Bull. Environ. Contam. Toxicol. (2000) 65:691–698

DOI: 10.1007/s001280000179

Bulletin of Environmental Contamination and Toxicology

Mutagenic Effects of Plant Growth Hormones with the Salmonella/Microsome Test and the SOS Chromotest

E. Öksüzoğlu, N. Diril, M. Durusoy

Hacettepe University of Science, Department of Biology, 06532 Beytepe, Turkey

Received: 25 April 2000/Accepted: 9 October 2000

Cancer is a dread disease worldwide. Mortality of individuals suffering from cancer is high despite the current improved methods of early detection, surgery and therapy. Prevention of cancer is the recognized goal of many activities in cancer research. This aim was recognized early to involve the bioassay of environmental chemicals or mixtures. Many in vitro short-term tests have been developed to identify chemicals that can damage cellular DNA or cause mutations and, secondarily, to identify potential carcinogens. The somatic mutation theory of carcinogenesis holds that mutations are a common first step in the development of a cancer cell; consequently, interest in these bacterial short- term tests has been fueled by their ability to identify potential carcinogens (Fetterman et al., 1997; Hofnung and Quillardet, 1986; Weisburger, 1999).

Since 1973, many bacterial short-term tests have been proposed, from which the most important are the Ames test, also called the Salmonella / microsome assay (Ames et al., 1973; Gee et al., 1994), the SOS chromotest (Quillardet et al., 1982), the *umu* test (Oda et al., 1985) and the Mutatox test (Ulitzur et al., 1980).

Salmonella / microsome test is the most popular of the bacterial test system. It detects mutagenic substances via their ability to revert histidine auxotrophs of *S. typhimurium* to wild-type. A homogenate of rat liver is added to the bacterial suspension to mimic of mammalian metabolism (Ames et al., 1973 ; Maron and Ames, 1983; Hofnung and Quillardet, 1986).

The SOS chromotest allows evaluation of the SOS response induced by genotoxic agents and is based on the special tester strain *E.coli* PQ 37 which has been derived from *E.coli* GC4436 harboring a *sfiA::lacZ* fusion gene (Huisman and d' Ari, 1985). *E.coli* PQ 37 is additionally characterized by the following important markers: *uvr* A , preventing excision repair ; *Pho C* , resulting in constitutive expression of alkaline phosphatase ; *rfa* , allowing enhanced diffusion of compounds through cell membranes (Ouillardet and Hofnung, 1985). As a measure of the induction of the SOS system the *sfiA* gene , one of the SOS regulon genes , linked β-galactosidase activity is determined. The alkaline phosphatase activity is a measure of protein synthesis and is used for toxicity correction.

Plant growth hormones are randomly and widely used to obtain high quality and yield productcs. Therefore in this study, the mutagenic potentials of some plant growth hormones have been investigated by using Salmonella / microsome test and SOS chromotest system.

Correspondence to: E. Öksüzoğlu

MATERIALS AND METHODS

The products were obtained commercially from the sources indicated: Indole-3-acetic acid and gibberellic acid (Powder), padomin (Sedar Ltd. Şti.), berelex (Zeneca Turkey Ltd.), sodium azide (Merk-Schuchardt, Darmstadt,F.R.G.), 2-aminoflouren and ampicilline trihydrate (Fluka), α-naphthaleneacetic acid, benzyladenine, D-glucose-6-P (monosodium salt), β-NADP (sodium salt), D-biotin, 4-nitroquinolin 1-oxide (4-NQO), O-nitrophenyl- β-D-galactopyranoside (ONPG), P-nitrophenyl phosphate disodium (PNPP), 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal), sodium dodecyl sulphate (SDS) and yeast extract (Sigma Chemical Co., St. Louis, U.S.A), L-histidine-HCl monohydrate (BDH), bacto-yeast extract, bacto tryptone and bacto agar (Difco), oxoid nutrient broth No:2 (Oxoid).

S. typhimurium TA 98 (*his Δ3052*, *rfa*, Δ*uvr*A, pKM 101) and TA 100 (*his G46*, Δ*uvr*B, *rfa*, pKM 101) were kindly provided by Dr. Bruce Ames (University of California, Berkeley Ca.,U.S.A). *E.coli* PQ 37 (*sfiA ::lacZ* , Δ*uvrA*, *Pho C*, *rfa*) by Dr.M.Hofnung (Institute Pasteur, Paris, France).

