

Mutagenic Activity of Chemical Carcinogens and Related Compounds in the Intraperitoneal Host-Mediated Assay^{1,2}

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ABSTRACT—The mutagenic activities of 79 carcinogens, non-carcinogens, and structurally related compounds toward *Salmonella typhimurium* strains TA1530, TA1535, and TA1538 and toward *Saccharomyces cerevisiae* D3 were investigated in the intraperitoneal host-mediated assay. Fewer than half of the carcinogens were mutagenic toward the *Salmonella* strains. The insensitivity of the system was most marked with the aromatic amine and polycyclic hydrocarbon precarcinogens. Under the test conditions, fewer than 10% of the carcinogens showed clear mutagenic activity toward *S. cerevisiae* D3. However, none of the non-carcinogens was significantly mutagenic toward either *S. typhimurium* or *S. cerevisiae* D3. Overall, the intraperitoneal host-mediated assay does not seem suitable for routine preliminary screening of large numbers of potential carcinogens. The median lethal doses to mice of 48 compounds were determined.—*J Natl Cancer Inst* 62: 911-918, 1979.

One of the methods proposed for routine prescreening of potential carcinogens has been the host-mediated bioassay (1). By this procedure sensitive microbial organisms or mammalian cells are implanted into animals and the animals are subsequently treated with the test chemical. The administered cells and their mutants are then recovered and counted. In this system the formation and transformation of the active form of the test chemical may be performed by the host. The host-mediated assay has been successfully used in the detection of a number of chemical carcinogens with widely varying structures. These include the nitrosamines (2-4), the nitrosamides (5), hydrazine (6), aryldialkyltriazenes (7), alkylating agents (8-10), cyclophosphamide (11), ethylene thiourea (12), and vinyl chloride (13). The detector organisms include yeast (14), *Neurospora* (15), *Salmonella typhimurium* (16), the murine leukemia cell line L5178y (17), *Escherichia coli* K12 and WP2 (8, 18), and bacteriophage T4 (19). Few studies have dealt with the mutagenic activities of the aromatic amines, the aminoazo dyes, and the polycyclic hydrocarbons in the host-mediated assay. Therefore, the mutagenic activities of a series of carcinogens and structurally related noncarcinogens in the host-mediated assay were determined.

MATERIALS AND METHODS

The experiments were conducted by two groups at separate laboratories: group I (Herbert S. Rosenkranz at Columbia University) and group II (Vincent F. Simmon at SRI International). With the exception of minor modifications (noted below), the protocols were essentially identical to those previously published in (1). Group I generally used *S. typhimurium* strains TA1530 and TA1538, whereas group II used strains

TA1535 and TA1538 as well as *Saccharomyces cerevisiae* D3.

Animals, chemicals, and administration of compounds.—Both groups used adult male Swiss-Webster mice (Division of Cancer Treatment, Mammalian Genetics and Animal Production Section, National Cancer Institute) weighing 25-30 g. The mice were fed laboratory chow ad libitum until they were treated with the test chemicals. The sources of the chemicals are listed in (20). The compounds were administered at levels of 2-5,500 mg/kg (generally 125 mg/kg) (group I) or at 24-hour LD50 (group II). When the 24-hour LD50 were not available in the literature, group II determined the LD50 according to the method of Weil (21) (see table 1).

Test organisms.—The strains of *S. typhimurium* and *S. cerevisiae* D3 were maintained and grown as described previously (22, 23). In each test group were 4-6 mice, and positive and negative control groups were used in each experiment. The test compound was given by im injection (group I) or as a single dose in 0.2 ml dimethyl sulfoxide by oral intubation (group II).

Two milliliters of an overnight culture of the test microorganism was injected ip into the mouse after the skin on the abdomen was washed with alcohol. After 4 hours, the mice were killed by cervical dislocation, and 2 ml sterile saline was injected into the peritoneal

ABBREVIATIONS USED: CFU=colony-forming units; LD50=median lethal dose(s).

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cavity to aid in the recovery of the microorganisms. After the skin on the abdomen was washed with alcohol, sterile forceps and surgical scissors were used to expose the peritoneum. The microorganisms were recovered with 1-ml tuberculin syringes (without needles). The peritoneal fluid was placed in sterile test tubes in an ice bath, and subsequent dilutions in saline were done in the ice bath.

