REFERENCES

Alok Sarkar, Biswajit Mukherjee and Malay Chatterjee. 1994. Inhibitory effect of β-carotene on chronic 2-acetylaminofluorene induced hepatocarcinogenesis in rat. **Carcinogenesis** 15 (5) : 1055-1060.

Ames, B.N. 1971. The detection of chemical mutagen with enteric bacterial. In : Chemical mutagen; Principle and method for their detection. Edited by Hollaender A. : Plenum Press, New York. 1 : 267-282.

_______, Durton, W.E., Yamasaki, E., and Lee, F.D. 1973a. Carcinogens are mutagens : a simple test system combinding liver homogenates for activation and bacteria for detection. **Proc. Natl. Acad. Sci.** 70 : 2280-2285.

_______, Kammen, H.O., and Yamasaki, E. 1975. Hair dyes are mutagens : identification of a variety of mutagenic ingredients. **Proc. Natl. Acad. Sci.** 72 : 2423-2427.

_______, Lee, F.D., and Durston, W.E. 1973b. An improved bacterial test system for the detection and classification of mutagens and carcinogens. **Proc. Natl. Acad. Sci.** 70 : 782-786.

_______ and McCann, J. 1981. Validation of the *Salmonella* test : A reply to Rinkus and Legator. **Cancer Res.** 41 : 4192-4196.

_______, McCann, J. and Yamasaki, E. 1975. Method of detecting carcinogens with the *Samonella*/mammalian-microsome mutagenicity test. **Mutat. Res.** 31 : 347-364.

Anderson, J.W. 1985. Physiologic and Metabolic Effect of Dietary Fiber. **Fed. Proc.** 44 : 2902-2906.

________, J.W. 1986. Fiber and Health : An Overview. **Am. J. Gastroenterol.** 81 (10) : 892-897.

Andrews, A.W., Fornwald, J.A. and Lijinsky, W. 1980. Nitrosation and Mutagenicity of some amine drugs.**Toxicol. Appl. Pharmacol.** 52 : 237-244.

Aoyagi, M. et al. 1980. Induction of liver tumors in Wistar rats by sodium nitrite given in pallet diet. **J. Natl. Cancer Inst.** 65 : 411-414.

Armijo, R., and Coulson, A. 1975. Epidemiology of stomach cancer in Chili, the role of nitrogen fertilizer. **Int J. Epidemiol.** 4 : 301-309.

Bartsch, H. *et al.*, 1980. Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V 79. Chinese hamster cell in the presence of various metabolizing systems. **Mutat. Res.** 76 : 1-50.

Bender,A.E. and Bender, D.A. 1982. Diet and Cancer. In : Nutrition for Medical Students : 186-196.

Brusich, D. 1982. Genetic Toxicology. In : Principles and Methods of Toxicology. y Hayes, A.W. : Raven Press, New York : 241-248.

Burkitt, D.P. 1971. Epidemiology of cancer of the colon and rectum. **Cancer** 28 : 3-13.

Burkitt, D.P. and Trowell, H.C. 1975. Refined carbohydrate food and disease. In : Some implication of dietary fiber. Academic Press, London.

Coulston, F. 1980. In : The Potential Carcinogenicity of Nitrosable Drugs. Edited by F. Coulston & J.F. Dunne. Proceedings of a WHO symposium. Geneva.

1978 : p. 8. Albox Publishing Co., Norwood. NJ. USA.

Cuello, C. *et al.* 1976. Study of gastric cancer in Columbia, I. Cancer risk and suspect environmental agents. **J.Natl. Cancer Inst**. 51 : 1015-1021.

Cummings, J.H. 1982. Consequences of the metabolism of fiber in the human large intestine. In : Dietary fiber in health and disease. Vanhouny, G.V. and Kritchevsky, D. : Plenum, New York : 9-22.

________, J.H. and Stephen, A.M. 1980. The role of dietary fiber in human colon. **Can. Med. Assoc. J.** 6:1109-1114.

De Serres, F.J. and Shelby, M.D. 1979. Recommendations on data production and analysis using the *Salmonella*/microsome mutagenicity assay. **Mutat. Res.** 64 : 159-165.

Deutsh, M.J. 1984. Vitamins and other nutrients. In : Official methods of analysis of the Association of official analytical chemists. Williams, S. 14th ed. : 834-842.

Eastwood, M.A., and Mitchell, W.D. 1976. Physical properties of fiber : A biological evaluation In : Fiber in human nutrition. Spiller, G.A. and Amen, R.G. : Plenum, New York : 118-119.

________, Brydon, W.G., and Tadesse, K. 1980. Effect of fiber on colon function In : Medical aspects of dietary fiber. Spiller G.A. and McPherson-Kay, R. Plenum, New York : 1-26

El Nahas, S.M., Globus, M., and Vethamany-Globus, S. 1984. Chromosomal aberrations induced by sodium nitrite in bone marrow of adult rats and liver cells of transplacentally exposed embryoes. **J. Toxicol. Environ. Health**

13 : 643-647.

Ershoff, B.H. 1974. Antitoxic effects of plant fiber. **Am. J. Clin. Nutr.** 27 : 1395-1398.

Felton, J.S. and Knize, M.G. 1991. Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food (Review). **Mutat. Res**. 259 (3-4) : 205-217.

Gillatt, P.N., Hart, R.J., and Walters, C.L. 1983. Susceptibilities of drug to nitrosation under standardized chemical conditions. **Food Chem. Toxicol.** 22 (4) : 269-274.

Glatt, H.R., Faigle, J.W., and Oesch, F. 1987. Tricyclic drugs : potent mutagenicity of traces of a nitroarene formed in the reaction of opipramol with nitrite. **Mutat. Res.** 190 (1) : 7-11.

