1,2-DIMETHYLHYDRAZINE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

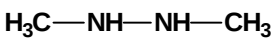
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 540-73-8
Chem. Abstr. Name: 1,2-Dimethylhydrazine
IUPAC Systematic Name: 1,2-Dimethylhydrazine
Synonyms: DMH; hydrazomethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_8N_2$ Relative molecular mass: 60.10

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Flammable, hygroscopic liquid. Fumes in air and gradually turns yellow. Characteristic ammonia-like odour of aliphatic hydrazines (Budavari, 1996)

(*b*) *Boiling-point*: 81°C (Lide, 1995)

(*c*) *Melting-point*: –9°C (United States National Library of Medicine, 1997)

(*d*) *Solubility*: Miscible with water with much evolution of heat. Also miscible with ethanol, diethyl ether, dimethylformamide and other hydrocarbons (Budavari, 1996)

(*e*) *Vapour pressure*: 9 kPa at 24.5°C (United States National Library of Medicine, 1997)

(*f*) *Conversion factor*: $mg/m^3 = 2.46 \times ppm$

1.2 Production and use

There are no known commercial uses for 1,2-dimethylhydrazine other than as a research chemical (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

Occupational exposures to 1,2-dimethylhydrazine may occur in the laboratory.

1.3.2 *Environmental occurrence*

The limited production and use of 1,2-dimethylhydrazine as a research chemical may result in its release to the environment in small quantities through various waste streams (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for 1,2-dimethylhydrazine in workplace air. Sweden and Switzerland have 8-h time-weighted average threshold limit values of 0.2 mg/m^3 and 1.2 mg/m^3, respectively, for exposure in workplace air, with a skin notation (International Labour Office, 1991).

No international guideline for 1,2-dimethylhydrazine in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2-Dimethylhydrazine was tested for carcinogenicity in mice, rats and hamsters following oral and subcutaneous or intramuscular administration, producing tumours at various sites (IARC, 1974).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female Swiss albino mice, six weeks of age, received 1,2-dimethylhydrazine continuously in the drinking-water for life at dose levels of 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10 or 20 mg/L (ppm). A positive dose–response relationship for the incidence of vascular tumours at various sites (muscle, liver, pararenal tissues) and an inverse relationship for the latency period were observed. In males and females combined, tumour incidences were: 1, 6, 9, 23, 61, 91, 95 and 79% at the above dose levels, respectively. The incidence of blood vessel tumours in controls was 1% in males and 3% in females. Tumours were angiomas and angiosarcomas; the percentage of the latter correlated positively with dose. Thus, at the four highest dose levels, almost all tumours were angiosarcomas, while at the low doses, 30–50% of all tumours were angiomas. In

addition, some animals developed lung tumours, but no association was observed between the dose of 1,2-dimethylhydrazine and lung tumour incidence (Toth & Patil, 1982).

Groups of 16 male and 16 female strain A/J mice received 1,2-dimethylhydrazine in the drinking-water at a dose of 240 mg/kg bw, the maximum tolerated dose, for eight weeks. All animals were killed 24 weeks after the initiation of the bioassay. All male and female mice surviving to the end of the experiment developed lung adenomas with an average of 13.6 ± 7.4 adenomas per mouse in males ($p < 0.001$ compared with untreated controls) and 29.4 ± 12.7 in females ($p < 0.001$ compared with untreated controls). The corresponding figures in untreated controls were 38% of males with 0.47 ± 0.63 adenomas per mouse and 25% of females with 0.29 ± 0.56 adenomas per mouse (Stoner *et al.*, 1986).

3.1.2 *Rat*

A group of 28 male Fischer rats, seven weeks of age, was treated by gavage with a single dose of 35 mg/kg bw 1,2-dimethylhydrazine hydrochloride dissolved in 0.1 M sodium acetate buffer. The animals were killed 1.5 years after treatment and 22/28 (78.6%) treated rats had colon epithelial tumours [detailed histological description not given]. One of the rats with colon tumours also developed 'a small intestinal tumour' and another had a tumour of the Zymbal gland (Schiller *et al.*, 1980).

Male and female rats of three inbred strains (DA, HS and AS2, as described by Festing & Staats, 1973), approximately 35–45 days of age, were treated at weekly intervals with 10 doses of 30 mg/kg bw 1,2-dimethylhydrazine hydrochloride in saline by gavage. Controls received saline alone. At intervals of 4, 9, 15 and 25 weeks after the first dose, three DA and three HS rats treated with 1,2-dimethylhydrazine were killed together with one control rat of each strain. The remaining surviving animals were killed 30 weeks after the first dose. Two DA and two HS rats died during the course of the experiment and all the others between weeks 20 and 30. All AS2 rats receiving 30 mg/kg bw 1,2-dimethylhydrazine died between day 6 and 59 after the first dose (profuse diarrhoea and rhinorrhoea developed within the first four days) and survival was only marginally improved by subcutaneous administration of this dose of 1,2-dimethylhydrazine. Consequently, a lower dose was used (10 mg/kg bw) and 12/20 AS2 rats survived to 30 weeks. Fifteen or more weeks after the end of 1,2-dimethylhydrazine treatment, 27.5% of treated DA rats developed diarrhoea, 10% rectal bleeding and 7.5% abdominal swelling. The incidence of large bowel tumours was 10/10 and 13/13 in male and female treated DA rats, with 6.8 tumours per rat in males and 2.8 tumours per rat in females. The incidence was somewhat lower in male HS rats (7/9) and even lower in female HS rats (5/14). The small bowel was affected less frequently than the large bowel ($p < 0.05$), particularly with regard to tumour multiplicity (see Table 1). Histologically, the intestinal tumours were classified as adenocarcinomas. In addition, one hepatocellular carcinoma, seven cholangiomas and three liver angiosarcomas and two Zymbal gland tumours developed in 12 AS2 rats [sex unspecified] treated with 10 mg/kg bw

Table 1. Incidence of intestinal tumours in DA, HS and AS2 rats 30 weeks after the beginning of 1,2-dimethylhydrazine treatment by gavage

	DA		HS		AS2	
	Males	Females	Males	Females	Males	Females
Rats with large bowel tumours	10/10	13/13	7/9	5/14	8/10	2/2
No. of tumours per rat	6.8	2.8	1.4	0.4	2.3	3.5
Rats with small bowel tumours	7/10	1/13	2/9	2/14	7/10	2/2
No. of tumours per rat	0.8	0.08	0.2	0.1	1.6	1.0

From Teague *et al*. (1981)

1,2-dimethylhydrazine. In DA rats, 7/21 males and 0/19 females developed Zymbal gland tumours, with invasion into the bone in all but one case (Teague *et al*., 1981).

In a study of the relationship between colon tumours and the gut-associated lymphoid tissue, 1,2-dimethylhydrazine hydrochloride dissolved in saline was administered by gavage to outbred male weanling Sprague-Dawley rats starting at seven weeks of age. In experiment 1, 20 rats received five weekly doses of 15 mg/kg bw 1,2-dimethylhydrazine with either 5% mixed fat or 24% corn oil in the diet and were killed after the last treatment. No intestinal tumours were found. In experiment 2, four groups of 10 rats received five weekly doses of 65 mg/kg bw 1,2-dimethylhydrazine and were fed diets containing different types and levels of fat (5% mixed fat, 24% beef tallow, 24% corn oil or 25% Crisco). The 33 surviving animals were killed four months after the first dose. There was no difference in the incidence of colon tumours between diet groups. A total of 49 colon adenocarcinomas developed in the 33 rats, 71% of which were polypoid and 29% sessile. Fifty per cent of the sessile tumours and none of the polypoid tumours were associated with colonic lymphoid aggregates ($p < 0.001$). In experiment 3, four groups of 60 rats were fed different diets and received 15 mg/kg bw 1,2-dimethylhydrazine once a week for five weeks. The animals were killed when moribund or when they showed clinical signs of intestinal tumours. A total of 165 colon tumours developed in 159 rats, 38% of which were polypoid and 62% sessile. As in experiment 2, a highly significant association between colonic tumours and the presence of lymphoid aggregates in a given segment of the colon was found only for sessile adenocarcinomas (28% versus 0 in polypoid adenocarcinomas) (Nauss *et al*., 1984). [The Working Group noted that only the total number of colon tumours but not the number of rats with tumours was indicated.]

In a study designed to investigate the role of caloric restriction, 38 male Fischer 344 rats (50 days of age) were given 30 mg/kg bw 1,2-dimethylhydrazine hydrochloride dissolved in saline by gavage once a week for six weeks. One week after the final dose, the rats were randomized into groups of 19 and fed one of two semi-purified diets. One group was fed *ad libitum*, with 30 g of food given to each rat. Rats in the calorically restricted group were given 60% of the weight of food that the rats fed *ad libitum* had

consumed the day before. Rats had an average weight of 257 g one week after the final dose of 1,2-dimethylhydrazine. Rats fed *ad libitum* had a final mean weight of 372 ± 6 g. The calorically restricted rats lost weight during the first four weeks and then reached a plateau with slight weight loss to a final weight of 216 ± 4 g ($p < 0.001$). The animals were killed after 28 weeks of feeding the semi-purified diets. All 19 rats (100%) fed *ad libitum* developed tumours of the colon versus 10/19 (53%) of the calorically restricted rats; the incidence of invasive carcinomas was 11% and 6%, respectively ($p < 0.05$). Extra-colonic tumours (those of the duodenum and ear canal) were seen in 32% and 11% ($p < 0.05$) of rats fed *ad libitum* and calorically restricted rats, respectively (Klurfeld *et al*., 1987).

3.2 Subcutaneous, intraperitoneal, intramuscular, intrarectal or intraplacental administration

3.2.1 *Mouse*

A group of 60 female CF_1 mice received six weekly subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine dihydrochloride. Forty-three survivors were killed 45 weeks after the onset of the experiment and 'over 83%' of them (i.e., 36 mice) developed visible colonic neoplasms with 2.3 tumours per animal. The 3-cm segment of the colon above the anus contained 61% of all neoplasms. Thirteen of the 43 surviving 1,2-dimethylhydrazine-treated mice demonstrated some degree of carpeting, i.e. uncountable numbers of tumours. Histologically, the tumours showed the full spectrum of neoplastic lesions from morphologically benign polyps to adenocarcinomas (Deschner & Long, 1977).

