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Validation of the Salmonella (SV50)/arabinose-resistant forward mutation assay system with 26 compounds

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Summary

Mutagenic sensitivity of the Salmonella/arabinose-resistant (Ara^r) assay system using the tester strain SV50 was evaluated with 26 compounds both by the preincubation and the standard plate incorporation tests. The mutagenic activity of all 26 compounds was also tested with TA98 and/or TA100 of the Ames Salmonella/microsome assay system. The results indicate that 13 and 10 of 26 compounds were mutagenic and nonmutagenic, respectively, in both assay systems. PR toxin and hydrogen peroxide were mutagenic only in the Ara^r assay, while 2-nitrofluorene was mutagenic only in the Ames assay. The results also show that the mutagenic response of SV50 to 13 of 15 mutagenic compounds was much higher (2.1–154-fold) if the compounds were tested with the preincubation rather than the plate incorporation test. The mutagenic activity of 4 compounds (diethyl sulfate, niridazole, PR toxin and hydrogen peroxide) in the Ara^r assay was detected only with the preincubation test.

Since the Ara^r assay using tester strain SV50 has similar mutagenic sensitivity as the Ames assay to chemicals with different modes of action and since it requires only one tester strain, we find this assay system to be useful for screening environmental mutagens. Based on the effectiveness of the preincubation test in this study, it is recommended that the preincubation test instead of the plate incorporation test be used for the Ara^r assay system with tester strain SV50.

In addition to the widely used Ames Salmonella/microsome assay (Ames et al., 1975), several bacterial mutagenesis test systems, including the Ara^r assay, have been developed for the detection of the mutagenic activity of chemical carcinogens and environmental agents (Hollstein et al., 1979; Ruiz-Vazquez et al., 1978). Unlike the Ames assay, the Ara^r assay of *Salmonella typhimurium*, developed by Ruiz-Vazquez et al. (1978), is a forward mutation assay system which can detect different types of gene mutations. The original tester, SV3, used in the system carries a mutation at the *araD* gene and is unable to convert L-ribulose 5-phosphate to D-xylulose 5-phosphate in the arabinose metabolic pathway. Accumulation of L-ribulose 5-phosphate, a presumptive toxic intermediate,

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Abbreviations: A-FAA, *N*-acetoxy-2-fluorenyl-acetamide; AFL B_2, aflatoxin B_2; AFL G_1, aflatoxin G_1; *araD*, arabinose D gene; Ara^r, arabinose resistant; Ara^s, arabinose sensitive; BaP, benzo[*a*]pyrene; DMSO, dimethyl sulfoxide; EMS, ethyl methanesulfonate; his^-, histidine deficient; his^+, histidine proficient; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; 2-NF, 2-nitrofluorene; NMU, *N*-nitroso-*N*-methylurea; 4NQO, 4-nitroquinoline-*N*-oxide; rfa^-, lipopolysaccharide barrier deficient; $uvrB^-$, excision-repair deficient.

leads to cell death. Therefore, SV3 is sensitive to L-arabinose. Cells become resistant, however, if forward mutations occur in one of at least 3 genetic loci prior to the *araD* gene in the arabinose operon (Englesberg et al., 1962; Pueyo and Lopez-Barea, 1979). It is possible that the conversion of arabinose sensitivity to arabinose resistance could be due to the reverse mutation of *araD* gene. However, the chance is much lower than that due to forward mutations.

To improve the assay system, Pueyo (1978) developed a set of new testers by incorporating *uvrB*$^-$ and/or *rfa*$^-$ genetic markers into SV3. With the new testers, the Ara^r assay system was shown to be sensitive to several mutagens in the suspension test, but not in the plate incorporation test. Whong et al. (1981a) developed a new tester, SV50, by transferring an R factor plasmid pKM101 into SV21, one of the testers derived from SV3 by Pueyo (1978). With SV50, Whong et al. (1981a, 1983) have shown that the Ara^r assay is sensitive to several chemical mutagens and complex mixtures with a standard plate incorporation test. Using the same tester, Felton et al. (1983) found that chemicals from beef after cooking had high mutagenic activity when tested in the Ara^r system. However, the compounds tested with the Ara^r assay system have not been extensive.

