CHAPTER II

REVIEW OF LITERATURES

Drug Treatment : Long term use

Great advances in the treatment and prevention of diseases by drugs have radically altered the practice of medicine during the last several years. To some extent the corresponding developments necessary in medical education have lagged behind, though the importance of ensuring that these powerful remedies are safety and effectively used by doctors is self-evident. Nevertheless, at clinical meeting the traditional emphasis on diagnosis and pathophysiology persists and drugs therapy and the problems that if may pose often relatively little attention or critical discussion. However, in recent years, there have been great advances in clinical pharmacology. Factors that influence to the absorption, distribution, metabolism, excretion, and interaction of drugs in man have been increasingly recognised and are made available to clinicians, who should at the same time appreciate its relevance to practical therapeutics.

There is no potent drug effective in the treatment of disease that does not at the same time carry with it the risk of producing adverse effects and these may arise in a multiplicity of different ways which must be understood, at least in principle, by the prescribling doctor. Early recognition of a toxic reaction is particularly important in safe guarding the health of the patient. No drug is completely free from hazard and

safety is always a relative matter in which the seriousness and natural course of the disease, the ability of the agent to produce benefit, and its liability to cause harmful effects, must all be taken into account.

Long term drug administrations are sometime, necessary for various chronic disease. They may be several months, several years or throughout of the life. These diseses ; such as cardiovascular disease, diabetes, tuberculosis, psychotropic disease and gastrointestinal disease are necessary to be treated by various drugs for long term periods of time. In addition many drugs are necessary to be administered with meal or after meal; therefore, they have chances to interact with various components in diet. Some of the interactive products are proved to increase carcinogenic risk to human, for example drug-nitrite interaction induced mutagenesis which is extensively discussed in this investigation (Takeda and Kanaya, 1982) .

Nitrite in Foods.

Nitrite is available either as a natural constituent after reduction of nitrate or as food additive in meats, fish, cheese and their products. Sodium and potassium nitrite are used to preserve meat products for the purpose of retaining color, improving flavor, and inhibiting growth and toxin formation by *Clostridium botulinum* (Olajos and Coulston, 1987). Bioconversion of nitrate to nitrite by nitrifying bacteria that may present in foodstuffs, saliva and in gastrointestinal tract (Figure 1) is also the other important source of nitrite.

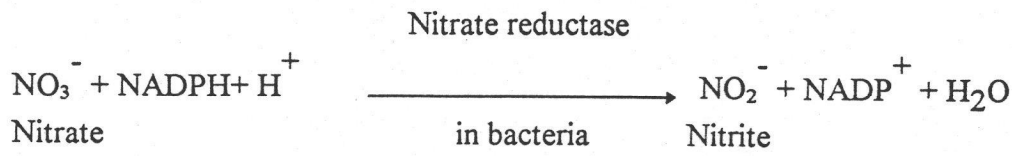

Figure 1 The conversion of nitrate to nitrite by nitrifying bacteria.

Nitrites can react *in vitro* with amines under acidic condition to produce nitroso compounds, which some of them are known to be carcinogenic in animals in extremely small concentrations (Bender and Bender, 1982). The conditions under which amines and nitrites react are similar to those found in human stomach. The small amounts of nitrites from the diet and saliva are present together in stomach for long enough, and at a sufficiently low pH, for significant amounts of nitrosamines to be formed, and whether such nitrosamines are stable under the conditions of small intestine long enough to have any harmful effect. Risk/benefit evaluation on the use of nirites must be balanced since nitrites are especially valuable as a mean of preventing the growth of *Clostridium botulinum,* the causative organism of one of the most severe forms of bacterial food poisoning. A balance must struck between the use of preservatives that may have some carcinogenic potential and the possibility of series outbreaks of botulinum toxin.(Bender and Bender, 1982).

Mutagenicity and carcinogenicity of nitrite Sodium nitrite induced reverse mutation in *Salmonella typhimurium* and causes chromosomal aberrations in culture Chinese hamster fibroblast cells (Ishidate *et al.*, 1984). Sodium nitrite did not cause an increase in single strand breaks in cultured mouse cells ; on the other hand, there

was a dose-ralated increase in gene mutations and chromosome aberrations at high concentrations, possible due to deamination of bases (Kodama *et al.*, 1976).