For assays with *Salmonella*, undiluted 0.2-ml aliquots were spread on five minimal agar plates containing biotin to determine the number of histidine-independent cells. An aliquot was diluted serially in saline to 10^{-7} , and 0.2-ml aliquots were spread on bacterial complete agar to determine the survival (or recovery) of *Salmonella*. The minimal agar plates were incubated for 2 days at 37°C , and the number of histidine-independent revertants was determined. The bacterial complete agar plates were incubated overnight, and the number of CFU per milliliter was determined.

In assays with *S. cerevisiae* D5 as the test organism, the overnight culture was diluted to 5×10^7 CFU/ml before injection of 0.2 ml into each mouse. The recovered peritoneal fluid was diluted to 10^{-5} . Aliquots (0.2 ml) of the 10^{-5} dilution were spread on 5 yeast agar plates (to determine the number of mitotic recombinants), and 0.2-ml aliquots of the 10^{-4} and 10^{-3} dilutions were each spread on 3 yeast agar plates to determine the survival (or recovery) of the *Saccharomyces*. The plates were incubated for 2 days at 30°C . Colonies on plates spread with the 10^{-4} or 10^{-3} dilution were counted to determine the CFU per milliliter. Plates spread with the 10^{-5} dilution were refrigerated to enhance the red pigment development indicative of mitotic recombination (adenine minus homology). After refrigeration for 2-3 days, the number of red colonies or red segments per plate was determined by scanning the plates with a $10\times$ dissecting microscope. The mutants per milliliter or mitotic recombinants per milliliter were then divided by the CFU per milliliter to determine the mutants (or mitotic recombinants) per 10^7 survivors (for *Salmonella*) or per 10^7 survivors (for *Saccharomyces*). Thus a mutation frequency was determined for cells recovered from each mouse.

Treatment of data and calculation of results.—In each experiment the mutant organisms per milliliter and the total organisms per milliliter were measured for each mouse; the former was divided by the latter to give an observed mutation frequency for each animal. The mean mutation frequency plus or minus standard error of the mean was thus calculated for each treated group of mice and compared to their simultaneously run controls. Treated animals, from which the total number of organisms per milliliter was less than 10% of the average total organisms per milliliter obtained from the negative control animals, were not used in the determinations of mutation frequencies. With *Salmonella* a compound was judged mutagenic if a two-tailed *t*-test of statistical comparison showed *P* at 0.05 or less. With *S. cerevisiae*, compounds were regarded as mutagenic only if the mutation frequency of the treated animals was at least ten times that of the

TABLE 1.—LD50 determinations

Compound	LD50*
Natulan	614 mg/kg
4-Aminoozobenzene	483 mg/kg
3-Methyl-4-(dimethylamino)azobenzene	1.53 g/kg
2,3-Dimethyl-4-aminobiphenyl	1.13 g/kg
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide	>1.6 g/kg
2-Nitronaphthalene	1.3 g/kg
4-Nitrobiphenyl	347 mg/kg
1-Anthramine	1.25 g/kg
N-Hydroxy-N-(2-fluorenyl)acetamide	1.5 g/kg
Hydrazine sulfate	418 mg/kg
Dimethylcarbamyl chloride	1 g/kg
1,8,9-Trihydroxyanthracene	>1.6 g/kg
1,2-Dimethylhydrazine	1 g/kg
Bromobenzene	1 g/kg
Acetamide	1 g/kg
Chrysene	>320 mg/kg
1-Naphthylamine	187 mg/kg
2-Naphthylamine	867 mg/kg
2-Anthramine	1.5 g/kg
Methotrexate	1.0 g/kg
2-Nitrofluorene	1.6 g/kg
α -Caprolactone	1.3 g/kg
Glycidaldehyde	200 mg/kg
Ethyl-p-toluenesulfonate	1 g/kg
5-Iodo-2'-deoxyuridine	1 g/kg
1,4-Butanesultone	138 mg/kg
2-Methyl-4-(dimethylamino)azobenzene	345 mg/kg
N-Nitrosodiethylamine	132 mg/kg
Thioacetamide	1.13 g/kg
1-Phenyl-3,3-dimethyltriazene	233 mg/kg
Thiourea	1 g/kg
3-Amino-1,2,4-triazole	>6 g/kg
Propyleneimine	355 mg/kg
β -Propiolactone	406 mg/kg
Lead acetate	3.5 g/kg
Titanocene dichloride	>660 mg/kg
Beryllium sulfate	1.2 g/kg
N-Methyl-N'-nitro-N-nitrosoguanidine	66 mg/kg
2-Fluorenamine	132 mg/kg
4-Nitroquinoline N-oxide	190 mg/kg
1,3-Propanesultone	467 mg/kg
Methylazoxymethanol acetate	105-160 mg/kg
N-Nitrosoethylurea	600 mg/kg
N-Acetoxy-N-2-fluorenylacetamide	>660 mg/kg
α -Naphthylisothiocyanate	152 mg/kg
Phenanthrene	700 mg/kg

* Determined by Dr. Simmon, unless otherwise indicated.