Hartman, P.E., Leviene, K., Hartman, Z., and Berger, H. 1971. Classification and mapping of spontaneous and induced mutagenicity in the histidine operon of *Salmonella* **Adv. Genetics** 16 : 1-34.

Hill, M.J. 1975. The etiology of colon cancer. **Crit. Rev. Toxicol.** 4 : 31-82.

IARC. 1979. Monographs on the evaluation of carcinogenic risk of chemical to humans, Supplement 1, Chemicals and industrials processes associates with cancer in human. Lyon International Agency for Research on Cancer : 1-20

____ . 1983. Monographs on the evaluation of carcinogenic risk of chemical to humans, Polycyclic aromatic hydrocarbon, Part 1, Chemical, Environmental and Experimental Data. Lyon International Agency for Research on Cancer : 32.

Inui, N., Nishi, Y., Taketomi, M., and Mori, M. 1979. Transplacentral action of sodium nitrite on embryonic cells of Syrian golden hamster. **Mutat. Res.** 66 : 149-158.

Ishidate, M. et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. **Food Chem. Toxicol.** 22:623-636.

Isono, K. and Yourno, J. 1974. Chemical carcinogens as frameshift mutagens : *Salmonella* DNA sequence sensitive to mutagenesis by polycyclic carcinogens. **Proc. Natl. Acad. Sci.** 71 : 1612-1617.

Iyer, V.N. and Szybalski, M. 1959. Mutagenic effect of azaserine in relation to azaserine resistance in *Escherichia coli.* **Science** 129 : 839-840.

Joanne, L.S. 1990. Dietary fiber : mechanisms or magic on disease prevention? **Nutrition today** Nov/Dec : 7-10.

Kakuda, Y., Gray, J.I. and Man, L.L. 1980. N-nitrosamines and their precursors in food systems. **J. Agric. Food Chem.** 28 : 588-595.

Kawtaluk, H. and Kopan, A.O. 1986. Meat. In : Food for today . Glenoc, Chicago : 357-360.

Kato, T. and Kikugawa, K. 1992. Protein and amino acids as scavengers of nitrite ; inhibitory effect on the formation of nitroso dimethylamine and diazoquinone. **Food Chem. Toxicol.** 30(7) : 617-626.

Kikugawa, K. Kato, T. and Takeda, Y. 1987. Formation of a highly mutagenic diazo compound from the bramethan-nitrite reaction. **Mutat. Res.** 177(1) : 35-43.

Kodama, F., Umeda, M., and Tsutsui, T. 1976. Mutagenic effect of sodium nitrite on cultured mouse cells. **Mutat. Res.** 40 : 119-124.

Kornhauser, A., Warmer, W.G., Lambert, L.A. and Wet, R.R. 1994. Beta-carotene inhibition of chemically induced toxicology *in vivo* and *in vitro*. **Food Chem. Toxicol**. 32(2) : 149-154.

Laohavechvanich, P. 1994. Nitrite Scavenging Activity of Fibers Derived from Fruits and Vegetables; and Their Antimutagen Formation of Aminopyrene and Nitrite in Simulated Gastric Condition using Ames test. Master's Thesis, Mahidol University.

Lathia, D. and Blum, A. 1989. Role of vitamin E as nitrite scavenger and N-nitrosamine inhibitor : a review. **Int. J. Vit. Nutr. Res**. 59(4) : 430-438.

Larsen, J.C. and Poulson, E. 1987. Mutagens and carcinogens in heat-processed food. In : Toxicological aspects fo foods. Miller, K. : Elsevier, England : 205-241.

Levin, D.E., Hollstein, M.C., Christman, M.F., Schwiers, F.A., and Ames, B.N. 1982 a. A new *Salmonella* tester strain (TA 102) with A : T base pairs at the site of mutation detects oxidative mutagens. **Proc. Natl. Acad. Sci. :** 312-332.

Levin, D.E., Yamasaki, E., and Ames, B.N. 1982b. A new *Salmonella* tester strain for the detection of frameshift mutagens : A run of cytosines as a mutational hot spot. **Mutat. Res.** 94 : 315-330.

Lucien, R.J. 1986. Relationships between dietary fiber and cancer : metabolic, physiological and cellular metabolism. **Proc. Soc. Exp. Bio. Med**. 183 : 299-310.

Luigi Robbiano, Pia Carlo, Renata Finollo, and Giovanni Brambilla. 1990. DNA Damage Induced in Rats by Oral Administration of Chlordiazepoxide Plus Sodium Nitrite or N-Nitrosochlordiazepoxide. **Toxicol. Appl. Pharmacol.**

102 : 186-190.

Macphee, D.G. 1973a. *Salmonella typhimurium* his G 46 (Rutrecht) possible use in screening mutagens and carcinogens. **Appl. Microbiol** 26 : 1004-1005.

Macphee,D.G. and Podger,D.M. 1977. Mutagenicity test on anthelmintics : Microsomal activation of virynium embonate to a mutagen. **Mutat. Res.** 48 : 307-312.

Manoonpol, K. 1994. Direct mutagenic potential of polycyclic aromatic hydrocarbon (PAHs) treated with nitrite in simulated gastric condition : comparison between authentic PAHs and PAH-containing fraction derived from smoked and charcoal-broiled foods using Ames test. Master's thesis, Mahidol University.

Maron, D.M. and Ames, B.N. 1983. Revised methods for the *Salmonella* mutagenicity test. **Mutat. Res.** 113 : 173-215.

Matsushima, T., Sugimuva, M., Nagao, T., Yahagi, T., Shirai, A., and Sawamure, M. 1980. Factors modulating mutagenicity in microbial tests. In : Short-term test systems for detecting carcinogens. Norphoth, K.H., Garner, R.C. Springer : Berling : 273-285.

