Genotoxicity of a Variety of Hydrazine Derivatives in the Hepatocyte Primary Culture/DNA Repair Test Using Rat and Mouse Hepatocytes

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The genotoxicity of a variety of hydrazine derivatives was examined in the DNA-repair test on rat or mouse hepatocytes. Out of 32 hydrazine derivatives, 6 chemicals, i.e., *N*-acetyl-4-(hydroxymethyl)phenylhydrazine, 1,2-dimethylhydrazine·2HCl, 1-hydrazinophthalazine·HCl, methylhydrazine·sulfate, *p,p'*-oxybisbenzene disulfonylhydrazide and phenylhydrazine·HCl, elicited positive DNA repair responses in the test on rat hepatocytes. In the test on mouse hepatocytes, 4 more hydrazine derivatives, i.e., 1,1-dimethylhydrazine, hydrazine hydrate, hydrazine sulfate and 2-methyl-4-chlorophenoxyacetic acid hydrazide·HCl also generated positive responses, in addition to the 6 positive compounds in the rat assay. These results suggest that mouse hepatocytes are more susceptible to the genotoxicity of hydrazine derivatives, and that the species differences in genotoxicity appear to be in agreement with the *in vivo* carcinogenicity of these agents.

Key words: Genotoxicity — Hydrazine derivatives — Hepatocyte/DNA repair test — Species differences

Hydrazine derivatives have been widely used in industrial, agricultural and medical fields, and they also exist naturally in some plants.^{1,2)} Since the first reports clarifying the carcinogenicity of isonicotinic acid hydrazide,^{3,4)} extensive studies on the carcinogenicity of this class of chemicals have been performed, and a number of hydrazine derivatives have been shown to be potentially carcinogenic in animals,⁵⁻²⁹⁾ in particular, in mice.^{5-13, 15, 16, 18, 20-25, 27-29)} Some of these studies have revealed a relationship between the chemical structures and the organotropic carcinogenicity of the derivatives.^{14, 30)} However, knowledge about the metabolism of these derivatives in mammalian cells is limited, and the modes of action of their carcinogenicity have not yet been clarified. Furthermore, it appears that none of the present short-term tests, including the bacterial mutagenicity assay,^{31, 32)} is able to predict the carcinogenic potentials of all of the carcinogenic hydrazine derivatives.

The hepatocyte/DNA repair test, which measures unscheduled DNA synthesis (UDS),³³⁾ is known to be sensitive to various classes of DNA-reactive carcinogens, and is regarded as a reliable short-term test for the detection of chemical carcinogens.³⁴⁻³⁸⁾ In this study, 32 hydrazine derivatives including those of unknown carcinogenicity were examined for genotoxicity by using the DNA repair test. In order to investigate possible species differences in carcinogenic potencies, the test was done using hepatocytes from rats and mice. Genotoxic carcinogens are currently of considerable interest because of their potential hazards to human health.³⁹⁾

MATERIALS AND METHODS

Chemicals 1,1-Dimethylhydrazine, guanylhypazine·HCl, hydrazine hydrate and isonicotinic acid hydrazide were purchased from Tokyo Kasei Chem. Co. (Tokyo). 2,4-Dinitrophenylhydrazine was provided by Kanto Chem. Co. (Tokyo). *p,p'*-Oxybisbenzene disulfonylhydrazide and 1,2-dimethylhydrazine·2HCl were purchased from Sankyo Chem. Co. (Tokyo) and Aldrich Chem. Co. (Milwaukee, WI), respectively. 2-Methyl-4-

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Table I. Chemical Structures of Tested Hydrazine Derivatives

