Mutagenicity Characteristics of 255 Environmental Chemicals

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The mutagenicity of 255 compounds were examined under the same conditions using the improved Ames test. These compounds were detected frequently in environment, were suspected of high toxicity, or were used as the positive standards for several toxicity tests. The relationships between the chemical structure and the strength of the mutagenicity were analyzed. Thirty compounds of the 255 tested compounds showed mutagenicity. It was found that the compounds, which are unintentionally formed, tended to show mutagenicity in a higher ratio but the artificially synthesized compounds tended to show it in a lower ratio. The number of compounds showed indirect mutagenicity (+S9) were more than the number of compounds showed direct mutagenicity (–S9) in the tested compounds. The mutagenicity strength was different by several hundred thousand times among the compounds. Condensed polycyclic aromatic nitrohydrocarbons, on the whole, showed very strong mutagenicity. The compounds were classified by the positive conditions. All of the tested condensed polycyclic aromatic nitrohydrocarbons accounted for the greatest majority of the compounds which showed mutagenicity under all the conditions of TA98 ± S9 and TA100 ± S9. Only two specific compounds showed mutagenicity under the three conditions except for TA98–S9. Some compounds showed mutagenicity only under the conditions of –S9 but there were various kinds of compounds which showed mutagenicity only under the conditions of +S9. The compounds which showed mutagenicity under only one condition showed weak mutagenicity.

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Key words —— mutagenicity strength, environmental chemicals, unintentionally formed compound, structure-activity relationship, Ames test

INTRODUCTION

The Ames mutagenicity test has many advantages such as a shorter test term, lower amount of test solution, a relatively higher sensitivity, better reproducibility and more quantitative estimation.[1,2] Therefore, this test is carried out by many research institutions[3–8] and is expected as a new evaluation method for testing the comprehensive toxicity of many pollutants.

However, though many compounds are evaluated by this test, there are few reports on the many environmental chemicals using the same method and conditions. Therefore, the mutagenicity of the compounds which are detected in environment samples are not apparent. Besides, most of the tested examples are qualitative or semi-quantitative. Therefore, we can not quantitatively compare the results in conventional reports.

In this study, we investigated the mutagenicity strength of 255 compounds using an improved Ames test,[9] and analyzed the relationships between the chemical structure and the mutagenicity strength.

MATERIALS AND METHODS

255 Compounds Tested —— The Ames test was carried out for 255 compounds as shown in Table 1. These compounds were detected frequently in environment, were suspected of high toxicity, or were used as the positive standards for several toxicity tests. These compounds were prepared from the project, "Research on the development of the total evaluation technique for the hazardous impact of chemical substances on humans and the ecology" supported by the Fundamental Research for the Environmental Future from the Environment Agency of Japan. These compounds were classified by their structures and expressed by the following symbols, I: Substitutes of benzene and naphthalene, II: Condensed polycyclic aromatic hydrocarbons (more than two rings) and their substitutes, III: Nitrogen-containing cyclic compounds, IV: Other cyclic compounds, V: Non-cyclic organic compounds, VI: Pesticides (complex formula compounds), and VII: In-