† Corrections from previous estimates.

‡ From Dr. Rosenkranz.

controls. The wide range of mutation frequencies (table 2) seen in the *S. cerevisiae* obtained from control mice precluded our calling compounds "mutagenic" that produce a twofold to tenfold increase in mutation frequencies.

RESULTS

Aromatic Amines

The host-mediated assay was relatively insensitive in the detection of carcinogenic aromatic amines (table 3). Fewer than half of the carcinogenic amines tested demonstrated mutagenic activity toward *S. typhimurium* TA1538 in this assay, whereas none of 20 carcino-

gens tested were clearly recombinogenic in assays with *S. cerevisiae* D3. However, none of the noncarcinogenic aromatic amines tested showed clear mutagenic activity toward either *S. cerevisiae* D3 or *S. typhimurium* TA1538 (table 3). Fourteen compounds were tested against *S. typhimurium* TA1538 in the two laboratories: 4 compounds [*N*-2-fluorenylacetamide, 1-naphthylamine, 2-nitronaphthalene, and 2-nitrofluorene] were mutagenic toward *S. typhimurium* TA1538 in one laboratory but not in the second (table 3). The different results may be due to the varying doses and modes of administration used by the two groups. A fifth compound, 2-methyl-4-(dimethylamino)azobenzene, was mutagenic toward *S. typhimurium* TA1538 in one test by group II but inactive in a second test by group II as well as in a single test by group I (table 3). The remaining 9 compounds tested in common gave the same results in both laboratories.

Polycyclic Aromatic Hydrocarbons

The host-mediated assay was also insensitive in detecting the carcinogenic polycyclic aromatic hydrocarbons (table 4). Only one of eight carcinogenic hydrocarbons tested showed mutagenic activity toward *S. typhimurium* TA1538, whereas none was active toward *S. cerevisiae* D3 (table 4). Neither of the two noncarcinogenic aromatic hydrocarbons tested showed clear mutagenic activity (table 4). However, anthracene did

give one positive response toward *S. typhimurium* TA1538 in the host-mediated assay, but this result was not reproducible in the same or in the second laboratory.

Nitrosamines, Nitrosamides, and Hydrazines

The host-mediated assay was relatively successful in detecting this class of chemical carcinogens. Of seven such carcinogens investigated, five were mutagenic toward *S. typhimurium* TA1530 or TA1535 in the host-mediated assay (table 5). However, group I detected four of five carcinogens, whereas group II detected two of six carcinogens tested against the base-pair substitution strains TA1530 and TA1535. Further, of four carcinogens tested in both laboratories, only *N*-nitrosoethylurea was found to be mutagenic to both *S. typhimurium* TA1530 and TA1535. The only procarcinogens not detected by either of these two strains were *N*-nitrosodiethylamine and natulan (table 5). Of the eight carcinogenic nitroso and hydrazine derivatives tested, only *N*-nitrosoethylurea and 1-phenyl-3,3-dimethyltriazene were active toward *S. cerevisiae* D3 in the host-mediated assay.

Alkylating Agents

Eight of ten alkylating carcinogens were mutagenic toward *S. typhimurium* TA1530 or TA1535 in the host-