No.	Name	Structure
1	N'-Acetyl-4-(hydroxymethyl)phenylhydrazine	$\text{HOCH}_2\text{-C}_6\text{H}_4\text{-NHNHCOCH}_3$
2	1-Acetyl-2-phenylhydrazine	$\text{C}_6\text{H}_5\text{-NHNHCOCH}_3$
3	4-Allylthiosemicarbazide	$\text{H}_2\text{CCHCH}_2\text{NHCSNHNH}_2$
4	Benzoylhydrazine	$\text{C}_6\text{H}_5\text{-CONHNH}_2$
5	Benzylhydrazine · 2HCl	$\text{C}_6\text{H}_5\text{-CH}_2\text{NHNH}_2 \cdot 2\text{HCl}$
6	Butylhydrazine oxalate	$\text{H}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CNHNH}_2(\text{COOH})_2$
7	Carbamylhydrazine · HCl	$\text{H}_2\text{NNHCONH}_2 \cdot \text{HCl}$
8	1-Carbamyl-2-phenylhydrazine	$\text{C}_6\text{H}_5\text{-NHNHCONH}_2$
9	1,2-Diethylhydrazine dicarboxalate	$\text{H}_3\text{C}_2\text{NHNHC}_2\text{H}_5 \cdot 2(\text{COOH})_2$
10	1,2-Diformylhydrazine	HOCNHNHCHO
11	1,1-Dimethylhydrazine	$(\text{CH}_3)_2\text{NNH}_2$
12	1,2-Dimethylhydrazine · 2HCl	$\text{CH}_3\text{NHNHCH}_3 \cdot 2\text{HCl}$
13	2,4-Dinitrophenylhydrazine	$\text{O}_2\text{N-C}_6\text{H}_3(\text{NO}_2)\text{-NHNH}_2$
14	Ethylhydrazine oxalate	$\text{H}_3\text{CH}_2\text{CNHNH}_2(\text{COOH})_2$
15	Formic acid hydrazide	H_2NNHCHO
16	Guanylhiazine · HCl	$\text{H}_2\text{NC(NH)NHNH}_2 \cdot \text{HCl}$
17	β -N- $\{\gamma$ -L-(+)-Glutamyl}-4-hydroxymethyl-phenylhydrazine (Agaritrine)	$\text{HOCH}_2\text{-C}_6\text{H}_4\text{-NHNHCOCH}_2\text{CH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$
18	Hydrazine hydrate	$\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$
19	Hydrazine sulfate	$\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{SO}_4$
20	1-Hydrazinophthalazine · HCl	$\text{NHNH}_2 \cdot \text{HCl}$
21	2-Hydroxyethylhydrazine	$\text{H}_2\text{NNHCH}_2\text{CH}_2\text{OH}$
22	Isonicotinic acid hydrazide	$\text{N} \equiv \text{C-C}_5\text{H}_4\text{-CONHNH}_2$
23	2-Methyl-4-chlorophenoxyacetic acid hydrazide · HCl	$\text{Cl-C}_6\text{H}_3(\text{CH}_3)\text{-OCH}_2\text{CONHNH}_2 \cdot \text{HCl}$
24	N-Methyl-N-formylhydrazine	$\text{H}_2\text{NNCH}_3\text{CHO}$
25	Methylhydrazine · sulfate	$\text{CH}_3\text{NHNH}_2 \cdot \text{H}_2\text{SO}_4$
26	4-Methylphenylhydrazine · HCl	$\text{H}_3\text{C-C}_6\text{H}_4\text{-NHNH}_2 \cdot \text{HCl}$
27	p,p'-Oxybisbenzene disulfonylhydrazide	$\text{H}_2\text{NNHSO}_2\text{-C}_6\text{H}_4\text{-O-C}_6\text{H}_4\text{-SO}_2\text{NHNH}_2$
28	β -Phenylethylhydrazine · sulfate	$\text{C}_6\text{H}_5\text{-CH}_2\text{CH}_2\text{NHNH}_2 \cdot \text{H}_2\text{SO}_4$
29	Phenylhydrazine · HCl	$\text{C}_6\text{H}_5\text{-NHNH}_2 \cdot \text{HCl}$
30	β -Phenylisopropylhydrazine	$\text{C}_6\text{H}_5\text{-NHNHCH}(\text{CH}_3)_2$
31	Succinic acid 2,2-dimethylhydrazide	$\text{HO}_2\text{CCH}_2\text{CHCONHN}(\text{CH}_3)_2$
32	Thiosemicarbazide	$\text{H}_2\text{NCSNHNH}_2$

