]	13 (65)[d]	NE
No. of tumour-bearing animals	2 (10)	17 (85)[d]	16 (84.2)[d]	17 (85)[d]	16 (80)[d]	NE
Kidney						
Adenoma	1 (5)	3 (15)	1 (5.2)	7 (35)[b]	3 (15)	
Adenocarcinoma	0	0	2 (10.5)	1 (5)	7 (35)[c]	$p < 0.01$
Nephroblastoma	4 (20)	0	4 (12.1)	6 (30)	9 (45)	$p < 0.05$
No. of tumour-bearing animals	5 (25)	3 (15)	6 (31.6)	13 (65)[b]	13 (65)[b]	$p < 0.001$
Liver						
Altered cell foci						
Clear-cell foci	10 (50)	12 (60)	14 (73.7)	19 (95)[c]	20 (100)[d]	$p < 0.001$
Basophilic foci	1 (5)	2 (10)	3 (15.8)	10 (50)[c]	17 (85)[d]	$p < 0.001$
Eosinophilic foci	1 (5)	2 (10)	8 (42.1)[c]	15 (75)[d]	16 (80)[d]	$p < 0.001$
Hyperplastic nodule	0	0	2 (10.5)	9 (45)[d]	7 (35)[c]	$p < 0.001$
Hepatocellular carcinoma	0	2 (10)	0	8 (40)[c]	8 (40)[c]	$p < 0.001$
Cholangioma	0	0	0	1 (5)	1 (5)	
Haemangioma	0	0	0	1 (5)	0	
No. of tumour-bearing animals	0	2 (10)	2 (10.5)	17 (85)[d]	13 (65)[d]	$p < 0.001$

Table 27 (contd)

Organ and finding	DMA^V					Two-tailed Cochran-Armitage analysis[a]
	0 ppm *n* = 20 (%)	50 ppm *n* = 20 (%)	100 ppm *n* = 19 (%)	200 ppm *n* = 20 (%)	400 ppm *n* = 20 (%)	
Thyroid gland						
Hyperplasia	3 (15)	4 (20)	2 (10.5)	13 (65)[c]	13 (65)[c]	$p < 0.001$
Adenoma	2 (10)	1 (5)	3 (15.8)	1 (5)	6 (30)	
Adenocarcinoma	1 (5)	1 (5)	5 (26.3)	5 (25)	4 (20)	
No. of tumour-bearing animals	3 (15)	2 (10)	8 (42.1)	6 (30)	9 (45)[b]	$p < 0.05$

From Yamamoto *et al.* (1995)

DMA^V, dimethylarsinic acid; DMBDD, *N*-nitrosodiethylamine + *N*-methyl-*N*-nitrosourea + 1,2-dimethylhydrazine chloride + *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine + *N*-bis(2-hydroxypropyl)nitrosamine; NE, not examined

[a] The significance of differences in the incidence of lesions between groups was assessed using the Fisher's exact probability test. To evaluate the dose–response relationships of the incidences in lesions in the kidney, liver and thyroid gland, two-tailed Cochran-Armitage analysis was used.

Significantly different from 0 ppm at [b] $p < 0.05$, [c] $p < 0.01$ and [d] $p < 0.001$

Development of preneoplastic lesions and tumours of the bladder (papillary or nodular hyperplasia, papillomas and carcinomas) was enhanced in a dose-dependent manner (see Table 28). Doses of 25, 50 and 100 ppm increased the incidences (%) and multiplicities (number per rat) of bladder papillomas and carcinomas. A significant increase in multiplicity of total tumours (papillomas plus carcinomas) was observed with doses as low as 10 ppm DMA^V ($p < 0.05$): 0 ppm, 0.20; 2 ppm, 0.20; 10 ppm, 0.55; 25 ppm, 1.47; 50 ppm, 2.30; 100 ppm, 2.40. Compared with controls, doses of 50 or 100 ppm significantly increased the incidence of papillary or nodular hyperplasia (Wanibuchi *et al*., 1996).