This report describes the mutagenic sensitivity of the Ara^r assay using tester SV50 for a series of 26 compounds known to be mutagenic in the Ames assay or mutagenic only in other assay systems (McCann et al., 1975a, b; Levin et al., 1982a; Douglas et al., 1982; Sacks and MacGregor, 1982). The Ames Salmonella/microsome assay with those 26 compounds was also employed. Both the plate incorporation and preincubation tests were compared simultaneously. The plate incorporation test was used because it is the most commonly adopted procedure; the preincubation test was used because it enhances the mutagenic activities of some chemicals or is necessary for detection of the mutagenicity of certain chemicals (Yahagi et al., 1977; Sugimura and Nagao, 1980).

Materials and methods

Test chemicals

Compounds tested were from the following sources: 4,5-dichlorocatechol, 4,5-dichloroguaiacol, 3-chloromuconic acid and 7-oxodehydroabietic acid were purchased from British Columbia Research, Vancouver, B.C., Canada; 4NQO, EMS, NMU, diethyl sulfate, nalidixic acid, actinomycin D, bleomycin sulfate, and methotrexate from Sigma Chemical Company, St. Louis, MO; diethylstilbestrol, acetovanillone, MMS, MNNG, and 2-NF from Aldrich Chemical Company, Milwaukee, WI; AFL B_2 and AFL G_1 from Calbiochem, San Diego, CA; captan from City Chemical Corporation, New York; A-FAA from Starks Associate, Buffalo, NY; hydrogen peroxide (H_2O_2) from Fisher Scientific Company, Fair Lawn, NJ. BaP was supplied by ICI Central Toxicology Laboratory, Alderly Park, Cheshire, England. PR toxin was provided by Dr. R. Wei, National Yang-ming Medical College, Taiwan. Niridazole was obtained from Dr. E. Bueding, The Johns Hopkins University, Baltimore, MD. Metronidazole was extracted from Flagyl tablets bought from local pharmacy.

Chemical stock solutions were freshly prepared for each experiment. Bleomycin, H_2O_2 and MNNG were dissolved in distilled water, and nalidixic acid and actinomycin D were dissolved in 95% ethanol. DMSO was used as solvent for the remaining compounds tested.

Media

Culture media used for TA98 and TA100 were prepared according to Ames et al. (1975). Soft agar for SV50 contained 0.6% Bacto-Difco agar and 0.5% NaCl. Before use 0.3 ml of 20% L-arabinose and 0.1 mg of glucose were added to 2 ml of molten soft agar. M9 medium was used as bottom agar for SV50, containing 1.5% Bacto-Difco agar, 1.5% supplement stock (2 mg tryptophan/ml, 2 mg threonine/ml, and 1 mg uracil/ml), 1.5% glycerol stock (20% glycerol) and 10% M9 salt stock (10 g NH_4Cl, 60 g Na_2HPO_4, 30 g KH_2PO_4, 50 g NaCl and 2.05 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water). SV50 medium for overnight culture contained the same components as those of M9 medium except agar. In addition, 0.1 ml of ampicillin stock (5 mg/ml) was added to 25 ml of SV50 medium.

Tester strains

In the Ames assay, the tester strains TA98 and

TA100 of *S. typhimurium* were used for detecting reverse mutations from *his*$^-$ to *his*$^+$ (Ames et al., 1975). Both testers were used in the assay except for compounds known to be mutagenic in the system. For these compounds, either TA98 or TA100 was used. These tester strains were generously provided by Professor B.N. Ames, University of California, Berkeley, CA.

In the Ara^r assay system, the Ara^s strain SV50 of *S. typhimurium* was employed for scoring forward and reverse mutations from Ara^s to Ara^r. The cell concentration of SV50 was determined with Spectronic 20 colorimeter (Bausch and Lomb) and adjusted to 1.5×10^7 cells/ml in distilled water prior to use.

S9 mix

Male CD rats, each approximately 200 g (Charles River laboratories, Wilmington, MA), were injected i.p. with Aroclor 1254 (500 mg/kg body weight) 5 days before they were sacrificed. The preparation of liver S9 homogenate and S9 mix (10% of liver S9) followed the procedure described by Ames et al. (1975).