McCann, J., and Ames, B.N. 1976. Detection of carcinogens as Mutagens in the *Slamonella*/microsome tests. Assay of 300 chemicals : Discussion. **Proc. Natl. Acad. Sci** 73 : 950-954.

_______, and Ames, B.N. 1977. The *Samonella*/microsome mutagenicity tests; Predictive value for animal carcinogenicity. In : Origins of human cancer Book C : Human risk assessment. Hiatt, H.H., Watson, J.D. and Winsten,

J.A. Cold Spring Harbor Laboratory, New york : 1431-1450.

_______ , Springarn, N.E., Kobori, J., and Ames, B.N. 1975. Detection of carcinogens as mutagens : bacterial tester strains with R factor plasmids. **Proc. Natl. Acad. Sci.** 72 : 979-983.

McPherson-Kay, R. 1982. Dietary fiber. **J. Lipd Res.** 23 : 221-242.

Mirvish, S.S. 1975. Formation of N-nitroso compounds chemistry, kinetics, and vivo occurrence. **Toxicology and applied Pharmacol.** 31 : 325-351.

_______ , Bulay, O., Runge, R.G., and Patil, K. 1980. Study of the carcinogenicity of large doses of dimethynitramine N-nitroso-L-proline and sodium nitrite administered in drinking water to rats. **J. Natl. Cancer. Inst.** 64 : 1435-1442.

Moller, M.E. Dahl, R. and Bockman, O.C. 1988. A possible role of the dietary fiber product , wheat bran, as a nitrite scavenger. **Food Chem. Toxicol.** 26(10) : 841-845

Mortelma, K.E. and Stoker, B.A.D. 1979. Segregation of the mutator property of plasmid R 46 from its ultraviolet protecting property. **Mol. Gen. Gent.** 167 : 317-327

Newberne, P.M., et al. 1987. Gastric carcinogenesis : a model for the identification of rish factors. **Cancer Lett.** 38 (1-2) : 49-63.

Ohshima, H., Furihata, C., Matsushima, T. and Bartsch, H. 1989. Evidence of potential tumor-inhibiting and tumor-promoting activities of hickory smoke condensate when given alone or with nitrite to rats. **Food Chem. Toxicol.** 27 (8) : 511-513.

Okinaka, R.T., Nickol, J.W., Whaley, T.W., and Strniste, G.F. 1986. 1-Nitropyrene : a mutagenic product induced by the action of near ultraviolet light on 1-aminopyrene. **Mutat. Res.** 163(1) : 81-89.

Olajos, E.J. and Coulston, F. 1987. Compative toxicology of N-nitroso compounds and their carcinogenic potential to man. **Ecotoxicol Environ. Safety** 2 : 317-367.

Prival, M.J., King, V.D., and Sheldon, A.T. 1979. The mutagenicity of dialkyl nitrosamines in the *Samonella* plate assay. **Environ. Mutagen** 1 : 95-104.

Purchase, I.F.H., et al. 1976. Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use. **Nature** 264 : 624-627.

Regina, G.Z. 1991. Vegetables, fruit, and carotenoids and the risk of cancer. **Am. J. Clin. Nutr.** 53 : 1S-9S.

Rinkus, S.J. and Legator, M.S. 1981. *Salmonella* revisited : A reply to Ames and McCann. **Cancer Res.** 41 : 4196-4203.

Risch, H.A. et al. 1985. Dietary factors and the incidence of cancer of the stomach. **Am. J. Epidemiol.** 122 : 947-953.

Roberfroid, M. 1993. Dietary fiber, Inulin, and Oligofructose : A Review Comparing their Physiological Effects. **Cnt. Rev. Food Sci. Nutr.** 33(2):103-148.

Rydning, A., Berstad, A., Aadland, E. and Udegard, B. 1982. Prophylactic effect of dietary fiber in duodenal ulcer disease. **Lancet II** 736-742..

Shenoy, N. R. and Choughuley, A.S. 1992. Inhibitory effect of diet related sulhydryl compounds on the formation of carcinogenic nitrosamines. **Cancer Lett**. 65

(3) : 227-232.

Shenoy, N. R., Eisenbrand, G., and Preussmann, R. 1976. Influenc of nitrite on nitrite content of human saliva : Possible relevande to in vivo formation of N-nitroso compounds. **Food Cosmet. Toxicol.** 14 : 545-549.

Sugimura, T., et al. 1976. Overlapping of carcinogens and mutagens. In : Fundament is in cancer prevention. Magee, P.N. Tokyo University of Tokyo Press. : 191-215.

Takeda,Y. and Kanaya,H. 1981. Formation of nitroso-compound and mutagens from tranquilizer by drug/nitrite interaction. **Cancer Lett.** 12 : 81-86.

Takeda,Y. and Kanaya,H. 1982. A Screening Procedure for the formation of Nitroso Derivatives and Mutagens by Drug-Nitrite Interaction. **Chem. Pharm. Bull.** 30 (9) : 3399-3404.

Thamavit, W., Moore, M.A., Hiasa, Y. and Ito, N. 1988. Generation of high yields of Syrian hamster cholangiocellular carinomas and hepatocellular nodules by combined nitrite and aminopyrene administration and opithorchis viverrini infection. **Jpn. J. Cancer Res.** 79 (8) : 909-916.

Thornton, J.R. 1981. High colonic pH promotes colorectal cancer. **Lancet** 1 : 1081-1083.

Tricker, A.R. and Preussmann, R. 1991. Carcinogenic N-nitrosamines in the diet : occurrence, formation mechanisms and carcinogenic potential. **Mutat. Res.** 259 : 277-289.

Vanschaik, N. and Graf, N. 1993. Structure-Activity- Relationships of Tricyclic Antidepressants and Related- Compounds in the Wing Somatic Mutation and

Recombination test of Drosophila-Melanogaster. **Mutat. Res**. 286 : 155-163.