Syrian hamsters were given sodium nitrite at doses of 125, 250, or 500 mg/kg body weight by gavage on day 11 and 12 of pregnancy, and embrionic cell cultures were prepared 24 hrs later. There was a dose-dependent increase in micronuclei and in 8-azaquanine- and ouabain-resistant mutants. Cell transformation was also observed *in vitro* and implication of transformed cells led to tumor development (Inui *et al.*, 1979). However, administration of sodium nitrite in drinking water at a concentration of 1250 mg/l to non-pregnant and pregnant rats (on days 5-18 of gestation) induced chromosomal aberrations in the adult bone marrow and in the embrionic livers. The ratio of number of metaphases with aberrations in treated and control animals was higher for embrionic livers compared to adult bone marrow. The higher incidence might result from the higher numbers of mitotic cells in embrionic tissue (El Nahas *et al.*, 1984). However, Wistar rats of both sexes were given sodium nitrite in distilled water at concentration of 0 to 3000 mg/l on 5 days / week for more than 100 weeks. Papilomas of the forestomach were seen in 8/45 treated animals (the group recieving sodium nitrite 3 g/l drinking water; total dose, 63 mg/kg B.W.) (Mirvish *et al.*, 1980).

Three groups of 24 males Wistar rats were given sodium nitrite in diet at concentrations of 0, 800, or 1600 mg/kg B.W. for 92 weeks. One rat in the lower dose group developed a benign liver tumor and 5 rats in the higher dose group developed liver tumor, three classified as benign and two as malignant. The tumors

were derived from parenchymal or hemangioendothelial cells. However, the nitrite-containing diets were found to contain NDMA (N-nitrosodimethylamine) and NPYR (N-nitroso pyrollidine) and the authors concluded that these preformed nitrosamines were probably the principle cause of the tumors (Aoyagi *et al.*, 1980)

Mutagenicity of nitrite and foods or drugs

1. N-nitroso compounds : Nitroso compounds can be produced by the acid catalyzed reaction of nitrite with certain nitrogen containing compounds (Mirvish, 1975). The compounds are devided into nitosamines and nitrosamides (with some related compounds) (Figure 2). Nitrosamines are N-nitroso derivatives of secondary amines, whereas nitrosamides are N-nitroso derivatives of substituted ureas, amides, carbamates, guanidines and similar compounds (Mirvish, 1975).

N-nitrosamines are relatively stable compounds under most conditions are found in foods and do not decompose during food processing or preparation. On the other hand, nitrosamides are less stable, especially at neutral to basic pH. Kakuda *et al,* (1980) studied the stability of nitrosamides in foods and concluded that it was unlikely that they would survive common cooking procedures. Nitrosation of some food component with nitrite occurs most rapidly at pH 2-4 (Kakuda *et al.*, 1980) and also occur under other conditions that are important in some foods. For example, certain oxides of nitrogen can react directly with amines without the requirement for acid as in the case of nitrite (Tricker and Preussmann, 1991).

N-nitrosamine N-nitrosamide*

*where:

Y	X	Compound
O	alkyl, aryl	N-nitrosamide
O	NH_2, NHR, NR_2	N-nitrosourea
O	RO	N-nitrosocarbamate
NH	NH_2, NHR, NR_2	N-nitrosoguanidine

R_1 and R_2 can be virtually any organic groups. There are two main categories of N-nitroso compounds; the relatively stable nitrosamines and the relatively unstable nitrosamides . For N-nitrosamines, R_1 and R_2 can be either alkyl, aryl groups, or in some cases, alicyclic. They can be devided from dialkyl-, diaryl-, or cyclic secondary amines, a closely related class of compounds, N-nitrosamides, where R_1 is alkyl or aryl group and R_2 is an acyl group, can be devided from N-alkylurea, N-alkylcarbamates and simple N-alkylamides.

Figure 2. Generalized structure of N-nitroso compound: N-nitrosamines and N-nitrosamides and related compounds.