Mutagenicity assays

All 26 compounds were tested simultaneously in the Ara^r assay system with SV50 and in the Ames assay with TA98 and/or TA100 using both the standard plate incorporation and preincubation tests.

The procedures of the plate incorporation test followed those described by Ames et al. (1975). In brief, 0.1 ml of tester cells from an overnight culture and the test compound were added to 2 ml of molten soft agar at 45°C. After mixing, the soft agar was overlayed onto a VB minimal agar plate for TA98 and TA100 or onto a M9 selective agar plate for SV50. With metabolic activation, 0.5 ml of S9 mix was also added to the soft agar.

Some modifications were made in the Ara^r assay system to reduce the spontaneous mutants, including adding 1.5×10^6 cells, 0.1 mg glucose and 0.3 ml of 20% L-arabinose to 2 ml of molten soft agar instead of 1.5×10^7 cells, 0.2 mg glucose and 0.2 ml of 20% L-arabinose, respectively, previously reported by Whong et al. (1981a,b).

The preincubation test was performed according to the method of Yahagi et al. (1977) with modification. In brief, the test compound was added to a 17 mm × 100 mm plastic tube containing 0.1 ml of overnight culture and 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4) or S9 mix. Each mixture in a tube was incubated at 37°C for 30 min with rotation in a roller drum. Thereafter, 2 ml of soft agar was added to each tube for overlaying as described above in the plate incorporation test.

All the overlayed plates were incubated at 37°C for 2 and 3 days for the Ames and Ara^r assays, respectively. Colonies on plates were counted with a Artek counter (model 880).

Each compound was assayed by at least 2 independent experiments in duplicate. Both positive and negative controls were routinely included in each experiment. Compounds were considered

TABLE 1

MUTAGENIC RESPONSE OF THE ARA^r AND AMES ASSAY SYSTEMS TO 26 COMPOUNDS

Chemical	Ara^r assay (SV50)	Ames assay (TA98 and/or TA100)
Acetovanillone	–	–
4,5-Dichlorocatechol	–	–
4,5-Dichloroguaiacol	–	–
3-Chloromuconic acid	–	–
7-Oxodehydroabietic acid	–	–
Nalidixic acid	–	–
Actinomycin D	–	–
Bleomycin sulfate	–	–
Methotrexate	–	–
Diethylstilbestrol	–	–
4NQO	+	+
Captan	+	+
MNNG	+	+
NMU	+	+
AFL G_1	+	+
A-FAA	+	+
AFL B_2	+	+
MMS	+	+
Metronidazole	+	+
EMS	+	+
BaP	+	+
Diethyl sulfate	+	+
Niridazole	+	+
PR toxin	+	–
H_2O_2	+	–
2NF	–	+

mutagenic when the average number of revertants or mutants obtained at any dose level was 2-fold or greater than the solvent control and showed a dose–response relationship.

Results

10 of 26 compounds tested gave negative responses in the Ara^r and Ames assays both in the plate incorporation test and preincubation test. They were acetovanillone, 4,5-dichlorocatechol, 4,5-dichloroguaiacol, 3-chloromuconic acid, 7-oxo-dehydroabietic acid, nalidixic acid, actinomycin D, bleomycin sulfate, methotrexate and diethylstilbestrol. 13 of 26 compounds were mutagenic with different degrees in both the Ara^r and Ames assay systems, including 4NQO, captan, MNNG, NMU, AFL B_2, A-FAA, AFL G_1, MMS, metronidazole, EMS, BaP, diethyl sulfate and niridazole (Table 1).

The results with PR toxin, H_2O_2 and 2-NF differed from those of the other compounds (Tables 1 and 2). PR toxin and H_2O_2 were mutagenic in the Ara^r assay with preincubation but gave negative responses in TA98 and TA100 using the Ames assay with either the plate incorporation or preincubation test. 2-NF was a potent mutagen in the Ames assay, but it was non-mutagenic in the Ara^r assay using strain SV50 at a dose of up to 20 μg/plate, a toxic dose (data not shown).

