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The Ames test: a methodological short review

Sebastian Tejs

Department of Environmental Biotechnology, University of Warmia and Mazury in Olsztyn, ul. Sloneczna 45G, 10–712 Olsztyn, Poland; e-mail sebastiantejs@op.pl

ABSTRACT

The Ames *Salmonella*/microsome mutagenicity assay (*Salmonella* test; Ames test) is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations. The test is used to evaluate the mutagenic properties of test articles. The Ames test uses amino acid-dependent strains of *Salmonella typhimurium* and *Escherichia coli*, each carrying different mutations in various genes in the histidine operon. These mutations act as hot spots for mutagens that cause DNA damage *via* different mechanisms. In the absence of an external histidine source, cells cannot grow and form colonies. Only those bacteria that revert to histidine independence (*his*$^+$) are able to form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant. However, when a mutagen is added to the plate, the number of revertant colonies per plate is increased, usually in a dose-related manner. The Ames test is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and drugs. The purpose of this publication is to help researchers who apply the Ames test in their studies.

INTRODUCTION

The identification of substances capable of inducing mutations has become an important procedure in safety assessment. Chemicals that can induce mutations can potentially damage the germ line leading to fertility problems and to mutations in future generations. Mutagenic chemicals are also capable of inducing cancer, and this concern has driven most of the mutagenicity testing programs.

Mutations can occur as gene (point) mutations (where only a single base is modified or one or a relatively few bases are inserted or deleted), as large deletions or rearrangements of DNA, as chromosome breaks or rearrangements, or as gain or loss of whole chromosomes (Ames et al. 1973a).

Gene mutations are readily measured in bacteria and other cell systems when they cause a change in the growth requirements of the cell, whereas chromosome damage in mammalian cells is typically measured by observing the cell's chromosomes under magnification for breaks or rearrangements. The *Salmonella typhimurium*/microsome assay (*Salmonella* test; Ames test) is a widely accepted short-term bacterial assay for identifying substances that can produce genetic damage that leads to gene mutations. The test uses a number of *Salmonella* strains with preexisting mutations that leave the bacteria unable to synthesize the required amino acid, histidine, and therefore unable to grow and form colonies in its absence. New mutations at the site of these preexisting mutations, or nearby in the genes, can restore the gene's function and allow the cells to synthesize histidine. These newly mutated cells can grow in the absence of histidine and form colonies. For this reason, the test is often referred to as a "reversion assay."

The *Salmonella* strains used in the test have different mutations in various genes in the histidine operon; each of these mutations is designed to be responsive to mutagens that act *via* different mechanisms. Additional mutations were engineered into these strains to make them more sensitive to a wide variety of substances.

The *Salmonella* mutagenicity test was specifically designed to detect chemically induced mutagenesis (Ames et al. 1975). Over the years its value as such has been recognized by the scientific community, and by government

agencies and corporations. The test is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and drugs because there is a high predictive value for rodent carcinogenicity when a mutagenic response is obtained (McCann et al. 1975; Sugimura et al. 1976; Zeiger 1985; Zeiger et al. 1990). International guidelines have also been developed (e.g. Organisation for Economic Co-operation and Development (OECD); International Commission on Harmonization (ICH)) for use by corporations and testing laboratories to ensure uniformity of testing procedures prior to submission of data to regulatory agencies for registration or acceptance of many chemicals, including drugs and biocides.

HISTORICAL ASPECTS

The Ames *Salmonella*/microsome mutagenicity assay evolved over the years from the initial screening of a number of histidine mutants which led to the selection of mutants that were highly sensitive to reversion by a variety of chemical mutagens (Ames 1971; Ames et al. 1973a; Levin et al. 1982a, b; Maron and Ames 1983). Because bacteria are unable to metabolize chemicals *via* cytochromes P450, as it happens in mammals and in other vertebrates, a key component for making the bacterial mutagenicity test useful was the inclusion of an exogenous mammalian metabolic activation system (Ames et al. 1973b; Malling 1971). At the same time, the development of the plate incorporation assay protocol to replace spot test or liquid suspension procedures was a major contributing factor to the success of the Ames test because it made the test easier to perform and reduced its cost.