Female mice of eight different strains were treated with 25 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base). Survivors were killed 50 weeks after the beginning of treatment. Colon tumours developed in all strains, with the highest incidence in BALB/c mice (93.3%) and the lowest incidence in C3HA mice (30.9%) (Table 2). Likewise, tumours of the anal region and clitoral glands developed in mice of all strains with incidence varying from 24% (F_1 hybrid and C57BL/6) to 63.3% (BALB/c strain). Sarcoma of the uterus developed in about 40% in C3H and CBA mice, 20.7% in F_1 hybrids, 7.7% in AKR mice, 2.7% in C57BL/6 mice, but no such tumours in BALB/c, DBA/2 and C3HA mice. Ovarian lesions (mainly big haemorrhagic cysts) were most frequent in BALB/c, DBA/2 and C3HA mice (40.7, 62.9 and 85.7, respectively) and rather rare in C3H and CBA mice (6% and 3.7%, respectively) (see Table 2). None of the above tumours was found in untreated mice of the same strains (Turusov *et al.*, 1982).

Groups of female CBA mice or male and female (C57BL × CBA)F_1 mice were given 1,2-dimethylhydrazine subcutaneously, intraperitoneally or by gavage daily, weekly or once every two weeks. When female CBA mice were given 1,2-dimethylhydrazine weekly subcutaneously, intraperitoneally or by gavage (groups 1, 2 and 3) at a dose of 8 mg/kg bw (base) for 25 weeks, survival was the same, as was the incidence of tumours in the intestine, uterus or anal region. The incidence of liver haemangioendothelioma

Table 2. Incidence (%) of tumours at various sites and ovarian lesions in eight strains of mice treated with 1,2-dimethylhydrazine (8 mg/kg bw for 25 weeks) subcutaneously

	C3H	CBA	(CBA×C57BL/6J)F_1	C57BL/6J	BALB/c	DBA/2	C3HA	AKR
Effective no.[a]	16	29	29	37	30	27	42	39
Colon tumours	75	70.4	79.3	59.4	93.3	55.5	30.9	53.8
Anal region/clitoral gland tumours	50	59.2	24.1	24.3	63.3	25.9	59.5	38.5
Uterine sarcomas	37.5	40.7	20.7	2.7	–	–	–	7.7
Ovarian lesions	6.3	3.7	17.2	35.1	46.7	62.9	85.7	10.2
Liver haemangio-endotheliomas	6.3	18.4	37.9	5.4	23.3	22.2	14.3	20.5

From Turusov *et al.* (1982)

[a] Number of mice surviving 20 weeks after the beginning of treatment with 1,2-dimethylhydrazine

was highest after intraperitoneal administration (13/39 versus 7/34 and 5/27 after oral gavage or subcutaneous administration, respectively). Groups 4, 5 and 6 received the same dose by subcutaneous administration either every two weeks (16 mg/kg bw as a single treatment), weekly (8 mg/kg bw) or daily (1.14 mg/kg bw) for 30 weeks. In the daily treatment group (group 6), all mice died by the end of 1,2-dimethylhydrazine treatment, with ascites and cirrhotic livers which were firm and reduced in size. No such liver damage was observed in groups 4 and 5. Tumour incidence was somewhat higher in group 5 (weekly treatment) than in group 4 (bi-weekly treatment). A comparison with group 6 (daily injections) is not possible because of the short lifespan. Nevertheless, the incidence of liver haemangioendotheliomas in group 6 was similar to that in group 5 (5/28 versus 8/29). Groups 7 and 8 received subcutaneous daily or weekly treatments at a total dose one-third that of groups 5 and 6 (for 10 weeks instead of 30 weeks). Consequently, the lifespan in both groups was similar (no considerable cirrhosis of the liver developed after daily treatment). The incidence of colon tumours was much lower in group 8 (3/69), receiving daily treatments, than in group 7 (12/36) that received weekly treatments ($p < 0.001$) and the same effect was observed with tumours of the anal region (Table 3). Daily treatment resulted in a much higher incidence of renal adenomas (43/69 versus 8/36 in groups 8 and 7, respectively) and liver haemangioendotheliomas (29/69 and 4/36, respectively) as compared to weekly treatment, with no change in the incidence of liver adenoma, lung adenoma and uterine sarcoma. When 1,2-dimethylhydrazine was given in the drinking-water (groups 12 and 13), survival was eight to nine weeks shorter than after administration of the same total dose by subcutaneous injection (groups 10 and 11). In spite of this, the incidence of vascular tumours of the renal capsule in males (group 13) was significantly higher (16/20) than that in males receiving subcutaneous injections (9/20; $p < 0.001$). The decrease in groups 12 and 13 (daily treatment) of the incidence of tumours of the colon and anal region (see Table 3) is still real when allowance is made for shorter survival. Thus, the negative correlation between the incidence of vascular and intestinal tumours is determined not by the route of 1,2-dimethylhydrazine administration but by the size of a single dose, with a predominance of vascular tumours at low single doses and that of intestinal tumours at high single doses (Turusov *et al.*, 1983).

Male mice of six different strains were treated with 15 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (base) and were killed 42–43 weeks after the beginning of treatment. Tumour incidence is given in Table 4. The incidence of colon tumours varied from 3–4% (C3H and C3HA strain) to 74% in C57BL mice. The incidence of kidney adenomas ranged from 13 and 14% in C3H and C3HA mice to 79% in CBA mice. Angiosarcomas of the renal capsule developed in almost all mice of the CBA strain (97%) and only in 4% and 7% in C57BL and C3HA mice (Table 4) (Turusov *et al.*, 1985).

Groups of 16 male and 16 female strain A/J mice received intraperitoneal injections of 48, 120 or 240 mg/kg bw 1,2-dimethylhydrazine three times per week for eight weeks, at which time all animals were killed. Lung adenomas were found in 63%, 63% and 94% of male mice at the three dose levels used, with average numbers of adenomas per mouse of

Table 3. Route of administration and dose fractionation in 1,2-dimethylhydrazine carcinogenesis in mice

Group	Strain	Sex	Treatment			Effective no. of mice	Mice with tumours of the									
			Route	Frequency	Duration (wks)		Colon	Anal region	Kidney (adenoma)	Liver		Uterus (sarcoma)	Lung	Blood vessels		
										Adenoma	HAE			Renal capsule	Ovary	Other sites
1	CBA	F	gavage	Weekly	25	34	23	14	–	–	7	16	2	–	–	–
2	CBA	F	i.p.	Weekly	25	39	24	22	–	–	13	11	–	3	2	–
3	CBA	F	s.c.	Weekly	25	27	19	16	–	2	5	11	–	–	–	–
4	CBA	F	s.c.	Bi-weekly	30	28	17	17	–	–	9	12	–	–	–	–
5	CBA	F	s.c.	Weekly	30	29	21	23	–	–	8	14	–	–	2	–
6	CBA	F	s.c.	Daily	30	28	–	6	–	–	5	–	–	–	–	–
7	CBA	F	s.c.	Weekly	10	36	12	21	8	16	4	6	7	–	–	–
8	CBA	F	s.c.	Daily	10	69	3	17	43	28	29	12	15	–	4	–
9	CBA	F	Control	–	–	40	–	–	–	1	–	–	–	–	–	–
10	F_1	F	s.c.	Weekly	25	33	24	10	6	3	16	19	3	1	5	2
11	F_1	M	s.c.	Weekly	20	48	41	14	21	11	6	–	3	9	–	4
12	F_1	F	d.w.	Continuous	25	19	–	–	9	–	11	2	1	4	3	6
13	F_1	M	d.w.	Continuous	20	19	–	–	2	–	7	–	1	16	–	5
14	F_1	F	–	–	–	20	–	–	–	–	–	–	–	–	–	–
15	F_1	M	–	–	–	19	–	–	–	–	–	–	4	–	–	–

From Turusov *et al*. (1983)
HAE, haemangioendothelioma; wks, weeks; i.p., intraperitoneal; s.c., subcutaneous; d.w., drinking-water

Table 4. Strain differences in tumour incidences in mice that received 15 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine base

Strain	Effective no. of mice	% Animals with				
		Renal capsule angiosarcomas	Renal adenomas	Colon tumours	Liver haemangioendotheliomas	Tumours of anal region
C3H	31	35	13	3	13	13
CBA	33	97	79	33	15	18
(CBA×C57BL)F_1	47	36	30	54	9	21
C57BL	47	4	23	74	0	32
BALB/c	23	13	43	26	9	48
C3HA	28	7	14	4	0	14

From Turusov *et al.* (1985)

0.81 ± 0.98, 1.06 ± 1.12 and 4.25 ± 2.21, respectively. The figures for females were 56%, 69% and 100% with average numbers of adenomas per mouse of 0.75 ± 0.86, 1.81 ± 1.87 and 10.63 ± 5.69. Corresponding figures for controls were 7% and 0.07 ± 0.27 in males and 47% and 0.60 ± 0.83 in females. The difference from controls was significant ($p < 0.05$ to 0.001) in all groups except females given the lowest dose (Stoner *et al*., 1986).

Groups of male and female CBA mice received 8, 16 or 32 subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base) and were allowed to live until their natural death or were killed when moribund. The incidence of tumours is presented in Table 5. Clear-cut dose–response relationships were observed in the incidence of colon tumours in females and, in part, in males; vascular kidney tumours (renal capsule angiosarcomas) in males; and liver haemangioendotheliomas, uterine sarcomas and ovarian lesions in females. Decreased tumour incidence at some sites (colon, anal region, liver adenomas) at the highest dose in males was due to the early mortality from vascular kidney tumours. In the same study, there were additional groups of mice of similar size receiving the same number of injections of deuterium-labelled 1,2-dimethylhydrazine. The influence of the deuterium-labelling on tumour development at various sites is shown in Table 6 (Turusov *et al.*, 1988).

In another experiment, 37–40 mice per group were given one, two or four injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base) and the mice lived until their natural death. The incidence of epithelial kidney tumours was 5.3%, 5.0%, 55.3% and 64.9% after 0, 1, 2 and 4 injections, respectively. All kidney tumours in the control group or after one injection of 1,2-dimethylhydrazine were small incidental adenomas, while half of the kidney tumours after two or four injections were fatal (Turusov *et al.*, 1990). One male receiving four injections of 1,2-dimethylhydrazine developed a nephroblastoma, a tumour which is exceedingly rare in mice (Turusov, 1992).