Table II. Results of DNA Repair Test on Rat or Mouse Hepatocytes

Chemicals	Dose (M)	Rat		Mouse	
		UDS grains/ nucleus ^{a)}	DNA repair ^{b)}	UDS grains/ nucleus ^{a)}	DNA repair ^{b)}
<i>N'</i> -Acetyl-4-(hydroxy- methyl)phenylhydrazine (1)	10 ⁻³	10.4±4.0 (89)		5.5±2.3 (61)	
	10 ⁻⁴	3.6±2.5 (27)	+	3.2±2.7 (14)	+
	10 ⁻⁵	1.2±1.6 (2)		-0.5±1.5 (0)	
1-Acetyl-2-phenyl- hydrazine (2)	10 ⁻³	-0.8±1.5 (0)	-	-0.6±1.3 (0)	-
	10 ⁻⁴	-0.9±1.6 (0)		-0.6±1.3 (0)	
	10 ⁻⁵	-0.7±1.5 (0)		-0.7±1.4 (0)	
4-Allylthiosemicarbazide (3)	10 ⁻³	-0.7±1.5 (0)	-	-0.6±1.4 (0)	-
	10 ⁻⁴	-0.6±1.3 (0)		-0.6±1.2 (0)	
	10 ⁻⁵	-0.4±1.3 (0)		-0.7±1.3 (0)	
Benzoylhydrazine (4)	10 ⁻³	-0.8±1.5 (0)	-	-1.0±1.2 (0)	-
	10 ⁻⁴	-0.9±1.6 (0)		-0.8±1.2 (0)	
	10 ⁻⁵	-0.7±1.5 (0)		-0.6±1.2 (0)	
Benzylhydrazine·2HCl (5)	10 ⁻³	Toxic	-	Toxic	-
	10 ⁻⁴	-0.7±1.4 (0)		-0.4±1.2 (0)	
	10 ⁻⁵	-0.8±1.5 (0)		-0.5±1.3 (0)	
Butylhydrazine oxalate (6)	10 ⁻³	Toxic	-	Toxic	-
	10 ⁻⁴	-0.4±1.4 (0)		-0.6±1.4 (0)	
	10 ⁻⁵	-0.7±1.4 (0)		-0.5±1.3 (0)	
Carbamylhydrazine·HCl (7)	10 ⁻³	-1.2±1.6 (0)	-	-0.6±1.3 (0)	-
	10 ⁻⁴	-0.4±1.5 (0)		-0.7±1.2 (0)	
	10 ⁻⁵	-1.2±1.7 (0)		-0.8±1.2 (0)	
1-Carbamyl-2-phenyl- hydrazine (8)	10 ⁻³	-0.8±1.5 (0)	-	-0.6±1.3 (0)	-
	10 ⁻⁴	-0.1±1.6 (0)		-0.6±1.2 (0)	
	10 ⁻⁵	-0.7±1.4 (0)		-0.8±1.5 (0)	
1,2-Diethylhydrazine dicarboxalate (9)	10 ⁻³	-0.7±1.5 (0)	-	-0.7±1.3 (0)	-
	10 ⁻⁴	-1.0±1.4 (0)		-0.8±1.3 (0)	
	10 ⁻⁵	-1.2±1.5 (0)		-0.6±1.3 (0)	
1,2-Diformylhydrazine (10)	10 ⁻³	-0.4±1.5 (0)	-	-0.7±1.3 (0)	-
	10 ⁻⁴	-1.0±1.4 (0)		-0.8±1.5 (0)	
	10 ⁻⁵	-1.2±1.5 (0)		-0.8±1.4 (0)	
1,1-Dimethylhydrazine (11)	10 ⁻³	1.0±1.5 (3)	-	5.2±2.6 (54)	+
	10 ⁻⁴	-0.8±1.5 (0)		2.2±1.5 (8)	
	10 ⁻⁵	-0.6±1.2 (0)		-0.6±1.4 (0)	
1,2-Dimethylhydrazine· 2HCl (12)	10 ⁻³	5.9±2.7 (61)	+	4.6±3.4 (40)	+
	10 ⁻⁴	3.7±2.2 (28)		2.1±1.8 (9)	
	10 ⁻⁵	2.7±2.0 (13)		0.2±1.4 (1)	
2,4-Dinitrophenylhydrazine (13)	10 ⁻³	Toxic	-	Toxic	-
	10 ⁻⁴	-1.0±1.5 (0)		-0.5±1.4 (0)	
	10 ⁻⁵	-0.5±1.4 (0)		-0.7±1.5 (0)	
Ethylhydrazine oxalate (14)	10 ⁻³	0.8±1.6 (2)	-	1.2±1.3 (2)	-
	10 ⁻⁴	0.1±1.5 (0)		0.6±1.3 (1)	
	10 ⁻⁵	-0.6±1.5 (0)		-0.6±1.5 (0)	
Formic acid hydrazide (15)	10 ⁻³	-0.8±1.5 (0)	-	-0.8±1.4 (0)	-
	10 ⁻⁴	-0.9±1.6 (0)		-0.8±1.3 (0)	
	10 ⁻⁵	-1.2±1.5 (0)		-0.6±1.4 (0)	
Guanylhiazine·HCl (16)	10 ⁻³	-1.1±1.4 (0)	-	-0.7±1.3 (0)	-
	10 ⁻⁴	-0.4±1.6 (0)		-0.7±1.4 (0)	
	10 ⁻⁵	-0.7±1.5 (0)		-0.8±1.3 (0)	
β -N-[γ -L-(+)-Glutamyl]- 4-hydroxymethylphenyl- hydrazine(Agaritine) (17)	10 ⁻³	-0.4±1.6 (0)	-	-0.7±1.4 (0)	-
	10 ⁻⁴	-0.6±1.5 (0)		-0.8±1.4 (0)	
	10 ⁻⁵	-0.6±1.5 (0)		-0.5±1.4 (0)	
Hydrazine hydrate (18)	10 ⁻³	-0.5±1.5 (0)	-	4.8±2.5 (46)	+
	10 ⁻⁴	-0.3±1.3 (0)		3.5±2.1 (32)	
	10 ⁻⁵	-0.8±1.6 (0)		0.7±1.3 (1)	
Hydrazine sulfate (19)	10 ⁻³	-0.6±1.5 (0)	-	10.8±4.9 (94)	+
	10 ⁻⁴	-0.6±1.6 (0)		4.9±2.3 (42)	
	10 ⁻⁵	-0.8±1.5 (0)		0.3±1.4 (0)	