Groups of 10 male Fischer 344 rats, 6 weeks of age, were given a single intraperitoneal injection of 0 (control) or 200 mg/kg bw NDEA in saline and 2 weeks later received 0, 25, 50 or 100 ppm [µg/mL] DMA^V in the drinking-water for 6 weeks. Partial hepatectomy was performed on all animals at the end of week 3. Final body weights were decreased dose-dependently but not significantly. No significant variation in relative liver weights was noted. Dose-dependent significant increases in both numbers and areas of glutathione *S*-transferase placental form (GST-P)-positive foci in the liver were observed after initiation with NDEA; the significance was evident at doses of 50 and 100 ppm ($p < 0.01$) for numbers and at all three doses ($p < 0.05$ or $p < 0.01$) for areas [exact values were not listed because of figure]. No GST-P-positive foci were observed in groups not initiated with NDEA (Wanibuchi *et al*., 1997).

Groups of eight male NCI-Black-Reiter rats (which lack α_{2u}-globulin), 9–14 weeks of age, received 0.05% BBN in the drinking-water for 4 weeks followed by 0 or 100 ppm [µg/mL] DMA^V for 32 weeks. When killed at the end of week 36, the incidence and multiplicity of papillary or nodular hyperplasia in the bladder was significantly increased in DMA^V-treated rats (6/8 [75%]; $p < 0.05$; number per rat, 1.1 ± 1.0, $p < 0.05$) compared with rats receiving BBN alone (0/8 [0%]; number per rat, 0). A 38% incidence of bladder papillomas or carcinomas was observed in DMA^V-treated but not in control animals (Li *et al*., 1998).

Groups of 20 male Fischer 344 rats, 10 weeks of age, received a single intraperitoneal injection of 200 mg/kg bw NDEA followed 2 weeks later by DMA^V, monomethylarsonic acid (MMA) or trimethylarsine oxide (TMAO) at a dose of 100 ppm in the drinking-water for 6 weeks. Numbers of GST-P-positive foci in the liver were significantly increased in rats treated with MMA, DMA^V and TMAO compared with the controls. Areas of GST-positive foci were also significantly increased in rats treated with MMA, DMA^V and TMAO compared with the controls (Nishikawa *et al*., 2002).

Table 28. Induction of urinary bladder lesions in Fischer 344 rats treated with BBN followed by DMA^V at various doses

DMA^V (ppm)	No. of rats examined	Papillary or nodular hyperplasia		Papilloma		Carcinoma	
		Incidence (%)	No./rat	Incidence (%)	No./rat	Incidence (%)	No./rat
0 (control)	20	14 (90)	1.05 ± 95[a]	3 (15)	0.15 ± 0.37	1 (5)	0.05 ± 0.22
2	20	13 (65)	1.30 ± 1.30	2 (10)	0.10 ± 0.31	2 (10)	0.10 ± 0.31
10	20	14 (70)	1.55 ± 1.47	7 (35)	0.40 ± 0.60	3 (15)	0.15 ± 0.37
25	19	18 (95)	2.37 ± 1.17[b]	11 (58)[c]	1.05 ± 1.18[b]	7 (37)[d]	0.42 ± 0.61[e]
50	20	20 (100)[d]	2.95 ± 1.88[b]	13 (65)[f]	1.50 ± 1.36[b]	10 (50)[f]	0.80 ± 0.95[c]
100	20	20 (100)[d]	4.10 ± 3.02[b]	17 (85)[g]	1.70 ± 1.17[b]	12 (60)[g]	0.70 ± 0.66[b]

From Wanibuchi *et al.* (1996)

BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DMA^V, dimethylarsinic acid

[a] Mean ± SD

[b] $p < 0.001$ (significantly different from control, Student's *t*-test); [c] $p < 0.01$; [d] $p < 0.05$; [e] $p < 0.05$; [f] $p < 0.01$; [g] $p < 0.001$ (significantly different from control, Fisher's exact probability test)