Table 1. Tested 255 Compounds

	Compound name		Compound name		Compound name
I	Nitrobenzene	I	Resorcinol	III	2-Amino-1-methyl-6-phenylimidazole[4,5-b]pyridine (PhIP)
	p-Nitrotoluene		Hydroquinone		3-Amino-1-methyl-5*H*-pyrido[4,3-b]indole (Trp-P-2)
	o-Chloronitrobenzene		2-Naphthol		2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)
	4-Chloronitrobenzene		Toluene		
	o-Nitrophenol		Benzylalcohol		
	m-Nitrophenol		Benzaldehyde		
	p-Nitrophenol		Benzoic acid		
	o-Dinitrobenzene		Ethyl benzene	IV	Epichlorohydrin
	1-Chloro-2,4-dinitrobenzene		Styrene monomer		2-Mercaptoimidazoline
	2,4-Dinitroaniline		Cumene		Cycrohexyl amine
	2,4-Dinitrophenol		α-Methylstyrene		Cyclohexanol
	Chlorobenzene		*n*-Butylbenzene		Cyclohexanone
	4-Chlorotoluene		*p*-Toluenesulfoneamide		Aplysiaterpenoid A
	4-Chloroaniline		Diethylbenzene, mixture		Isophorone
	p-Chlorophenol		*p*-*t*-Butylbenzoic acid		1,4-Dioxane
	4-Chloro-3-methylphenol		Terephthalic acid		Morpholine
	o-Dichlorobenzene		Diethyl phthalate		Cyclophosphamide
	1,4-Dichlorobenzene		Dibutyl phthalate (DIBP)		1,2-Epoxyethylbenzene
	3,4-Dichloroaniline		Di-2-ethylhexyl phthalate		Dicyclohexylamine
	2,4-Dichloroaniline		Naphthalene		Biphenyl
	2,5-Dichloroaniline		1-Methylnaphthalene		Diphenylmethane
	2,4-Dichlorophenol		1,2-Dimethylnaphthalene		Benzophenone
	2,5-Dichlorophenol		1,8-Dimethylnaphthalene		Dibenzyl ether
	1,2,3-Trichlorobenzene		2,6-Dimethylnapthalene		2,2-Bis(3,5-dibromo-4-hydroxyphenyl)propane
	1,2,4-Trichlorobenzene		Menadione		Hexachlorophene
	2,4,5-Trichlorophenol				*N*-Nitrosodiphenylamine
	2,4,6-Trichlorophenol	II	Anthracene		*o*-Tolidine
	Aniline		1,2-Benzanthracene		Bis-phenol-A
	Phenylhydrazine		Pyrene		Diethylstilbestrol
	N-Methylaniline		Benzo[*b*]fluoranthene		Triphenyltin(IV) chloride
	N,*N*-Dimethylaniline		Benzo[*k*]fluoranthene (0.2 mM)		Genistein
	N-Ethylaniline		Benzo[*e*]pyrene		β-Estradiol-17-acetate
	o-Toluidine		Benzo[*a*]pyrene		17-α-Ethynylestradiol
	m-Aminophenol		1,2;5,6-Dibenzanthracene		Dexamethasone
	2-Phenylene diamine		Benzo[*ghi*]perylene		Coumestrol (0.5 mM)
	2,4-Diaminotoluene		2-Nitrofluorene		Aflatoxin B1 (0.2 mM)
	N-Phenyl-1-naphthylamine		3-Nitrofluoranthene		Coumestrin (0.3 mM)
	N-Phenyl-2-naphthylamine		1-Nitropyrene		Okadaic acid (0.05 mM)
	Phenol		1,8-Dinitropyrene (0.1 mM)		Cucumechinoside D (0.01 mM)
	2-Methylphenol		1,6-Dinitropyrene (0.1 mM)		Marthasteroside A1 (0.01 mM)
	p-Cresol		2-Aminoanthracene		Microcystin RR (0.1 mM)
	Pentylphenol		2-Aminoanthraquinone		Cyclosporin A (0.5 mM)
	p-Nonylphenol		3-Methylcholanthrene		
	2,4-Dimethylphenol			V	Tris(2-chloroethyl)phosphate
	2,4,6-Trimethylphenol	III	2-Methylpyridine		Tris(2,3-dibromopropyl)phosphate
	2,6-Di-*t*-butyl-4-methylphenol		Melamine		Tributyl phosphate
	p-Bromophenol		2-Mercaptobenzothiazole		Tris(butoxyethyl)phosphate
	2,4,6-Tribromophenol		Quinoline		Methylmercury Chloride
	Catechol		4-Nitroquinoline-*N*-oxide		Bromodichloromethane

Concentrations in bracket are exceptional maximum test concentrations. I: The substitutes of benzene and naphthalene. II: Condensed polycyclic aromatic hydrocarbons and their substitutes. III: Nitrogen-containing cyclic compounds. IV: Other cyclic compounds. V: Non-cyclic organic compounds. VI: Pesticides (Complex formula compounds). VII: Inorganic compounds.