Table 5. Incidence of tumours in CBA mice that received 8, 16 or 32 weekly subcutaneous injections of 8 mg/kg (base) bw 1,2-dimethylhydrazine

No. of injections	Sex	Effective no. of mice	Kidney tumours		Colon tumours (%)	Tumours of anal region[a] (%)	Tumours of the liver		Uterine sarcomas (%)	Lesions[b] of ovary (%)
			Vascular (%)	Epithelial (%)			Haemangio-endotheliomas (%)	Adeno-mas (%)		
0	F	44	0	0	0	0	0	27.3	2.3	6.8
8	F	29	3.4	17.2	20.7	44.3	24.1	13.8	31.0	0
16	F	25	0	16.0	44.0	44.0	24.0	12.0	60.0	12.0
32	F	25	4.0	16.0	56.0	44.0	36.0	4.0	52.0	20.0
0	M	48	0	4.2	0	0	0	64.6		
8	M	20	45.0	75.0	40.0	15.0	30.0	20.0		
16	M	20	75.0	55.0	65.0	55.0	30.0	20.0		
32	M	20	85.0	40.0	25.0	15.0	15.0	10.0		

From Turusov *et al.* (1988)

[a] Clitoral or preputial gland

[b] Haemorrhagic cysts or angiomas

Table 6. Combined relative risks (odds ratios)[a] of various tumours in CBA mice that received 1,2-dimethylhydrazine versus those that received 1,2-di-[$^2H_3$]methylhydrazine

Tumour type	Females	Males
Vascular renal tumours	1.90	0.48
Epithelial renal tumours	0.79	1.07
Tumours of the anal region	2.50[b]	2.60[b]
Colon tumours	1.63	5.85[c]
Liver haemangioendotheliomas	0.77	1.45
Hepatomas	0.94	0.27[c]
Uterine sarcomas	1.54	
Ovarian angiomas	0.95	
Lung adenomas	0.59	

From Turusov *et al.* (1988)

[a] Relative risk was calculated by using the one-tailed exact method for the combination of 2×2 tables.

[b] Relative risk differed significantly from 1, $p < 0.05$

[c] Relative risk differed significantly from 1, $p < 0.01$

3.2.2 *Rat*

Subcutaneous administration

Groups of BD IX rats [sex not specified] received a single subcutaneous injection of 40 mg/kg bw 1,2-dimethylhydrazine at the age of one day (11 rats), 10 days (7 rats) or 30 days (13 rats). The rats lived until their natural death. Among 11 rats treated at one day of age, one developed a colon carcinoma, with no dysplasia or hyperplasia in other rats. Among rats treated at the age of 10 days, 3/7 developed 4 colon carcinomas. One rat had a dysplasia and another had a hyperplasia. Four of 13 rats treated at the age of 30 days developed 13 carcinomas and three rats developed carcinomas of the small bowel. Six rats had dysplastic and four rats hyperplastic colon lesions. One rat had a dysplastic lesion and one a hyperplastic lesion of the small bowel (Martin *et al.*, 1974).

Groups of seven-week-old (12 conventional and 12 germ-free) female Fischer rats received 20 weekly subcutaneous injections of 10 mg/kg bw 1,2-dimethylhydrazine and were autopsied one week after the last injection. Another group of 24 conventional and 18 germ-free rats received the same treatment but were killed 20 weeks after the last injection. None of the germ-free rats that were autopsied after the last injection of 1,2-dimethylhydrazine had developed colon tumours, while 2/12 conventional rats showed adenocarcinoma. At 40 weeks, all colon tumours in germ-free rats were adenomas, whereas in conventional rats, four out of a total of six colon tumours were adenocarcinomas (Table 7) (Reddy *et al.*, 1974).

Groups of 15 conventional and 24 germ-free female Fischer rats, 50 days of age, were given 20 weekly subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine

Table 7. Incidence of colon tumours in germ-free and conventional rats treated with 10 mg/kg bw 1,2-dimethylhydrazine subcutaneously

Status	Study duration (weeks)	No. of animals with colon tumours	Tumour	
			No. of adeno-carcinomas	No. of adenomas
Germ-free[a]	20	0/12	0	0
Conventional[a]	20	2/12	2	0
Germ-free[b]	40	2/18	0	2
Conventional[b]	40	6/24	4	2

From Reddy *et al.* (1974)

[a] Animals received subcutaneous injections of 10 mg/kg bw weekly for 20 weeks and then were autopsied.

[b] Animals received subcutaneous injections of 10 mg/kg bw weekly for 20 weeks and were autopsied 20 weeks after last injection (40 weeks total).

dihydrochloride. The rats were killed 15 weeks after the last injection. 1,2-Dimethylhydrazine induced squamous-cell carcinomas of the ear duct, mesenchymal tumours of the kidney, adenomas and adenocarcinomas of the small intestine (mainly in the duodenum) in conventional rats but not in germ-free animals. The multiplicity of colon tumours was much higher in conventional rats (Table 8) (Reddy *et al.*, 1975).

A total of 25 male and 16 female Wistar-Furth (WF) rats, 10 weeks of age, were divided into two groups [number of rats per group not specified], one of which was treated with 1,2-dimethylhydrazine and the other served as untreated control. Nine male and 16 female of Long-Evans (LE) rats of the same age were also given subcutaneous doses of 20 mg/kg bw 1,2-dimethylhydrazine weekly for 20 weeks. Three to six rats of each sex were killed at the 10th, 15th and 25th week after the last injection. The total incidence of intestinal tumours was 70% (12/17) in WF rats and 100% (17/17) in LE rats. The number of intestinal tumours per rat was 1.5 (26/17) for all tumours, 1.2 for carcinomas in WF rats and 5.7 (97/17) and 2.7, respectively, in LE rats; metastatic deposits were found in 3/17 WF rats and 8/17 LE rats. Autoradiographic study did not reveal any difference between the two strains of either the treated group or non-treated controls in relation to the number of [^{3}H]thymidine-labelled cells or of mitotic cells in the mucosa of the descending colon (Takizawa *et al.*, 1978). [The Working Group noted the small number of animals and the lack of information on distribution by sex.]

A group of 15 male Fischer 344 rats, 11 weeks old, received 20 weekly subcutaneous injections of 10 mg/kg bw 1,2-dimethylhydrazine dihydrochloride. The rats were killed 44 weeks after the first injection. A second group of 28 male Fischer rats received 20 weekly subcutaneous injections of 20 mg/kg bw and the rats were killed 31 weeks after the first injection. Five animals of Group 1 developed large bowel adenocarcinomas (1.2 tumour/colon-tumour-bearing rat or 0.4 tumour per rat in the whole group). Twenty-two

Table 8. Tumour incidence in germ-free and conventional rats treated with 20 mg/kg bw 1,2-dimethylhydrazine for 20 weeks

Status	No. of rats	No. of animals with tumours				Multiplicity of colon tumours (per rat)		
		Ear canal	Kidney	Small intestine	Colon	Total tumours	Adenocarcinoma	Adenoma
Germ-free	24	0	0	0	5	$0.2 \pm 0.1^{a,b}$	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}
Conventional	15	13	3	12	14	2.1 ± 0.4	1.2 ± 0.2	0.9 ± 0.3

From Reddy *et al.* (1975)

[a] Mean ± S.E.

[b] Significantly different from conventional group: $p < 0.01$

animals in Group 2 developed large bowel tumours (total 54 or 2.5 tumours per colon-tumour-bearing rat or 1.9 per rat in the group). There were four small bowel adenocarcinomas in Group 1 and 12 small bowel adenocarcinomas in Group 2. One rat (6%) in Group 1 and 21 rats (75%) in Group 2 developed squamous-cell carcinomas of the ear canal (Hagihara *et al.*, 1980).

A group of 60 female Wistar rats, 15 weeks of age, received two subcutaneous injections of 120 mg/kg bw 1,2-dimethylhydrazine (calculated as base) at 10-day intervals. Twenty-three animals were killed 30 weeks after the first injection. Thirteen of 23 animals exhibited tumours of the colon and one a tumour of the small intestine. Twenty-one (91%) of 23 rats developed renal tumours; in 10 rats, they were bilateral and in six rats, there was more than one tumour per affected kidney. All neoplasms were diagnosed as mesenchymal kidney tumours (Sunter & Senior, 1983).

A group of 40 weanling outbred female Wistar rats received a single subcutaneous injection of 200 mg/kg bw 1,2-dimethylhydrazine. Four rats died within four days after the injection. Twenty-five of 36 rats that survived for more than four days and were killed within one year after 1,2-dimethylhydrazine administration developed renal tumours, nine of them bilateral. Pulmonary metastases were found in one animal. Twenty-five (73%) of 34 tumours examined histologically were diagnosed as nephroblastoma-like tumours similar to human Wilms' tumour, three were mesenchymal tumours, three were adenomas, two were adenocarcinomas and one was unclassifiable (Sadrudin *et al.*, 1985).

Five groups of 34 male weanling Sprague-Dawley rats received either fibre-free basal diet or the basal diet uniformly diluted by the addition of a citrus pectin, guar gum or cellulose, so that the final diet consisted of 10% (by weight) fibre source and 90% basal diet. Oat bran, containing 26% fibre, was added into the diet of the fourth experimental group which thus was fed only a 5.2% fibre diet. Twenty-four rats in each diet group received 12 weekly subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine. The control rats were killed at 35 weeks and all carcinogen-treated rats were killed 30 weeks after the start of the study (only four rats did not survive until this time). The percentage of rats developing large bowel tumours was higher in all groups fed diets supplemented with fibre, but the increase was only significant in the group given guar gum (62.5% versus 33.4% in the fibre-free group, $p < 0.05$). There was a four-fold increase in the percentage of rats with proximal colonic adenocarcinomas in the groups fed oat bran, pectin and guar gum as compared to the fibre-free controls ($p < 0.025$) [data presented graphically] (Jacobs & Lupton, 1986).