Mutagenic sensitivity of the Ara^r assay system to 13 compounds positive in both assay systems using the plate incorporation and preincubation tests was shown in Table 3. 11 of 13 compounds were mutagenic in both the plate incorporation and preincubation tests. 2 compounds, diethyl sulfate and niridazole, were mutagenic only with the preincubation test. The comparison of the mutagenic sensitivity between the preincubation and plate tests in the Ara^r assay was expressed as a fold increase. The fold was obtained from dividing the largest increase in the mutant ratio (experimental/control) in the preincubation test by the mutant ratio at the same dose level in the plate test. The comparative results demonstrated that the preincubation test enhanced the sensitivity of

TABLE 2

MUTAGENIC ACTIVITY OF COMPOUNDS POSITIVE IN EITHER THE ARA^r OR AMES ASSAY[a]

Chemical	Dose (μg/plate)	Ara^r mutants/plate		*his*$^+$ revertants/plate			
		SV50		TA98		TA100	
		Plate test	Preincubation test	Plate test	preincubation test	Plate test	Preincubation test
PR toxin	0	23 (1.0)[b]	28 (1.0)[b]	17 (1.0)[b]	16 (1.0)[b]	177 (1.0)[b]	143 (1.0)[b]
	0.15	33 (1.4)	37 (1.3)	19 (1.1)	23 (1.4)	173 (1.0)	160 (1.1)
	0.31	26 (1.1)	48 (1.7)	19 (1.1)	19 (1.2)	149 (0.8)	159 (1.1)
	0.625	31 (1.3)	61 (2.2)	16 (0.9)	23 (1.4)	188 (1.1)	147 (1.0)
	1.25	28 (1.2)	90 (3.2)	21 (1.2)	20 (1.3)	195 (1.1)	146 (1.0)
H_2O_2[c]	0	29 (1.0)	23 (1.0)	19 (1.0)	21 (1.0)	179 (1.0)	209 (1.0)
	0.00015	34 (1.2)	35 (1.5)	17 (0.9)	20 (1.0)	198 (1.1)	206 (1.0)
	0.00031	42 (1.4)	86 (3.7)	23 (1.2)	21 (1.0)	194 (1.1)	197 (0.9)
	0.000625	46 (1.6)	126 (5.5)	28 (1.5)	23 (1.1)	211 (1.2)	213 (1.0)
	0.00125	39 (1.3)	105 (4.6)	20 (1.1)	23 (1.1)	188 (1.1)	199 (1.0)
2-NF	0	37 (1.0)	34 (1.0)	20 (1.0)	23 (1.0)		
	0.63	35 (0.9)	39 (1.1)	103 (5.2)	233 (10.1)		
	1.25	41 (1.1)	49 (1.4)	191 (9.6)	383 (16.7)		
	2.5	37 (1.0)	45 (1.3)	410 (20.5)	407 (17.7)		
	5.0	40 (1.1)	44 (1.3)	506 (25.3)	483 (21.0)		

[a] Results are mean values from at least 2 Expts.

[b] Revertant or mutant ratio (experimental/control).

[c] Doses expressed in μl/plate.

TABLE 3

MUTAGENIC ACTIVITY OF 13 COMPOUNDS POSITIVE IN THE ARA^r ASSAY SYSTEM [a]

