

Comparison of responses of base-specific Salmonella tester strains with the traditional strains for identifying mutagens: the results of a validation study

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Abstract

The ability of a TA7000 series of Salmonella *his*⁻ mutant tester strains to detect mutagens as classified by the traditional tester strains (TA100, TA98, TA1535, TA1537, TA97, TA102 and TA104) was evaluated using 30 coded chemicals, 5 of which were duplicates with different code numbers. The TA7000 series of tester strains were TA7001, TA7002, TA7003, TA7004, TA7005 and TA7006, each of which reverts by a specific base substitution. In addition, each chemical was tested in a mixture of the base-specific strains (the Mix), plus the traditional strains, TA98 and TA1537. A liquid version of the Salmonella mutagenicity assay was performed in microtiter plates to allow partial automation for increased throughput. The results were compared to those in the National Toxicology Program (NTP) database, which were obtained from the traditional strains in the preincubation assay. In the two strains common to both protocols, TA98 and TA1537, the agreement was 80% and 85%, respectively. When compared to the NTP results for TA100, the Mix gave a 72% concordance, while the addition of the frameshift tester strain, TA98, increased the agreement to 76%. The overall agreement on positive or negative classifications of mutagenicity was 88% for the 25 chemicals tested. There were three notable exceptions to the overall agreement. Benzaldehyde was detected as a mutagen in TA7005 in contrast to its classification as a non-mutagen in the NTP database. This does not necessarily contradict the NTP results because the base-specific strains may respond to different mutagens. Two weak mutagens in the NTP database, 1-chloro-2-propanol and isobutyl nitrite, were not detected as mutagens in the base-specific new strains in the liquid protocol. While there are a number of major differences in the two assays, it was concluded that the results from each procedure are comparable. © 1998 Elsevier Science B.V.

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1. Introduction

The Salmonella/microsome reversion assay has been used extensively in genetic toxicology testing [1–3]. The tester strains that are used traditionally to

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identify mutagens that induce reversion of specific base-pair substitution and small frameshift mutations in the *his* operon. In order to be sensitive to a broad range of chemicals, the *his*⁻ strains chosen for routine use carry target sites that revert by numerous pathways of mutagenesis. As a result of this selection of strains, one can conclude that a mutagenic event such as a point mutation at the target site or suppression of point mutations was caused by a chemical [4]. However, the specific base mutation (in the case of base-pair substitutions) can not be discerned without molecular analyses of the reverted and mutant cells [5–8].

To identify base substitutions easily, a set of 6 *his*⁻ mutant strains (TA7001, TA7002, TA7003, TA7004, TA7005, and TA7006) was developed to revert by unique base-pair substitutions [9]. Each strain detects one, and only one, of 6 possible base substitutions and therefore results from testing with this set of strains can be used to determine the mutational spectrum caused by the mutagenic agent. Strains, TA7001, TA7002, and TA7003 detect base changes at A:T base pairs, while TA7004, TA7005, and TA7006, detect base changes at G:C base pairs. Because the reversion pathway for each strain is restricted to a specific base change, the spontaneous reversion frequencies are low (from less than 1 to about 25 revertants per plate).

There are many different procedures for performing bacterial mutagenicity tests. Several protocols deviate from the 'standard' agar plate incorporation or preincubation assay [10]. The use of solid agar plating was retained in an automated spiral application of test agent and bacterial culture to the standard agar plates [11,12] and in an automated liquid preincubation exposure protocol [13] to increase throughput. Attempts have been made to automate parts or all of these procedures for reductions in the time and cost/chemical, and to allow the test to be adapted to high throughput screening. We have modified the fluctuation assay [14–16] to allow automation of plating the exposed cells in selective media using the TA7000 series of tester strains which have been designated 'Ames II™' (Xenometrix, Boulder, CO, USA).

A validation study was performed to compare the performance of the TA7000-series tester strains with respect to the detection of mutagens identified by the

traditional tester strains. In this study, 30 coded chemicals (5 of which were duplicates with different code numbers) were tested in the individual strains TA7001–TA7006, to identify mutagens that produced base-pair substitutions, in a mixture of these 6 strains (the Mix), and in strains TA98 and TA1537 to detect frameshift mutagens.