The two major sources of oxides of nitrogen (nitrosating agents) result from (I) addition of nitrate and/or nitrite to foods, and (II) the heating and/or drying of foods in combustion gases in which molecular nitrogen can be oxidized to oxides of nitrogens. Nitrosation reaction can be influenced by the presence of nitrosation inhibitors (redox compounds such as ascorbate and vitamin E) and catalysts (metal ions, carbonyl compounds and nucleophilic anions such as CL^-, I^- and SCN^-. In plant-based foods, phenolic compounds can catalyse and inhibit nitrosation depending on their structure (Tricker and Preussmann, 1991).

2. Non - nitroso compounds : Polycyclic aromatic hydrocarbons (PAHs) are other group of toxicants capable of reacting with nitrite. They are highly lipophilic chemicals and present ubiquitously in the environments as pollutants or as products of pyrolysis of organic matter (Larsen and Poulsen, 1987). They have long been of concern as a potential human health hazard, since many members of this class are tumor initiators or promoters or tumorigenic and/or mutagenic in vitro and in vivo (IARC, 1979). Humans are exposed to PAHs by three routes: (1) respiratory exposure such as tobacco smoke and urban air pollution, (2) skin contact for example cosmetic and medicinal products including occupational exposure, and (3) ingestion (IARC, 1983). Two major sources exist for the occurrence of PAHs in foods. The first is the deposition and uptake of PAHs from polluted air on food crops such as cereals, vegetables, fruits and vegetable oils. The second significant source is the formation and deposition of PAHs on foods during heat processing methods such as roasting, smoking, and grilling. Curing smoke is normally produced from wood by the

initial pyrolytic changes of lignin, hemicellulose and cellulose, followed by secondary reaction leading to the formation of PAHs and a variety of different chemical compounds (Larsen and Poulsen, 1987).

Nitro-containing (nitro) PAHs are group of fused ring aromatic hydrocarbon which contain one or more nitro molecules covalently linked to cyclic carbon atoms. An examination of the mutagenic and genotoxic properties of the nitro-PAHs appears to be timely in view of the recent recognition that this group of chemicals may well have a widespread distribution in the environment (Burkitt, 1971).

The consumption of smoked fish and meat products is associated with an increase risk of stomach cancer. A commercial hickory smoke condensate (HSC) was evaluated for its tumor-initiating and/or promoting activities in the glandular stomach using short-term methods *in vivo*. The administration of HSC with nitrite generated new substance(s) also induced unscheduled DNA synthesis in the pyloric mucosa (Oshima *et al.*, 1989).

Nitrite scavengers

Several chemicals presented in diet can modify levels of endogenously formed nitrosamines by acting as catalyst or inhibitors (Shenoy and Choughuley, 1992).

Proteins and amino acids : High concentrations of protein similar to those found in the digestive tract effectively scavenged nitrite and thus inhibited the formation of mutagen due to nitrite. Kato and Kikugawa (1992) stated that some

amino acids could also convert nitrite into nitrogen gas ; proline was converted into non-mutagenic nitrosoproline, thiocysteine to S-nitrosocysteine, tryptophan to weakly mutagenic nitrosotryptophan and tyrosine to non-mutagenic diazotyrosine.

Sulhydryl compounds : Nitrite scavenging nature of sulhydryl compounds was reported. Shenoy and Choughuley (1992), studied the modifying effect of sulhydryl compounds e.g., cysteine, cystine, glutathione, cysteamine, cystamine, cysteic acid and thioglycolic acid on the nitrosation of model amines e.g., pyrrolidine, piperidine and morpholine. Many of these compounds are normally present in food. The inhibitory effect of onion and garlic juices on the nitrosation reaction was also described. Both onion and garlic are known to contain sulphur compounds. Most of these compounds behave as antinitrosating agents and their inhibitory activity towards formation of carcinogenic nitrosamines.

Para-aminobenzoic acid : p-aminobenzoic acid (PABA) reduced mutagenicity of nitrite treatment of various foodstuffs (e.g., chicken, bloater, the soybean flour 'kinako', and Ban-Ban-Chi sauce) on *Salmonella typhimurium* TA 100. In analysis of the reactions of PABA and sodium nitrite under acidic conditions (pH 3.0), p-hydroxybenzoic acid (PHBA) was identified as major reaction product. The reaction seemed to involve two steps, diazotization and diazonium substitution. PABA was not mutagenic to four strains (TA 97, TA 98, TA 100 and TA 102) of *S. typhimurium* with or without metabolic activation (Kato and Kikugawa, 1992).