Sprague-Dawley male rats were used for the preparation of liver S-9 fraction. 3-methylcholanthrene and phenobarbital were used for the induction of rat liver enzymes. 3-methylcholanthrene was diluted in corn oil (125mg/kg body weight) and injected intraperitoneally to each rat five days before sacrifice. The rats were given drinking water ad libitum for five days. Phenobarbital was added to drinking water (0,1%g/l) and given for five days before sacrifice. Preparation of the liver S-9 fraction was based on the procedure of Garner et al. (1972). The protein concentration of S-9 fraction was determined by the procedure of Lowry et al. (1951). The protein content of S-9 fraction was found to be amount 9mg/ml.

The amounts of test compounds to be used in the Salmonella mutation assays were selected based on cytotoxicity assay. 0,1 ml of a suitable dilution of an overnight bacterial culture was added to 2 ml top agar together with different concentration the compounds in distilled water. The top agar was poured onto nutrient agar plates and assessment of cytotoxicity was made after 24 h incubation at 37^{o}C (Dean et al., 1985).

The Salmonella/microsome test system was performed basically the same as described by Maron and Ames (1983). Briefly 0.1 ml of bacterial tester strain and sample to be tested was added to 2 ml of molted top agar. The contents were mixed and poured on agar plates. After 2-3 days of incubation, revertant colonies were counted. At least four plates were used for each dose and each experiments was repeated three times. The number of spontaneous revertant colonies for *S. typhimurium* strains were found to be 28± 9 for TA 98 and 145±45 for TA 100.

In Salmonella / microsome test system, data are interpreted on the basis of a consistent doubling of the spontaneous reversion frequency confirmed by a dose-response relationship. Where the number of induced revartants is less than twice the spontaneous rate, but a reproducible dose-related increase in revertants is detected, this is also interpreted as a positive response (Dean et al., 1985).

SOS chromotest was conducted according to the method described by Quillardet and Hofnung (1985). The experiments were performed at the concentrations of chemicals at which a decrease of the activity of alkaline phosphatase was not observed. When a chemical did not shown this decrease, the SOS chromotest was carried out at the highest soluble dose. The plant growth hormones were tested at each concentrations at least in triplicate. Tester bacteria were exposed to the different concentration the plant growth hormones during 2h. Then β-galactosidase and alkaline phosphatase activities were estimated according to Miller (1972). As a measure of SOS-inducing effect ,the induction factor (IF) was calculated. This is the ratio of the β-galactosidase

and alkaline phosphatase activities (R_C) at the certain concentrations of chemical tested to their values for negative (solvent) control (R_O) ; IF= R_C / R_O

According to the criteria of Quillardet and Hofnung (1985) a compound is considered genotoxic when a dose- dependent increase in IF and also in β- galactosidase activity is observed and IF is at least 1.5 . According to the others a compound was classified as "non-genotoxic" if the induction factor remained < 1.5, as "marginal" if the induction factor was between 1.5 and 2.0 , and as"genotoxic" if the induction factor was in excess of 2.0 (Mersch-Sundermann et al.,1992).

4-nitroquinoline-1-oxide (4NQO) was used as positive control. 4NQO is genotoxic in most in vivo and in vitro test system and carcinogenic in mammals (Purchase et al., 1981). It produces several adducts with DNA bases (Sugimura et al., 1980). It induces the SOS repair systems in *E. coli*, by blocking the DNA polymerase (Le Curieux et al., 1993).

RESULTS AND DISCUSSION

In this study , the mutagenic potentials of some plant growth hormones were investigated by Salmonella / microsome test and SOS chromotest. Table 1 shows the results obtained from the experiments using *S. typhimurium* TA 98 and TA 100 strains with and without S9 mix . Among the plant growth hormones tested only benzyladenine was weakly mutagenic in the presence of S9 fraction in S. typhimurium TA98 strain at concentrations 0,062 mg/plate and 0,250mg /plate. Table 2 shows the results of SOS chromotest in the absence of S9 fraction. All compound tested in this test system gave negative results. In the SOS chromotest system, in the presence of indole-3-acetic acid (12 mg/ml) and padomine (21 mg/ml and 28 mg/ml) although the induction factor was >1,5 (Table2), these concentrations were cytotoxic. These results were evaluated as negative since alkaline phosphatase activity decreased appreciably and β-galactosidase decreased compared to control at these concentrations. 4NQO used in this system as positive control increased induction factor (>1,5) and β-galactosidase activity significantly compared to control.