In 1973 Ames et al. (1973a, b) developed the plate incorporation assay procedure which is more sensitive and quantitative than the spot test. The procedure consists of adding the buffer or S-9 mix, the histidine dependent bacteria (about 10^8) and test chemical to 2ml of top agar containing biotin and a trace amount of histidine (0.05mM each). The mixture is then gently mixed and poured on glucose minimal (GM) agar plates. When the top agar has solidified the plates are incubated in an inverted position in a 37°C incubator for 48h at which time the histidine revertant colonies are counted. The assay procedure is depicted in Figure 1.

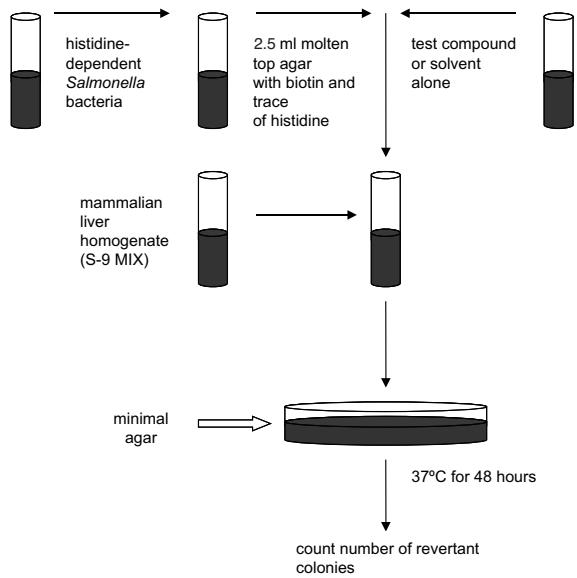

Figure 1. Diagram depicting the steps involved in the plate incorporation assay.

METABOLIC AND ACTIVATION SYSTEM

Some carcinogenic chemicals, such as aromatic amines or polycyclic aromatic hydrocarbons, are biologically inactive unless they are metabolized to active forms. In humans and lower animals, the cytochrome-based P450 metabolic oxidation system, which is present mainly in the liver and to a lesser extent in the lungs and kidneys, is capable of metabolizing a large number of these chemicals to DNA-reactive, electrophilic forms. Some of the intermediate metabolites are potent mutagens in the Ames *Salmonella* assay. Since bacteria do not have this metabolic capability, an exogenous mammalian organ activation system needs to be added to the large Petri plate together with the test chemical and the bacteria. For this purpose, a rodent metabolic activation system was introduced into the test system (Ames et al. 1973b; Garner et al. 1972; Malling 1971; Miller and Miller 1971). The metabolic activation system usually consists of a S-9 microsomal supernatant fraction of a rat liver homogenate, which is delivered to the test system in the presence of NADP and cofactors for NADPH-supported oxidation (S-9 mix). To increase the level of metabolizing enzymes, the animals are pretreated with the mixed-function oxidase inducer Aroclor 1254. Other inducers, such as phenobarbital and 3-naphthoflavone, can also be used (Maron and Ames 1983).

The metabolic activation system can also consist of a reductive enzyme system for classes of chemicals containing azo and diazo bonds. Reduction of chemical substances can occur in mammals, including humans, by anaerobic intestinal microflora, and very likely by mammalian reductases in the intestinal wall or in the liver (Glowienke 2005; Henderson et al. 2000; Prival and Mitchell 1982; Prival et al. 1984; Reid et al. 1983, 1984; Zeiger 2001).

THE SALMONELLA TESTER STRAINS

The genotypes of the most commonly used *Salmonella* tester strains are listed in Table 1.

Table 1. Genotype of the most commonly used *Salmonella* tester strains, mutations in their histidine operon plus additional mutations. All strains have LipoPolySaccharide (LPS) defect caused by *rfa* mutation.