Visek, W.J. 1978. Diet and cell growth modulation by ammonia. **Am. J. Clin. Nutr.** 31 : 216-220S.

Williams, J.S. and Louis, S.V. 1975. Drug Induced Biogenesis of nitrosamines. **Res. Communicat. Chem. Pathol. Pharmacol.** 12(3) : 605-608.

Yahagi, T., Nagao, M., Seino, Y., Matsushima, T., Sugimura, T., and Okada, M. 1977. Mutagenicity of N-nitrosamines on *Salmonella.* **Mutat. Res.** 48 : 121-130.

Yamanaka, H., Nagao, M., Sugimura, T., Furaya, T., Shirai, A., and Matsushima, T. 1979. Mutagenicity of pyrrolizidine alkaloids in the *Samonella*/mammalian microsome test. **Mutat. Res.** 68 : 211-216.

Yamagushi,, T. 1992. Inhibitory activity of heat treated vegetables and indigestible polysaccharides on mutagenicity. **Mutat. Res.** 284 : 205-213.

Zeiger, E. 1993. Mutagenicity of chemical added to food. **Mutat. Res.** Nov; 290 (1) : 53-61.

APPENDIX

1. preparation of stock solutions and media (Maron and Ames, 1983)

1.1 Vogel-Bonner medium E stock salt solution (VB salt)

Use : Minimal agar

Ingredient	1 Liter	200 ml
Warm distilled water (45°C)	672 ml	134 ml
Magnesium sulfate ($MgSO_4 . 7H_2O$)	10 g	2 g
Citric acid monohydrate	100 g	20 g
Potassium phosphate, dibasic (anhydrous) (K_2HPO_4)	500 g	100 g
Sodium ammonium phosphate ($NaHNH_4PO_4 . 4H_2O$)	175 g	35 g

Add salts in the order indicated to warm water in beaker placed on a magnetic stirring hot plate. Allow each salt to dissolve completely before adding the next. Adjust the volume and filter the solutions into the glass bottles with screw caps and then autoclave at 121°C for 15 min.

1.2 Minimal glucose agar plate

Use : Mutagenicity assay

Ingredient	1 Liter	350 ml
Bacto agar	15 g	5.25 g
Distilled water	930 ml	330 ml
VB salts	20 ml	7 ml
40% glucose	50 ml	17.5 ml

Add agar to distilled water in a glass bottle. Autoclave at 121°C for 15 min using slow exhaust. When the solution has cooled slightly, add sterile VB salt and

sterile 40% glucose. After all the ingredients have been added, the solution should be swirled thoroughly. Pour 30 ml into each sterile petri plate. Minimal glucose agar plate were kept in incubator at 37°C before using.

note : The VB salts and 40% glucose should be autoclaved separately.

1.3 Oxoid nutrient broth No.2

Use : Growing culture

Dissolve 2.5 g of Oxoid nutrient broth No. 2 in 100 ml distilled water. Transfer 12 ml of nutrient broth for each 50 ml Erlenmeyer flask. Autoclave at 121°C for 15 min.

1.4 1 M L-histidine HCl stock

Use : Fortification of minimal agar plate

Ingredient	1 Liter	10 ml
L-Histidine HCl	2.096 g	209.6 mg
Distilled water	100 ml	10 ml

Dissolve L-histidine HCl (MW 209.63) in distilled water. Dilute 1 ml of 0.1 M L-histidine HCL in 99 ml of distilled water.

1.5 1mM biotin stock

Use : Fortification of minimal agar plate

Ingredient	100 ml
Biotin	24.43 mg
Distilled water	100 ml

Dissolve biotin (MW 244.3) in distilled water. Warm it until dissolve completely. Autoclave at 121°C for 15 min.

1.6 0.5 mM L-histidine/biotin solution

Use : Mutagenicity assay (add 10 ml to 100 ml of Top agar)

Ingredient	200 ml
1 mM L-histidine HCl	100 ml
1 mM biotin	100 ml

Mix and autoclave at 121°C for 15 min.

1.7 Top agar

Use : Mutagenicity assay

Ingredient	1 Liter	100 ml
bacto agar	6 g	0.6 g
sodium chloride	5 g	0.5 g
distilled water	1000 ml	100 ml

Dissolve ingredients in distilled water. Store in a glass bottle. Autoclave for 15 min at 121°C and then add 0.5 mM L-histidine/biotin solution (10 ml for 100 ml of Top agar).

1.8 1M potassium chloride

Use : Na_3PO_4 - KCl buffer

Ingredient	1 Liter	100 ml
Potassium chloride	74.56 g	7.456 g
Distilled water	1000 ml	100 ml

Mix and autoclave at 121°C for 15 min.

1.9 0.5 M sodium phosphate pH 7.4

Use : Na_3PO_4 - KCl buffer for mutagenicity assay

Ingredient

0.5 M Sodium dihydrogen phosphate (NaH_2PO_4)
(30 g / 500 ml)

0.5 M Disodium hydrogen phosphate ($Na_2HPO_4 . 2H_2O$)
(45.5 g / 500 ml)

Dissolve 45.5 g disodium hydrogen phosphate in 300 ml of distilled water. Add 0.5 M sodium dihydrogen phosphate until to pH 7.4, then adjust volume to 500 ml. Sterilize by autoclaving for 15 min at 121°C.

1.10 Na_3PO_4 - KCl buffer

Use : mutagenicity assay

Ingredient	330 ml
0.5 M Na_3PO_4 pH 7.4	100 ml
1 M KCl	16.5 ml
Distilled H_2O	213.5 ml

Autocave for 15 min at 121°C.

2. Recipes for some reagents and test chemicals

2.1 2 M sodium nitrite

Use : Nitrosation

Ingredient	1000 ml	10 ml
Soduim nitrite	138 g	1.38 g
Distilled water to	1000 ml	10 ml

Autoclave for 15 min at 121°C.