Table 1. Continued

	Compound name		Compound name		Compound name
V	Chlorodibromethane	V	Sodium lauryl sulfate	VI	2,4,-Dichlorophenyl 4-nitrophenyl ether (NIP)
	Bromoform		Di-2-ethylhexyl adipate		Bifenox
	Monochloroacetic acid				Paraquat
	1,2-Dibromoethane	VI	Methomyl		Permethorin
	1,1,1,2-Tetrachloroethane		Aldicarb		2,4,5-Trichlorophenoxyacetic acid
	Tetrachloroethylene		2-*s*-Butylphenyl methylcarbamate (BPMC)		Captans
	1,3-Dichloro-2-propanol		1-Naphthyl methylcarbamate (NAC)		4-Nitrophenyl 2,4,6-trichlorophenyl ether (CNP)
	1,3-Dichloropropene,mixture		Thiophanate-methyl		Methoxychlor
	1,2,3-Trichloropropane		Thiobencarb		Tetrachloroisophthalonitrile (TPN)
	1,2-Dibromo-3-chloropropane		Molinate		Fthalide
	2-Chloro-1,1,2-trifluoroethyl ethyl ether		Maneb		Pentachloronitrobenzene (PCNB)
	Hexachloro-1,3-butadiene		Manzeb		Pentachlorophenol
	Tributyltin chloride		Ziram		Kelthane
	Thiourea		Thiram		1,2,3,4,5,6-Hexachlorocyclohexane
	N-Nitrosodimethylamine		Simazine		*n*-Decyl alcohol
	2-Aminoethanol		Simetryne		3-Amino-1*H*-1,2,4-triazole
	Acetamide		2,2-Dichlorovinyl dimethyl phosphate (DDVP)		Trifluralin
	N,*N*-Dimethylformamide		Glyphosate		Dimethyl phthalate
	1,3-Dimethyl-2-thiourea		Acephate		Dibutyl phthalate
	Ethyl carbamate		Dimethoate		Dicyclopentadiene
	Acrylamide		Ethylthiomethone		Diphenylamine
	N-Nitrosodiethylamine		Malathion		Diquat dibromide monohydrate
	Triethylenetetramine		*O*,*O*-Dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate (MEP)	VII	Potassium dichromate (VI)
	Triethylamine		*O*,*O*-Dimethyl *O*-4-methyltio-*m*-tolyl phosphorothioate (MPP)		Nickel(II)chloride
	2,2′,2″-Nitrilotriethanol		Ethyl dimethoxyphosphinothioylthio-(phenyl)acetate (PAP)		Copper(II)sulfate
	Nitrilotriacetic acid		*S*-Benzyl *O*,*O*-di-isopropyl phosphorothioate (IBP)		Zinc nitrate hexahydrate
	Tetraethylenepentamine		Diazinon		Sodium arsenite
	EDTA 2Na		Isoxathion		Sodium selenate
	Formaldehyde		*O*-Ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN)		Sodium molybdate
	Ethylene glycol		*O*-Ethyl *S*,*S*-diphenyl phosphorodithioate (EDDP)		Cadmium chloride
	Acetaldehyde		Alachlor		Antimony(III)chloride
	Glyoxal		Bis(2-chloroethyl) ether		Mercury(II)chloride
	Propylene glycol		3′,4′-Dichloropropionanilide (DCPA)		Thallium(I)chloride
	Methylglyoxal		2,4-Dichlorophenoxy acetic acid		Lead nitrate
	Ethylene glycol monoethyl ether		Vinclozolin		Boric acid
	2-Methyl-1-propanol				Hydroxyl ammonium sulfate
	1-Butanol				Potassium cyanide
	Vinylacetic acid				Barium nitrate
	Diethylene glycol				
	Diethyl sulfate				
	Adipic acid				
	n-Butyl acrylate				
	1-Nonanol				

organic compounds. These compounds were also classified in much detail within each group. Namely, the compounds in Group II were expressed with the following symbols, IIa: Condensed polycyclic aromatic hydrocarbons, IIb: Their nitro compounds, and IIc: The other substitutes.

Test Method for the 255 Compounds —— The improved Ames tests[9] were carried out using *Salmonella typhimurium* TA98 and TA100 without (–S9) and with (+S9) a metabolic activator S9 mix. The TA98 strain and TA100 strain, which were obtained from the National Institute of Public Health

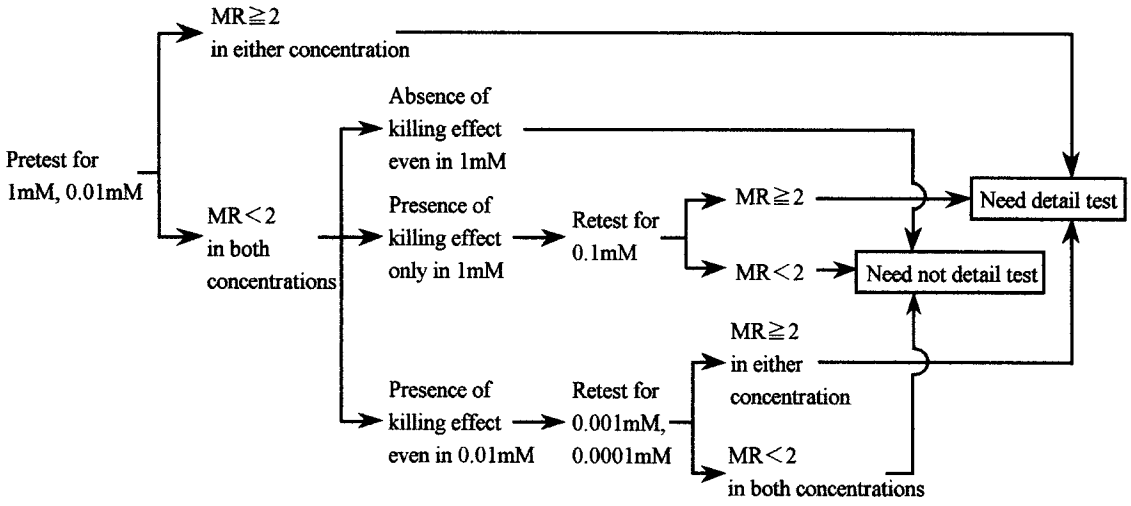

Fig. 1. Flow Pattem for Jdging Ncessity of Detailed Test

Japan, were used for detecting the mutagens that cause frameshifts and base-pair substitutions to DNA, respectively. The S9 was prepared using rat liver S9 induced by phenobarbital and 5,6-benzoflavone. The S9 and cofactor were purchased from the Oriental Yeast Co., Tokyo, Japan.