Genotype	Strain	Histidine operon mutations (*bio, chlD, uvrB, gal*)	Additional mutations (plasmid)
hisG46	TA 1535 TA 100	Deletion mutation Deletion mutation	No plasmid pKM101
hisD3052	TA 1538 TA 98	Deletion mutation Deletion mutation	No plasmid pKM101
hisC3076	TA 1537	Deletion mutation	No plasmid
hisD6610	TA 97	Deletion mutation	pKM101
hisG428	TA 104 TA 102	Deletion mutation Wild type	No plasmid pKM101, pAQ1

All strains are histidine dependent by virtue of a mutation in the histidine operon. Additional mutations/genetic alterations that have made the tester strains more sensitive to chemical mutagens are listed below:

- A deletion mutation through the *uvrB-bio* genes in all strains, except TA102. The *uvrB* deletion mutation eliminates the accurate excision repair mechanism, thereby allowing more DNA lesions to be repaired by the error-prone DNA repair mechanism. The deletion through the biotin gene makes the bacteria biotin dependent (Ames et al. 1973a).
- A mutation (*rfa*) in all strains that leads to a defective lipopolysaccharide (LPS) layer that coats the bacterial surface, making the bacteria more permeable to bulky chemicals (Ames et al. 1973a).
- Introduction of plasmid pKM101 in strains TA1535 and TA1538 resulting in the corresponding isogenic strains TA100 and TA98 (Ames et al. 1975) and in strains TA97 (Levin et al. 1982a) and TA102 and TA104 (Levin et al. 1982b). Plasmid pKM101 enhances chemical and UV-induced mutagenesis *via* an increase in the recombination DNA repair pathway (McCann et al. 1975; Shanabruch and Walker 1980; Walker and Dobson 1979). The plasmid confers ampicilline resistance, which is a convenient marker to detect the presence of the plasmid (Mortelmans and Stocker 1979).
- Insertion of the mutation *hisG428* on the multi-copy plasmid pAQl which was introduced in strain TA102 with the aim of amplifying the number of target sites. To enhance the ability of this strain to detect DNA cross-linking agents, the *uvrB* gene was retained making the bacterium DNA repair proficient (Czyz et al. 2002; Fluckiger-Isler et al. 2004; Levin et al. 1982b).

Table 2. DNA sequence specificity of the *Salmonella* tester strains; mutated genes and mutation types.

Allele	Strain	DNA target	Reversion event	Reference
hisG46	TA 100 TA 1535	-G-G-G-	Base-pair substitution	Maron and Ames 1983
hisD3052	TA 98 TA 1538	-C-G-C-G-C-G-C-G-	Frameshifts	Simmon et al. 1977
hisC3076	TA 1537	+ 1 frameshift (near –C-C-C- run)	Frameshifts	Zeiger 1985
hisD6610	TA 97	-C-C-C-C-C-C- (+ 1 cytosine at run of C's)	Frameshifts	Mortelmans and Cox 1992
hisG428	TA 102 TA 104	TAA (ochre)	Transitions/transversions	Venitt and Bosworth 1983

SPECIFIC TARGET DNA SEQUENCES

Table 2 shows the DNA sequences of the target mutations in the commonly used *Salmonella* test strains.

MODIFICATIONS OF THE STANDARD PLATE INCORPORATION ASSAY

Over the years, modifications to the standard plate incorporation assay have been developed by different researchers that enhanced the sensitivity of the test and allowed the testing of a wider range of chemicals, including gases and volatile chemicals. The most commonly used modifications are described below.

The preincubation assay

In the preincubation assay, the tester strains are exposed to the chemical for a short time (20 to 30min) in a small volume (0.5ml) of either buffer or S-9 mix, prior to plating on glucose agar minimal medium (GM agar) supplemented with a trace amount of histidine. With few exceptions it is believed that this assay is more sensitive than the plate incorporation assay, because short-lived mutagenic metabolites may have a better chance reacting with the tester strains in the small volume of preincubation mixture, and the effective concentration of S-9 mix in the preincubation volume is higher than that on the plate (Haworth et al. 1983; Mortelmans et al. 1986; Yahagi et al. 1975; Zeiger et al. 1987, 1988).

The desiccator assay for liquids and gases

The use of a closed chamber is recommended for testing highly volatile chemicals and gases (Araki et al. 1994; Hughes et al. 1987; Simmon et al. 1977; Zeiger et al. 1992).

The Kado *Salmonella* microsuspension assay for testing samples of small volumes

This procedure was designed to detect mutagenic metabolites in urine samples obtained from animals treated with test chemicals (Kado et al. 1983) because of the relatively small sample volumes obtainable in such studies.