Chemical	Dose (μg/plate)	Ara^r mutants/plate	
		Plate incorporation test	Preincubation test
4NQO	0	42 (1.0) [b]	20 (1.0) [b]
	0.00375	325 (7.7)	1006(50.3)
	0.0075	673(16.0)	1256(62.8)
	0.015	1080(25.7)	1345(67.3)
	0.030	1413(33.6)	740(37.0)
Captan	0	36 (1.0)	27 (1.0)
	0.31	–	234 (8.7)
	0.625	467(13.0)	566(21.0)
	1.25	539(15.0)	620(23.0)
	2.5	822(22.8)	226 (8.4)
	5.0	494(13.7)	32 (1.2)
MNNG	0	45 (1.0)	36 (1.0)
	0.31	60 (1.3)	1444(40.1)
	0.62	64 (1.4)	2124(59.0)
	1.25	194 (4.3)	1538(42.7)
	2.5	901(20.0)	1181(32.8)
NMU	0	48 (1.0)	30 (1.0)
	15.6	42 (0.9)	281 (9.4)
	31.25	34 (0.7)	1227(40.9)
	62.5	39 (0.8)	1566(52.2)
	125	68 (1.4)	1444(48.1)
	250	367 (7.7)	795(26.5)
AFL G_1 [c]	0	38 (1.0)	29 (1.0)
	0.031	47 (1.2)	325(11.2)
	0.063	88 (2.3)	359(12.4)
	0.125	127 (3.3)	332(11.4)
	0.25	168 (4.4)	289(10.0)
	0.5	231 (6.1)	263 (9.1)
A-FAA	0	60 (1.0)	44 (1.0)
	3.1	53 (0.9)	142 (3.2)
	6.25	100 (1.7)	343 (7.8)
	12.5	184 (3.1)	679(15.4)
	25.0	364 (6.1)	704(16.0)
AFL B_2 [c]	0	59 (1.0)	45 (1.0)
	0.37	27 (0.5)	223 (5.0)
	0.75	117 (2.0)	371 (8.2)
	1.5	104 (1.8)	384 (8.5)
	3.125	142 (2.4)	158 (3.5)
	6.25	156 (2.6)	61 (1.4)
	12.5	255 (4.3)	46 (1.0)
MMS [d]	0	55 (1.0)	30 (1.0)
	0.125	60 (1.1)	94 (3.1)
	0.25	80 (1.5)	153 (5.1)
	0.5	95 (1.7)	226 (7.5)
	1.0	118 (2.2)	208 (6.9)

TABLE 3 (continued)

Chemical	Dose (μg/plate)	Ara^r mutants/plate	
		Plate incorporation test	Preincubation test
Metronidazole	0	48 (1.0)	39 (1.0)
	62.5	101 (2.1)	261 (6.7)
	125	198 (4.1)	418(10.7)
	250	302 (6.3)	516 (13.2) }
	500	288 (6.0)	395 (10.1) }
EMS [d]	0	44 (1.0)	36 (1.0)
	2.5	57 (1.3)	918(25.5)
	5.0	73 (1.7)	2356(65.4)
	10.0	105 (2.4)	2035(56.5)
	20.0	525 (11.9)	707(19.6)
BaP [c]	0	51 (1.0)	24 (1.0)
	0.5	158 (3.1)	106 (4.4)
	1.0	272 (5.3)	156 (6.5)
	2.0	301 (5.9)	166 (6.9)
	4.0	328 (6.4)	165 (6.9)
Diethyl sulfate [d]	0	57 (1.0)	36 (1.0)
	0.125	54 (0.9)	102 (2.8)
	0.25	52 (0.9)	688(19.1)
	0.5	30 (0.5)	1979(55.0)
	1.0	24 (0.4)	2217(61.6)
	2.0	38 (0.7)	1384(38.4)
Niridazole	0	56 (1.0)	43 (1.0)
	0.0125	74 (1.3)	172 (4.0)
	0.025	87 (1.6)	174 (4.0)
	0.05	79 (1.4)	106 (2.5)
	0.1	77 (1.4)	87 (2.0)

[a] Results are mean values from at least 2 Expts.
[b] Mutant ratio (experimental/control).
[c] Mutagenicity assayed with S9.
[d] Doses expressed in μl/plate.

the Ara^r assay (Table 4). 13 of 15 compounds were increased in mutagenicity with the preincubation test. The fold increase was 2.1–154.

Discussion

The results demonstrated that, in general, the mutagenic response of the Ara^r assay to 26 compounds tested was similar to that of the Ames assay, since 10 and 13 of 26 compounds tested were negative and positive, respectively, in both assay systems. The results of the Ames assay are in agreement with those reported in the literature. PR toxin and H_2O_2 were only mutagenic in the Ara^r assay and 2-NF was only mutagenic in the Ames assay. It should be noted, however, that PR toxin has been shown to be weakly mutagenic using TA97 in the Ames assay (Levin et al., 1982a). New Ames *his*$^-$ testers which are capable of detecting the mutagenic activity of oxidative mutagens, including bleomycin, H_2O_2 etc., have also been developed (Levin et al., 1982b).