Procedure for the test is as follows. The tester strains were precultured for 24 hr at 25°C in the Oxoid nutrient broth. A 0.1 ml test solution and 0.5 ml of the phosphate buffer or 0.5 ml of the S9 mix, and 0.1 ml of the culture of the tester strains were placed in the tubes. After preincubation at 25°C for 20 min with vortexing, 2 ml of the top agar was mixed with the solution in the tubes. The mixture was poured onto the minimal glucose agar plate and then evenly extended. After incubation for 48 hr at 37°C, the number of revertant colonies were counted.

This method is almost the same as the conventional Ames test method.[1,2,10] The difference from the conventional method is that the temperature of the preculture and the preincubation is reduced to 25°C in order to avoid hard work from early morning or till night and to carry out the test within the usual working time.[9] The pretests for the 255 compounds were carried out before detailed tests to judge the necessity for the detailed test as shown in Fig. 1. However, 11 compounds which could not be obtained in 1 mM solutions were pretested in the concentrations shown in Table 1 and their 1/100 instead of 1 mM and 0.01 mM.

As for the compounds judged positive by the pretests, the detailed tests were done based on the results of the pretests. The numbers of net revertant colonies for a 10^{-9} mol dose (net rev./nmol) were calculated from the slopes of the straight parts of the dose–response curves. If the slope of the dose–response curve was clearly large from midstream, the slope in the higher dose area was used for the calculation.

The mean values and standard deviations of the mutagenicity strength for the positive controls in 16 times tests were 330 ± 19 net rev./nmol for TA98–S9, 89 ± 6 net rev./nmol for TA98+S9, 2300 ± 260 net rev./nmol for TA100–S9 and 200 ± 27 net rev./nmol for TA100+S9, respectively. 4-nitroquinoline-*N*-oxide and 2-aminoanthracene were used as the positive controls for the –S9 and +S9 test, respectively.

RESULTS AND DISCUSSION

Chemical Structures and Mutagenicity Existence of 255 Compounds

Thirty compounds were judged positive in the 255 tested compounds by the pretests.

Table 2 shows the ratio of the positive compounds to the tested compounds for each structural group. The 73 compounds in the 74 tested substitutes of benzene and naphthalene (Group I) did not show any mutagenicity, and only one of chloronitrobenzenes showed mutagenicity. On the other hand, 15 compounds in the 17 tested condensed polycyclic aromatic hydrocarbons and their substitutes (Group II), which are formed by combustion processes, showed mutagenicity while only anthracene and benzo[*e*]pyrene did not show any mutagenicity. Their nitro compounds and the other sub-

Table 2. Positive Ratio for the Each Structure Group of the Tested 255 Compounds

Symbol	Structure group	Mutagenic compounds /Tested compounds
I	Substitutes of benzene and naphthalene	1/ 74 (1.4%)
	Their nitro compounds	1/11
	Their chlorinated compounds	0/16
	Their amino compounds	0/11
	Their hydroxy compounds	0/14
	The other substitutes or naphthalene	0/22
II	Condensed polycyclic aromatic hydrocarbons (more than two rings) and their substitutes	15/ 17 (88%)
IIa	Condensed polycyclic aromatic hydrocarbons	7/ 9
IIb	Their nitro compounds	5/ 5
IIc	The other substitutes	3/ 3
III	Nitrogen-containing cyclic compounds	5/ 8 (63%)
IV	Other cyclic compounds	2/ 35 (5.7%)
V	Non-cyclic organic compounds	3/ 51 (5.9%)
	Their alkylphosphated compounds	1/ 4
	Their halogenated compounds	2/15
	Their nitro compounds	0/15
	The other substitutes	0/17
VI	Pesticides (complex formula compounds)	3/ 54 (5.6%)
	Carbamate	0/ 5
	Thiocarbamate	2/ 6
	Triazine	0/ 2
	Organophosphorus	0/14
	Organohalogen	1/19
	The other pesticides	0/ 8
VII	Inorganic compounds	1/ 16 (6.3%)
	Heavy metal salts	1/12
	The other inorganic compounds	0/ 4
	Total	30/255 (12%)