2.2 2 M ammonium sulphamate

Use : reaction mixture

Ingredient	1000 ml	50 ml
Ammonium sulfamate	228.24 g	11.41 g
Distilled water to	1000 ml	50 ml

Dissolve ammonium sulfamate in distilled water and adjust volume. Autoclave for 15 min at 121°C.

2.3 0.2 N hydrochloric acid

Use : reaction mixture

Ingredient	1000 ml	100 ml
Conc. Hydrochloric acid	15.36 ml	1.54 ml
Sterile distilled water	984.64 ml	98.46 ml

Dissolve conc hydrochloric acid in sterile water. Store in sterile glass tubes or bottles with screw caps.

Note : Preparation of 0.2 N HCl must be used sterile technique because hydrochloric acid cannot be autoclaved.

2.4 0.3 mg/ml aminopyrene

Use : standard solution for mutagenicity assay

Ingredient	1 ml
Aminopyrene	3 mg
Aetonitrile	1 ml

Dissolve aminopyrene in acetonitrile. Store in sterile vial with screw caps in the freezer. This preparation must be used sterile technique.

Ingredient	1 ml
3 mg/ml aminopyrene	0.1 ml
Acetonitrile	0.9 ml

Dissolve 3 mg/ml aminopyrene in acetonitrile. Store in sterile vial with screw cap in the freezer. This preparation must be used sterile technique.

2.5 8 mg/ml ampicillin solution

Ingredient	10 ml
Ampicillin (sodium)	800 mg
0.02 N sodium hydroxide	10 ml

2.7 0.1% crystal violet

Ingredient	10 ml
Crystal violet	10 mg
Distilled water to	10 ml

2.6 and 2.7 : Store at 0°C in glass bottle with screw cap.

2.8 Gastric condition mixture

Ingredient	100 ml
Sodium chloride	0.2 g
Bovine serum albumin	30 mg
Sodium thiocyanate	2.43 mg
Distilled water to	100 ml

3. Procedure for Reisolation and Growing Culture

Tester strains, TA 98 and TA 100 are grown in Oxoid nutrient broth No.2 and incubated overnight in a 37°C shaking water bath. The growth period should not exceed 16 hr (Ames *et al.*, 1973a). These cultures are reisolated by streaking on minimal glucose agar plates which the surface were spread with 0.1 ml of 8 mg/ml amipicllin. 0.3 ml of 0.1 M histidine HCl and 0.1 ml of 1 mM biotin. These plates are incubated at 37°C for 48 hr. After incubation, the 5 single colonies per strain TA 98 and TA 100 are picked up and grown in Oxoid nutrient broth No. 2 overnight 37° in shaking water bath. Each culture is confirmed genotypes of the strains and kept the cultures as the source of bacteria for mutagenicity testing. For each 1.0 ml of culture, add 0.09 ml of spectrophotometric grade DMSO. Combine the culture and DMSO in a sterile tube and distribute 400 µl of the culture aseptically into sterile cryotubes (Nunc). The tubes should be filled nearly full and then transfer to a -80°C freezer.

Confirming Genotype of Tester Strains The broth cultures of TA 98 and TA 100 are used to confirm genotypes in the following ways.

Histidine requirement The His+ character of the strains is confirmed by demonstrating the histidine requirement for growth on the minimal glucose agar plates enriched with histidine and biotin.

Procedure :plate a no histidine and biotin

plate b 0.1 ml of 1 mM biotin

plate c 0.3 ml of 0.1 m His-HCl

plate d 0.3 ml of 0.1 M His-HCl + 0.1 ml of 1 mM biotin

Four minimal glucose agar plates is required for each tester strains. Each of the plates is applied on the surface with 0.1 ml of 1 mM biotin, 0.3 ml of 0.1 M His-HCl, 0.3 ml of 0.2 M His-HCl plus 0.1 ml of 1 mM biotin and no application (plate b,c,d,a respectively). Made a single streak of each strains across these plates. Five strains could be tested on the same plate. Incubated at 37°C for 48 hr. The growth of bacteria in histidine plus biotin plate is the result of histidine requirement.

R Factor The R-factor strains (TA 977, TA 98, TA 100 and TA 102) should be tested routinely for the presence of the ampicillin resistance factor because the plasmid is somewhat unstable and can be lost from the bacteria.

Procedure : For each tester strain, add 0.3 ml of fresh overnight culture to a tube containing 0.1 ml of 0.2 M histidine-HCl followed by adding 2.0 ml of molten top agar containing 0.5 mM histidine and 0.5 mM biotin. Mixed and poured on a minimal glucose agar plate. Rotated the plate to distribute the mixtures and allowed several minutes for agar to become firm. R factor and rfa mutation (see the next section) are

performed in the same plate by dividing the plate into 2 areas, one for R factor and the other for rfa mutation. For R factor, commercial ampicillin disc or filter paper disc containing 8 mg/ml ampicillin is applied on the surface of the agar by using sterile forceps. The disc is pressed lightly to embed in the overlay. The plates are incubated at 37°C for 24 hr. The absence of the clear zones of inhibition around the discs indicate resistance to ampicillin.

rfa Mutation Strains having the deep rough (rfa) character should be tested for crystal violet sensitivity .

Procedure : Pipetted 0.1% solution of crystal violet to the sterile filter paper disc (1.4 inch) and transfered the disc to plates, seed with bacteria (the procedure is similar to R factor). Incubated at 37°C for 48 hr. The clear zone appeared around the disc indicated the presence of the rfa mutation that permitted crystal violet to enter and kill bacteria.