GENOTOXICITY OF HYDRAZINE DERIVATIVES

Table II — continued

Chemicals	Dose (M)	Rat		Mouse	
		UDS grains/ nucleus ^{a)}	DNA repair ^{b)}	UDS grains/ nucleus ^{a)}	DNA repair ^{b)}
1-Hydrazinophthalazine· HCl (20)	10 ⁻³	5.3±2.8 (50)	+	3.5±2.1 (32)	+
	10 ⁻⁴	4.6±2.3 (45)		6.7±3.7 (69)	
	10 ⁻⁵	1.3±2.4 (8)		1.3±1.4 (4)	
2-Hydroxyethylhydrazine (21)	10 ⁻³	-0.6±1.9 (0)	—	-0.5±1.4 (0)	—
	10 ⁻⁴	-0.8±1.8 (0)		-0.4±1.2 (0)	
	10 ⁻⁵	-0.7±1.7 (0)		-0.6±1.5 (0)	
Isonicotinic acid hydrazide (22)	10 ⁻³	-1.2±1.3 (0)	—	-0.9±1.3 (0)	—
	10 ⁻⁴	-1.1±1.5 (0)		-0.8±1.6 (0)	
	10 ⁻⁵	-0.7±1.4 (0)		-0.7±1.3 (0)	
2-Methyl-4-chlorophenoxy- acetic acid hydrazide· HCl (23)	10 ⁻³	1.3±1.5 (2)	—	9.4±4.7 (91)	+
	10 ⁻⁴	-0.7±1.5 (0)		3.4±1.9 (28)	
	10 ⁻⁵	-0.9±1.3 (0)		0.4±1.3 (1)	
N-Methyl-N-formyl- hydrazine (24)	10 ⁻³	-0.5±1.5 (0)	—	-0.6±1.2 (0)	—
	10 ⁻⁴	-1.2±1.4 (0)		-0.5±1.2 (0)	
	10 ⁻⁵	-0.7±1.5 (0)		-0.6±1.3 (0)	
Methylhydrazine·sulfate (25)	10 ⁻³	Toxic	+	9.6±3.8 (92)	+
	10 ⁻⁴	5.7±2.8 (58)		4.9±2.5 (47)	
	10 ⁻⁵	3.0±1.9 (21)		-0.4±1.3 (0)	
4-Methylphenylhydrazine· HCl (26)	10 ⁻³	0.7±1.4 (2)	—	Toxic	—
	10 ⁻⁴	-0.7±1.5 (0)		-0.5±1.0 (0)	
	10 ⁻⁵	-0.5±1.6 (0)		-0.5±1.3 (0)	
<i>p,p'</i> -Oxybisbenzene disulfonylhydrazide (27)	10 ⁻³	9.4±3.6 (78)	+	6.5±2.8 (71)	+
	10 ⁻⁴	2.1±1.9 (13)		3.6±2.0 (30)	
	10 ⁻⁵	0.1±1.3 (0)		-0.4±1.0 (0)	
β -Phenylethylhydrazine· sulfate (28)	10 ⁻³	Toxic	—	Toxic	—
	10 ⁻⁴	-0.2±1.4 (0)		-0.3±1.4 (0)	
	10 ⁻⁵	-0.1±1.4 (1)		-0.4±1.4 (0)	
Phenylhydrazine·HCl (29)	10 ⁻³	9.6±3.8 (85)	+	Toxic	+
	10 ⁻⁴	5.7±3.1 (60)		5.7±2.1 (60)	
	10 ⁻⁵	1.6±1.9 (6)		2.1±1.5 (7)	
β -Phenylisopropyl- hydrazine (30)	10 ⁻³	Toxic	—	Toxic	—
	10 ⁻⁴	1.1±1.4 (3)		-0.6±1.3 (0)	
	10 ⁻⁵	-0.8±1.4 (0)		-0.7±1.4 (0)	
Succinic acid 2,2-di- methylhydrazine (31)	10 ⁻³	-0.3±1.3 (0)	—	-0.6±1.3 (0)	—
	10 ⁻⁴	-0.6±1.5 (0)		-0.7±1.5 (0)	
	10 ⁻⁵	-0.7±1.4 (0)		-0.8±1.6 (0)	
Thiosemicarbazide (32)	10 ⁻³	-1.0±1.5 (0)	—	-0.7±1.2 (0)	—
	10 ⁻⁴	-1.2±1.4 (0)		-0.7±1.4 (0)	
	10 ⁻⁵	-0.8±1.3 (0)		-0.7±1.5 (0)	
N-2-Fluorenylacetamide (positive control)	10 ⁻⁵	43.2±9.2 (98)	+		
	10 ⁻⁶	15.6±4.3 (87)			
7,12-Dimethylbenz[<i>a</i>]an- thracene (positive control)	10 ⁻⁴			12.3±5.2 (82)	+
	10 ⁻⁵			4.9±3.2 (46)	
Dimethylsulfoxide (solvent control)		-0.8±1.6 (0)	—	-0.9±1.6 (0)	—
Distilled water (solvent control)		-0.7±1.5 (0)	—	-0.8±1.4 (0)	—