stitutes of them showed mutagenicity in all the 8 tested compounds. This was in contrast to the results of the nitro compounds of benzene and naphthalene. Nitrogen-containing cyclic compounds (Group III), which exist in burnt foods, also showed mutagenicity in the high ratio of 5 in 8. However, the 33 other cyclic compounds (Group IV) did not show any mutagenicity except for specific compounds such as *N*-nitrosodiphenylamine and aflatoxin B1. The 48 compounds in the 51 tested non-cyclic organic compounds (Group V) did not show any mutagenicity, and only tris(2,3-dibromopropyl)phosphate, 1,2,3-trichloropropane and 1,2-dibromo-3-chloropropane showed mutagenicity. Furthermore, the 51 compounds in the 54 tested pesticides (Group VI) did not show any mutagenicity, while only ziram, thiram and captans showed mutagenicity. Also, 15 compounds in the 16 tested inorganic compounds (Group VII) did not show any mutagenicity, while only potassium dichromate (VI) showed mutagenicity.

Thus, interestingly, the compounds which are unintentionally formed, such as the nitrogen-containing cyclic compounds, condensed polycyclic aromatic hydrocarbons and their substitutes, tended to show mutagenicity in a higher ratio but the artificial synthesized compounds including the pesticides tended to show it in a lower ratio. These unintentionally formed compounds were not individually managed, because there are too many kinds of compounds and each of them can hardly be determined. Therefore, the bioassay methods, which are able to perform inclusive evaluations, are very useful to manage these unintentionally formed compounds.

Mutagenicity Strength and Positive Conditions of 30 Positive Compounds

Examples of the dose–response curves are shown

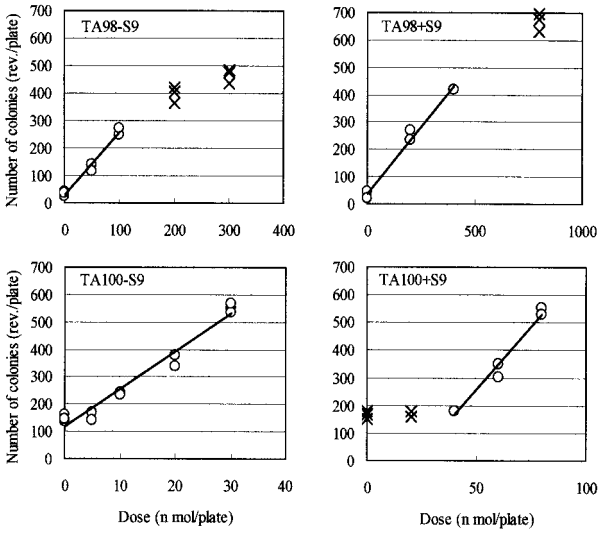

Fig. 2. Examples of the Dose–Response Curves (1-Chloro-2,4-dinitrobenzene)

in Fig. 2. The mutagenicity strength of the 30 positive compounds obtained from the dose–response curves by the detailed test using the same method and conditions are summarized in Table 3. The mutagenicity strength were in the wide range of several hundred thousand times. The condensed polycyclic aromatic nitrohydrocarbons (Group IIb), on the whole, showed much stronger mutagenicity. The number of compounds showed indirect mutagenicity (+S9) were more than the number of compounds showed direct mutagenicity (–S9) in the tested compounds.

The mutagenicity levels were classified by the sum of the mutagenicity strength SM (net rev./nmol) with and without the S9 mix for each strain, TA98 and TA100, and expressed in the following rankings: Rank SA is $SM > 10^5$, Rank A is $10^5 \geq SM > 10^4$, Rank B is $10^4 \geq SM > 10^3$, Rank C is $10^3 \geq SM > 10^2$, Rank D is $10^2 \geq SM > 10$, and Rank E is $10 \geq SM >$ N.D. The compounds were summarized by the positive conditions, and the rankings of the mutagenicity were classified in Table 4 from the results shown in Table 3.

All of the tested condensed polycyclic aromatic nitrohydrocarbons (Group IIb) showed mutagenicity under all the conditions of TA98 ± S9 and TA100 ± S9, and the dinitropyrenes showed very strong mutagenicity. These compounds accounted for the greatest majority of the compounds which showed mutagenicity under all the conditions. Only two specific compounds, aflatoxin B1 and captans appeared under three conditions but TA98–S9, and aflatoxin B1 showed strong mutagenicity. The compounds which showed mutagenicity only under the conditions of –S9 were only pyrene for TA98 and TA100 and *N*-nitrosodiphenylamine for TA100. Ten compounds showed only under the conditions of +S9 for TA98 and TA100, and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) showed strong mutagenicity. The compound that showed mutagenicity only under TA98+S9 was 2-aminoanthraquinone. Eight compounds, especially four compounds of polycyclic aromatic hydrocarbons (Group IIa) were positive only under the conditions of TA100+S9, but all of these compounds showed relatively weak mutagenicity. All the compounds which showed mutagenicity under only one condition showed weak mutagenicity.