Three groups of male Sprague-Dawley rats, weighing approximately 200 g, received subcutaneous injections of 21 mg/kg bw 1,2-dimethylhydrazine either as a single treatment (109 rats) or as weekly injections for three months (97 rats) or as weekly injections for six months (100 rats). Among rats receiving a single treatment, seven developed colonic carcinomas which all originated in the areas of lymphoid patches, this being considered by the authors as a proof of carcinoma development *de novo*. No adenomas were observed in this group. After weekly treatment for three months, 54 colonic tumours developed (4 adenomas and 50 adenocarcinomas). In 23 of the latter (46%), the

adenocarcinoma was seen to originate in a pre-existing adenoma and in three (6%) from discrete lymphoid patches. In the 100 rats treated for six months, 65 colonic tumours developed (8 adenomas and 57 adenocarcinomas). In 25 of 57 adenocarcinomas (43.9%), these were seen to originate from a pre-existing adenoma and in 2 (3.5%) from discrete lymphoid patches. In the remaining 30 adenocarcinomas of this group and in 21 adenocarcinomas of the three-month 1,2-dimethylhydrazine-treatment group, the origin of invasive growth could not be established (Rubio *et al.*, 1986).

Groups of male and female outbred SPF rats of the Riph:Wist stock were given subcutaneous injections of 1,2-dimethylhydrazine dihydrochloride starting from the first day of life up to 5, 10, 15 or 20 days after birth. The total dose of 1,2-dimethylhydrazine ranged from 0.36 to 2.16 mg per animal. [The interval between injections is not indicated, but probably these were daily treatments]. The animals were killed when moribund and the maximum survival was one year. No malignant tumour was found in control rats (5 males and 6 females). Hepatocellular carcinomas and kidney (mesenchymal) tumours developed in all groups of rats treated with 1,2-dimethylhydrazine. Thus in the group treated from day 1 to day 15, five of six survivors had liver carcinoma and all six rats had kidney mesenchymal tumours. The numbers of rats at risk in various groups varied from 1 to 11. Of a total of 73 rats, 32 developed malignant liver tumours and 50 developed mesenchymal kidney tumours, 22 of them being bilateral tumours (Sykora *et al.*, 1986).

Eight groups of 10–20 weanling female Wistar rats received subcutaneous injections of 1,2-dimethylhydrazine according to various protocols. All rats were killed when moribund and the experiment was terminated 27 months after the first injection. In Group A receiving 10 weekly injections of 15 mg/kg bw 1,2-dimethylhydrazine, 7 out of 14 rats examined developed intestinal carcinomas (3 colonic and 4 small intestinal carcinomas), 10 kidney fibrosarcomas, 3 hepatocarcinomas and 3 ear duct carcinomas. In Group B, given quarterly injections of 15 mg/kg bw 1,2-dimethylhydrazine, five out of seven rats examined developed intestinal carcinomas and one kidney fibrosarcoma, two hepatocarcinomas, one ear duct carcinoma and two mammary carcinomas were also seen. In Group C that received 27 weekly injections of 1.5 mg/kg bw 1,2-dimethylhydrazine, 1/16 rats had a colonic carcinoma and two rats had hepatocholangioma. Fifteen rats did not show any colonic lesion. In Group D, that received a single injection of 40 mg/kg bw 1,2-dimethylhydrazine, 1/7 rats had a colonic carcinoma. Twelve epithelial hyperplasias and 51 dysplasias were also found. In Group E, receiving eight quarterly injections of 5 mg/kg bw 1,2-dimethylhydrazine, 2/17 rats had colonic carcinomas; two dysplasias were also found. In Group F, that received a single injection of 20 mg/kg bw, 3/9 rats had colonic carcinomas, one of these rats having three carcinomas. Seventeen epithelial hyperplasias and twenty dysplasias were also found. In group G, receiving eight quarterly injections of 1 mg/kg bw 1,2-dimethylhydrazine, no carcinoma developed in 10 rats. No histologically detectable lesions were found in 19 control rats (Decaens *et al.*, 1989).

Eighteen Sprague-Dawley rats [sex not specified] received 19 weekly subcutaneous injections of 21 mg/kg bw 1,2-dimethylhydrazine and were killed 24 and 26 weeks after

the first injection. Twenty-three colon tumours and 10 tumours of the small bowel were observed. In addition, 15 tumours of the auditory canal were found in 10 rats: four squamous carcinomas, 10 papillomas and a pseudoepitheliomatous hyperplasia (Viñas-Salas *et al.*, 1992).

Methylazoxymethanol acetate

Groups of 10 male and female (combined) Sprague-Dawley rats, 40 days of age, susceptible to 1,2-dimethylhydrazine-induced colon tumours and a similar group of Lobund Wistar rats resistant to such induction received 10 weekly subcutaneous injections of 30 mg/kg bw methylazoxymethanol acetate, a metabolite of 1,2-dimethylhydrazine. All surviving rats were killed 20 weeks after the first injection. The carcinogenic effect in the Sprague-Dawley rats was much stronger than in Lobund Wistar rats (see Table 9). The authors concluded that the difference in susceptibility to colon tumour induction between the strains is partially due to inability to metabolize 1,2-dimethylhydrazine but also to some other endogenous factors (Pollard & Zedeck, 1978).

Table 9. Effect of methylazoxymethanol acetate on Sprague-Dawley and Lobund Wistar rats

Rat	No. of rats with tumours/no. of rats examined	No. of tumours in:			Total no. of tumours	No. of tumours/ rat
		Rectum	Colon	Duodenum		
Lobund Wistar	7/7[a]	2	11	9	22	3.1
Sprague-Dawley	10/10	4	84	116	204	20.4

From Pollard & Zedeck (1978)

[a] Three rats died soon after the onset of the experiment.

Intramuscular administration

A group of 25 male Fischer 344 rats, eight weeks of age, received weekly intramuscular injections of 20 mg/kg bw 1,2-dimethylhydrazine for 20 weeks. Rats were killed eight months after the beginning of treatment. Twenty-two (88%) rats developed large bowel tumours and 22 (88%) developed tumours of the small bowel. The average number of tumours per rat was 3.5. The tumours occurred most frequently in the descending colon followed by the transverse colon. A similar group of 98 rats received implantations of large bowel mucosa into the glandular stomach followed by the same treatment with 1,2-dimethylhydrazine as above. Forty-seven of 60 rats with successful grafts in the stomach developed tumours in the grafted colorectal tissue. Most successful were the grafts from the transverse and descending colon (100% and 93%, respectively) and the least successful were those from the proximal ascending colon (33%); grafts from the ascending colon (62%) and rectum (76%) had intermediate values. The percentage of poorly differentiated tumours was much higher in the colorectal tissue grafted from any

site of the large bowel compared to the intrinsic frequency, with an especially large increase in the grafts from the rectum. In both groups treated with 1,2-dimethylhydrazine, ear duct tumours were observed, their total incidence being 37% (Nakagawa *et al.*, 1992).

Intrarectal administration

Groups of 28 conventional and 28 germ-free Fischer rats received weekly intrarectal injections of 20 mg/kg bw 1,2-dimethylhydrazine for 20 weeks. The rats were killed 15 weeks after the last 1,2-dimethylhydrazine injection. A much higher incidence of squamous-cell carcinomas of the ear canal, mesenchymal kidney tumours, carcinomas of the small intestine and colon was observed in conventional rats than in germ-free rats (Table 10). (Reddy *et al.*, 1976).

3.2.3 *Hamster*

A group of 40 male Syrian golden hamsters received weekly intramuscular injections of 43 mg/kg bw 1,2-dimethylhydrazine dihydrochloride (total dose, 142.2 mg/kg bw). A group of 12 hamsters served as controls. All animals were observed until natural death. Fifteen treated animals died of toxicity before the end of dosing. Among the 25 surviving animals, nine developed gastrointestinal tumours (one stomach, one adenocarcinoma of the duodenum, four adenocarcinomas of the ileum and three adenocarcinomas of the colon). Five animals developed hepatocellular carcinomas (Osswald & Krüger, 1969).

3.2.4 *Monkey*

1,2-Dimethylhydrazine was administered subcutaneously to nine *Macaca fascicularis* monkeys (6 males and 3 females) at doses of 16 mg/kg bw, three times per month for up to two years. The experiment was terminated 275 weeks after the first injection, when three surviving monkeys were killed under ether. The total doses of 1,2-dimethylhydrazine received over the entire period ranged from 198 to 7143 mg. Tumours were found in three monkeys: two males developed tumours in the colon and one female in the uterus. Colon tumours were observed 34 and 47 weeks after the beginning of treatment, when the total doses of 1,2-dimethylhydrazine were 1080 mg (528 mg/kg bw) and 3696 mg (400 mg/kg bw), respectively. The death of these animals was preceded by loss of weight, absence of appetite and diarrhoea. Infiltration in the abdomen was palpated. The tumours in both cases were located in the ascending colon. Histologically, the colon tumours had the structure of adenocarcinomas, in one case with complexes of tumour cells in lymphatic vessels. No metastases were found. No intestinal tumours were found in the untreated monkeys. A female that received a total dose of 3648 mg (608 mg/kg bw) 1,2-dimethylhydrazine died of uterine bleeding 55 weeks after the onset of the experiment. At necropsy, a tumour of the corpus uteri was found; the tumour was a fibromyoma with very rare mitoses (Beniashvili *et al.*, 1992).

Table 10. Tumour incidence in germ-free and conventional Fischer rats treated intrarectally with 1,2-dimethylhydrazine

Status	No. of rats	No. of animals with tumours				Multiplicity of colon tumours (per rat)[a]		
		Ear canal	Kidney	Small intestine	Colon	Total tumours	Adeno-carcinoma	Adenoma
Germ-free	28	2	0	3	12	1.0[b] ± 0.1	0.50 ± 0.05	0.50 ± 0.06
Conventional	28	14	10	10	24	2.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1

From Reddy *et al.* (1976)

[a] Mean ± SE

[b] Significantly different from conventional group, $p < 0.05$

3.3 Transplacental exposure

Six female and three male *Macaca fascicularis* monkeys received 16 mg/kg bw 1,2-dimethylhydrazine by subcutaneous injection twice a month for 12 months. Males and females were kept together. Six female monkeys became pregnant while receiving 1,2-dimethylhydrazine; the deliveries took place 182, 237, 248, 305, 312 and 327 days after the first dose of carcinogen. The total amount of 1,2-dimethylhydrazine varied from 527 to 725 mg; 9 or 10 doses were given. Kept with their mothers during the rearing period, the three male and three female offspring were not subjected to any intervention, whereas the mothers continued to receive 1,2-dimethylhydrazine. Development of the offspring was normal, and all the animals were followed for over seven years. Two offspring died and postmortem examination revealed kidney tumours in both. Of these, monkey No. 1 was a 19-month old female; the amount of 1,2-dimethylhydrazine received by the mother was 177 mg before fertilization, 527 mg during the pregnancy and 710 mg during the neonatal and early development period. The cause of death was bilateral pneumonia. Histologically, the tumour consisted of mesenchymal and epithelial components, the latter resembling embryonal glomeruli. No tumour at any other site was found. Monkey No. 2 was a 14-month-old female. The mother received 560 mg 1,2-dimethylhydrazine before fertilization, 725 mg during embryogenesis and 640 mg during lactation. The monkey died of respiratory failure. Postmortem examination revealed a dense tumour in the cortex of the right kidney and nodes in both lungs. Histologically, the kidney tumour had a variable structure with mesenchymal and epithelial components. The epithelial component contained structures resembling embryonal glomeruli and tubule areas. Pulmonary metastases had the same structure. In both cases, a diagnosis of nephroblastoma was established (Beniashvili, 1989).