3- methylcholantrene and phenobarbital were used as inducers in obtaining S9 fraction from rat liver. Inducers were given to the animal at the same time. When two inducers were given to the animal simultaneously both cyt P-450 and cyt P-448 were activated (Singer and Grunberger, 1983). Inducer agents used by others were Aroclor-1254, ethanol, β-naphtaflavene, 5,6-benzoflavone (Bernacchi et al., 1996; Shane and Winston, 1997; Shimada and Guengerich, 1990). When different inducer were used, different cytochrome isozymes were activated. (Paolini and Cantelli-Forti, 1997).

Salmonella / microsome test system is in itself a mini battery test system. *S. typhimurium* TA98 strain detects frame-shift mutagens. On the other hand, *S. typhimurium* TA100 detects mutagens that cause base-pair substitutions. For this reason, different mutagenic agents have a possibility of being detected by using the Salmonella / microsome test system (Maron and Ames,1983). In our study, it was shown that benzyladenine cause frame-shift mutation.

The reason we used both test systems together is that in Ames test system the capacity to detect mutagen as non-carcinogens is high, so they complement each others.(Rosenkranz et al., 1999). In our study the plant growth hormones tested gave negative results in the absence S9 fraction in the both test systems, only benzyladenine was weakly mutagen in *S.typhimurium* TA98 strain in the presence S9 fraction. In a review study, the results of Salmonella / microsome and SOS chromotest have been evaluated (Quillardet and Hofnung, 1993). Among 452 chemicals tested, 375 (% 82) of them gave similar results in both test systems, 79 (%18) of them gave different results. These results confirm that there is a high correlation between both test systems. If a chemical is mutagenic it also induces SOS systems.

Table 1. Mutagenic potentials of some plant growth hormones against *S.typhimurium* TA 98 and TA 100 in the presence of S9-fractions induced with phenobarbital and 3-methylcholantrene*

Compound	Conc. of comp.	Revertant Colony Numbers			
		TA 98		TA 100	
		S9 (-) mean+SD	S9 (+) mean+SD	S9 (-) mean+SD	S9 (+) mean+SD
Control	0	28 ± 9	28 ± 9	145 ± 45	145 ± 45
Indole-3-acetic acid	1	21 ± 3	30 ± 6	157 ± 45	193 ± 59
(mg/plate)	2	19 ± 3	28 ± 5	145 ± 45	188 ± 25
	4	19 ± 6	30 ± 5	161 ± 23	179 ± 32
	8	19 ± 0.5	32 ± 5	153 ± 35	166 ± 31
	10	20 ± 4	28 ± 4	125 ± 31	158 ± 37
Gibberellic acid	10	24 ± 6	29 ± 8	139 ± 44	201 ± 24
(mg/plate)	15	22 ± 4	27 ± 10	139 ± 63	188 ± 14
	20	20 ± 4	28 ± 4	130 ± 27	194 ± 15
	30	21 ± 6	26 ± 4	143 ± 18	198 ± 20
	40	20 ± 4	24 ± 5	114 ± 43	191 ± 23
Padomin	0.726	30 + 9	28 + 3	142 ± 45	201 + 24
(µg/plate)	1.453	30 ± 9	28 ± 6	135 ± 40	188 ± 14
	7.260	25 ± 11	24 ± 6	148 ± 35	194 ± 15
	14.53	30 ± 13	26 ± 6	138 ± 49	198 ± 20
	145.3	32 ± 12	26 ± 6	145 ± 43	191 ± 23
Berelex	0.5	29 ± 12	26 ± 12	134 ± 36	195 ± 19
(mg/plate)	1	31 ± 13	30 ± 7	132 ± 38	168 ± 10
	2	30 ± 11	22 ± 5	137 ± 43	170 ± 18
	3	29 ± 14	-	-	-
	4	-	26 ± 8	148 ± 42	174 ± 18
	10	32 ± 13	26 ± 3	101 ± 47	171 ± 12
α-Naphthalene	0.040	21 ± 5	35 ± 5	144 ± 55	138 ± 12
acetic acid	0.080	23 ± 6	39 ± 7	155 ± 53	125 ± 45
(mg/plate)	0.169	23 ± 6	40 ± 7	156 ± 65	137 ± 11
	0.330	27 ± 10	33 ± 8	157 ± 50	142 ± 10
	0.660	25 ± 8	33 ± 3	165 ± 84	119 ± 56
Benzyladenine	0.062	22 ± 11	40 ± 2	139 ± 41	138 ± 11
(mg/plate)	0.125	27 ± 8	39 ± 6	152 ± 57	133 ± 8
	0.250	27 ± 8	41 ± 1	155 ± 43	115 ± 46
	0.500	29 ± 8	36 ± 4	147 ± 62	124 ± 42
	1.000	27 ± 9	36 ± 6	156 ± 58	144 ± 9
Positive Controls					
2-Aminofluorene (TA 98)	10 µg	29 ± 8	354 ± 45	-	-
Sodium azide (TA 100)	1.5 µg	-	-	1387 ± 600	1222 ± 656