Testing chemicals in a reduced oxygen atmosphere

Anaerobic environments, such as anaerobic chambers, have been used to study mutagenicity of chemicals and fecal samples under reduced oxygen levels (Bruyninck et al. 1978; Hartman et al. 1984; MacPhee and Jolly 1985; Mortelmans and Cox 1992; Venitt and Bosworth 1983).

There is more information of the Ames test modifications concerning Ames II test, liquid microtiter modification, *Vibrio harveyi* strains, and mini Ames test (Czyz et al. 2002; Flamand et al. 2001; Fluckier-Isler et al. 2004).

GENETIC ANALYSIS

It is recommended that the tester strains be analyzed for their genetic integrity and spontaneous mutation rate when frozen cultures are prepared. A strain check should also be performed whenever an experiment is performed. The strain check is usually performed with the nutrient broth overnight cultures.

The steps given below should be followed for a complete strain check.

- Histidine dependence (*his*): streak a loopful of the culture across a GM agar plate supplemented with an excess of biotin. Because all the *Salmonella* strains are histidine dependent, there should be no growth on the plates.
- Biotin dependence (*bio*): streak a loopful of the culture across a GM agar plate supplemented with an excess of histidine. There should be no growth on the plate except for strain TA102 which is biotin independent.
- Biotin and histidine dependence (*bio*, *his*): streak a loopful of the culture across a GM agar plate supplemented with an excess of biotin and histidine. Growth should be observed with all strains.
- *rfa* marker: streak a loopful of the culture across a GM agar plate supplemented with an excess of biotin and histidine. Apply 10 μl of a sterile 0.1% crystal violet solution. All *Salmonella* strains should show a zone of growth inhibition.
- Presence of plasmid pKM101 (ampicilline resistance): apply in the center of a plate 10 μl of ampicilline solution. Streak a loopful of the pKM101-carrying *Salmonella* culture across an agar plate supplemented with an excess of histidine and biotin. Growth should be observed.
- Spontaneous mutant frequency: use the standard plate incorporation assay procedure without the inclusion of a solvent for determining the spontaneous mutant frequency (negative control) of each of the tester strains. When the spontaneous control values fall outside an acceptable range (Table 3) the genetic integrity of the strain is considered compromised, and a new culture should be isolated. There may be non-strain related reason for a spontaneous value that is too high or too low. These should be considered before a new culture is isolated (Mielzynska et al. 1998).

If one or more of the above strain checks fails, the culture is considered unacceptable for use. If such a culture was used for performing an experiment, the results should be considered invalid and the experiment should be repeated. In case the strain check was performed when the strains were frozen (permanent or working cultures) and yielded unacceptable results, the strain(s) should be re-isolated following the procedures outlined above (Zeiger et al. 1981).

SPONTANEOUS CONTROL VALUES

Each tester strain has a characteristic spontaneous mutant frequency. There is usually some day-to-day and laboratory-to-laboratory variation in the number of spontaneous revertant colonies. Choice of solvent may also affect the spontaneous mutant frequency (Maron et al. 1981). Each laboratory has a characteristic range of revertant colonies for each strain which is referred to as "historical control values". The spontaneous mutant frequency obtained when the strain check is performed should be compared to the laboratory's historical control values. Table 3 presents a range of spontaneous histidine revertant (negative solvent) control values per plate with and without metabolic activation considered valid in the authors' laboratories. The values obtained in the presence of a metabolic activation system (microsomal fraction) includes both rat and hamster liver S-9. Some of the strains (e.g. TA97, TA102, TA104) are highly sensitive to S-9 and their spontaneous reversion values will increase with the increasing S-9 concentration. Other acceptable ranges of background revertant counts have been published by Ames et al. 1975; Kier et al. 1986; Maron and Ames 1983.

ASSAY PROCEDURES

Standard plate incorporation assay

The standard plate incorporation assay consists of exposing the tester strain(s) to the test chemical directly on a minimal glucose agar plate (GM plate) usually in the presence and absence of a metabolic activation system. The different components are first added to sterile test tubes containing 2.5ml of molten top agar supplemented with limited histidine and biotin. It is important to maintain the top agar at a temperature between 43°C and 48°C and to minimize prolonged exposure to avoid killing of the tester strains. The contents of the tubes are mixed and poured on glucose minimal agar plates. After the top agar has hardened the plates are inverted and incubated at 37°C for 48h, at which time histidine-revertant colonies are counted on all plates. The number of colonies on the test plates are compared to those on the (negative) solvent control plates (Mielzynska et al. 1998).