The study reported here addressed several aspects of these new tester strains and their proposed uses.

- The effectiveness of the individual TA7000 strains, or in the Mix, for identifying mutagens, as compared to different combinations of the traditional *Salmonella* tester strains.
- The usefulness of these base-specific strains, which measure only base-pair substitution mutations, in combination with frameshift-responding strains, to identify mutagens.
- A comparison of the individual strains and the Mix for identifying mutagens.
- The Mix was evaluated for its the degree of sensitivity in detecting mutagens when used to enhance the efficiency of screening.
- Comparisons of the various combinations of these new strains with the standard *Salmonella* strains currently used by the NTP for identifying mutagens.

The results from this study were compared with the results of testing these same chemicals using a preincubation procedure by the US National Toxicology Program (NTP).

2. Methods and materials

2.1. Bacterial strains

Eight individual *Salmonella typhimurium* strains and a mixture of the base-specific strains (Mix) were treated with the test chemicals. The strains and their genotypes are described in Table 1. Each of the 6 base-specific strains (TA7001–7006) carry a target missense mutation in the histidine biosynthetic operon that reverts to prototrophy by base-substitution events unique to each strain. Strains TA7001, TA7002, and TA7003 were developed to detect point substitutions at A:T base pairs while TA7004, TA7005, and TA7006 detect base changes at G:C base pairs. Both TA98 and TA1537 were used to

Table 1
Bacterial strains used, and the mixture

Strains	Genotypes	Mutation detected	Reference
TA7001	<i>hisG1775 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1041/pKM101</i>	T:A → C:G	[9]
TA7002	<i>hisC9138 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1042/pKM101</i>	T:A → A:T	[9]
TA7003	<i>hisG9074 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1043/pKM101</i>	T:A → G:C	[9]
TA7004	<i>hisG9133 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1044/pKM101</i>	C:G → T:A	[9]
TA7005	<i>hisG9130 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1045/pKM101</i>	C:G → A:T	[9]
TA7006	<i>hisC9070 Δara9 Δchl1004 (bio chlD uvrB chlA) galE503 rfa1046/pKM101</i>	C:G → G:C	[9]
Mix	TA7001, TA7002, TA7003, TA7004, TA7005, TA7006	Missense	[9]
TA1537	<i>hisC3076 Δara9 Δchl1007 (bio chl uvrB gal)rfa1003 (+1 G)</i>	Frameshifts	[41]
TA98	<i>hisD3052 Δara9 Δchl1008 (bio chl uvrB gal) rfa1004/pKM101 (-1 C)</i>	Frameshifts	[41]

detect frameshift mutations. The Mix consisted of TA7001, TA7002, TA7003, TA7004, TA7005 and TA7006 in equal proportions and was stored as a mixture at -70°C . This was used as the source inoculum and treated as if it were an individual strain throughout this study. During overnight growth of the Mix(ed) culture each strain was present, however each may not be represented in equal proportions due small differences in growth rates. Two to 8 μl of a freshly thawed frozen stock were inoculated into 5 to 15 ml, respectively, of Growth Media (Oxoid Broth, UnipathOxoid, Basingstoke, UK made for Xenometrix). These cultures were grown overnight (12 to 15 h) at 37°C in an environmental shaker at 250 rpm (New Brunswick Scientific, Edison, NJ) in the presence of 25 $\mu\text{g}/\text{ml}$ ampicillin (Xenometrix). TA1537 cultures were grown in the absence of ampicillin because this strain does not carry pKM101 which contains an ampicillin resistance gene.

2.2. Chemicals

Twenty-five chemicals were selected for this study from the NTP database of more than 1700 chemicals. Although all test results are, to some extent protocol-dependent, an attempt was made to remove all those chemicals where the positive response was highly dependent on methodology (i.e. those requiring reductive metabolism; gases and highly volatile; metal salts; etc.). Also excluded were chemicals not easily obtainable (or expensive), controlled substances, undefined substances or mixtures, mixtures of positional isomers, insolubles, inorganics, organometallics and chemicals with conflicting re-

sponses in different tests using the same protocol. Only chemicals with published results were considered [2,17–28] (see Table 2).