Since positive DNA repair responses were not present at 2 doses (10⁻⁶, 10⁻⁷M), only the data at 3 doses (10⁻³–10⁻⁵M) of each tested chemical are shown.

a) Mean±standard deviation of 3 coverslips. b) +, Induced DNA repair; —, failed to induce DNA repair. Numbers in parentheses are % of UDS positive cells.

chlorophenoxyacetic acid hydrazide·HCl was provided by Dainippon Chem. Co. (Tokyo). The other 25 hydrazine derivatives were all kindly donated by Dr. Bela Toth (the Epply Institute for Research in Cancer). The chemical structures are shown in Table I.

Hepatocyte/DNA Repair Test The test was performed basically in accordance with the methods of Williams *et al.*³⁵⁾ Hepatocytes were isolated from the livers of ACl/N rats weighing 200-250 g and C3H/HeN mice weighing about 20 g. The isolated hepatocytes were allowed to attach for 2 hr to plastic coverslips in primary culture using Williams' Medium E. The cultures were then washed and exposed to the test compounds and [Me-³H]thymidine (10 μ Ci/ml; 49 Ci/mmol; Amersham International, Amersham, UK) for 20 hr.³⁴⁾ 4-Allylthiosemicarbazide, 1,2-diformylhydrazine, 2,4-dinitrophenylhydrazine and *p,p'*-oxybisbenzene disulfonylhydrazide were dissolved in dimethylsulfoxide (Nakarai Chem. Co., Tokyo). Other hydrazine derivatives were all dissolved in distilled water. All of the tested chemicals were logarithmically diluted before addition to the cultures (10^{-3} – 10^{-7} M). In principle, the concentration tested included the highest soluble nontoxic dose.^{34, 35)} As a positive control, *N*-2-fluorenylacetyl-amide (Nakarai Chem.) was used in the assay with rat hepatocytes, and 9,10-dimethyl-1,2-benz[*a*]anthracene (Nakarai Chem.) was used in the assay with mouse hepatocytes. At the end of incubation, the cultures were washed in Williams' Medium E, and the coverslips were mounted on glass slides.³⁵⁾ The slides were dipped in Sakura NR-M2 photographic emulsions and exposed for 14 days. Autoradiographic grains were counted on a CRT display (Olympus, type S) with a microscopic attachment. The net nuclear grain counts of repair synthesis were obtained by subtracting the highest cytoplasmic area count from the nuclear area count. The data were expressed as the average net counts/nucleus for 3 coverslips $\pm$ the standard deviation (50 cells/coverslip). Each cell nucleus was considered positive when the net nuclear grain count was more than 5 grains above the cytoplasmic background.³⁴⁾ The test compounds were judged positive when the mean nuclear grain count was more than 5 grains or the positive cells were more than 33%. Two assays were performed on every test compound with hepatocytes of each species.