Experimental procedure

1. Steps taken prior to performing the experiment:
   - Inoculate *Salmonella* cultures 12h prior to performing the experiment.
   - Label an appropriate number of GM agar plates and sterile test tubes for each test chemical.
   - Prepare metabolic activation system and keep on ice until use.
   - Prepare chemical dilutions.
   - Melt top agar supplemented with 0.05mM histidine and biotin and maintain at 43°C to 48°C.
2. To the 100ml sterile tubes maintained at 43°C, add in the following order with mixing after each addition:
   - 0.01ml of the test chemical dilution.
   - 2.5ml of molten top agar with overnight culture of the *Salmonella* strain (about $1\text{-}2\cdot10^8$ bacteria per tube).
   - 0.5ml of metabolic activation (S-9) mix.
3. The contents of the test tubes are then mixed and poured onto the surface of GM agar plates.
4. When the top agar has hardened (2-3min), the plates are inverted and placed in a 37°C incubator for 48h.
5. The colonies are then counted and the results are expressed as the number of revertant colonies per plate.

Table 3. Spontaneous revertant control values; strain types and number of revertants (Maron and Ames 1983; Mortelmans and Zeiger 2000).

Strain	Number of revertants	
	without S-9	with S-9
TA97	75-200	100-200
TA98	20-50	20-50
TA100	75-200	75-200
TA102	100-300	200-400
TA104	200-300	300-400
TA1535	5-20	5-20
TA1537	5-20	5-20
TA1538	5-20	5-20

INTERPRETATION AND REPORTING

Counting of histidine revertant colonies

After the plates are removed from the incubator, the colonies are counted and the results are expressed as revertant colonies per plate. For this purpose, an electronic counter is a convenient way to count the colonies, especially for strains TA100 and TA97 which usually have a spontaneous background above 100 colonies·plate^{-1}. However, hand-counting is required when strains TA102 and TA104 are used because of the high number of spontaneous revertant colonies, usually above 200 colonies·plate^{-1}. Hand-counting is also required when precipitate is present on the plate, when there is poor contrast between the colony and the agar, or when a test chemical discolors the agar which prevents sufficient light from passing through the agar. If precipitate is present on the plates at the higher dose levels, all plates, including the solvent control plates, should be hand-counted. Also, all plates from a given experiment, including positive and negative control plates, should be counted using the same method at the same time (Mielzynska et al. 1998).

Data reporting

The results are generally reported as a mean number of revertant colonies per plate with the S.D. or S.E.M. for the test chemical and for the controls. The concentration of the test chemical is expressed as mg or μg·plate^{-1}, or milliliter or mole in the desiccator procedure for volatile liquids and gases, respectively. Information regarding toxicity and/or precipitation of the test chemical should also be included.

A number of statistical programs have been developed for analyzing *Salmonella* mutagenicity data, and all have their strengths and weaknesses (Bernstein et al. 1982; Edler 1992; Katz 1979; Margolin et al. 1981; Stead et al. 1981; Venitt and Crofton-Sleigh 1979; Weinstein and Lewinson 1978).

A non-statistical procedure has been established to evaluate the results of *Salmonella* experiments (Zeiger et al. 1992).

SAFETY CONSIDERATIONS

It is important that basic bacteriological laboratory procedures be used to minimize exposure to the *Salmonella* tester strains. Surface areas must be properly disinfected before and after use. Though wild-type *S. typhimurium* can cause diarrhea and food poisoning, the *gal* and *rfa* (deep rough) mutations that are present in all the *Salmonella* tester strains described here eliminate, to different levels, the polysaccharide side chain of the LPS layer that coats the bacterial surface which makes the bacteria non-pathogenic. It is nevertheless prudent to use caution at all times and to practice standard laboratory safety procedures such as using plugged pipettes and autoclaving all contaminated material. Mouth-pipetting as a general rule should never be practiced. Aseptic techniques, being part of basic bacteriological laboratory procedures, are also essential to prevent contamination of the overnight cultures, GM agar plates, and the solutions and reagents. Contaminated frozen permanent and working cultures of the *Salmonella* tester strains will be useless and should be discarded (Ames et al. 1973a; Wilkinson et al. 1972). As a general rule, it is prudent to consider all chemicals as if they were mutagens and carcinogens. All handling of chemicals, as well as the test itself, should be performed in a chemical safety cabinet. Workers should protect themselves from chemical exposure by wearing gowns, eye glasses and gloves. Wearers of contact lenses should wear regular eye glasses since some volatile chemicals might react with the contact lens. All contaminated material (e.g., test tubes, pipettes and pipette tips, gowns and gloves) should be properly disposed of as well as the unused chemical dilutions and stock solutions of the test chemical, and positive control chemicals.