TABLE 2.—Typical experiments listed to illustrate variation in data collected

Group	Microbial strain	Compound	Dose, mg/kg	Mouse No.	Total population, CFU/ml. $\times 10^{-7}$	<i>His</i> ⁺ revertants/ml	<i>His</i> ⁺ revertants/ 10^8 cells	Average mutation frequency, $\pm$ SEM, $\times 10^4$				
I	<i>S. typhimurium</i> TA1538	Control	125	1	9.3	55	5.91	6.03 $\pm$ 0.26				
				2	8.4	55	6.54					
				3	6.2	35	5.65					
		2-Anthramine		1	7.4	70	9.46	9.61 $\pm$ 0.14				
				2	7.9	75	9.49					
				3	9.1	90	9.89					
II	<i>S. typhimurium</i> TA1535	Control	420	1	4.82	24	4.97	9.36 $\pm$ 1.92				
				2	2.62	36	13.74					
				3	2.88	22	7.64					
		Hydrazine sulfate		4	2.88	32	11.11	41.5 $\pm$ 7.1				
				1	4.20	189	45.0					
				2	1.72	37	21.5					
				3	1.36	58	42.9					
				4	4.45	251	56.4					
									Total population, CFU/ml. $\times 10^{-7}$	<i>Ade</i> ⁺ recombinants/ml. $\times 10^{-7}$	<i>Ade</i> ⁺ recombinants/ 10^8 cells	Average mutation frequency, $\pm$ SEM, $\times 10^4$
				II	<i>S. cerevisiae</i> D3	Control	1,300		1	6.45	2	3.10
2	4.15	4	9.64									
3	10.33	1	0.97									
4	9.65	0	0.00									
5	11.10	4	3.60									
6	8.87	1	1.13									
2-Nitronaphthalene	1	0.60	0.1			1.67		2.43 $\pm$ 0.57				
	2	7.38	2			2.71						
	3	8.85	4			4.52						
	4	1.56	0.3			1.92						
	5	7.53	1	1.33								

mediated assay (table 5). The alkylating carcinogens not mutagenic toward these two *Salmonella* strains were benzyl chloride and ethyl-*p*-toluenesulfonate. *S. cerevisiae* D3 was even less sensitive in host-mediated assays with alkylating carcinogens; only methylazoxymethanol acetate increased mitotic recombinations in this assay (table 5). No well-categorized noncarcinogens were studied in this assay.

Miscellaneous Carcinogens

These compounds have been arbitrarily subdivided into heterocyclic compounds; amides, ureas, and azoating agents; antimetabolites; inorganic compounds; and promoters. Few of the carcinogens tested in any of these categories showed clear mutagenic activity toward any of the strains tested (table 6).

TABLE 3.—Mutagenic activities of carcinogenic and noncarcinogenic aromatic amines in the host-mediated assay^a

Compound	Carcino- genic activity ^a	Dose, mg/kg	Group I ^b		Dose, mg/kg	Group II ^b	
			Increase in mutation frequency, ^c <i>S. typhimurium</i>			Increase in mutation frequency ^c	
			TA1530	TA1538		<i>S. typhimurium</i> TA1538	<i>S. ce revisiae</i>
<i>N</i> -Acetoxy- <i>N</i> -2-fluorenylacetamide	UC	25	0.9	28.6 (25.5±2.1 vs. 0.9±0.1) ^f	660	5.9 (7.13±1.40 vs. 1.23±0.59) ^f	
4-Aminoazobenzene	PC	125	1.8 (1.39±9 vs. 0.73±0.05) ^f	0.9	500	1.4	0.
2-Aminobiphenyl	PC	12	0.8		495	0.7	1.1
4-Aminobiphenyl					132	1.8 (2.29±1.21 vs. 1.23±0.59)	2.1
1-Anthramine	NC				1,250	0.6	1
2-Anthramine	PC	125	1.0	1.6 (9.6±0.1 vs. 6.0±0.3) ^f	1,500	11.5 (45.8±12.0 vs. 3.98±0.86) ^f	(
Auramine	PC				660		1.1
Bis- <i>p</i> -(dimethylamino)diphenyl- methane	PC	125	1.4	2.7 (26.3±2.3 vs. 9.7±1.6) ^f	1,600		1
2,3-Dimethyl-4-aminobiphenyl	PC	125	1.1		1,050	1.0	(
2-Fluorenamine	PC	2		7.0 (5.1±0.3 vs. 0.7±0.1) ^f	132	34.6 (50.1±17.1 vs. 1.45±0.77) ^f	1.
		12	0.6				
<i>N</i> -2-Fluorenylacetamide	PC	125	1.0	1.3	495	2.0 (4.80±0.23 vs. 2.40±1.00) ^f	
<i>N</i> -4-Fluorenylacetamide	NC				495	0.4	
<i>N</i> -Hydroxy- <i>N</i> -(2-fluorenyl)acetamide	PC				1,500		2
3-Methoxy-4-aminoazobenzene	PC	125	2.7 (2.0±0.1 vs. 0.73±0.05) ^f	9.2 (34.0±8.8 vs. 3.7±1.5) ^f	50	9.5 (15.1±5.8 vs. 1.6±0.4) ^f	
2-Methyl-4-(dimethylamino)azo- benzene	PC	125	1.1	1.1	340	6.3 (10.0±4.0 vs. 1.6±0.4) ^f	
						1.2	
3-Methyl-4-(dimethylamino)azo- benzene	PC	125	1.1	0.5	1,500	0.3	
1-Naphthylamine	NC	125	1.6	1.0	167	3.1 (11.0±4.0 vs. 3.5±0.4) ^f	1.2
2-Naphthylamine	PC	125	2.7 (15.1±4.4 vs. 5.5±0.6) ^f	1.0	867	1.1	1
<i>N</i> -1-Naphthylhydroxylamine	UC				396	1.1	0.
<i>N</i> -2-Naphthylhydroxylamine	UC				500	2.7 (2.7±0.5 vs. 1.0±0.2) ^f	0.
α -Naphthylisothiocyanate	NC	125		1.0	165		0.
4-Nitrobiphenyl	PC	125	1.1	1.0	1,600	0.8	
2-Nitrofluorene	PC	125	1.2	13.7 (49.2±3.2 vs. 3.6±0.2) ^f	1,600	3.1 (7.0±2.7 vs. 2.3±1.0)	
2-Nitronaphthalene	PC	125	1.8 (9.8±0.4 vs. 5.5±0.6) ^f	6.5 (23.7±6.1 vs. 3.6±0.2) ^f	1,300	0.4	(
<i>p</i> -Rosaniline	PC	125	0.7	1.2	1,600	0.5	
4-(<i>o</i> -Tolylazo)- <i>o</i> -toluidine	PC	125	1.2		1,250	0.9	