3.4 Administration with modifying agents

3.4.1 *Mouse*

A group of 30 female CBA mice (susceptible to 1,2-dimethylhydrazine-induction of uterine sarcomas), 2–3 months of age, received 10 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine base (group 1). In three other groups, oestradiol dipropionate dissolved in olive oil was administered subcutaneously one day before each injection of 1,2-dimethylhydrazine, for a total of 10, 20 or 25 treatments. A fifth group received 25 injections of oestradiol propionate without 1,2-dimethylhydrazine. Mice were killed 50 weeks after the first treatment. The incidence of uterine sarcomas was 1/30, 7/28, 17/26 and 19/26 in groups 1, 2, 3 and 4, respectively. The difference between group 1 (1,2-dimethylhydrazine alone) and the groups in which 1,2-dimethylhydrazine was combined with oestradiol propionate was significant ($p < 0.05$ to $p < 0.001$). There was a negative dose–response relationship in the time of the observation of the first uterine sarcoma at necropsy (Table 11). No uterine tumours were observed in group 5, which received oestradiol propionate alone (Turusov *et al.*, 1980).

Two groups of 36 and 40 female C3HA mice (a strain resistant to 1,2-dimethylhydrazine induction of uterine sarcomas), 2–3 months of age, received 20 weekly sub-

Table 11. Incidence of uterine sarcomas in CBA mice given 1,2-dimethylhydrazine combined with oestradiol dipropionate subcutaneously 50 weeks after the start of experiment

Group	Treatment	Effective no. of mice	Mice with uterine sarcomas		*p* value compared with group 1	Time (weeks) of observation of the first uterine sarcoma
			No.	%		
1	DMH	30	1	3.3		49
2	DMH + EP 10 weeks	28	7	25.0	0.05	40
3	DMH + EP 20 weeks	26	17	65.4	0.001	17
4	DMH + EP 25 weeks	26	19	73.0	0.001	18
5	EP 25 weeks	27	0	0		

From Turusov *et al.* (1980)
DMH, 1,2-dimethylhydrazine; EP, oestradiol dipropionate

cutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base). One group received subcutaneous injections of 10 µg/mouse oestradiol dipropionate dissolved in olive oil each time one day before 1,2-dimethylhydrazine administration and weekly for 10 weeks after 1,2-dimethylhydrazine treatment was stopped. The incidence of uterine sarcomas was 0/36 after administration of 1,2-dimethylhydrazine alone (group 1) and 11/40 in the group with 1,2-dimethylhydrazine plus oestradiol propionate (group 2) ($p = 0.008$). Haemorrhagic ovarian lesions were found in 20/34 animals in group 1 and 0/31 in group 2; tumours of the clitoral gland were found in 23/36 and 7/37 ($p = 0.026$) and colon tumours in 3/29 and 12/27 ($p = 0.057$), respectively. One colon polyp (6%) was found in a group receiving oestradiol propionate alone. No uterine or colon tumours or ovarian lesions were found in 29 controls or in 28 mice receiving oestradiol propionate alone (Turusov *et al.*, 1994).

Male and female newborn CBA mice received a single subcutaneous injection of 0.5 mg testosterone propionate in olive oil. At the age of two months, 30 male and 37 female neonatally androgenized and 30 male and 27 female control mice started receiving weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (total, 20 injections). All the surviving females were killed 30 weeks after the beginning of 1,2-dimethylhydrazine treatment and the surviving males at 34 weeks. There were also 30 androgenized and 20 intact females and 29 androgenized and 25 intact males that served as non-1,2-dimethylhydrazine-treated controls. By the end of the experiment, 27 females had palpable uterine tumours (up to 2 cm) out of 28 androgenized females treated with 1,2-dimethylhydrazine. Two out of 22 intact females treated with 1,2-dimethylhydrazine developed small (up to 0.5 cm) uterine sarcomas detected in mice killed at the end of the experiment ($p < 0.0001$). The incidence of renal capsule angiosarcomas (22/28) and colon tumours (20/28) was significantly higher in neonatally androgenized males treated

with 1,2-dimethylhydrazine than in control males treated with 1,2-dimethylhydrazine (7/28, $p < 0.0002$; and 9/28, $p < 0.005$, respectively) (Smirnova & Turusov, 1988).

A group of 90 male CD-1 mice, 40 days of age, received 20 weekly subcutaneous injections of 20 mg/kg 1,2-dimethylhydrazine dihydrochloride alone (group 1) or in combination with epidermal growth factor (EGF) (group 2). EGF was injected subcutaneously at a dose of 5 μg in 0.25 mL water on alternate days, through weeks 20 to 22. Each mouse received a total of 35 μg EGF. Mice were killed at 30 weeks. Colon tumours were found in 13/20 (mean, 2.25 ± 0.54) mice of group 1 (1,2-dimethylhydrazine alone) versus 18/24 (mean, 2.64 ± 0.65) of group 2 (1,2-dimethylhydrazine plus EGF) ($p > 0.05$). Anal tumours were present in 2/20 (mean, 0.1 ± 0.07) mice of group 1 versus 8/24 (mean 0.33 ± 0.1) ($p < 0.05$) mice of group 2 (Kingnorth *et al.*, 1985).

Groups of male CD-1 mice, 35 days of age, received six weekly subcutaneous injections of 15 mg/kg bw 1,2-dimethylhydrazine. Colitis was produced by seven weekly rectal instillations of 10 mM formyl-norleucylphenylalanine (FNLP) dissolved in dimethylsulfoxide after the mice had been anaesthetized by intramuscular injections of ketamine. Three days after the last injection of 1,2-dimethylhydrazine, all animals received the first of seven enemas of FNLP. Surviving mice were killed 21 weeks after the last injection of 1,2-dimethylhydrazine. At this time, 18/40 mice in the 1,2-dimethylhydrazine plus FNLP group, 5/39 mice receiving 1,2-dimethylhydrazine plus control enema and 6/15 mice receiving FNLP alone had died, either from 1,2-dimethylhydrazine-induced hepatotoxicity, colonic distension or perforation or from anaesthetic complications. In 5/22 animals receiving 1,2-dimethylhydrazine plus FNLP and 1/34 mice receiving 1,2-dimethylhydrazine and control enema ($p = 0.025$), adenocarcinomas developed in the descending colon. None of the mice developed multiple tumours and no tumours occurred in mice receiving FNLP alone. Colitis was confirmed histologically (Chester *et al.*, 1989).

3.4.2 *Rat*

Groups of 12, 7, 5, 8 and 16 male Fischer 34 rats [age unspecified] received a single intraperitoneal injection of 0, 25, 50, 75 or 100 mg/kg bw 1,2-dimethylhydrazine, respectively. Twelve hours after 1,2-dimethylhydrazine treatment, the rats were subjected to either partial hepatectomy or sham hepatectomy. After a two-week period, all the animals were placed on a basal diet containing 0.02% 2-acetylaminofluorene (2-AAF) for two weeks. One week after beginning the 2-AAF diet, the rats were given a single intraperitoneal injection of 2 mg/kg bw carbon tetrachloride in corn oil. After 14 days on the 2-AAF diet, the rats were placed on basal diet for an extra week and were then killed. Liver sections were stained for γ-glutamyltranspeptidase (γ-GT) histochemically. The numbers of γ-GT-positive foci per cm^2 in the liver were: 1, 2, 6, 10 and 19 in the 0-, 25-, 50-, 75- and 100-mg/kg bw groups, respectively [numbers taken from graphs]. The difference between the rats subjected to partial hepatectomy and those with sham hepatectomy in the number of γ-GT-positive foci at the 100 mg/kg bw dose of 1,2-dimethylhydrazine was highly significant ($p < 0.001$): 19.3 ± 2.6, 3.6 ± 0.8 and

0.8 ± 0.2 in rats receiving 100 mg/kg bw 1,2-dimethylhydrazine and subjected to partial hepatectomy, rats receiving 1,2-dimethylhydrazine and subjected to sham hepatectomy and rats that received a vehicle control and were subjected to partial hepatectomy, respectively (Ying *et al.*, 1979).

In another study, 3-aminobenzamide (an inhibitor of poly(ADP-ribose)polymerase) enhanced the induction of γ-GT-positive foci produced in the liver by 1,2-dimethylhydrazine in Wistar rats but not in Fischer rats. Rats were administered a single intraperitoneal injection of 100 mg/kg bw 1,2-dimethylhydrazine hydrochloride dissolved in 0.4 mM EDTA followed 4 h later by an intraperitoneal injection of either 600 mg/kg bw 3-aminobenzamide in dimethylsulfoxide or dimethylsulfoxide alone. Two weeks later, the rats were placed on a diet containing 0.02% 2-AAF for two weeks; in the middle of this period, a single intragastric dose of carbon tetrachloride was administered. The rats were killed five weeks after 1,2-dimethylhydrazine administration. The number of γ-GT positive foci per cm^2, their size and the area occupied by foci were significantly higher in Wistar rats treated with 1,2-dimethylhydrazine and 3-aminobenzamide than in those given only 1,2-dimethylhydrazine and dimethylsulfoxide. This effect was not seen in similarly treated Fischer rats (see Table 12) (Denda *et al.*, 1988).