Spontaneous Reversion Spontaneous reversion of the tester strains to histidine independence is measured routinely in mutagenicity experiments and is expressed as the number of spontaneous revertants per plate. The revertant colonies are clearly visible in a uniform background lawn of auxotrophic bacteria. Each tester strain reverts spontaneously at a frequency that is characteristic of the strain. Nevertheless, there is variability in the number of spontaneous revertants from one experiment to another and from one plate to another, and it is advisible to include at least 2-3 spontaneous mutation control plates for each strain in a mutagenicity assay.

Procedure : 0.1 ml of DMSO (Solvent in the experiment) is added to capped culture tube. Add 0.5 ml of Na_3PO_4-KCl buffer pH 7.4 in the absence of metabolic activation, 0.1 ml of fresh overnight culture of TA 98 or TA 100, followed by 2.0 ml of molten top agar. Mixed and then poured on minimal glucose agar plate. Rotated plates and left it to become harden. Incubated at 37°C for 48 hr and the His+ revertant colonies were counted.

The Response to Standard Mutagen

Standard mutagens or positive mutagens are used routinely in mutagenicity experiments to confirm the reversion property and specificity of each strain. The standard mutagen used in these experiments is aminopyrene in the absence of metabolic activation. Tester strain which highly response to positive mutagens must be collected.

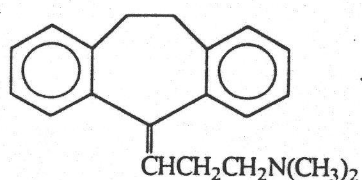
Procedure : The procedure is as described in spontaneous reversion except aminopyrene (0.06, 0.12 and 0.24 μg/plate for TA 100 and TA 98, respectively) are used instead of DMSO in the absence of S9 mix, respectively. The characteristic of the stock culture for TA 98 and TA 100 as the source of bacteria for mutagenicity is

a. contained R factor (pKM 101) and rfa mutation
b. His^+ requirement
c. low spontaneous reversion
d. highly response to standard carcinogen

After the characteristic of the culture was tested, the mutagenicity test was started

4. Chemical structure and specific action of drug samples

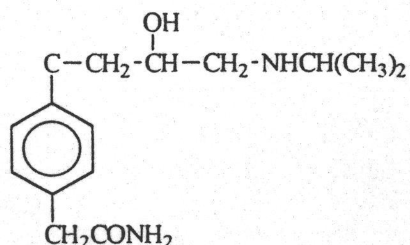
4.1 Amitriptyline (Tryptanol® Merck Sharp & Dohme)



· HCl

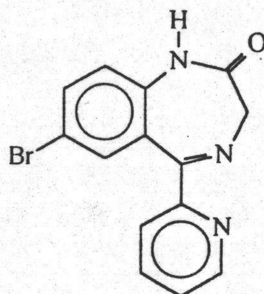
Antidepressant

4.2 Atenolol (Oraday® Biolab)



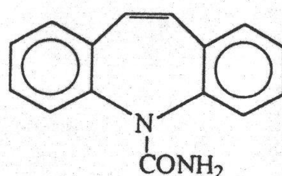
Beta- blocker

4.3 Bromazepam (Lexotan® Roche)



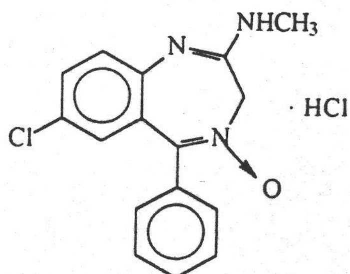
Minor tranquilizer

4.4 Carbamazepine (Tegretol® Ciba-Geigy)



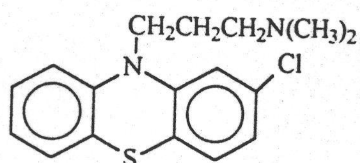
Anticonvulsant

4.5 Chlordiazepoxide (Benpine® Atlantic)



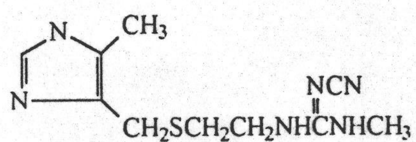
Minor tranquilizer

4.6 Chlorpromazine (Matcine® Atlantic)



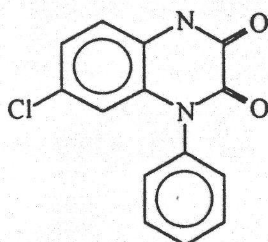
Major tranquilizer

4.7 Cimetidine (Cimulcer® Biolab)



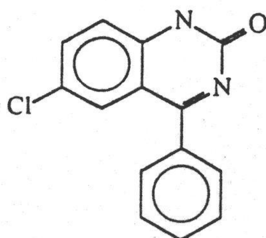
Antiulcerant

4.8 Clobazam (Frisium® Hoechst)



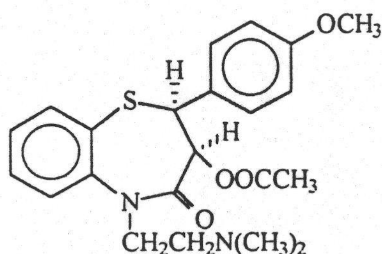
Minor tranquilizer

4.9 Diazepam (Valium® Roche)



Minor tranquilizer

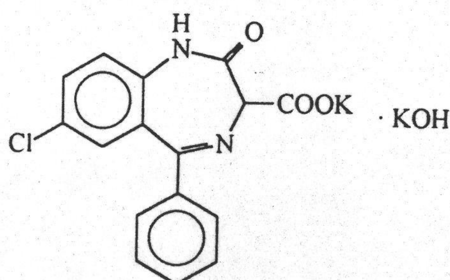
4.10 Diltiazem HCl (Herbessor® Tanabe seiyaku)