Natural antioxidant : Vitamin E was effective in preventing the nitrosation of amino substrates under physiological conditions. Both vitamin E and vitamin C together have a stronger inhibiting effect on the formation of N-nitrosamine (Lathia and Blum ,1989). They suggested that vitamin E when ingested simutaneously with food, might reduce human exposure to carcinogenic N-nitrosamines.

Dietary fiber : Moller and his colleagues (1988) found that wheat bran acted as nitrite scavenger under conditions similar to those that exist in the human stomach. Wheat bran, at a concentration equivalent to that in the stomach after ingestion of about two pieces of whole-wheat bread, reduced the nitrite concentration from 20 μM to about 10 μM in 60 min at pH 3.5 and 37°C. At pH 1.5 and 2.5, most of the nitrite had disappeared in 10 min. At pH less than or equal to 2.5 the nitrite scavenging effect of bran was as efficient as that of ascorbic acid. Ferulic acid, a component of bran, reacted rapidly with 20 μM- nitrite both at pH 3.5 and 1.5, whereas phenolic lignin model compounds only reacted at pH 1.5. In 1994, Laohavechvanich reported several types of plant fiber were efficient nitrite scavengers under conditions similar to those prevailing in the normal human stomach and using realistic nitrite concentrations.

Mutagenicity of drug -nitrite interaction

Nitrogen containing drugs are among the nitrosatable precursors to which humans are exposed, and the formation of hazardous substances by drug-nitrite interaction is an important problem in the evaluation of the safety of drugs. Chemical and biological studies on the nitrosation products of some chemicals have been

reported, however, ones a few drugs have any information on the possible human risk from drug-nitrite interaction. (Takeda and Kanaya, 1982).

Drugs containing a secondary amino or amido group are potentially able of reacting with nitrous acid to form a relatively complex N-nitroso derivative. This phenomena also occur to other drugs containing tertiary amines with either a dialkylamino substituent or an alkyl or other group attached to a ring tertiary nitrogen atom. (Gillat *et al.*, 1983). In a report of a WHO meeting held in Geniva in 1978, Coulston (1980) stated that the development of a systemic approach to the investigation of the potential hazard of nitrosatable drugs must be based on two considerations, namely the relative speed at which drugs undergo N-nitrosation under standardized condition *in vitro* and the carcinogenicity of the more strongly-reacting compound in suitable animal models. Further criteria upon which the selection of drugs for evaluating should be made are those of doses and period of use, particularly when children and elderly are involved.

Several studies have suggested that carcinogenic N-nitroso compounds are formed *in vivo* from the reaction of nitrosatable drugs and nitrosating agents (Mirvish, 1975) . The formation of a mutagen other than N-nitrosodimethylamine is suggested, a reaction product of aminopyrine with nitrite, has recently been shown to have mutagenic activity (Takeda and Kanaya, 1982). Chlordiazepoxide, a benzodiazepine used for the treatment of anxiety, reacts with sodium nitrite in both acetic acid and an aqueous HCl solution yielding a nitrosamidine that has been found to produce *in vitro* genotoxic effect in both bacterial and human mammalian systems.

(Brambilla *et al.*, 1989). Luigi and his colleagues (1990) demonstrated that oral administration of high doses of chlordiazepoxide and sodium nitrite induced DNA fragmentation in rat liver. The reaction of nitrite and tranquilizers resulted in varying yields of nitroso compounds, e.g., fluipentixol, chlordiazepoxide, spiperone, thiothixene and chlorpromazine more than 40% yield. (Takeda and Kanaya, 1981). Reaction products of some tranquilizers were mutagenic. The tricyclic psychotropic drugs opipramol reacted *in vitro* with sodium nitrite in acidic solution to form products including mutagens for *S. typhimurium* TA 98 and TA 100 (Glatt *et al.*, 1987). The strong mutagenicity of the crude reaction mixture was almost exclusively due to a compound which was present only in trace quanlities (less than 0.1%). In 1987, Kikugawa found that a variety of cardiovascular drugs treated with nitrite in acidic solution, a preparation of bamethan showed strikingly high mutagenicity by treatment toward *S. typhimurium* TA 98 and TA 100 strains. It was noted that after nitrosation this drug produced strong mutagenic diazo-compound.