Most of the condensed polycyclic aromatic hydrocarbons (Group IIa) and their substitutes except for the nitro substitutes (Group IIc) showed mutagenicity under the condition of +S9, but only pyrene did not show mutagenicity with metabolism. Most of the nitrogen-containing cyclic compounds (Group III) also showed mutagenicity only under the condition of +S9 though only 4-nitroquinoline-*N*-oxide (4NQO), which is widely used as a positive standard compound, showed mutagenicity under all the conditions.

These fundamental data can serve to estimate what compounds contribute largely to mutagenicity strength of environmental samples and to estimate emission sources of the mutagens in environment.

Comparison with Literature Results

Of the 255 tested compounds, the mutagenicity data of 160 compounds have already been reported,[2,11–17] and 48 compounds of the 160 compounds were reported as positive under at least one condition of TA98 ± S9 and TA100 ± S9.

Of the 30 compounds which were judged positive in this investigation, 18 compounds were found in these 160 compounds. Sixteen of the 18 compounds were judged positive and 2 compounds were judged negative in the literature. Fourteen of the 16 compounds were judged positive and 2 compounds were judged negative from the data in the literature[11–13] using the same criterion as in this investigation. Two compounds, *N*-nitrosodiphenylamine and pyrene, were judged positive in this investigation, though they were judged negative in the literature.[16,17] Though the number of revertant colonies obtained in several doses was shown, the compounds were only judged positive or negative and

Table 3. Mutagenicity Strength of the Positive Compounds (net rev./nmol)

	Compound name	Mutagenicity strength			
		TA98		TA100	
		−S9	+S9	−S9	+S9
I	1-Chloro-2,4-dinitrobenzene	2.2	0.99	14	9.1[a)]
IIa	1,2-Benzanthracene	N	0.47	N	15
	Pyrene	0.87	N	1.5	N
	Benzo[*k*]fluoranthene	N*	N*	N*	38
	Benzo[*b*]fluoranthene	N	N	N	35
	Benzo[*a*]pyrene	N	7.7	N	120
	1,2;5,6-Dibenzanthracene	N	N	N	21
	Benzo[*ghi*]perylene	N	N	N	4.4
IIb	2-Nitrofluorene	5.0	4.3	31	41
	3-Nitrofluoranthene	1200	130[a)]	2200	420[a)]
	1-Nitropyrene	490	64	280	120[a)]
	1,8-Dinitropyrene	120000	2000[a)]	53000	1300[a)]
	1,6-Dinitropyrene	55000	1100[a)]	31000	810[a)]
IIc	2-Aminoanthracene	N	88	N	230
	2-Aminoanthraquinone	N	2.1	N	N
	3-Methylcholanthrene	N	6.3	N	83
III	Quinoline	N	N	N	1.1
	4-Nitroquinoline-*N*-oxide	340	15[a)]	2600	23
	PhIP	N	320	N	52
	MeIQx	N	25000	N	1300
	Trp-P-2	N	7500	N	220
IV	*N*-Nitrosodiphenylamine	N	N	3.0	N
	Aflatoxin B1	N*	8200	14	56000
V	Tris(2,3-dibromopropyl)phosphate	N	2.0	N	13
	1,2,3-Trichloropropane	N	N	N	2.2
	1,2-Dibromo-3-chloropropane	N	0.71	N	38
VI	Ziram	N	N	N	1.5
	Thiram	N	N	N	2.2
	Captans	N	4.3[a)]	51	28[a)]
VII	Potassium dichromate (VI)	N	0.32	N	1.6
	Number of positive compounds	8	20	11	27

N: The amount of addition which becomes MR = 2 is more than 100 nmol/plate, or the compound is not mutagenic under this condition. N*: The amount of addition which becomes MR = 2 is more than 20 nmol/plate, or the compound is not mutagenic under this condition. *a*) The slope of the dose-response curve in high dose area was used for the calculation because the slope became large from midstream.

their mutagenicity strength was not calculated using their slopes of the dose–response curves in most of the reports. Therefore, we roughly estimated the mutagenicity strength of the 2 compounds using the data from the literature. The mutagenicity strength of *N*-nitrosodiphenylamine in this investigation was 3.0 net rev./nmol under TA100–S9, but the value which was calculated using the data from the literature[16] was less than 0.49 net rev./nmol. The mutagenicity strength of pyrene in this investigation was 0.87 net rev./nmol under TA98–S9 and 1.5 net rev./nmol under TA100–S9, but the values calculated