The 25 chemicals selected included 18 mutagens and 7 non-mutagens as classified by results from the traditional strains. Factors in the selection of the chemicals included chemical structure, responses in the standard Salmonella strains, and consistency of responses in replicate experiments, especially where weak. Preference was given to chemicals among the 114 evaluated by Tennant et al. [23] and Zeiger et al. [18]. The chemicals selected were the same salt and isomer as was tested by the NTP.

The purities, sources, and the dose ranges tested are listed in Table 2. Approximately 2–5 g, or 2–5 ml of each sample were distributed into vials, and coded with a 4-digit random number at NIEHS, prior to shipping to Xenometrix. Five chemicals were aliquoted in two different vials and treated as two independent samples for coding and shipping purposes. Sealed envelopes containing the chemical name, CAS no., and available safety information were sent with each shipment of chemicals to be opened only in the event of a spill or personnel exposure. All coded, 'unknown' chemicals were handled by the experimentalist as if they were carcinogenic and mutagenic, and since there were no incidents regarding safety these envelopes were not opened until after the chemicals were decoded.

2.3. Control chemicals

Positive control chemicals including: N^4 -aminocytidine (N4AC), methyl methanesulfonate (MMS), streptonigrin (STN), 4-nitroquinoline-*N*-oxide

Table 2
Chemicals tested

Chemical	CAS No.	Supplier	Purity ^a (%)	Solvent used	Dose range (per ml)	References ^b
9-Aminoacridine · HCl · H ₂ O	52417-22-8	Aldrich	98	DMSO	5–100 µg	[17]
2-Amino-5-nitrophenol	121-88-0	Aldrich	90 (tech.)	DMSO	1–100 µg	[18,19]
5-Azacytidine	320-67-2	N.I.E.H.S.	Unknown	DMSO	5–100 µg	[2]
Benzaldehyde	100-52-7	Aldrich	99 +	DMSO	50–1000 µg	[20]
Benzo[<i>a</i>]pyrene	50-32-8	Sigma	Practical	DMSO	5–100 µg	[20,21]
Benzyl chloride ^c	100-44-7	Aldrich	99 ^c	DMSO	50–5000 µg	[19]
1-Chloro-2-propanol	127-00-4	Fluka	97 +	Water	50–1000 µg	[19]
Coumarin	91-64-5	Rhone-Poulenc	Unknown	DMSO	5–1000 µg	[20]
Crotonaldehyde	4170-30-3	Aldrich	99 +	DMSO	50–1000 µg	[20]
Cumene hydroperoxide	80-15-9	Aldrich	80 (tech.)	DMSO	1–100 µg	[17]
Dicumyl peroxide	80-43-3	Aldrich	98	DMSO	50–5000 µg	[22]
Di(2-ethylhexyl)phthalate	117-81-7	Aldrich	99.5	DMSO	0.5–1000 µg	[23,24]
Dimethyl sulfoxide	67-68-5	Fisher	Certified	Water	50–5000 µg	[2]
1,2-Epoxybutane	106-88-7	TCI America	99 +	DMSO	50–5000 µg	[21,25,26]
Ethylenediamine	107-15-3	Aldrich	Unknown	Water	50–1000 µg	[20]
8-Hydroxyquinoline	148-24-3	Aldrich	99.5	DMSO	1–50 µg	[22]
Isobutyl nitrite	542-56-3	King's Labs.	Unknown	Ethanol	1–1000 µg	[17]
Nitrofurantoin	67-20-9	Norwich/Eaton	Unknown	DMSO	0.1–5 µg	[20]
4,4'-Oxydianiline	101-80-4	Aldrich	97	DMSO	10–500 µg	[22]
Phenol	108-95-2	Aldrich	99 +	DMSO	50–5000 µg	[20]
Proflavin HCl · 1/2 H ₂ O	952-23-8	Aldrich	~ 95	Water	0.5–10 µg	[27]
Quercetin	117-39-5	Freeman Industries	97	DMSO	5–100 µg	[2]
Tetracycline HCl	64-75-5	Aldrich	98	Water	0.5–10 µg	[28]
Trichloroacetonitrile	545-06-2	Aldrich	98	DMSO	50–1000 µg	[17]
Tricresyl phosphate	1330-78-5	Stauffer	Unknown	DMSO	10–1000 µg	[20]

^a Purity (%): suppliers' stated purity; where there is no entry, purity is not known or reported.