· HCl

Calcium antagonist

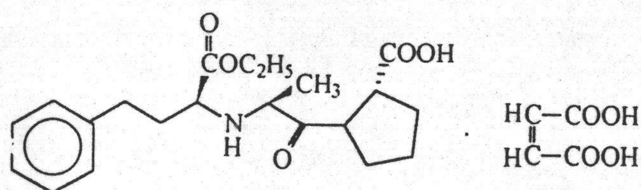
4.11 Dipotassium chlorazepate (Tranxene® Sandoz)



· KOH

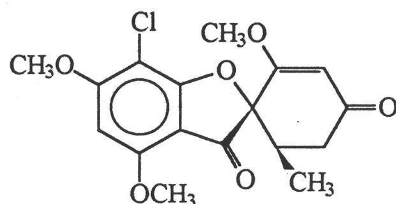
Minor tranquilizer

4.12 Enalapril maleate (Enaril® Biolab)



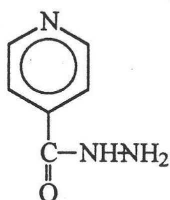
ACE inhibitor

4.13 Griseofulvin (Fulcin® ICI)



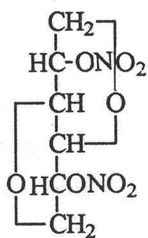
Antifungal

4.14 Isoniazid (Isoniazid® Atlantic)



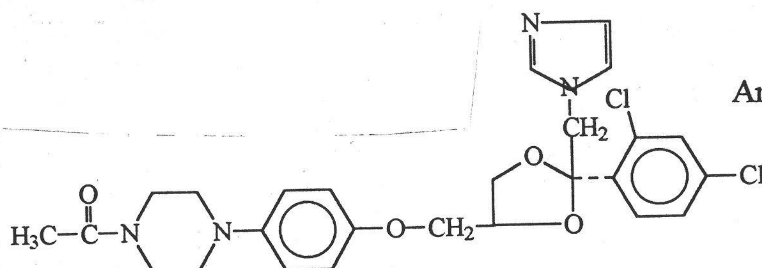
Antituberculous agent

4.15 Isosorbide dinitrate (Isordil® Wyeth-Ayerst)



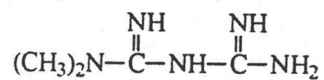
Cardiac drug

4.16 Ketoconazole (Fungazol® biolab)



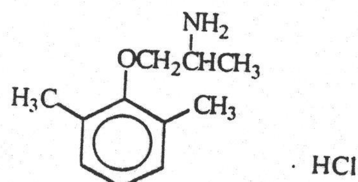
Antifungal

4.17 metformin HCl (Glucophage® Lipha/ E Merck)