Table 4. Positive Conditions and Levels of Mutagenicity for the Positive Compounds

Positive conditions				Ranks of the mutagenicity strength		Compound name	Structure group
TA98		TA100					
−S9	+S9	−S9	+S9	TA98	TA100		
+	+	+	+	SA	A	1,8-Dinitropyrene	IIb
				A	A	1,6-Dinitropyrene	IIb
				B	B	3-Nitrofluoranthene	IIb
				C	B	4-Nitroquinoline-*N*-oxide	III
				C	C	1-Nitropyrene	IIb
				E	D	1-Chloro-2,4-dinitrobenzene	I
				E	D	2-Nitrofluorene	IIb
−	+	+	+	B	A	Aflatoxin B1	IV
				E	D	Captans	VI
+	−	+	−	E	E	Pyrene	IIa
−	+	−	+	A	B	MeIQx	III
				B	C	Trp-P-2	III
				C	D	PhIP	III
				D	C	2-Aminoanthracene	IIc
				E	C	Benzo[*a*]pyrene	IIa
				E	D	1,2-Benzanthracene	IIa
				E	D	3-Methylcholanthrene	IIc
				E	D	Tris(2,3-dibromopropyl)phosphate	V
				E	D	1,2-Dibromo-3-chloropropane	V
				E	E	Potassium dichromate (VI)	VII
−	−	+	−	N.D.	E	*N*-Nitrosodiphenylamine	IV
−	+	−	−	E	N.D.	2-Aminoanthraquinone	IIc
−	−	−	+	N.D.	D	Benzo[*k*]fluoranthene	IIa
				N.D.	D	Benzo[*b*]fluoranthene	IIa
				N.D.	D	1,2;5,6-Dibenzanthracene	IIa
				N.D.	E	Benzo[*ghi*]perylene	IIa
				N.D.	E	Quinoline	III
				N.D.	E	1,2,3-Trichloropropane	V
				N.D.	E	Ziram	VI
				N.D.	E	Thiram	VI

Mutagenicity ranks were classified using the following symbols. Rank SA: $SM > 10^5$, Rank A: $10^5 \geq SM > 10^4$, Rank B: $10^4 \geq SM > 10^3$, Rank C: $10^3 \geq SM > 10^2$, Rank D: $10^2 \geq SM > 10$, Rank E: $10 \geq SM >$ N.D. N.D.: The amount of addition which becomes MR = 2 is more than 20 or 100 nmol/plate, or the compounds is not mutagenic under both of the ± S9 conditions.

from the literature[17] were less than 0.010 net rev./nmol under TA98–S9 and less than 0.063 net rev./nmol under TA100–S9. Thus the values calculated from the literature were several times or several tens lower than the values from this investigation. Therefore, it was considered that there were not only differences in the activities of the strains and the test procedure, but some mistakes such as in sample preparation. Therefore, these compounds were judged positive in this investigation but negative in the literature.

Two compounds, quinoline and 1,2,3-trichloropropane, were judged positive in this investigation and the literature,[11–13] though their data in the literature were judged negative by the same criterion as in this investigation. The mutagenicity

strength of quinoline in this investigation was 1.1 net rev./nmol under TA100+S9, but the values calculated from the literature were different, *i.e.*, 1.3 net rev./nmol,[11] 1.4 net rev./nmol,[11] 0.56 net rev./nmol[13] and 0.12 net rev./nmol.[12] Here, the S9 mix conditions of each literature were compared. Rat liver S9 induced by phenobarbital and 5,6-benzoflavone was used in the literature,[11] and rate of the S9 in S9 mix was 10% similar to this investigation. However, the rat liver S9 induced by aroclor 1254 was used in the literature[13] though the rate of S9 in the S9 mix was same as in this investigation. Rat liver S9 induced by PCB was 30% in the S9 mix in the literature.[12] Therefore, the reasons for the ten times variation were considered to be that the conditions of the S9 mix and activities of the strains and the test procedure were different. The mutagenicity strength of 1,2,3-trichloropropane in this investigation was 2.2 net rev./nmol under TA100+S9, but the value calculated from the literature[13] was 0.22 net rev./nmol. The reason for this difference was considered that the S9 was induced by aroclor 1254 or that there were some mistakes such as sample preparation for the ten times difference.