RESULTS

In this study, virtually identical results were obtained in 2 replicate experiments, and therefore only the data from one experiment are presented. In the DNA repair test with rat

hepatocytes, the test dose of hydrazine derivatives at the highest concentration (10^{-3} M) was toxic in the cases of benzylhydrazine·2HCl, butylhydrazine oxalate, 2,4-dinitrophenylhydrazine, methylhydrazine·sulfate, β -phenylethylhydrazine·sulfate and β -phenylisopropylhydrazine. In the assay, 6 of 32 compounds, i.e., *N'*-acetyl-4-(hydroxymethyl)phenylhydrazine, 1,2-dimethylhydrazine·2HCl, 1-hydrazinophthalazine·HCl, methylhydrazine·sulfate, *p,p'*-oxybisbenzene disulfonylhydrazide and phenylhydrazine·HCl, elicited positive DNA repair synthesis (Table II). Among the positive compounds, the nuclear grain counts were roughly related to the doses. In the DNA repair test with mouse hepatocytes, the highest dose was also toxic in the cases of 7 hydrazine derivatives. Ten hydrazine derivatives, i.e., *N'*-acetyl-4-(hydroxymethyl)phenylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine·2HCl, hydrazine hydrate, hydrazine sulfate, 1-hydrazinophthalazine·HCl, 2-methyl-4-chlorophenoxyacetic acid hydrazide·HCl, methylhydrazine·sulfate, *p,p'*-oxybisbenzene disulfonylhydrazide and phenylhydrazine·HCl, were positive in the test with mouse hepatocytes (Table II). All of the 6 positive chemicals in the rat assay also showed positive response in the test with mouse hepatocytes.

As shown in Table II, almost all numbers of UDS grains/nucleus of negative compounds or controls were negative values. The results may reflect an inappropriate evaluation of background grains, since the nuclear area has less cytoplasm over and beneath the nucleus than the cytoplasmic area resulting in less non-specific binding of tritiated thymidine. Table III summarizes the correlation between the results of the DNA repair test and the bacterial mutagenicity as well as the carcinogenicity. Four hydrazine derivatives, i.e., hydrazine hydrate, hydrazine sulfate, 1-hydrazinophthalazine·HCl and phenylhydrazine·HCl were positive with respect to genotoxicity in the DNA repair test with hepatocytes of either one or both species, mutagenicity in *Salmonella typhimurium* and carcinogenicity. Ten chemicals of known carcinogenicity, i.e., 1-acetyl-2-phenylhydrazine, benzoylhydrazine, benzylhydrazine·2HCl, carbamylhydrazine·HCl, 1-carbamyl-2-phenylhydrazine, 1,2-diformylhydrazine, ethylhydrazine oxa-

Table III. Genotoxicity, Mutagenicity and Carcinogenicity of Tested Hydrazine Derivatives