^a The detector organisms were injected ip, whereas the chemicals were administered either im or orally.

^b Data from Dr. Rosenkrantz; 3 mice were in each group, and the compounds were administered im.

^c Data from Dr. Simmon; 4-6 mice were in each group, and the compounds were administered orally.

^d UC=ultimate carcinogen, PC=procarcinogen, NC=noncarcinogen.

^e Mutation frequency of the experimental group/mutation frequency of the control group. The average mutation frequency ($\times 10^6$) for *S. typhimurium* TA1530, TA1535, and TA1538 for all positive or borderline compounds are in parentheses; corresponding control values. Because of the excessively low mutation frequencies of the negative compounds, such values are not given for *S. cerevisiae*.

^f $P \leq 0.01$ in a two-tailed *t*-test of statistical comparison.

^g $0.01 \leq P \leq 0.05$ in a two-tailed *t*-test of statistical comparison.

TABLE 4.—Mutagenic activities of carcinogenic and noncarcinogenic aromatic hydrocarbons in the host-mediated assay*

Compound	Carcino- genic activity ^c	Dose, mg/kg	Group I ^b		Dose, mg/kg	Group II ^b		
			Increase in mutation frequency, ^a <i>S. typhimurium</i>			Increase in mutation frequency ^a		
			TA1530	TA1538		<i>S. typhimurium</i>		<i>S. cere- visiae</i> D3
						TA1535	TA1538	
Anthracene	NC	125	1.1	1.0	1,600	0.9	0.9	2.9
			1.1	5.1 (50.1±1.5 vs. 9.9±1.9) ^d				
Benz[a]anthracene	PC	125	1.1	0.9	1,600		2.7 (4.1±1.0 vs. 1.5±0.3) ^d	1.8
Benz[a]pyrene	PC	125	1.0	1.1	496		0.7	1.6
Benz[e]pyrene	PC				500		0.7	
7-Bromomethyl-12- methylbenz[a]an- thracene	UC				500		1.0	1.4
Chrysene	PC	125	0.9	0.5	330		0.7	0.6
7,12-Dimethylbenz- [a]anthracene	PC	125	0.9	1.2	1,600		0.7	0.8
3-Hydroxybenz- [a]pyrene	PC				23		1.5	2.5
7-(Hydroxymethyl)- 12-methylbenz- [a]anthracene	PC				500		0.1	0.6
Phenanthrene	NC	125	0.5	0.9	700	1.0		0.2

* The detector organisms were injected ip, whereas the chemicals were administered either im or orally.

^b Data from Dr. Rosenkrantz: 3 mice were in each group, and the compounds were administered im.^c Data from Dr. Simmon: 4-6 mice were in each group, and the compounds were administered orally.^d NC=noncarcinogen, PC=procarcinogen, UC=ultimate carcinogen.^e Mutation frequency of the experimental group/mutation frequency of the control group. The average mutation frequency ±SEM (×10⁶) for *S. typhimurium* TA1530, TA1535, and TA1538 for all positive or borderline compounds are in parentheses vs. the corresponding control values. Because of the excessively low mutation frequencies of the negative compounds, such values are not listed for *S. cerevisiae*.^f P≤0.01 in a two-tailed t-test of statistical comparison.^g 0.01≤P≤0.05 in a two-tailed t-test of statistical comparison.