On the other hand, 32 of the 48 compounds, which were judged positive in the literature, were not judged positive in this investigation. Three of the 32 compounds could be regarded as positive by the same criterion as in this investigation and the mutagenicity strength of 29 compounds were weak and were judged negative by the same criterion as in this investigation. Three compounds that included benzo[*e*]pyrene, *o*-tolidine and methylglyoxal were not judged positive in this investigation, but were judged positive in the literature[11–13,17] by the same criterion as in this investigation. The mutagenicity strength of benzo[*e*]pyrene in this investigation was less than 0.33 net rev./nmol under TA98+S9 and less than 1.3 net rev./nmol under TA100+S9, but the values calculated from the literature[17] were 0.98 net rev./nmol under TA98+S9 and 3.2 net rev./nmol under TA100+S9. The mutagenicity strength of *o*-tolidine in this investigation was less than 0.42 net rev./nmol under TA98+S9, but the values calculated from the literature were 2.1 net rev./nmol[11] and 1.0 net rev./nmol.[13] The mutagenicity strength of methylglyoxal in this investigation was less than 1.2 net rev./nmol under TA100–S9, but the value calculated from the literature[12] was 2.9 net rev./nmol. These differences might be caused by the differences in the activities of the strains, the test procedure and rat liver S9.[13,17]

Thus, the values of mutagenicity strength for a compound in previous reports were rather different by test institutions because they were obtained under various conditions. Therefore, our data obtained under the same conditions enabled us to compare mutagenicity strength of many compounds.

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REFERENCES

1) Ministry of Labor Japan (1991) *Mutagenicity assay for the Industrial Safety and Health Act: Test guide line and GLP*, Japan Industrial Safety and Health, Tokyo.
2) Urano, K., Takanashi, H. and Igarashi, I. (1997) Guidance manual of the Ames mutagenicity assay for water sample (I) (II) (III). *J. Water and Waste*, **39**, 163–168, 255–259, 342–349.
3) Yoshino, H. and Urano, K. (1998) Mutagenic activities of exhaust gas and ash from sludge incineration plants. *Sci. Total Environ.*, **215**, 41–49.
4) Crebelli, R., Fuselli, S., Baldassarri, L. T., Ziemacki, G., Carere, A. and Benigni, R. (1995) Genotoxicity of urban air particulate matter : correlations between mutagenicity data, airborne micropollutants, and meteorological parameters. *Int. J. Environ. Health Res.*, **5**, 19–34.
5) Utsumi, H., Hamada, A., Hayatsu, H., Hashimoto, T. and Aizawa, Y. (1990) Seasonal and basinal variations of mutagenicity of river water. *Japan J. Water Poll. Res.*, **13**, 227–234.
6) Kool, H. J. and Van Kreijl, C. F. (1984) Formation and removal of mutagenic activity during drinking water preparation. *Water Res.*, **18**, 1011–1016.
7) Takanashi, H., Urano, K. and Ohgaki, S. (2000) Analysis of mutagen formation potential on chlorination of wastewater in Japan. *J. Japan Soc. on Water Environ.*, **23**, 352–359.
8) Abe, A. and Urano, K. (1996) Characteristics of lethal substances and the removal effect of molecular sulfur in the Salmonella mutagenicity test for river sediments. *Mutat. Res.*, **351**, 61–66.
9) Kubo, T., Chikasada, K., Kameya, T. and Urano, K. (1999) Study of efficient preculture method for Ames mutagenicity test and mutagenicity analysis of chemicals. In *Proceeding of the 33rd annual meeting of Japan Society on Water Environment*, p. 503.
10) Maron, D. M. and Ames, B. N. (1983) Revised methods for the Salmonella mutagenicity test. *Mutat.*

Res., **113**, 173–215.
11) Ministry of Labor Japan (1996) *Mutagenicity test data of existing chemical substances based on the toxicity investigation system of the Industrial Safety and Health Law*, Japan Chemical Industry Ecology-Toxicology & Information Center, Tokyo.
12) Ishidate, M., Nohmi, T. and Matsui, M. (1991) *Mutagenicity Test Data Used Microorganism*, Life-Science Information Center, Tokyo.
13) Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. and Zeiger, E. (1983) Salmonella Mutagenicity Test Results for 250 Chemicals. *Environ. Mutagen*., Supplement 1, 3–142.
14) Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. and Zeiger, E. (1986) Salmonella Mutagenicity Tests:II. Results From the Testing of 270 Chemicals. *Environ. Mutagen*., Supplement 7, 1–119.
15) Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. and Speck, W. (1987) Salmonella Mutagenicity Tests:III. Results From the Testing of 255 Chemicals. *Environ. Mutagen*., Supplement 9, 1–110.
16) Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. and Mortelmans, K. (1988) Salmonella Mutagenicity Tests:IV. Results From the Testing of 300 Chemicals. *Environ. Mol. Mutagen*., Supplement 12, 1–158.
17) Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. and Mortelmans, K. (1992) Salmonella Mutagenicity Tests:V. Results from the Testing of 311 Chemicals. *Environ. Mol. Mutagen*., Supplement 21, 2–141.