However, from the practical point of view of nitrosamine biogenesis, the amine component is perhaps the most important because the chemical nature (and therefore biological activity) of the nitrosamines formed in any particular environment are determined exclusively by the amine component. One important source of free nitrosatable amines in the environment is the large number of amine drugs (mostly tertiary amines) which are commonly ingested, some of which are taken in high doses over long periods. The potential hazards associated with the long term clinical use of such drugs are interesting to study and concern.

The Protective Role of Dietary Fiber

The cause of large bowel cancer have more complex network of interrelated factors. Cocarcinogen and promoting factors play an important role in the subsequent stages of carcinogenesis. Initially, the protective effect of dietary fiber was seen in its ability to increase stool bulk and decrease transit time (Burkitt, 1971) and thus to affect the amount, concentration and activity of noxious substances in the faeces through dilution, decreased exposure time and altered faecal bacterial flora.

Plant fiber as antitoxicant formation

Some evidences suggested that diets high in carbohydrate and fiber but low in fat are valuable in prevention of diabetes, cardiovascular disease, obesity, certain gastrointestinal disease, and perhaps cancer (Anderson, 1986). Burkitt and Trowell (1975) postulated the dietary fiber hypothesis, linking low-fiber intake to high incidence of colon cancer, coronary heart disease, obesity, diabetes, hypertension and certain other disease among Western people. Fiber was described in the context of a hypothesis that considered a diet rich in material derived from plant cell walls as potentially protective against some diseases, dietary fiber has become a nutrient of importance on its own right (Roberfroid, 1993).

Plant fiber are refered to the non starch polysaccharide and lignin portions of plant foods not digested in the human small intestine. Dietary fiber is a more general term refering to all nondigestible cell wall components, including cutins waxes, and other coat materials. The nondigestible polysaccharide fractions, however, account for

most of the physiological and therapeutic effect of dietary fiber (Anderson, 1985). Laohavechvanich (1994) found that plant fiber especially from ivygourd was good in the prevention of mutagen formation in aminopyrene-nitrite model (Table1).

Table 1 Effect of ivygourd fiber on the mutagenicity of incubation mixture of amino-pyrene and nitrite. Data expressed as means and standard deviation (in parenthesis) of revertants per plate of the bacterial testers.

Fiber source	TA 98 *			TA100 **		
	Amount of fiber (cal. µg/ plate)	No. of revertants/plate Unprocessed	Processed	Amount of fiber (cal. µg/ plate)	No. of revertants/plate Unprocessed	Processed
Ivygourd	0	648 (54)	648 (54)	0	724 (5)	724 (5)
	37.5	376 (6)	365 (47)	62.5	758 (45)	702 (112)
	75	86 (3)	64 (11)	125	739 (14)	671 (16)
	150	26 (3)	32 (11)	250	202 (22)	330 (6)
	300	20 (3)	23 (1)	500	184 (6)	161 (10)
	450	24 (11)	20 (9)	750	212 (6)	218 (5)
	Spontaneous reversion	16(2)	16 (2)	Spontaneous reversion	189 (13)	189 (13)

* result of 15 µl of incubation mixture per plate

** result of 25 µl of incubation mixture per platef

The stool-bulking effect of dietary fiber is well established through numerous clinical studies in healthy humans. Cereal fibers have more pronounced bulking effect than fermentable vegetable and fruit fibers, whereas almost fermented gums do not increase fecal weight markedly (Eastwood *et al.*, 1980). Fermentable fibers stimulate bacterial growth and thus contribute to faecal weight increase indirectly (Cummings and Stephen, 1980). Both these bulking mechanisms lead to dilution of solutes in the faeces, unless there is compensation by increasing the absolute amounts of excreted solutes, such as observed for sodium and potassium (Cummings, 1982).