Twenty-four male Sprague-Dawley rats were given eight weekly subcutaneous injections of 1,2-dimethylhydrazine at a dose of 9.5 mg/kg bw (calculated as base). Rats were subdivided into three groups of eight rats per group and were kept on a diet containing either 0, 5 or 15% cellulose. Rats were killed two weeks after the last injection of 1,2-dimethylhydrazine. Three other groups of rats were kept on the same diets but not treated with 1,2-dimethylhydrazine. The animals of control and 1,2-dimethylhydrazine-treated

Table 12. Effect of 3-aminobenzamide on the induction by 100 mg/kg bw 1,2-dimethylhydrazine of γ-GT positive foci in Fischer 344 and Wistar rats

Strain of rat	Treatment	No. of rats	γ-GT-positive foci		
			No./cm^2	Size of foci (mm^2)	Area occupied by foci (%)
Fischer 344	DMH + ABA	9	8.1 ± 2.3	0.52 ± 0.55	4.2 ± 2.2
	DMH + DMSO	9	6.8 ± 1.4	0.39 ± 0.14	2.7 ± 1.2
Wistar	DMH + ABA	10	5.8 ± 2.9[a]	0.13 ± 0.12	0.8 ± 0.7[b]
	DMH + DMSO	10	1.3 ± 1.0	0.8 ± 0.05	0.1 ± 0.1

From Denda *et al.* (1988)

[a] $p < 0.001$ compared to rats treated with DMH + DMSO

[b] $p < 0.01$ compared to rats treated with DMH + DMSO

γ-GT, γ-glutamyltranspeptidase; ABA, 3-aminobenzamide; DMH, 1,2-dimethylhydrazine; DMSO, dimethylsulfoxide

groups were maintained on the same caloric intake. 1,2-Dimethylhydrazine induced a significant increase in the mitotic activity as measured by the number of metaphase figures per crypt. The dietary cellulose caused a significant suppression of the 1,2-dimethylhydrazine-induced increase in crypt mitotic activity. Numbers of metaphase figures per crypt in the control rats were as follows: 3.45, 3.20 and 2.71 in rats given diet with 0%, 5% and 15% cellulose, respectively. The corresponding figures for 1,2-dimethylhydrazine-treated rats were 5.46, 3.56 and 3.63, respectively (Cameron *et al.*, 1989).

Male Sprague-Dawley rats, seven weeks of age, received eight weekly subcutaneous injections of 12 mg/kg bw 1,2-dimethylhydrazine (calculated as base). At week 9, pairs of rats (with or without 1,2-dimethylhydrazine treatment) were subdivided into groups and placed on seven different diets for 32 weeks. There was no significant difference in the mean total number of aberrant crypt foci per rat between animals which did or did not develop an adenocarcinoma in the descending colon, suggesting that this marker is not by itself a reliable predictor of the colon adenocarcinoma incidence (Table 13) (Hardman *et al.*, 1991).

Four groups of 30–32 male Sprague-Dawley rats each received subcutaneous injections of 12 mg/kg bw 1,2-dimethylhydrazine (calculated as base) weekly for eight weeks (defined as the initiation stage of the experiment). During the initiation period, all rats received standard food with 5% cellulose and 5% corn oil. The experimental diets were started one week after the last injection of 1,2-dimethylhydrazine. The rats were killed during week 32; 3 hours before scheduled sacrifice, each rat was given an intraperitoneal injection of 1 mg/kg bw colchicine. Neither a diet containing 10% pectin and

Table 13. Incidence of adenocarcinomas of the descending colon and the mean number of aberrant crypt foci in rats given subcutaneous injections of 1,2-dimethylhydrazine and different diets

Dietary group	Adenocarcinomas per rat	Mean no. ± SE of aberrant crypt foci per rat	
		With adenocarcinoma	Without adenocarcinoma
0% fibre	0.47	50.67 ± 7.5	50.13 ± 7.7
5% guar gum	0.33	42.20 ± 10.8	42.56 ± 6.5
10% guar gum	0.33	39.80 ± 6.4	33.77 ± 7.6
5% guar gum/5% pectin	0.37	38.00 ± 9.6	39.88 ± 6.6
10% pectin/5% corn oil	0.23[a]	22.43 ± 5.5	37.71 ± 4.6
10% pectin/10% corn oil	0.37	29.10 ± 7.3	29.06 ± 5.8
10% pectin/20% corn oil	0.06[a]	19.00 ± 19.00	31.32 ± 4.9

From Hardman *et al.* (1991)

[a] Significantly less than in rats on the 0% fibre diet

5% corn oil nor a diet containing 10% pectin and 20% corn oil suppressed the incidence of adenocarcinomas in the ascending colon, but both dietary modifications suppressed the incidence of adenocarcinomas in the descending colon. The decrease in adenocarcinoma incidence, due to addition of pectin, was attributed to a significant ($p < 0.05$) decrease in the incidence of adenocarcinomas in the area of the aggregates of lymphoid nodules, while the incidence of adenocarcinomas in locations distant from aggregates of lymphoid nodules was not significantly altered. The reduction in the risk for colon cancer was not necessarily accompanied by suppression of cell proliferation in the colonic crypts. The ability of a diet containing 10% or 20% corn oil to suppress cell proliferation was altered by the location of the crypt in the colon and by 1,2-dimethylhydrazine treatment (Hardman & Cameron, 1995).

3.5 Genetic studies

3.5.1 *Mouse*

The 100% susceptible ICR/Ha strain and the resistant C57BL/Ha strain were used in a genetic analysis of colon tumour induction by 1,2-dimethylhydrazine. Starting at 12 to 14 weeks of age, all mice received 22 subcutaneous weekly injections of 15 mg/kg bw 1,2-dimethylhydrazine. The mice were then observed up to 44 weeks. The percentages of colon tumours developing in the parental and hybrid mice are summarized in Table 14.

Table 14. 1,2-Dimethylhydrazine-induced colon tumour incidence in two inbred mouse strains and four types of hybrid showing inheritance of a major dominant susceptibility gene

Genotype	No. with tumour/ no. tested	% with tumour	
		Observed	Expected
ICR (M and F)	60/60	100	100
C57BL (M and F)	0/90	0	0
F_1 (M and F)	68/68	100	100
BCS[a] (M and F)	42/42	100	100
F_2 (M and F)	94/120	78	75[c]
BCR[b] (M and F)	46/117	39	50[c]
BCR (males only)	27/57	47	50[c]
BCR (females only)	19/60	32	50

From Evans *et al.* (1977)

[a] Susceptible backcross ($F_1 \times$ ICR)

[b] Resistant backcross ($F_1 \times$ C57BL)

[c] χ^2 test for goodness of titre to 1-gene expectation: F_2, $\chi^2 = 0.70$, $p > 0.4$; male BCR, $\chi^2 = 0.16$, $p > 0.6$. The combined BCR data fall somewhat short of the expected tumour incidence because of the incomplete penetrance in BCR females.

The 100% incidence in the large bowel of F_1 and susceptible backcross hybrids indicated dominance of the ICR-derived susceptibility to carcinogenesis by 1,2-dimethylhydrazine. Findings in the F_2 and resistant backcross hybrids are mutually supportive and in agreement with the respective 75% and 50% tumour incidences expected if a single dominant susceptibility gene is inherited from the ICR grandparent (Evans *et al.*, 1977).

Hybrid crosses were bred from a highly sensitive strain SWR/J mice and AKR/J mice highly resistant to 1,2-dimethylhydrazine carcinogenesis. 1,2-Dimethylhydrazine was injected subcutaneously at a dose of 20 mg/kg bw weekly with a total of 10 injections. Mice were killed 27 weeks after the first injection of 1,2-dimethylhydrazine. The colon tumour incidence in the hybrids and backcrosses is given in Table 15. The interpretation of results by the authors was that susceptibility to 1,2-dimethylhydrazine-induced tumorigenesis is not inherited as a dominant trait and that the kinetic properties of the epithelial cells are not linked to 1,2-dimethylhydrazine susceptibility (Deschner *et al.*, 1988).

Table 15. Incidence of 1,2-dimethylhydrazine-induced colonic tumours 27 weeks after the first injection

Cross	No. of mice	No. (%) of tumour-bearing mice
F_1 (SWR/J × AKR/J)	71	31 (43.7)
F_2	98	52 (52.0)
Backcross, susceptible	73	72 (98.6)
Backcross, resistant	68	5 (7.3)

From Deschner *et al.* (1988)

Groups of A/J and C57BL/6J strains, their F_1 progeny and 23 recombinant inbred strains, at eight weeks of age, received 20 weekly subcutaneous injections of 15 mg/kg bw 1,2-dimethylhydrazine. The animals were killed six weeks after the last 1,2-dimethylhydrazine injection. The analysis of colon tumour incidence led to the conclusion that there are several or many mechanisms in colon carcinogenesis and each of them requires several if not many genes for its functioning (Fleiszer *et al.*, 1988).

Inbred 1,2-dimethylhydrazine-susceptible ICR/Ha female mice were mated to inbred C57BL/6Ha males to produce 18 F_1 progeny (12 males and 6 females). These mice were backcrossed in both orientations to the resistant C57BL/6Ha strain, producing 126 (ICR/Ha × C57BL/6Ha)F_1 × C57BL/6Ha progeny. At the age of eight weeks, mice were given 25 weekly subcutaneous injections of 1,2-dimethylhydrazine at a dose of 20 mg/kg bw. The animals were killed one week after the last injection. Tumours were found in 40/122 progeny from a backcross to the resistant strain. Progeny animals were examined for segregation of 177 genetic markers distributed at intervals of 5–30 cM on all mouse chromosomes. Multiple loci were shown to contribute to the phenotype, with significant linkage to a novel locus, Ccs1, between D12Mit5 and D12Mit6 on mouse chromosome 12. Comparative maps suggested, in the authors' opinion, that the human homologue of Ccs1 is near FOS on human chromosome 14q (Jacoby *et al.*, 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Human colon microsomal cytochrome P450 enzymes can demethylate 1,2-dimethylhydrazine, yielding formaldehyde, and activities in the descending colon are always higher than in the ascending or transverse colon (Newaz *et al.*, 1983). *N*-Demethylation was also detected in microsomes from a human colon tumour line, adenocarcinoma LS 174T cells. The activity was inducible by phenobarbital and/or hydrocortisone.

4.1.2 *Experimental systems*

1,2-Dimethylhydrazine is well absorbed from the colon of the rat, as shown by an in-vivo perfusion technique (Meshkinpour *et al.*, 1985). The absorption was enhanced significantly by bile acids and by hydroxy-fatty acids; fatty acids had no significant effect.