^b Publication containing the original NTP Salmonella mutagenicity test results. Some of these chemicals had additional, unpublished, tests performed.

^c Contains 0.25% propylene oxide.

(4NQO) and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical (St. Louis, MO), while 2-nitrofluorene (2NF) and 2-aminoanthracene (2AA) were purchased from Aldrich Chemical (Milwaukee, WI). Ethanol was obtained from Aaper Alcohol and Chemical (Shelbyville, KY). N4AC was prepared in sterile deionized water ($\Omega \geq 10^{15}$ ohms) for a final concentration of 50 µg/ml and used as a positive control chemical for TA7001. STN was dissolved in DMSO and used at a final concentration of 5 ng/ml as a positive control for TA7003. MMS was used as a positive control chemical for TA7002, TA7004, TA7005, TA7006, and the Mix at a final concentration of 500 µg/ml. In experiments where MMS was not consistent in reverting the cultures, 4NQO was used as a positive control chemical for strains, TA7004, TA7005, TA7006, and the Mix at a final concentration of 1 µg/ml dissolved in DMSO. The

positive control chemicals used were those shown to be mutagenic by Gee et al. [9]. 2NF was prepared in DMSO at a final concentration of 1 µg/ml as a positive control for both TA98 and TA1537. 2AA was prepared in DMSO at a final concentration of 5.0 µg/ml and used as the positive control in the presence of S9 fraction. The degree of reversion in response to these control chemicals was within the limits typical of results observed over a minimum of 10 experiments performed over the previous year. In cases where the results were ambiguous or negative with respect to these control chemicals, the entire set of experiments were repeated before the test chemicals were decoded.

The final concentration of solvent in each experiment was 2% in a final exposure volume of 0.5 ml. The range of doses used was determined by a combination of viability measured in the number of posi-

tive wells in non-selective media and the optical density of exposed cultures after 90 min of incubation at 37°C in an environmental shaker at 250 rpm. Where there was no detectable toxicity and no limitations in the solubility of the chemical, a maximal concentration of 5 mg/ml was tested. The density of unknown liquids was assumed to be 1.0 at the time of testing and concentrations were adjusted after decoding for Table 2.

2.4. Study design

Data were collected for a total of 4 doses of each test chemical plus a solvent (zero dose) and positive control. Each culture was treated and dispensed into microtiter plates in triplicate. Three independent cultures were dosed either on the same day and/or up to 3 weeks later. Each culture came from a separate frozen vial, although all the vials were from the same production lot. Therefore, 9 measurements were obtained at each dose for each chemical. Doses were selected using a preliminary range-finding experiment with the Mix. All strains and the Mix were used at the same time. Approximately 2–8 chemicals were tested in 1 day.

2.5. Liquid exposure

The chemicals were tested in the Ames II strains using a liquid fluctuation test (the AMAX test procedure: Ames II™ Mutagenicity Assays by Xenometrix). Unless stated otherwise, all procedures were performed manually (i.e. without using the robotics station). In the absence of S9 fraction, 9 ml of Ames II Exposure medium (Xenometrix), and 1.0 ml of each overnight culture were mixed gently. In experiments where the S9 fraction was used, the Exposure medium was decreased to 7.5 ml to accommodate 1.5 ml of the S9 reagent to provide a final concentration of 4.5% S9 fraction. The S9 mix, in a total volume of 20 ml, contained: 0.66 ml of 1 M KCl (J.T. Baker, Phillipsburg, NJ), 0.64 ml of 0.25 M MgCl₂ (J.T. Baker), 0.50 ml of 0.2 M glucose-6-phosphate (Sigma), 2 ml of 0.04 M NADP (Sigma), 10 ml of 0.2 M NaHPO₄ buffer (J.T. Baker) and 6 ml of rat liver S9 fraction induced by Aroclor 1254 (Molecular Toxicology, Annapolis, MD, or Microbiological Associates, Rockville, MD).