*The results are means of four separate experiments with three plates each

Table 2. Induction of SOS repair in *E. coli* PQ37 of some plant growth hormones. *

Compound	Conc. of comp. (μg/assay)	ß -Gal. (Unit)	Al.phos. (Unit)	Induction factor
Indole-3-acetic acid	0.095(4NQO)	9.50	7.07	4.18
	0.19 (4NQO)	16.56	8.43	6.13
	0.00	2.98	9.25	1.00
	0.30	3.04	10.05	0.94
	0.60	2.92	10.10	0.91
	1.25	2.95	9.64	0.97
	2.50	2.93	9.44	0.97
	5.00	2.83	9.73	0.91
	10.00	2.81	9.42	0.94
	20.00	2.75	9.38	0.91
	40.00	2.26	8.13	0.88
	80.00	1.52	5.17	0.91
	120.00	0.95	1.96	1.50
	160.00	0.48	1.89	0.78
Padomin	0.095 (4NQO)	7.69	11.32	2.62
	0.19 (4NQO)	11.16	12.05	3.58
	0	3.23	12.33	1
	0.6	3.57	12.81	1.08
	1.12	3.43	12.64	1.04
	2.25	3.42	12.44	1.04
	4.5	3.21	12.48	1
	9	3.15	12.38	0.96
	18	3.07	12.02	1
	36	2.94	11	1.04
	70	2.38	8	1.15
	140	1.64	5.57	1.12
	210	1.43	2.77	2
	280	1.44	2.74	2.03
α - Napthalenacetic acid	0.095 (4NQO)	34.07	13.3	1.58
	0.19 (4NQO)	50.25	15.48	2
	0	25.5	15.7	1
	0.5	25.14	16.54	0.94
	1	24.79	16.19	0.94
	2	25.5	16.16	0.97
	4	25.32	15.65	1
	8	24.14	15.65	0.95
	16.5	24.35	14.32	1.04
	33	21.28	13.51	0.97
	49.5	20.07	11.24	1.09
	66	17.92	11.32	0.98
	99	18.17	10.24	1.09
	132	15.92	8.48	1.15

Compound	Conc. of comp. (μg/assay)	ß-Gal. (Unit)	Al.phos. (Unit)	Induction factor
Gibberellic acid	0.095(4NQO)	6.08	7.87	2.20
	0.19 (4NQO)	18.95	7.59	7.03
	0.00	2.99	8.58	1.00
	6.20	2.82	8.89	0.91
	12.50	3.05	8.67	1.00
	25.00	2.81	8.60	0.94
	50.00	2.87	8.52	0.97
	100.00	3.05	8.48	1.03
	200.00	3.05	8.36	1.03
	400.00	3.13	8.27	1.09
	800.00	3.08	8.16	1.09
	1200.00	2.89	7.69	1.09
	1600.00	2.71	7.49	1.03
	2400.00	2.12	5.48	1.11
	3200.00	1.50	5.67	0.74
Berelex	0.095 (4NQO)	7.3	11.13	3.3
	0.19 (4NQO)	11.21	12.66	4.45
	0	2.6	13.11	1
	14	2.32	14.75	0.8
	29	2.51	13.84	0.9
	58	2.56	13.85	0.9
	117	2.32	13.95	0.85
	234	2.54	14.08	0.9
	468	2.68	13.66	1
	937	2.59	14.26	0.9
	1875	2.7	14.73	0.9
	3750	2.68	14.45	0.95
	7500	2.11	11.54	0.9
	10000	1.56	7.87	1
	15000	1.44	6.83	1.05
Benzyladenine	0.095(4NQO)	48.07	41.23	1.67
	0.19 (4NQO)	75.05	42.34	2.53
	0	29.14	41.54	1
	0.375	29.19	44.28	0.94
	0.75	29.78	42.55	0.98
	1.55	29.877	42.7	0.98
	3.1	29.27	40.76	1.02
	6.25	28.57	40.14	1.01
	12.5	26.32	31.26	1.2
	25	23.57	27.48	1.22
	37.5	23.39	26.22	1.27
	50	23.8	25.58	1.32
	75	23.05	25.27	1.3
	100	22.73	23.75	1.37

* Each value is the mean of two replicates from each of three separate experiments.