Among the heterocyclic compounds, only safrole, 4-nitroquinoline *N*-oxide, *N*-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide, and 4-(hydroxyamino)quinoline *N*-oxide showed evident mutagenic activity toward *Salmonella* (table 6). Safrole, when administered at a dose of 5,500 mg/kg, was mutagenic to *S. typhimurium* TA1538 (table 5) but was inactive at the lower dose of 869 mg/kg. Group I found that *N*-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide (5-12 mg/kg) was mutagenic to *S. typhimurium* TA1538; group II found that it was inactive even at a dose of 1,600 mg/kg. It is difficult to reconcile this difference. 3-Amino-1,2,4-triazole appeared to be mutagenic to *S. typhimurium* TA1530 and TA1538 at low doses. It also appeared to possess borderline activity toward *S. cerevisiae* D3. Acridine orange, aflatoxins B₁ and B₂, and 7,9-dimethylbenz[*c*]acridine showed no mutagenic activity in any test studied.

Among the amides, ureas, and acylating agents, dimethylcarbamyl chloride was clearly mutagenic toward TA1535 at a dose of 1,000 mg/kg (table 6); however, at a dose of 125 mg/kg, it was inactive toward *S. typhimurium* TA1530. Acetamide and urethan were completely inactive toward *S. typhimurium* TA1535 and *S. cerevisiae* D3. Both thioacetamide and thiourea appeared to be weakly mutagenic in the host-mediated assay. At doses of 125 mg/kg, both compounds produced significant increases in the mutation frequencies observed with

S. typhimurium TA1530 and TA1538; however, doses of 1,000-1,450 mg/kg had no observable effect on the mutation frequencies of *S. typhimurium* TA1535 or the mitotic recombination frequency of *S. cerevisiae* D3 (table 6). None of the antimetabolites or metals showed any significant mutagenic activity toward any of the test strains (table 6). The apparent recombinogenic activity of 5-iodo-2'-deoxyuridine toward *S. cerevisiae* D3 was not significant. Similarly, the promoter 1,8,9-trihydroxyanthracene showed no mutagenic activity in the host-mediated assay toward either *S. typhimurium* TA1538 or *S. cerevisiae* D3.

DISCUSSION

The present results indicate that the host-mediated assay is insufficiently sensitive for the routine preliminary screening of large numbers of potential carcinogens. Even when the metals and promoters are excluded the host-mediated assay detected only about 50% of the carcinogens tested. Forty-three percent of the carcinogenic aromatic amines and 13% of the carcinogenic hydrocarbons were clearly mutagenic toward *S. typhimurium* TA1538 in the host-mediated assay. However, 71% of the carcinogenic nitrosamine derivatives and 80% of the alkylating carcinogens were mutagenic to *S. typhimurium* TA1530 or TA1535 in the same assay.

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TABLE 5.—Mutagenic activities of carcinogenic and noncarcinogenic nitrosamines, nitrosamides, hydrazines, and alkylating agents in the host-mediated assay*