Chemicals	Hepatocyte/DNA repair		Bacterial mutagenicity ^{a)}	Carcinogenicity
	rat	mouse		
<i>N'</i> -Acetyl-4-(hydroxymethyl)-phenylhydrazine (1)	+	+	— (31)	+ (5)
1-Acetyl-2-phenylhydrazine (2)	—	—	— (31)	+ (6)
4-Allylthiosemicarbazide (3)	—	—	+ (31)	?
Benzoylhydrazine (4)	—	—	— (31)	+ (7)
Benzylhydrazine·2HCl (5)	—	—	— (31)	+ (8)
Butylhydrazine oxalate (6)	—	—	+ (31)	+ (9)
Carbamylhydrazine·HCl (7)	—	—	— (31)	+ (10)
1-Carbamyl-2-phenylhydrazine (8)	—	—	— (31)	+ (11)
1,2-Diethylhydrazine dicarboxalate (9)	—	—	— (31)	?
1,2-Diformylhydrazine (10)	—	—	— (31)	+ (12)
1,1-Dimethylhydrazine (11)	—	+	— (31)	+ (13)
1,2-Dimethylhydrazine·2HCl (12)	+	+	— (31)	+ (14, 15)
2,4-Dinitrophenylhydrazine (13)	—	—	+ (31)	?
Ethylhydrazine oxalate (14)	—	—	— (31)	+ (16)
Formic acid hydrazide (15)	—	—	— (31)	+ (17)
Guanylhiazine·HCl (16)	—	—	— (31)	?
β - <i>N</i> -[γ -L-(+)-Glutamyl]-4-hydroxymethyl-phenylhydrazine (Agaritrine) (17)	—	—	— (31)	?
Hydrazine hydrate (18)	—	+	+ (31)	+ (18)
Hydrazine sulfate (19)	—	+	+ (31)	+ (19, 20)
1-Hydrazinophthalazine·HCl (20)	+	+	+ (31)	+ (21)
2-Hydroxyethylhydrazine (21)	—	—	+ (31)	+ (22)
Isonicotinic acid hydrazide (22)	—	—	+ (31)	+ (3, 4)
2-Methyl-4-chlorophenoxyacetic acid hydrazide·HCl (23)	—	+	— (31)	+ (23)
<i>N</i> -Methyl- <i>N</i> -formylhydrazine (24)	—	—	— (31)	+ (24)
Methylhydrazine·sulfate (25)	+	+	— (31)	+ (25, 26)
4-Methylphenylhydrazine·HCl (26)	—	—	+ (31)	+ (27)
<i>p,p'</i> -Oxybisbenzene disulfonylhydrazide (27)	+	+	+ (31)	?
β -Phenylethylhydrazine·sulfate (28)	—	—	+ (31)	+ (28)
Phenylhydrazine·HCl (29)	+	+	+ (31)	+ (8)
β -Phenylisopropylhydrazine (30)	—	—	— (31)	?
Succinic acid 2,2-dimethylhydrazine (31)	—	—	— (31)	+ (29)
Thiosemicarbazide (32)	—	—	— (31)	?

a) In *Salmonella typhimurium*.

late, formic acid hydrazide, *N*-methyl-*N*-formylhydrazine and succinic acid 2,2-dimethylhydrazine have negative hepatocyte/DNA repair response and bacterial mutagenicity.

DISCUSSION

The hepatocyte/DNA repair test combines intact cell metabolism with the broadest capability for xenobiotic biotransformation, to-

gether with a biochemical end-point for genotoxicity.³⁵⁾

In the present DNA repair test with rat hepatocytes, 6 of 32 hydrazine derivatives elicited positive DNA repair responses. However, 4 more hydrazine derivatives, in addition to the 6 positive derivatives in the rat assay, generated repairing UDS in the test with mouse hepatocytes. These data clearly indicate a species difference in the genotox-

icity of the hydrazine derivatives, and the data appear to be consistent with the susceptibility of mice to the carcinogenicity of hydrazine derivatives.^{5-13, 15, 16, 18, 20-25, 27-29)} In the present DNA repair test, the number of positive compounds among the tested hydrazine derivatives was relatively small. Furthermore, the UDS level of the positive chemicals was relatively low compared to that of other types of carcinogenic chemicals.³⁴⁻³⁸⁾ These data are in agreement with the results of bacterial mutagenicity assay of these hydrazine derivatives, and the data also suggest a mode of action for the tumorigenicity of these chemicals.

The carcinogenic hydrazine derivatives such as 1-acetyl-2-phenylhydrazine and benzoylhydrazine, which lack genotoxicity in the DNA repair test as well as mutagenicity in the *Salmonella typhimurium* assay^{31, 32)} might be carcinogenic through some nongenotoxic mechanism. *p, p'*-Oxybisbenzene disulfonylhydrazide which has not yet been shown to be carcinogenic, gave a clearly positive response in the test with rat hepatocytes as well as in the mouse assay. The bacterial mutagenicity of this chemical has also been confirmed.³¹⁾ In view of the reliability of both systems in detecting carcinogens, the chemical is strongly suspected of being carcinogenic. An *in vivo* test of this compound for carcinogenicity is now being performed.

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