Aliquots of the appropriate stock concentrations of each test chemical, control chemicals, and solvents were dispensed into individual wells of 24-well, microtiter plates (not treated for tissue culture; Falcon, Becton Dickinson Labware, Costar, Cambridge, MA; Nunc, Roskilde, Denmark) to a final volume of 500 µl per well. The 24-well plates were incubated at 37°C for 90 min, with shaking at 250 rpm. The S9 reagent was stored on ice until it was added to the culture mixture.

2.6. Prototrophic selection

After the 90-min incubation, the 24-well plates were removed from the incubator to room temperature. An aliquot of 2.5 ml of Ames II Reversion Indicator medium (Xenometrix) was pipeted manually or by a programmable automated pipeting station (Hamilton, Reno, NV) into each well of the 24-well plates containing chemically treated cultures. The histidine-deficient Indicator medium which selects for prototrophic reversion was mixed gently by pipeting and dispensing the volume in place several times, either manually or in the pipeting station. When adequately mixed, the contents of each well of a 24-well microtiter plate were distributed in 50-µl aliquots over 48 wells of a 384-well microtiter plate (Nunc) by Eppendorf Repeater Plus/8™ pipets (Brinkmann, Westbury, NY) manually or by the robotics pipeting station. Each column (4 wells) of the 24-well plate was transferred into one-half of a 384-well plate, effectively dividing each sample among 48 wells of the plate. The 384-well microtiter plates were sealed in Ziplock® plastic bags to prevent evaporation, and incubated at 37°C for 48 h.

2.7. Robotics

Several robots were evaluated for their ability to distribute the exposed liquid cultures into 384-well microtiter plates for prototrophic selection. Many criteria, including speed of pipeting, adequate mixing, minimal cross-contamination of cultures and chemicals without disposable pipet tips, flexibility and most importantly, the cost of the instrument, operating supplies and long-term maintenance, were considered. We selected the ML 2200 pipeting station manufactured by Hamilton (Reno, NV) to re-

duce the time needed for the performance of the study.

The ML 2200 pipeting station used a liquid level detector and wash station to minimize carry-over from well to well of the 24-well and 384-well plates. The system was controlled by ECLIPSE software (Hamilton) from a Hewlett-Packard Vectra 486/25N computer (Grenoble, France). The pipeting head consists of 8 Teflon-lined probes for aspiration and dispensation of liquids. Liquid was transferred by powered precision syringe pumps which controlled by DOS-based software. Each probe was programmed to flush with 5 ml of 70% ethanol, followed by 5 ml of sterile deionized water ($\Omega \geq 10^{15}$ ohms) 8 to 10 times between pipeting steps. The Indicator medium was dispensed into each well of the 24-well microtiter plate. Probes were rinsed twice with sterile deionized water before each transfer of Indicator medium from a sterile stock. The probes mixed Indicator media with the exposed culture three times before distributing 50 μ l into each well of a 384-well microtiter plate. The 384-well plates were covered, removed from the platform of the pipeting station, and incubated at 37°C as described above. The entire robotics workstation was enclosed in an HEPA-filtered positive pressure bubble (Biobubble, Ft. Collins, CO) to minimize contamination.

2.8. Data acquisition

Bromocresol purple, an essential constituent of the Indicator media, turns yellow as the pH drops ($pK_a \cong 5.2$) as catabolites accumulate from the metabolic activity of revertant cells which grow in the absence of histidine. The number of positive wells out of a total of 48 wells is an indication of the frequency of reversion per replicate per dose, and was compared to the number of spontaneous revertant wells obtained in the solvent control sections. Each 48-well section of the 384-well plates was scored for the number of revertant wells (yellow) by a SLT Spectra Image plate reader (Tecan U.S., Research Triangle Park, NC) at $OD_{492\text{ nm}}$ normalized at $OD_{623\text{ nm}}$ as a reference wavelength. The optical density was digitized by the SLT data Capture software and exported to Microsoft Excel, Version 7.0 (Microsoft, Bothell, WA) for data analysis. The digitized optical densities were converted to positive (1)

or negative (0) wells and summated by macros written in Excel to determine the number of positive wells for each sample. The data were organized further into summary tables of triplicate data per culture and pooled data per three independent cultures for each dose.