MUTAGENICITY INFORMATION

There are several databases publicly available through the internet. The Environmental Mutagen Information Center (EMIC) database can be entered through http://toxnet.nlm.nih.gov/servlets/simple-search. In this database, information can be retrieved regarding the test organisms used and end-points examined. The other database is that of the U.S. National Toxicology Program's genetic toxicology testing program. Results have been published on more than 1500 chemicals. The summary of the test results for specific chemicals, published and unpublished, can be retrieved from http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ Res_Stat/iH _Res_Stat_Frames.html (Zeiger 1997).

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REFERENCES

Ames, B.N. 1971. The detection of chemical mutagens with enteric bacteria. In: Chemical Mutagens, Principles and Methods for Their Detection vol. 1 (ed. A. Hollaender), pp. 267-282. Plenum, New York.

Ames, B.N., F.D. Lee, W.E. Durston. 1973a. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proceedings of the National Academy of Science U.S.A. 70: 2281-2285.

Ames, B.N., W.E. Durston, E. Yamasaki, F.D. Lee. 1973b. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proceedings of the National Academy of Science U.S.A. 70: 2281–2285.

Ames, B.N., J. McCann, E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research 31: 347–364.

Araki, A., T. Noguchi, F. Kato, T. Matsushima. 1994. Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag. Mutation Research 307: 335–344.

Bernstein, L.S., J. Kaldor, J. McCann, M.C. Pike. 1982. An empirical approach to the statistical analysis of mutagenesis data from the *Salmonella* test. Mutation Research 97: 267–281.

Bruyninck, W.J., H.S. Mason, S.A. Morse. 1978. Are physiological oxygen concentrations mutagenic? Nature 274: 606–607.

Czyz, A., H. Szpilewska, R. Dutkiewicz, W. Kowalska, A. Biniewska-Godlewska, G. Węgrzyn. 2002. Comparison of the Ames test and a newly developed assay for detection of mutagenic pollution of marine environments. Mutation Research 519: 67-74.

Edler, L. 1992. Statistical methods for short-term tests in genetic toxicology: the first fifteen years. Mutation Research 277: 11–33.

Flamand, N., J.R. Meunier, P.A. Meunier, C. Agapakis-Causse. 2001. Mini mutagenicity test: a miniaturized version of the Ames test used in a prescreening assay for point mutagenesis assessment. Toxicology in Vitro 15: 105-114.

Fluckiger-Isler, S., M. Baumeister, K. Braun, V. Gervais, N. Hasler-Nguyen, R. Reimann, J. Van Gompel, H.,G. Wunderlich, G. Engelhardt. 2004. Assessment of the performance of the Ames II assay: a collaborative study with 19 coded compounds. Mutation Research 558: 181-197.

Garner, R.C., E.C. Miller, J.A. Miller. 1972. Liver microsomal metabolism of aflatoxin B1 to a reactive derivative toxic to *Salmonella typhimurium* TA1530. Cancer Research 32: 2058–2066.

Glowienke, S., W. Frieauff, T. Allmendinger, H.J. Martus, W. Suter, L. Mueller. 2005. Structure-activity considerations and in vitro approaches to assess the genotoxicity of 19 methane-, benzene-, and toluene-sulfonic acid esters. Mutation Research 581: 23-34.

Hartman, Z., P.E. Hartman, W.M. Barnes, E. Tuley. 1984. Spontaneous mutation frequencies in *Salmonella*: enhancement of G/C to A/T transitions and depression of deletion and frameshift mutation frequencies afforded by anoxic incubation. Environmental and Molecular Mutagenesis 6: 633–650.

Haworth, S., T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger. 1983. *Salmonella* mutagenicity results for 250 chemicals. Environmental Mutagenesis 5 (Suppl.): 3–142.