Pueyo (1978) showed that the Ara^r system was very sensitive to mutagens in the suspension test. With the new tester, Whong et al. (1981a, b; 1983) showed that the mutagenicity of a number of known mutagens and complex mixtures can be detected by the plate incorporation test. It seems

TABLE 4

INCREASE IN THE MUTAGENIC RESPONSE OF SV50 TO 15 COMPOUNDS WITH THE PREINCUBATION TEST

Chemical	Fold increase
4NQO	2.6
Captan	1.5
MNNG	42.1
NMU	65.3
AFL G_1	5.4
A-FAA	2.6
AFL B_2	4.7
MMS	4.4
Metronidazole	2.1
EMS	38.5
BaP	1.2
Diethyl sulfate	154.0
Niridazole	3.1
PR toxin	2.7
H_2O_2	3.4

[a] The fold increase was obtained from dividing the largest increase in the mutant ratio (experimental/control) in the preincubation test by the mutant ratio at the same dose level in the plate incorporation test derived from Tables 2 and 3.

that the preincubation test, which is a simplified combination of the suspension test and the plate incorporation test, might be a better test protocol for the Ara^r assay system with SV50. Data from the present study strongly support this idea. Preincubation enhanced the sensitivity of the Ara^r assay to all 15 mutagenic compound tested at least 2-fold except for BaP (1.2-fold) and captan (1.5-fold). The mutagenicity of some mutagenic compounds such as NMU and diethyl sulfate tested in the preincubation test was 65.3 and 154 times higher than that in the plate test. If the sensitivity was expressed in terms of the absolute numbers of induced mutants, the mutagenic response of SV50 to 14 of 15 mutagenic compounds was higher (except BaP) in the preincubation test than in the plate incorporation test. In addition, preincubation made it possible to detect the mutagenicity of all compounds tested at a much lower dose level, e.g., NMU had 7.7-fold increase at a dose of 250 μg/plate in the plate incorporation test, while it had 52.2-fold increase using only 62.5 μg/plate in the preincubation test. Comparable results were found with all the remaining compounds. For compounds, such as diethyl sulfate, niridazole, PR toxin and H_2O_2, preincubation was essential in the Ara^r assay, i.e., these 4 compounds negative in the plate test were easily detected in the preincubation test. The enhanced sensitivity in the preincubation test may be due to higher concentrations of compounds reacting with the tester cells, or to increased contact between tester cells and mutagen caused by the rotary agitation during preincubation.

Pueyo (1978) indicated that none of the SV3, SV20 and SV21 strains increased significantly the number of mutants with 2-NF in the Ara^r assay system using the plate incorporation test, but mutagenic response to 2-NF was observed with the suspension test using SV20 (carries *rfa*$^-$ mutation). The response further increased with SV21 (carries both *rfa*$^-$ and *uvrB*$^-$). However, in our studies no mutagenic response to 2-NF was detected either in the plate incorporation or preincubation test with strain SV50, which differed from its original Ara^s strain (SV21) in carrying an additional R factor. It seems that the R factor is responsible for the negative result with 2-NF. This is in agreement with that reported by Castellino et al. (1978). They noted that with the 8-azaguanine-resistant forward mutation assay, 2-NF was highly mutagenic in strains TA1535, TA1537 and TA1538, but not in the R-factor-containing strains TA98 and TA100. However, potent mutagenicity of 2-NF was observed in TA98 with the Ames reversion assay. It appears that the presence of R factor in strains SV50 or TA98 and TA100 could in some way prevent this compound from being mutagenic in the forward mutation assay system but not in the reverse mutation assay. The reason why the R factor behaves differently in the forward and reverse mutations is unknown.

These studies further indicate that the Ara^r forward mutation assay using strain SV50 is a useful assay system for screening mutagenicity of compounds with different modes of action. In addition, use of the Ara^r assay makes it possible to reduce the working time and the amount of chemicals or the sample size of complex environmental mixtures used for testing. Based on the increased sensitivity with preincubation in the Ara^r assay and the simplicity of its procedure, the use of the preincubation test instead of the standard plate

incorporation test is recommended for mutagenicity screening with the Ara^r assay system using tester SV50.

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References

Ames, B.N., J. McCann and E. Yamasaki (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test, Mutation Res., 31, 347–363.