The mutagenic and carcinogenic actions of these hormones have been investigated in several studies. Indole-3–acedic acid (IAA) and indole-3-butyric acid (IBA) were found to induce tumors in experimental animals (Pesonen,1950; Dunning and Curtis, 1958). The mutagenic effects of IAA and IBA in Salmonella / microsome test system have also been investigated and were found to be non-mutagenic in several *S. typhimurium* strains. Howewer, IAA and IBA were highly recombinogenic in *Aspergillus nidulans* (Kappas, 1988). Both IAA and IBA induce mitotic-crossing over and chromosomal non-disjunction in *A. nidulans* and in the presence of S-9 fraction, mitotic-crossingover were rapid (Kappas,1983). Moriya and coworkers showed that IAA and IBA to be non-mutagenic in strains of *S. typhimurium* and *E. coli* strain (Moriya et al., 1988). Our study confirm the results of Moriya (1988).

It is known that some chemicals wouldn't be mutagenic in the standard test procedure but would be mutagenic in these test system only after special treatments. We found indole-3-acetic acid, α-naphthaleneacetic acid , padomin, gibberellic acid and berelex to be non-mutagenic in standard test procedure. These chemicals should be exposed to special treatments (Von der Hude et al., 1988; Shibuya et al., 1997; Wagner et al., 1996; Mersch-Sundermann et al., 1991; Mersch-Sundermann et al.,1993) in order to prove their non-mutagenicity in all conditions.

Acknowledgments: The study was supported by TUBITAK (Project No : TBAG- AY/25). We would like to thank Dr. B. Ames and Dr. M. Hofnung for providing *S. typhimurium* TA98, TA100 and *E. coli* PQ 37 strain respectively, Assistant Miss Kamile Öztürk for her helpful and Dr. Tülay Saraçcıbaşı for statistical assistance.

REFERENCES

Ames BN, FD. Lee and Durston WE (1973) An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc Natl Acad Sci USA 70: 782-786

Bernacchi F, Ponzanelli I, Barale R and Loprieno N (1996) Mutagenic activity of some coal-derived humid compounds evaluated by the Ames test. Mutat Res 369: 107- 112

Dean BJ, Brooks TM, Hodson- Walker G, Hutson DH, (1985) Genetic toxicology testing of 41 industrial chemicals. Mutat Res 153: 57-77

Dunning WF and Curtes MR (1958) The role of indole in incidence of 2-acetylaminoflurence-induced bladder cancer in rats. Proc Soc Exp Biol Med 99: 91-95

Fetterman BA, Kim BS, Margolin BH, Schildcrout JS, Smith MG, Wagner SM, and Zeiger E (1997) Predicting rodent carcinogenicity from mutagenic potency measured in the Ames *Salmonella* Assay. Environ Mol Mutagen 29: 312-322

Garner RC, Miller EC, Miller JA (1972) Liver microsomal metabolism aflatoxin B1 to a reactive derivate toxic to *Salmonella typhimurium* TA 1530.Cancer Res 32: 2058-2066

Gee PDM, Maron and BN, Ames (1994) Detection and classification of mutagens : a set of base-specific *Salmonella* tester strains. Proc Natl Acad Sci USA 91:11606-11610

Hofnung M, and Quillardet P (1986) Recent development in bacterial short-term tests for the detection of genotoxic agents. Mutagenesis 1: 319-330

Huisman O and d ' Ari (1981) An inducible DNA replication- cell division coupling mechanism in *E. coli*, Nature (London) 290: 797-799

Kappas A (1983) Genotoxic activity of plant growth-regulating hormones in *Aspergillus nidulans*. Carcinogenesis 4:1409-1411

Kappas A (1988) On the mutagenic and recombinogenic activity of certain herbicides in *Salmonella typhimurium* and in *Aspergillus nidulans*. Mutat Res 204:615-621

Le Curieux F, Marzin D, and Erb F (1993) Comparison of three short - term assay: results on seven chemicals. Mutat Res 319: 223-236

Lowry DH, Rosebrough NJ, Farr A L, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193 :265-2751