Henderson, L., S. Albertini, M. Aardema. 2000. Thresholds in genotoxicity responses. Mutation Research 464: 123-128.

Hughes, T.J., D.M. Simmons, L.G. Monteith, L.D. Claxton. 1987. Vaporization technique to measure mutagenic activity of volatile organic chemicals in the Ames/*Salmonella* assay. Environmental Mutagenesis 9: 421–441.

Kado, N.Y., D. Langley, E. Eisenstadt. 1983. A simple modification of the *Salmonella* liquid incubation assay: increased sensitivity for detecting mutagens in human urine. Mutation Research 122: 25–32.

Katz, A.J. 1979. Design and analysis of experiments on mutagenicity: II. Assays involving microorganisms. Mutation Research 64: 61–77.

Kier, L.E., D.J. Brusick, A.E. Auletta, E.S. Von Halle, M.M. Brown, V.F. Simmon, V. Dunkel, J. McCann, K.Mortelmans, M. Prival, T.K. Rao, V. Ray. 1986. The *Salmonella typhimurium*/mammalian microsomal assay. A report to the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Research 168: 69–240.

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

Levin, D.E., M.C. Hollstein, M.F. Christman, E.A. Schwiers, B.N. Ames. 1982b. A new *Salmonella* tester strain (TA102) with A:T base pairs at the site of mutation detects oxidative mutagens. Proceedings of the National Academy of Science U.S.A. 79: 7445–7449.

MacPhee, D.G., L.D. Jolly. 1985. Mutagenesis of anaerobic cultures of *Salmonella typhimurium* by nitrosoguanidine, diethyl sulfate and 9-aminoacridine. Mutation Research 143: 181–186.

Malling, H.V. 1971. Dimethylnitrosamine: formation of mutagenic compounds by interaction with mouse liver microsomes. Mutation Research 13: 425–429.

Margolin, B.H., N. Kaplan, E. Zeiger. 1981. Statistical analysis of the Ames *Salmonella*/microsome test. Proceedings of the National Academy of Science U.S.A. 78: 3779–3783.

Maron, D., B.N. Ames. 1983. Revised methods for the *Salmonella* mutagenicity test. Mutation Research 113: 173–215.

Maron, D., J. Katzenellenbogen, B.N. Ames. 1981. Compatibility of organic solvents with the *Salmonella*/microsome test. Mutation Research 88: 343–350.

McCann, J., E. Choi, E. Yamasaki, B.N. Ames. 1975. Detection of carcinogens in the *Salmonella*/microsome test. Assay of 300 chemicals. Proceedings of the National Academy of Science U.S.A. 72: 5135–5139.

Mielzynska, D., E. Siwinska, A. Bubak. 1998. Genotoxicity of 13 polycyclic aromatic hydrocarbons in chosen in vitro systems. [Genotoksyczność 13 wielopierścieniowych węglowodorów aromatycznych w wybranych systemach in vitro]. IMPZŚ w Sosnowcu, pp. 46-52 (in Polish).

Miller, E.C., J.A. Miller. 1971. The mutagenicity of chemical carcinogens: correlations, problems and interpretations. In: Chemical Mutagens, vol. 1 (ed. A. Hollaender), pp. 83-119. Plenum, New York.

Mortelmans, K., M. Cox. 1992. Evidence that inhibitor(s) are formed which may interfere with the growth of revertant colonies in the Ames *Salmonella* and the *E. coli* tryptophan reverse mutation assays when strict anaerobic conditions are used. Environmental and Molecular Mutagenesis 20: 44–52.

Mortelmans, K., B.A.D. Stocker. 1979. Segregation of the mutator property of plasmid R46 from its ultraviolet-protection properties. Molecular and General Genetics 167: 317–327.

Mortelmans, K., E. Zeiger. 2000. The Ames *Salmonella*/microsome mutagenicity assay. Mutation Research 455: 29-60.

Mortelmans, K., S. Haworth, T. Lawlor, W. Speck, B. Tainer, E. Zeiger. 1986. *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis 8 (Supplement 7): 1–119.

Prival, M.J., S.J. Bell, V.D. Mitchell, M.D. Peiperl, V.L. Vaughan. 1984. Mutagenicity of benzidine and benzidine-congenor dyes and selected monoazo dyes in a modified *Salmonella* assay. Mutation Research 136: 33–47.