Compound	Carcino- genic activity ^a	Dose, mg/kg	Group I ^b		Dose, mg/kg	Group II ^b			
			Increase in mutation frequency, ^c <i>S. typhimurium</i>			Increase in mutation frequency, ^c <i>S. typhimurium</i>		<i>S. cere- visiae</i> D3	
			TA1530	TA1538		TA1535	TA1538		
Nitrosamines, nitro- samides, and hydrazines									
Dimethylhydrazine	PC	125	2.5 (10.9±1.0 vs. 4.4±0.6) ^f	1.2	995	1.7			1.7
Diphenylnitro- samine	NC	125	1.0	1.0	5,000	1.3			0.4
Hydrazine sulfate	PC	125	0.6	1.1	200	1.0			
					420	4.4 (41±7 vs. 9±2) ^f			
<i>N</i> -Methyl- <i>N</i> '-nitro- <i>N</i> -nitroso- guanidine	UC	5	3.6 (26.5±11.6 vs. 7.3±0.4)	9.7 (3.2±0.6 vs. 0.3±0.1) ^f	66	0.8			
		30	44.3 (61.5±0.7 vs. 1.4±0.2) ^f	11.8 (3.9±0.1 vs. 0.3±0.1) ^f	66				0.1
Natulan	PC	125	0.6	1.6 (0.57±0.08 vs. 0.35±0.05) ^f	600		0.7		1.9
<i>N</i> -Nitrosodiethyl- amine	PC	377		0.8, 1.5	132	0.6			0.2
<i>N</i> -Nitrosoethyl- urea	UC	12	0.8	2.6 (0.8±0.1 vs. 0.3±0.1) ^f	602	32.8 (410±244 vs. 12.5±1.8) ^f	16.4 (180±76 vs. 11.0±4.3) ^f		257
		120	27.4 (37.8±0.8 vs. 1.3±0.2) ^f						
1-Phenyl-3,3-di- methyltriazene	PC	12	14.7 (127±17 vs. 8.7±1.9) ^f	1.0	198	0.6			34.3
Alkylating agents									
Benzyl chloride	UC	4,400	1.0	1.1	450	1.1			0.5
Bromobenzene	—	600	1.0	1.1	1,000		0.3		
1,4-Butanesultone	UC	106.4	16.5 (132±11 vs. 8.0±0.3) ^f	7.4 (63.4±11.0 vs. 8.6±0.2) ^f	188	164.0 (833±311 vs. 5.4±1.2) ^f			
ε-Caprolactone	—	432	1.1	0.9	1,300	1.4			1.3
1,2:3,4-Diepoxy- butane	UC	444	2.8 (20.8±1.5 vs. 7.5±0.2) ^f		56				4.7
Ethyl- <i>p</i> -toluene- sulfonate	UC	468	1.0	0.9	1,000		0.6		1.5
Glycidaldehyde	UC	456	2.5 (13.5±1.3 vs. 7.5±0.2) ^f	1.0	200	2.8 (14.8±4.7 vs. 5.3±1.5) ^f			0.7
Methylamry- methanol acetate	PC	469	672 (665±92 vs. 1.0±0.5) ^f	148 (260±28 vs. 1.8±0.7) ^f	132				9.6
1,3-Propanesultone	UC	12	548 (472±40 vs. 0.8±0.2) ^f	6.3 (62.1±8.9 vs. 9.9±1.8) ^f	467	269 (485±218 vs. 1.8±0.6) ^f			4.2
						2.2 (3.9±0.7 vs. 1.8±0) ^f			
β-Propiolactone	UC				405	3.9 (21.1±7.5 vs. 5.4±1.2) ^f			0.1
Propyleneimine	UC				355	3.1 (5.6±0.8 vs. 1.8±0.6) ^f			6.9
Uracil mustard	UC	12	3.7 (43.7±2.4 vs. 11.8±0.4) ^f	1.1					

* The detector organisms were injected ip, whereas the chemicals were administered either im or orally.

^b Data from Dr. Rosenkrantz: 3 mice were in each group, and the compounds were administered im.^c Data from Dr. Simmon: 4-6 mice were in each group, and the compounds were administered orally.^d PC=procarcinogen, NC=noncarcinogen, UC=ultimate carcinogen.^e Mutation frequency of the experimental group/mutation frequency of the control group. The average mutation frequency ±SEM (×10⁶) for *S. typhimurium* TA1530, TA1535, and TA1538 for all positive or borderline compounds are in parentheses vs. the corresponding control values. Because of the excessively low mutation frequencies of the negative compounds, such values are not listed for *S. cerevisiae*.^f P≤0.01 in a two-tailed t-test of statistical comparison.^g 0.01≤P≤0.05 in a two-tailed t-test of statistical comparison.

S. cerevisiae D3 organisms were generally very insensitive to the activity of the carcinogens, especially the aromatic amines and the miscellaneous carcinogens. Also, the *Salmonella* strains used in the host-mediated

assay detected a greater percentage of ultimate carcinogens than procarcinogens (67 vs. 38%).

The results from this study generally agree well with previous findings. The high percentage of the nitro-

TABLE 6.—Mutagenic activities of carcinogenic and noncarcinogenic heterocyclic compounds, amides, antimetabolites, metals, and promoters in the host-mediated assay^a