Transit time and stool bulk are inversely related, but Burkitt's suggestion that rapid transit would decrease time for intestinal carcinogen formation and action (Burkitt, 1971) has been vigorously challenged by Hill in 1975. Transit time is nevertheless important in controlling several aspects of fiber metabolism (Cummings, 1982), although its relevance for large bowel cancer may be a more indirect one.

The anaerobic fermentation of dietary fiber leads to volatile fatty acid (VFA), gases and energy for microbial growth. The VFA, mainly acetic, propionic and butyric acids can reduce colonic pH and hence free ammonia concentration, the latter being further reduced by its consumption for microbial synthesis. Since ammonia increases cell turnover and is suspected to favour growth of malignant cells in preference to normal cell (Visek, 1978), a low colonic ammonia concentration would be a desirable goal to achieve. Butyrate especially has been shown to modify the metabolism of a wide range of cell types and to affect the activity of many enzymes but a direct

relevance to colon cancer of butyrate formation through fiber fermentation has not been demonstrated yet (Cummings, 1982).

Low colonic pH inhibits 7-dehydroxylation of bile acids and is thought to have a number of positive influences in relation with cancer (Thornton, 1981). The bile acids are thought to play a central role in the etiology of large bowel cancer (Hill, 1975). Deoxycholic and lithocholic acid, the two main secondary bile acids have been shown to be cocarcinogenic in the rat, but no active carcinogen derived from bile acids has yet been identified in human faeces. High fat diets stimulate bile secretion leading to high faecal output and concentration of bile acids whereas many dietary fiber types markedly dilute faecal bile acid concentration even though they may increase daily excretion (McPherson-Kay, 1982).

Dietary fibers also exhibit adsorption properties which are modified through fermentation, so that their nature in the colon remains obscure. Adsorption phenomena may be responsible for detoxification virtues attributed to dietary fiber (Ershoff, 1974).

Finally, dietary fiber could modulate the number and ratio of colonic bacteria species and their enzymatic activity. Thus, nuclear dehydrogenating (NDH) *Clostridia*, bacteria capable of dehydrogenating bile acids, and bacterial enzymes such as beta-glucuronidase, nitroreductase and azoreductase which could convert procarcinogens to proximal carcinogens have been implicated in colon cancer etiology.

Mutagenic screening : Ames Test

The *Salmonella* Mutagenic Assay (Ames Test)

The simplicity, sensitivity, and accuracy of this method for screening large numbers of environmental sources of potential carcinogens has resulted in its current use in over 1,000 government, industrial, and academic laboratories throughout the world. The potential of this method for use as a bioassay for the development of safe, useful chemicals raised many questions about the extend to which this kind of approach should be used in a program aimed at cancer prevention. McCann and Ames (1977), discussed several aspects of the experimental basis for their current assessment of the value of the test as a useful predictive tools:

1. The predictive value of the test as an indicator of carcinogenic potential, including both the strength and weakness of the test at this stage in its development.

2. Current applications of the test method to problems that were not approachable using conventional animal test methods.

3. Some of the environmental chemicals that were pinpointed as potential carcinogens by the test and the current status of carcinogenicity tests of these chemicals in animals.

4. The evidence that the correlation between carcinogenicity and mutagenicity in the *Salmonella* test reflected more than a useful coincidence and fitted into a compelling collection of evidence supporting a central role for somatic mutation in the initiation of human cancer.

The *Salmonella* test was first validated in a study of 300 chemicals, most of which were known carcinogens (McCann and Ames, 1977). It was subsequently validated in studies by the Imperial Chemical Industries (Purchase *et al.*, 1976), the National Cancer Center Research Institute in Tokyo (Sugimura *et al.*, 1976), and the International Agency for Research on Cancer (Bartsch *et al.*, 1980). Nearly 90% of the carcinogens tested were mutagenic in these studies. However, Ames and McCann (1981) estimated the correlation to about 83%. All the validations show that the test fails to detect a few classes of carcinogens such as polychlorinated pesticides (Rinkus. and Legator, 1981). Prior to the initial development of the *Salmonella/* microsome assay there were several studies that employed bacterial systems to detect mutagenic agents (Iyer and Szybalski, 1959) . However, one of the problems with these earlier approaches was the use of screening techniques that did not employ bacterial strains designed to detect broad range of mutagenic mechanism. Therefore, Ames (1971) developed a set of *S. typhimurium* strains that were permeable to a wide range of chemicals and also were partially deficient in DNA repair.