Prival, M.J., V.D. Mitchell. 1982. Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S9. Mutation Research 97: 103–116.

Reid, T.M., C.Y. Wang, C.M. King, K.C. Morton. 1983. Conversion of congo red and 2-azoxyfluorene to mutagens following in vitro reduction by whole-cell rat caecal bacteria. Mutation Research 117: 105-112.

Reid, T.M., K.C. Morton, C.Y. Wang, CM. King. 1984. Mutagenesis of azo dyes following metabolism by different reductive/oxidative systems. Environmental Mutagenesis 6: 247-259.

Shanabruch, W.G., G.C Walker. 1980. Localization of the plasmid (pKMIOl) gene(s) involved in *recA*$^+$ /*ex4*$^+$-dependent mutagenesis. Molecular and General Genetics 179: 289-297.

Simmon, V.F., K. Kauhanen, R.G. Tardiff. 1977. Mutagenic activities of chemicals identified in drinking water. In: Progress in Genetic Toxicology (ed. D. Scott, B.A. Bridges, F.H. Sobels), pp. 249-258. Elsevier/North-Holland, Amsterdam.

Stead, A.G., J.R. Hasselbald, L. Claxton. 1981. Modeling the Ames test. Mutation Research 85: 13-27.

Sugimura, T., S. Sato, M. Nagao, T. Yahagi, T. Matsushima, Y. Seino, M. Takeuchi, T. Kawachi. 1976. Overlapping of carcinogens and mutagens. In: Fundamental of Cancer Prevention (ed. R.N. Magee, S. Takayama, T. Sugimura, T. Matsushima), pp. 191-215. University Park Press, Baltimore.

Venitt, S., D. Bosworth. 1983. The development of anaerobic methods for bacterial mutation assays: aerobic and anaerobic fluctuations tests of human faecal extracts and reference mutagens. Carcinogenesis 4: 339-345.

Venitt, S., C. Crofton-Sleigh. 1979. Bacterial mutagenicity tests of phenazine methosulfate and three tetrazolium salts. Mutation Research 68: 107-116.

Walker, G.C., P.P. Dobson. 1979. Mutagenesis and repair deficiencies of *Escherichia coli umuC* mutants are suppressed by the plasmid pKM101. Molecular and General Genetics 172: 17–24.

Weinstein, D., T.M. Lewinson. 1978. A statistical treatment of the Ames mutagenicity assay. Mutation Research 51: 433–434.

Wilkinson, R.G., P. Gemski Jr., B.A.D. Stocker. 1972. Non-smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. Journal of General Microbiology 70: 527–554.

Yahagi, T., W. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, Y. Hashimoto. 1975. Mutagenicity of carcinogenic azo dyes and their derivatives. Cancer Letters 1: 91–97.

Zeiger, E. 1985. The *Salmonella* mutagenicity assay for identification of presumptive carcinogens. In: Handbook of Carcinogen Testing (ed. H.A. Milman, E.K. Weisburger), pp. 83-99. Noyes Publishers, Park Ridge, NJ.

Zeiger, E. 1997. Genotoxicity data base. In: Handbook of Carcinogenic Potency and Genotoxicity Databases (ed. L.S. Gold, E. Zeiger), pp. 687-729. CRC Press, Boca Raton, FL.

Zeiger, E. 2001. Mutagens that are not carcinogens: faulty theory or faulty tests? Mutation Research 492: 29-38.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, W. Speck. 1987. *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. Environmental Mutagenesis 9 (Supplement 9): 1–110.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. Environmental Mutagenesis 9 (Supplement 12): 1–158.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans. 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. Environmental and Molecular Mutagenesis 19 (Supplement 21): 1–141.

Zeiger, E., J.K. Haseman, M.D. Shelby, B.H. Margolin, R.W. Tennant. 1990. Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: confirmation of earlier results with 41 additional chemicals. Environmental and Molecular Mutagenesis 16 (Supplement 18): 1–14.

Zeiger, E., D.A. Pagano, I.G.C. Robertson. 1981. A rapid and simple scheme for confirmation of *Salmonella* tester strain phenotype. Environmental Mutagenesis 3: 205–209.