Compound	Carcino- genic activity ^a	Dose, mg/kg	Group I ^c		Dose, mg/kg		Group II ^c		S. cere- visiae D3
			Increase in mutation frequency, ^d <i>S. typhimurium</i>				Increase in mutation frequency ^d <i>S. typhimurium</i>		
			TA1530	TA1538			TA1535	TA1538	
Heterocyclics									
Acridine orange	PC				495		1.8 (4.3±1.2 vs. 2.4±1.0)	0.7	
Aflatoxin B ₁	PC				33		3.2 (5.1±1.6 vs. 1.6±0.4)	1.8	
Aflatoxin B ₁	PC				2		0.7, 1.4	1.4	
3-Amino-1,2,4- triazole	PC	12	4.0 (8.5±1.4 vs. 2.1±0.2) ^f	1.1	1,585	0.9			
		125	1.8 (1.8±0.1 vs. 1.0±0.2) ^f	1.2 (1.1±0.0 vs. 0.92±0.09) ^f	5,000			7.5	
		250	1.0	1.0					
7,9-Dimethyl- benz[<i>c</i>]acridine	PC	125	1.0	1.0	500		1.3	0.6	
4-(Hydroxyamino)- quinoline N-oxide	UC	12	3.7 (4.0±0.2 vs. 1.1±0.2) ^f	20.3 (84±23 vs. 4.1±0.3) ^f	1,600		4.7 (10.8±5.7 vs. 2.3±1.0) ^f	0.8	
N-[4-(5-Nitro-2- furyl)-2-thiazo- lyl]formamide	PC	5		231 (201±5 vs. 0.9±0.2) ^f	1,600		1.6	0.5	
		12	21.1 (24.8±0.7 vs. 1.2±0.1) ^f	108 (368.4±2.0 vs. 3.4±0.1) ^f					
4-Nitroquinoline N-oxide	PC	12	3.4 (7.2±5.5 vs. 2.1±0.2) ^f	150 (132±10 vs. 0.9±0.5) ^f	190		11.4 (14.0±2.5 vs. 1.2±0.6) ^f	93.5	
Safrole	PC	5,500	0.8	2.2 (3.8±0.5 vs. 1.8±0.1) ^f	869		0.9	20.9	
								0.5	
Amides									
Acetamide	PC				1,000	1.0		0.4	
								1.7	
Dimethylcarbamyl chloride	UC	125	1.0	1.1	1,000	3.5 (22.1±6.9 vs. 6.4±0.5) ^f		1.4	
Succinic anhydride	—	125	0.9	1.2	869	0.6		2.4	
Thioacetamide	PC	125	2.3 (1.9±0.3 vs. 0.9±0.3) ^f	2.8 (2.6±0.4 vs. 0.9±0.0) ^f	1,130	0.5		5.0	
			2.3 (2.3±0.4 vs. 1.0±0.5) ^f	4.2 (7.5±0.7 vs. 1.8±0.0) ^f				0.9	
			1.6 (1.4±0.1 vs. 0.9±0.0) ^f	3.5 (2.3±0.3 vs. 0.7±0.0) ^f					
			2.0 (2.0±0.5 vs. 1.0±0.5) ^f	2.0 (3.6±0.01 vs. 1.8±0.02) ^f	1,000	0.7		1.7	
			1.3	1.4 (0.92±0.06 vs. 0.65±0.01) ^f					
			1.6 (1.64±0.13 vs. 1.01±0.22) ^f	1.7 (1.58±0.14 vs. 0.92±0.04) ^f					
Thiourea	PC	125							

^a The detector organisms were injected ip, whereas the chemicals were administered either im or orally.^b Data from Dr. Rosenkrantz: 3 mice were in each group, and the compounds were administered im.^c Data from Dr. Simmon: 4-6 mice were in each group, and the compounds were administered orally.^d PC=procarcinogen, UC=ultimate carcinogen, NC=noncarcinogen.^e Mutation frequency of the experimental group/mutation frequency of the control group. The average mutation frequency $\pm$ SEM ($\times 10^4$) for *S. typhimurium* TA1530, TA1535, and TA1538 for all positive or borderline compounds are in parentheses vs. the corresponding control values. Because of the excessively low mutation frequencies of the negative compounds, such values are not listed for *S. cerevisiae*.^f $P \leq 0.01$ in a two-tailed *t*-test of statistical comparison.^g $0.01 \leq P \leq 0.05$ in a two-tailed *t*-test of statistical comparison.

samine derivatives showing mutagenic activity by the host-mediated assay in the present studies confirms previous observations (2-5). Similarly, several alkylating agents are mutagenic in the assay, both in the present and previous studies (8-11). In this study thioacetamide and thiourea were weakly mutagenic; a close structural analog, ethylene thiourea, is also mutagenic in the host-mediated assay (12). The two major classes of chemical carcinogens that show the least mutagenic activity in the host-mediated assay, the aromatic amines and the polycyclic hydrocarbons, had not been well investigated previously.

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