The tissue distribution and covalent binding of ^{3}H was examined in rats given [^{3}H]1,2-dimethylhydrazine according to various dose schedules (Pozharisski *et al.*, 1977). There was widespread covalent binding to protein, DNA and RNA of liver, kidney, duodenum, ileum and colon and this was greater when a large dose was given weekly than when the same dose was divided and given daily. However, while daily dosing resulted in a loss of cytochrome P450 in hepatic microsomes to 47% of control levels, weekly dosing had no effect.

1,2-Dimethylhydrazine is metabolized by a sequence of oxidation steps, first dehydrogenation to azomethane, *N*-oxidation of this to azoxymethane and finally a *C*-oxidation to methylazoxymethanol (Fiala, 1975, 1977). This last metabolite decomposes to give the highly reactive methyldiazonium ion to which the carcinogenicity of the compound has been attributed. The sequential nature of these oxidation steps has been shown in the isolated perfused rat liver (Wolter & Frank, 1982). Fiala (1977) showed that the *C*-oxidation of azoxymethane to methylazoxymethanol is catalysed by hepatic microsomes, while Schoental (1973) found that methylazoxymethanol was converted to the corresponding aldehyde by an NAD-dependent dehydrogenase.

In addition to this pathway of metabolism and activation, methyl radical intermediates may also be involved in the toxicity and metabolism of 1,2-dimethylhydrazine catalysed by haemoglobin, peroxidases and cytochrome P450 (Augusto *et al.*, 1985).

A further pathway of 1,2-dimethylhydrazine metabolism is *N*-demethylation, yielding monomethylhydrazine and formaldehyde. This can be catalysed by the mitochondrial enzyme monoamine oxidase (Coomes & Prough, 1983) and, most probably, by microsomal cytochrome P450 (Fiala, 1977; Hietanen *et al.*, 1986).

In cultured colon epithelial cells from conventional and gnotobiotic Sprague-Dawley rats, some 50% of 1,2-dimethylhydrazine was present in the medium as azoxymethane (minor) and methylazoxymethanol (major) and 50% was unchanged. Release of volatile

metabolites (presumably azomethane) was a very minor pathway. Although production of the soluble metabolites was unaltered, both DNA binding and levels of volatile metabolites were appreciably higher in cells from gnotobiotic rats (Glauert & Bennink, 1983).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Subcutaneous administration of radioactively labelled 1,2-dimethylhydrazine to albino rats resulted in a high degree of DNA, RNA and protein methylation in the intestinal mucosa, liver and kidney (Pozharisski *et al*., 1975). A regenerative response of the colonic mucosa in CF_1 mice was seen two to four days after a single subcutaneous injection of 20 mg/kg bw 1,2-dimethylhydrazine (Deschner, 1978). After repeated injections, focal atypias and an expansion of the proliferative compartment in the mid and upper portions of the crypts were noted. Wargovich *et al.* (1983a) described an initial abrupt reduction in colonic DNA synthesis followed by regenerative increases in proliferation in 1,2-dimethylhydrazine-treated C57BL/6 and CF_1 mice. Following intraperitoneal injection of 60 mg/kg bw and 200 mg/kg bw 1,2-dimethylhydrazine in female HalCR mice, DNA synthesis was transiently inhibited in the descending and ascending colon and, to a lower degree, in a number of other tissues (Koval, 1984). The sensitivity of two mouse strains to long-term 1,2-dimethylhydrazine exposure was found to be related to the indigenous proliferative characteristics of the distal colonic mucosa (Glickman *et al*., 1987).

In the vilus–crypt axis of the descending colon of 1,2-dimethylhydrazine-treated female Wistar rats, mucosal regeneration and preneoplastic alterations were reported by Delapierre *et al.* (1981). In male Wistar rats, subcutaneous treatment with 25 mg/kg bw 1,2-dimethylhydrazine twice weekly for two months and once a week thereafter for up to six months led to a significant increase in the number of epithelial cells in the villi and crypts and in the mitotic pool along the small intestine (Altmann & Snow, 1984). Similar proliferative responses to 1,2-dimethylhydrazine were observed between the proximal and distal colon of male Sprague-Dawley rats (McGarrity *et al*., 1988). Intragastric treatment with 1,2-dimethylhydrazine (25, 50 or 100 mg/kg bw), given twice four days apart, led to a dose-dependent increase in the number of aberrant crypts in the colon of Wistar rats (Bilbin *et al*., 1992). Jacobs and Lupton (1986) reported that cellulose failed to protect against an increased frequency of proximal colonic adenocarcinomas induced by 1,2-dimethylhydrazine administered to Sprague-Dawley rats. In another study, increasing dietary levels of cellulose suppressed significantly the increase in mitotic activity in the colonic crypts of male Sprague-Dawley rats treated with 1,2-dimethylhydrazine (Cameron *et al*., 1989). Food deprivation was reported to increase apoptotic cell counts in rat descending colonic and rectal crypts after subcutaneous injection of 100 mg/kg bw 1,2-dimethylhydrazine (Ishizuka *et al*., 1994).

Mayer *et al*. (1987) reported enlarged mucus-rich crypts with marked hypercellularity appearing very early in the colonic mucosa of male Sprague-Dawley rats given 16 weekly subcutaneous injections of 15 mg/kg bw 1,2-dimethylhydrazine. Hyperbasophilic crypts lacking mucus production were observed later and showed a loss of glucose-6-phosphatase, but marked increases in glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. Increased γ-glutamyltranspeptidase activity was observed throughout both the proximal and distal colon of 1,2-dimethylhydrazine-treated female Sprague-Dawley rats before the appearance of macroscopic tumours (Traynor *et al.*, 1988). Caderni *et al*. (1995) found that sulfomucin-producing cells usually found in normal distal colon were progressively reduced in aberrant crypts of 1,2-dimethylhydrazine-treated female Sprague-Dawley rats, whereas the number of cells containing sialomucins was increased. However, no correlation was found between the number of aberrant crypt foci and the presence of tumours, as was also observed in male Sprague-Dawley rats (Hardman *et al.*, 1991).

In rat liver, 1,2-dimethylhydrazine had no consistent effect on the relative focal volume of γ-glutamyltranspeptidase-positive preneoplastic foci (Denda *et al.*, 1988). Locniskar *et al*. (1985) treated Brown-Norway and Fischer rats with 150 mg/kg bw 1,2-dimethylhydrazine by gavage five times over a three-week period. Five months after the final treatment, isolated splenic, colonic intraperithelial lymphocytes and lamina propria lymphocytes from the Brown-Norway strain exhibited low natural killer cell activity and reduced splenic T-lymphocyte proliferation in response to autologous non-T lymphocytes. Furthermore, colonic lamina propria lymphocyte proliferation was low, and Brown-Norway rats had a low incidence of 1,2-dimethylhydrazine-induced colonic neoplasms (7%) in comparison with Fischer rats, which had more effective splenic and colonic intraperithelial lymphocyte natural killer cell activity, enhanced splenic autologous mixed lymphocyte response, enhanced colonic lamina propria lymphocyte proliferation and a higher incidence of colon tumours (20%).

An association between intestinal carcinoma and the occurrence of intestinal lymphoid patches was found in 1,2-dimethylhydrazine-treated male Sprague-Dawley rats (Martin *et al.*, 1986).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Keller *et al*. (1984) investigated the embryotoxicity and teratogenicity of 1,2-dimethylhydrazine in pregnant Fischer 344 rats. In the high-dose group treated intraperitoneally with 10 mg/kg bw 1,2-dimethylhydrazine per day on days 6–15 of gestation, maternal weight gain during the early part of gestation and mean fetal weights were significantly reduced. The number of implants and viable fetuses per litter were also lower than those in controls, and the number of malformations (retained testicle, anophthalmia or

severe microphthalmia, unfused ossification centres of vertebrae and sternebrae) was slightly increased. At a daily dose of 5 mg/kg bw, these effects were not observed.

4.4 Genetic and related effects

4.4.1 *Humans*

Autrup *et al.* (1980a) examined the metabolism and DNA binding of [^{14}C]1,2-dimethylhydrazine in cultured human colon explants from 120 patients. The method used did not distinguish between the covalent binding of reactive metabolites and incorporation of label into DNA: the former accounted for 60–80% of the DNA binding observed. There was a 100-fold variation in the DNA binding among the 120 people studied (mean, 940 pmol/10 mg DNA; range, 50–5600). In further work, Autrup *et al.* (1980b) characterized the alkylated DNA bases produced by cultured human colon explants. Of the total of 39 alkylated bases per 10^6 nucleotides (range 3.7–167), 46% were *N*7-methylguanine, 9% were *N*3-methyladenine and 23% were *O*^{6}-methylguanine. This pattern of alkylation was essentially identical in the various anatomical segments of the colon examined, although the extent varied.

4.4.2 *Experimental systems* (see Table 16 for references)

There is conflicting evidence concerning the mutagenicity of 1,2-dimethylhydrazine to bacteria. In a single study, it induced recombination in *Saccharomyces cerevisiae*. *In vitro*, 1,2-dimethylhydrazine formed DNA adducts in human bronchial cells, provoked unscheduled DNA synthesis in rat hepatocytes and induced gene mutation in mammalian cells. It gave positive results in rodents in microbial host-mediated assays. 1,2-Dimethylhydrazine induced micronucleus formation, gene mutation, nuclear aberrations and DNA strand breaks and formed DNA adducts in rodents *in vivo*.

Autrup *et al*. (1980b) examined the pattern of DNA alkylation produced in rat colon explants in culture. Total DNA binding was 1353 ± 32 pmol/10 mg DNA, of the same order as in human colon (620 pmol ± 642/10 mg DNA, range < 100–3270). Some 40% of this represented phosphate-bound radioactivity and the pattern of alkylation (*N*7-methylguanine > *O*^{6}-methylguanine >> *N*3-methyladenine) was very similar to that in human tissue, the *O*^{6}-methylguanine/*N*7-methylguanine ratio being 0.54 in rat and 0.49 in human.