Castellino, S., R. Lorenzetti, L. Ravenna and I. de Carneri (1978) Forward mutation assays using bacteria and 8-azaguanine, Ecotoxicol. Environ. Safety, 2, 277–299.

Douglas, G.R., E.R. Nestmann, A.B. McKague, O.P. Kamra, E.G.H. Lee, J.A. Ellenton, R. Bell, D. Kowbel, V. Liu and J. Pooley (1982) Mutagenicity of pulp and paper mill effluent: A comprehensive study of complex mixtures, in: M. Waters, S. Sandu, J. Lewtas, L. Claxton, N. Chernoff and S. Nestow (Eds.), Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III, Plenum, New York, pp. 431–459.

Englesberg, E., R.L. Adderson, R. Weinberg, N. Lee, P. Hoffel, G. Huttenhauer and H. Boyer (1962) L-Arabinose-sensitive L-ribulose-5-phosphate-4-epimerase-deficient mutants of *Escherichia coli*, J. Bacteriol., 84, 137–146.

Felton, J., S. Healy, L. Avalos and B. Wuebbles (1983) Comparison of mutagenicity of two cooking mutagens with three microbial bioassays, in: The 14th Annual Meeting of Environmental Mutagen Society (U.S.A.), Program and Abstracts, p. 135.

Hollstein, M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979) Short-term tests for carcinogens and mutagens, Mutation Res., 65, 133–226.

Levin, D.E., E. Yamasaki and B.N. Ames (1982a) A new Salmonella tester strain, TA97, for the detection of frameshift mutagens, Mutation Res., 94, 315–330.

Levin, D.E., M. Hollstein, M.F. Christman, E.A. Schwiers and B.N. Ames (1982b) A new Salmonella tester strain (TA102) with A·T base pairs at the site of mutation detects oxidative mutagens, Proc. Natl. Acad. Sci. (U.S.A.), 79, 7445–7449.

McCann, J., E. Choi, E. Yamasaki and B.N. Ames (1975a) Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals, Proc. Natl. Acad. Sci. (U.S.A.), 72, 5135–5139.

McCann, J., N.E. Spingarn, J. Kobori and B.N. Ames (1975b) Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids, Proc. Natl. Acad. Sci. (U.S.A.), 72, 979–983.

Pueyo, C. (1978) Forward mutations to arabinose resistance in *Salmonella typhimurium* strains: A sensitive assay for mutagenicity testing, Mutation Res., 54, 311–321.

Pueyo, C., and J. Lopez-Barea (1979) The L-arabinose-resistance test with *Salmonella typhimurium* strain SV3 selects forward mutations at several *ara* genes, Mutation Res., 64, 249–258.

Ruiz-Vazquez, R., C. Pueyo and E. Cerda-Olmedo (1978) A mutagen assay detecting forward mutations is an arabinose sensitive strain of *Salmonella typhimurium*, Mutation Res., 54, 121–129.

Sacks, L.E., and J.T. MacGregor (1982) The *B. subtilis* multigene sporulation test for mutagens: Detection of mutagens inactive in the Salmonella *his* reversion test, Mutation Res., 95, 191–202.

Sugimura, J., and M. Nagao (1980) Modification of mutagenic activity, in: F.J. de Serres and A. Hollaender (Eds.), Chemical Mutagens, Principles and Methods for their Detection, Vol. 6, Plenum, New York, pp. 41–60.

Whong, W.Z., J. Stewart and T.M. Ong (1981a) Use of the improved arabinose-resistant assay system of *Salmonella typhimurium* for mutagenesis testing, Environ. Mutagen., 3, 95–99.

Whong, W.Z., J. Stewart, M. McCawley, P. Major, J.A. Merchant and T. Ong (1981b) Mutagenicity of airborne particles from a nonindustrial town, Environ. Mutagen., 3, 617–626.

Whong, W.Z., J.D. Stewart, D.C. Adamo and T. Ong (1983) Mutagenic detection of complex environmental mixtures using the Salmonella/arabinose-resistant assay system, Mutation Res., 120, 13–19.

Yahagi, T., M. Nagao, Y. Seino, T. Matsushima, T. Sugimura and M. Ikada (1977) Mutagenicities of *N*-nitrosamines in Salmonella, Mutation Res., 48, 121–130.