The *Salmonella* tester strains The reverse mutation system of *S.typhimurium* uses the genetically well-defined histidine requiring mutants was developed by Ames and his colleagues (1973a). The original *Salmonella* histidine reverse mutation was based on the use of several selected *S. typhimurium* strains that revert from histidine dependence (auxotroph) to histidine independence (prototroph). Ames et al.(1973a) collected and characterized a large number of *S. typhimurium* strains containing mutations in different gene of the histidine operon (Table 2). *Salmonella* tester strains

were developed to make this method more effective in detecting mutagens that were not previously detected with the original strains. The current standard tester strains contain other mutations that greatly increase their ability to detect mutagens such as

- rfa mutation which causes partial loss of the lipopolysaccharide barrier that coat the surface of the bacteria and increases permeability to large molecules that do not penetrate the normal cell wall.

- uvr B mutation is a deletion of a gene coding for the DNA excision repair system, resulting increase sensitivity in detecting many mutagens.

- R-factor plasmid (pKM 101) The strains containing the plasmid show greatly enhanced response to chemical shown to be mutagenic and also give clear positive response to chemical describe as weak, borderline or nonmutagens with the original set of tester strains (Mortelma and Stocker, 1979). Furthermore, MacPhee (1973) reported that pKM 101 contains gene products associated with error-prone repair which may be responsible for the enhance sensitively seen in these strains.

Table 2. Genotypes of the TA strains used for mutagenesis testing.

Histidine mutation				LPS	Repair	R-factor
his D6610 his 01242 =TA 88	his D3052 (pAQ)	his G46	his G428			
TA 90	TA 1538	TA 1535	-	rfa	□ uvr B	-R
[TA 97]	[TA 98]	[TA 100]	-	rfa	□ uvr B	+R
-	TA 1978	TA 1975	-	rfa	+	-R
TA 110	TA 94	TA 92	-	+	+	+R
-	TA 1534	TA 1950	-	+	□ uvr B	-R
-	-	TA 2410	-	+	□ uvr B	+R
TA 89	TA 1964	TA 1530	-	□ gal	□ uvr B	+R
-	TA 2641	TA 2631	-	□ gal	□ uvr B	+R
-	-	-	[TA 102]	rfa	+	+R

Tester strains in bracket are recommended for general mutagenesis testing + indicates wild-types genes. The deletion (□) through uvr B also includes the nitrate reductase (chl) and biotin (bio) genes, whereas the gal strains and rfa/uvr B strains have a single deletion through gal chl bio uvr B.

Genotypes of the *Salmonella typhimurium* strains used for mutagenesis testing are showed in Table 2. The standard tester strains, TA 97, TA 98, TA100 and TA 102 contain the R-factor plasmid pKM 101. These R-factor strains are reverted by a number of mutagens that are detected weakly or not at all with the non R-factor parent strains (McCann *et al.*, 1975; Levin *et al.*, 1982b). These standard tester strains are recommended for general mutagenesis testing. TA 98 was derived from TA 1538 by introduction of plasmid pKM 101. It can detect mutagens that cause frameshif mutation with a DNA sequence -CGCGCGCG-, which can be reverted to histidine independence by a variety of mutagens that act by adding or deleting base pairs (Hartman, *et al.*, 1971; Isono and Yourno, 1974). While TA 100, the R-factor plasmid derivative TA 1535, can detect mutagens that cause base-pairs substitutions. The others *Salmonella* strains related to these 4 strains but with different characteristics in terms of DNA-repair capacity cell permeability and the presence of plasmid pKM 101 also are available and have been described (Levin et al., 1982a).

It is indicated that some mutagens affect only one strain of frameshift mutation strains (TA 1538 or TA 98) or only base-pair substitution strains (TA 1535 or TA 100), thus imparting a degree of mutagen class specificity to the assay. But, many or even most mutagens can affect both types of strains although the effective dose will often be higher for one type of strain than for the another .