Mouse uterine tumours were examined for genetic alterations in the *p53* tumour suppressor gene (Trukhanova *et al.*, 1998). Fourteen uterine sarcomas, including three primary and seven transplanted malignant fibrous histiocytomas, three stromal sarcomas and one undifferentiated sarcoma, from 1,2-dimethylhydrazine-treated mice were first screened by immunohistochemistry for *p53* missense mutations, followed by single strand conformation polymorphism analysis and DNA sequencing for the identification of point mutations. There was 100% correlation between p53 protein immunopositivity and subsequent detection of *p53* mutations in malignant fibrous histiocytomas had a characteristic *p53* G:C→A:T transition mutation, consistent with *O*^{6}-methylguanine mispairing with thymine.

Table 16. Genetic and related effects of 1,2-dimethylhydrazine

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1500	Von Wright & Tikkanen (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	570	Parodi *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	3	Malaveille *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	150	Wilpart *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	300	Matsushita *et al.* (1993)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	–	NG	Matsushita *et al.* (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	+	3	Malaveille *et al.* (1983)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	NG	Wilpart *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	3	Malaveille *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Wilpart *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Wilpart *et al.* (1983)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	NT	120	Von Wright & Tikkanen (1980)
SCH, *Saccharomyces cerevisiae* (RS112), homozygosis by mitotic recombination or gene conversion	+	NT	1000	Schiestl *et al.* (1989)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	60	Mori *et al.* (1988)
UIA, Unscheduled DNA synthesis, mouse hepatocytes *in vitro*	+	NT	60	Mori *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	6	Rogers & Back (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance, thioguanine resistance, cytosine arabinoside resistance *in vitro*	–	NT	300	Rogers & Back (1981)

Table 16 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BFA, Bile from Sprague-Dawley rats, *Salmonella typhimurium* G46 and TA100 mutagenicity *in vivo*	–		60 sc × 1	Moriya *et al.* (1979)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1950 in NMRI mice *in vivo*	+		100 po × 1	Von Wright & Tikkanen (1980)
HMM, Host-mediated assay, *Salmonella typhimurium* G46 in ICR mice *in vivo*	+		7.5 sc × 1	Moriya *et al.* (1979)
HMM, Host-mediated assay, *Escherichia coli* 343/113 in Swiss albino mice *in vivo*	+		60 ip × 1	Kerklaan *et al.* (1986)
DVA, DNA strand breaks, BALB/c mouse liver, colon, stomach, lung, kidney *in vivo*	+		12.5 po or sc × 1	Brambilla *et al.* (1978)
DVA, DNA fragmentation, Swiss albino mouse liver, lung *in vivo*	+		14 ip × 5	Parodi *et al.* (1981)
DVA, DNA strand breaks, AKR/J, DBA/2, CD1, C57BL/6N, SWR/J, B6D2F_1 mouse liver, kidney, colon *in vivo*	+		50 ip × 1	Bolognesi & Boffa (1986)
DVA, DNA strand breaks, Sprague-Dawley rat liver *in vivo*	+		0.45 po × 2	Kitchin & Brown (1994)
DVA, DNA strand breaks, Sprague-Dawley rat liver *in vivo*	+		1 po × 2	Kitchin & Brown (1996)
RVA, DNA repair exclusive of unscheduled DNA synthesis, Fischer 344 rat hepatocytes and liver nonparenchymal cells *in vivo*	+		2 drink, 4 wk	Bedell *et al.* (1982)
RVA, DNA repair exclusive of unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	+		20 sc × 1	Richardson *et al.* (1985)
RVA, DNA repair exclusive of unscheduled DNA synthesis, rat liver cells *in vivo*	+		1.7 sc × 1	O'Toole *et al.* (1993)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	+		20 po × 1	Mirsalis *et al.* (1982)
UVR, Unscheduled DNA synthesis, Fischer 344 rat kidney cells *in vivo*	–		50 ip × 1	Tyson & Mirsalis (1985)
GVA, Gene mutation, (C57BL/6J × SWR F_1) mouse intestine, *Dlb-1* locus *in vivo*	+		20 sc × 10	Winton *et al.* (1990)

Table 16 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GVA, Gene mutation, (C57BL/6J × SWR F_1) mouse intestine, *Dlb-1* locus *in vivo*	–		30 ip × 1	Tao & Heddle (1994)
GVA, Gene mutation, (C57BL/6J × SWR F_1) mouse intestine, *Dlb-1* locus *in vivo*	(+)		10 ip × 10	Tao & Heddle (1994)
GVA, Gene mutation, CD-1 mouse liver cells, *p53* intron 6 *in vivo*	+		20 po × 2	Jenkins *et al.* (1997)
SVA, Sister chromatid exchange, C57BL/6J mouse colonic epithelial cells *in vivo*	+		10 ip × 1	Couch *et al.* (1986)
SVA, Sister chromatid exchange, C57BL/6J mouse bone-marrow cells *in vivo*	–		40 ip × 1	Couch *et al.* (1986)
MVM, Micronucleus test, C57BL/6J mouse colonic epithelial cells *in vivo*	+		15 ip × 1	Goldberg *et al.* (1983)
MVM, Micronucleus test, C57BL/6J mouse bone-marrow cells *in vivo*	–		45 ip × 1	Goldberg *et al.* (1983)
MVM, Micronucleus test, CBA mouse bone-marrow cells *in vivo*	+		10 po × 1	Ashby & Mirkova (1987)
MVM, Micronucleus test, $CCBF_1$, CBA, C57BL/6J mouse bone marrow *in vivo*	+		50 po × 1	Albanese *et al.* (1988)
MVM, Micronucleus test, CBA mouse bone marrow *in vivo*	+		25 po × 1	Morrison & Ashby (1995)
MVR, Micronucleus test, Alderley Park rat bone marrow *in vivo*	–		80 po × 1	Albanese *et al.* (1988)
BID, Binding (covalent) to DNA and proteins, human bronchial cells *in vitro*	+	NT	77	Harris *et al.* (1977)
BVD, Binding (covalent) to DNA, various BD-VI rat tissues *in vivo*	+		300 sc	Likhachev *et al.* (1977)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes and liver nonparenchymal cells *in vivo*	+		1.7 drink, 4 wk	Bedell *et al.* (1982)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes and liver nonparenchymal cells *in vivo*	+		1.7 drink, 4 wk	Lewis & Swenberg (1983)
BVD, Binding (covalent) to DNA, ICR/Ha mouse colon *in vivo*	+		20 ip × 1	James & Autrup (1983)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes *in vivo*	+		20 sc × 1	Richardson *et al.* (1985)

Table 16 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to DNA, Wistar rat intestine and liver *in vivo*	+		20 sc × 1	Tacchi-Bedford *et al.* (1988)
BVD, Binding (covalent) to DNA, Wistar or Sprague-Dawley rat intestine and liver *in vivo*	+		300 ip × 1	Netto *et al.* (1992)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver *in vivo*	+		1.7 sc × 1	O'Toole *et al.* (1993)
Colonic nuclear aberration assay in C57BL/6J mice *in vivo*	+		12 po × 1	Wargovich *et al.* (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; sc, subcutaneously; ip, intraperitoneally; po, oral

4.4.3 *Mechanistic considerations*

The conflicting set of results from bacterial mutagenicity tests is probably due to a lack of specific metabolic enzymes in microsomal preparations which were present in intact hepatocytes.

1,2-Dimethylhydrazine forms DNA adducts in various rodent tissues (including the colon and liver) after its metabolic activation *in vivo*. Adducts include *N*7-methylguanine, O^6-methylguanine and O^4-methylthymidine. In one study *C*8-methylguanine was also identified in DNA of liver and colon from rats treated with 1,2-dimethylhydrazine (Netto *et al.*, 1992). Of these adducts, O^6-methylguanine and O^4-methylthymidine are considered to be miscoding bases, since they can act as adenine and cytosine respectively without distortion of the DNA helix during DNA replication (Lawley, 1984). The formation, persistence and repair of such adducts in various tissues may influence the probability of mutation and carcinogenesis and may help to explain the organ-specific carcinogenicity of 1,2-dimethylhydrazine (Zedeck, 1984; Pegg, 1990).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,2-Dimethylhydrazine is believed to be used only as a laboratory chemical. No information on potential human exposure is available.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,2-Dimethylhydrazine was studied for carcinogenicity in many experiments in rats and mice, mainly by subcutaneous, infrequently by oral and rarely by other routes of administration.

Whatever the route of administration, 1,2-dimethylhydrazine, if given at an appropriate dosage, produced in mice and rats a high incidence of adenomas and adenocarcinomas of the colon and, to a lesser extent, of the small bowel. When given with drinking water or by gavage at low single doses, it produced a high incidence of vascular tumours.

In some experiments in rats, it produced ear duct papillomas and carcinomas, hepatocarcinomas, kidney adenomas, carcinomas and fibrosarcomas. When given to rats at very high single doses, it produced high incidences of nephroblastomas.

In some strains of mice, it produced a high incidence of hormone-dependent angiosarcomas of the kidney capsule (males only), uterine sarcomas or vascular tumours and tumour-like lesions of the ovary.

5.4 Other relevant data

1,2-Dimethylhydrazine is readily absorbed. It can be *N*-demethylated, yielding formaldehyde, and can be oxidized through several steps to yield methylazoxymethanol. It binds covalently to protein, DNA and RNA in many mammalian tissues. The colon of rats is a target organ for 1,2-dimethylhydrazine toxicity, where it can produce aberrant crypts. In developmental studies, it is embryo- and feto-toxic in rats.

1,2-Dimethylhydrazine formed DNA adducts and induced gene mutations, DNA breaks and micronuclei *in vitro* and *in vivo* in rodents. *In vitro* it formed DNA adducts and induced unscheduled DNA synthesis and gene mutations in mammalian cells. Conflicting evidence has been obtained for its genotoxicity in bacteria.

Although the activating pathway has not been clarified in detail, there is good evidence that human tissues, cells and subcellular preparations can activate 1,2-dimethylhydrazine in a similar manner to the corresponding rodent models.

1,2-Dimethylhydrazine requires bioactivation to become mutagenic and alkylates DNA in several species *in vivo*. It is not genotoxic in bacteria, but it is mutagenic for various endpoints in virtually all somatic test systems examined *in vitro* and *in vivo*.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2-dimethylhydrazine were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,2-dimethylhydrazine.

Overall evaluation

1,2-Dimethylhydrazine is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into account that 1,2-dimethylhydrazine is consistently mutagenic in a wide range of test systems and gives rise to a similar pattern of DNA damage in human and animal tissues *in vitro.*

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