Maron DM, Ames BN (1983) Revised methods for the mutagenicity test. Mutat Res 113: 173-215

Mersch-Sundermann V, Wintermann F, Kern S, and Hof H (1993) Influence of S9 mix composition on the SOS response in *E. coli* PQ37 by polycyclic aromatic hydrocarbons. Mutat Res 291: 53-60

Mersch-Sundermann V, Kevekorder S and Mochayedi S (1991) Sources of variability of the *E. coli* PQ 37 genotoxicity assay (SOS Chromotest). Mutat Res 252: 51-60

Mersch-Sundermann V, Mochayede S and Kevekorder S (1992) Genotoxicity of polycyclic aromatic hydrocarbons in *Escherichia coli* PQ37. Mutat Res 278: 1-9

Miller JH (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.

Moriya MT, Ohta K, Watanabe T, Miyazawa T, Kato and Shirasu Y (1983) Further mutagenicity studies on pesticides in bacterial reversion assay system. Mutat Res 116: 185-216

Oda Y, Nakamura I, Oki T, Kato and Stinagawa (1985) Evaluation of a new system (*Umu*-test) for the detection of environmental mutagens and carcinogens. Mutat Res 147: 219-229

Paolini M and Cantelli- Forti G (1997) On the metabolizing systems for short-term genotoxicity assays: A review. Mutat Res 387: 17-34

Pesonen S (1950) Tumours resulting from parthenogenesis induced by plant hormones in albino rats. Acta Endocrinol 5: 409-412

Purchase IHF, Clayson DB, Preussmannand R, Tomatis L (1981) Activity of 42 compounds in animal carcinogenecity studies. in: F.J. de Serres and J. Ashby (Eds), Progress In Mutation Res.,Vol. 1, Elsevier/Norts-Holland, New York, p 21-32

Quillardet P and Hofnung M (1985) The SOS chromotest a colorimetric bacterial assay for genotoxins : procedures. Mutat Res 147: 65-78

Quillardet P and Hofnung M (1993) The SOS chromotest: A review. Mutat Res 297:235-297

Quillardet P, Huisman RD' Ari and M Hofnung (1982) SOS chromotest a direct assay of induction of an SOS function in *Escherichia coli* K12 to measure genotoxicity. Proc Natl Acad Sci USA 79: 5971-5975

Rosenkranz HS, Mersch-Sundermann V, Klopman G (1999) SOS chromotest and mutagenicity in Salmonella: evidence for mechanistic differences. Mutat Res 431: 31-38

Shane BS and Winston GW (1997) Activation and detoxification of dinitropyrenes by cytosol and microsomes from Aroclor-pretreated rats in the Ames and *umu* assays. Environ Mol Mutagen 30: 303-311

Shibuya N, Nakadaria H, Ohta T, Nakamura YH, Yamamoto M, Saitoh Y and Ogashi K (1997) Co-mutagenicity of glyco- and tauro-deoxycholicacids in the Ames test. Mutat Res 395: 1-7

Shimada T and Guengerich FP (1990) Inactivation of 1,3-1,6 and 1,8-dinitropyrene by cytochrome P-450 in human rat liver microsomes. Cancer Res 50: 2036-204

Singer B, Grunberger D (1983) Molecular biology of mutagens and carcinogens. Plenum Press New York , p 347

Sugimura T, Wakabayashi M, Yamada M, Nagao and Fujino T (1980) Activation of chemicals to proximal carcinogen. in: B. Holmstedt, R. Lauwerys, M. Mercier and M. Robertfroid (Eds.), Mechanisms of Toxicity and Hazard Evaluation, Development in toxicity and Environmental Sciences, Vol 8 Elsevier/North-Holland, New York

Ulitzur S, Weiser I and Yannai S (1980) A new sensitive and simple bioluminescence test for mutagenic compounds. Mutat Res 74 : 113-124

von der Hude W, Behm C, Gürtler R, Basler A (1988) Evaluation of the SOS chromotest. Mutat Res 203: 81-94

Wagner ED, Cebulska-Wasilewska A, Connolly S, Plewa MJ (1996) Mutagenic analysis of 2,3-diaminophenazine and 2-amino-3-hydroxyphenazine in *Salmonella* strains expressing different levels of O-acetyltransferase with and without plant and mammalian activation. Mutat Res 37: 65-74

Weisburger JH (1999) Carcinogenicity and mutagenicity testing, then and now. Mutat Res 437: 105-112