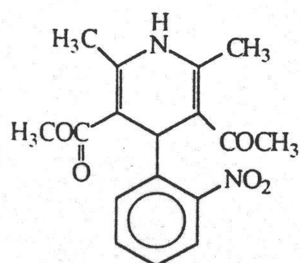
Oral hypoglycemic agent

4.18 Mexilitine HCl (Mexitil® Boehringer Ingelheim)



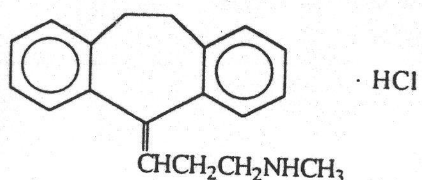
Cardiac drug

4.19 Nifedipine (Adalat® Bayer)



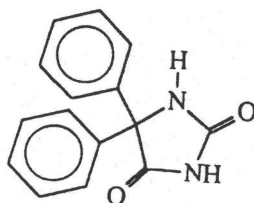
Calcium antagonist

4.20 Nortriptyline (Nortilen® Lundbeck)



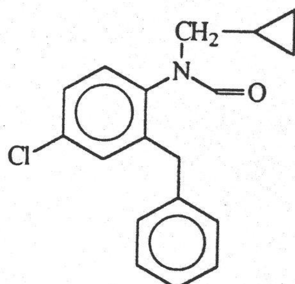
Antidepressant

4.21 Phenytoin (Dilatin® Parke-David)



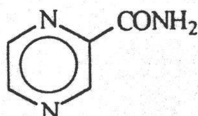
Anticonvulsant

4.22 Prazepam (Prasepine® Parke-David)



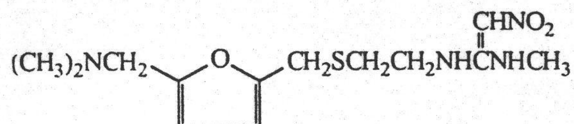
Minor tranquilizer

4.23 Pyrazinamide (Pyrazinamide® GPO)



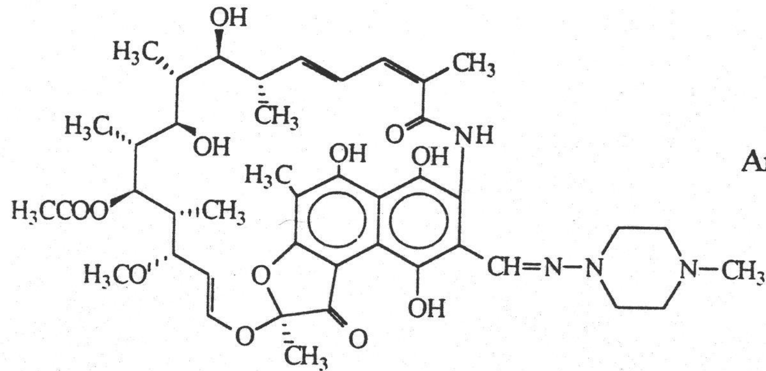
Antituberculous agent

4.24 Ranitidine (Ranidine® Biolab)



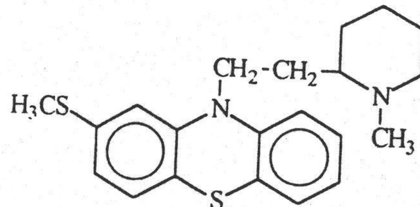
Antiulcerant

4.25 Rifampicin (Rimactan® Ciba-Geigy)



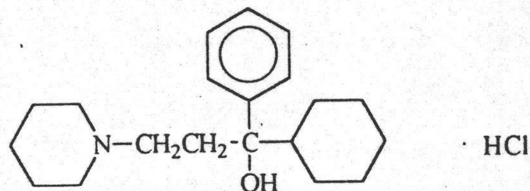
Antituberculous agent

4.26 Thiridazine (Melleril® Sandoz)



Major tranquilizer

4.27 Trihexyphenidyl HCl (Artane® Lederle)



· HCl

Antiparkinson

5. The mutagenicity test using *Salmonella typhimurium.*

Plate incorporation test The test is the standard method that has been used for test the mutagenicity of chemicals. This test consists of combining the test compound and the bacterial tester strain in soft agar which is poured onto a minimal agar plate. Positive and negative controls are also included in each assay. After incubation at 37°C for 48 hr revertant colonies are counted (Ames et al., 1973b). For initial screening chemicals were tested in concentrations over a three-log dose. A positive or questionable result should be confirmed by demonstrating a dose-response relationship using a narrower range of concentrations. For most mutagens, there is a concentration range that produces a linear dose-response curve and the number of revertants per plate reported for a mutagen should be taken from the region of the curve. However, a few mutagens such as 9-aminoacridline, MNNG, diethylsulfate and ethylmethanesulfonate produce non liner dose-response curve (McCann et al., 1975). The compounds that are negative can be retested using the preincubation method.

Preincubation method Some mutagens, such as dimethyl- and diethyl-nitrosamine are poorly detected in the standard plate incorporation assay and should be tested using a modification of the standard procedure. The most widely used test modification is the preincubation assay first described by Yahagi et al, 1975, in which carcinogenic azo dyes were found to be mutagenic. They incubated the mutagen and bacteria for 20-30 min at 37°C and then added the top agar. The assay has been also used to detect the mutagenicity of 10 carcinogenic nitrosamines and several carcinogenic alkaloids (Yamanaka et al., 1979). The mutagenic activity of aflatoxin

B1, benzidine, benzo(a)pyrene and methylmethanesulfonate has been determined using both plate incorporation and preincubation procedures and in all cases the preincubation assay is of equal or greater sensitivity than the plate incorporation assay (Matsushima et al., 1980). The increased activity is attributed to the fact that the test compound and bacteria are incubated at higher concentration in the preincubation assay than in the standard plate incorporation test (Prival et al., 1979). The procedure described below is based on recommendation of Matsushima et al., 1980.

The preincubation modification can be used routinely or when inconclusive results are obtained in the standard plate incorporation assay. This assay requires an extra step and therefore involves more work than the standard test but many laboratories use it routinely because of the increased sensitivity of some compounds. Its use in screening assays has been recommended by De Serres and Shelby (1979).

Spot test The spot test is the simplest way to test compounds for mutagenicity and is useful for the initial rapid screening of large numbers of compounds. Ames et al.(1975) have tested 169 different hair dyes for mutagenicity using this method. This test has several advantages. A few crystals of a solid mutagen or µl of a liquid mutagen can be put directly on the agar surface, thus eliminating the time-consuming preparation of solutions of the chemicals to be tested. As the test compound diffuse out from the central spot, a range of concentrations is tested simultaneously.

This test is primarily a qualitative test and has distinct limitations. It can be used only for testing chemicals that are diffusable in the agar. It is much less sensitive than the standard plate incorporation test. Mutagenicity should be confirmed by demonstrating a dose-response relationship using the standard plate incorporation test.

Positive control (diagnostic mutagens) In each experiment positive mutagenesis controls using diagnostic mutagens to confirm the reversion properties and specificity of each strain. The characteristic reversion patterns of the standard strains to some diagnostic mutagens are described by Maron and Ames, 1983.

Evaluation criteria for Ames assay Because the procedures to be used to evaluate the mutagenicity of the test article are semiquantitative. Each tester strian is specific to each type of mutation such as frameshift mutation, base pair substitute or oxidative mutation etc. The criteria used to determine positive effects are, therefore, inherently subjective and based primarily on the information shown in Table 2. Most data sets should be evaluated using the following criteria (Kowtaluk and Kopan, 1986).

a. Strains TA 1535, TA 1537, and TA 1538. If the solvent control value is within the typical range for the laboratory, a test article that produces apositive dose response over three concentrations, with the highest increase equal to three times the solvent control value, is considered mutagenic.

b. Strains TA 98 and TA 100. If the solvent control value is within the normal range for the laboratory, a test article that produces a positive dose response over three concentration, with the highest increase equal to twice the solvent control

value, is considered mutagenic. Occasionally a doubling is not necessary for TA 100 if a clear dose-related pattern is observed over several concentrations.

c. Pattern. Because TA 1535 and TA 100 are derived from the same parental strain (G46), and TA 1538 and TA 98 are derived from the same parental strain (D3052), to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen, and such a pattern is sought. Generally, if a strain responds to a mutagen in nonactivation tests, it should do so in activation tests.

d. Reproducibility. If a test article produces a response in a single test that cannot be reproduced in additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute and other extenuation factors may enter into a final evaluation decision. However, these criteria can be applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established. It must be emphasized that modifications of the procedure involving preincubation conditions is necessary for evaluation of specific chemicals or classes of chemicals.

VITA

Miss Suyanee Suharitdamrong was born on July 14, 1967 in Bangkok, Thailand. She recieved her Bachelor of Science in Pharmacy in 1990 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. At present, she is a pharmacist at Phya-Thai I Hospital, Bangkok, Thailand.