The average number of wells containing revertants per culture per dose were calculated from the triplicates, and the mutational ratios and increases above the zero dose were determined at each dose of test chemical. If the mean spontaneous reversion was 1 well or greater out of 48 wells, the mutational ratio was calculated as a ratio of the mean number of wells of revertants in the test dose divided by the mean number of wells in the zero/solvent dose control. If the mean spontaneous reversion was zero then the absolute difference between the numbers of revertants in the test dose and the zero dose control was used to look for a dose effect. These increases and ratios were tested for preliminary statistical significance by the Student's *t*-test at $p < 0.05$. Those chemicals that were negative based on these preliminary results were tested again in the presence of the S9 fraction.

2.9. Complementary-log generalized linear model (GLiM)

To ask whether there was a dose effect significant from the control cultures, the data were analyzed according to a model that assumed mutagenic events followed a Poisson process ([29–31]; W. Piegorsch and B. Margolin, personal communication). In preparation for these analyses, the variability among sets of triplicate plates and from culture to culture were determined.

The number of positive wells (containing revertants) from each of the triplicate plates within each given culture per dose were tested for excessive plate-to-plate variability [32,33]. Since there were no consistent patterns of significant extra binomial variation at the plate-to-plate level, there was sufficient homogeneity to pool these data sets. The pooled triplicates were analyzed for excessive variability amongst three independent cultures of each tester strain for each chemical tested with and without S9 fraction. There were consistent patterns of significant extra-binomial variation at the culture-to-culture level

due to jackpot mutations that were observed at every dose level for some cultures.

In these analyses, it was assumed that there were no culture-by-dose interactions present in these experiments. One data set for a chemical chosen at random, was tested for such possible interactions. Significant interactions at $\alpha = 0.05$ were found in two of the 18 possible strain and S9 conditions. This was thought to be an insubstantial interactive effect; therefore the results were not modified for a possible culture-by-dose interaction for this chemical, nor for any others. The small-sample operating characteristics of this form of likelihood ratio test under a complementary-log GLiM are not known, and further applied statistical research is required before this methodology can be recommended unequivocally. Because of this concern, only p -values lower than the extreme value of $\alpha = 0.001$ were chosen as a reasonable limit for significance.

The statistical treatment of variability among replicates within a culture, and among cultures will be addressed elsewhere (Piegorsch et al., in preparation).

2.10. NTP test results

Detailed descriptions of the test protocols and criteria for evaluation of the data can be found in Zeiger et al. [2,20,34,35]. Briefly, chemicals were tested under code using a preincubation procedure, and the data evaluated prior to deciphering the code. All chemicals were tested up to a toxic dose or to a dose limited by solubility. In the absence of toxicity, the highest dose tested was 10 mg/plate. The strains used were TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537. Not all strains were used for all chemicals. However, all chemicals were tested in TA98 and TA100; only 1 chemical was tested in TA102 and TA104. All tests contained 5 chemical concentrations at half-log intervals, in triplicate, and concurrent solvent and positive controls. Chemicals were tested without metabolic activation, and with 10% and/or 30% S9 from Aroclor-induced rat and hamster livers. All positive results were repeated. In general, a chemical was judged mutagenic if a reproducible, dose-related response was obtained, and statistical procedures were not used. A non-mutagenic chemical was one that was judged negative in at

least 4 tester strains (TA98, TA100, TA1535, and TA97 or TA1537).

For the purposes of this evaluation chemicals were selected which had been judged mutagenic (+), weakly mutagenic (+w), and non-mutagenic (-). A mutagenic response was defined as a reproducible, dose-related response. A weakly mutagenic response was a low-level, reproducible, dose-related response. The difference between a response judged mutagenic or weakly mutagenic was highly subjective. It was not necessary to achieve a 2-fold increase over background for a chemical to be considered (+) or (+w) [2,20,34,35].

3. Results

A summary of the qualitative results from the Ames II test and the NTP database are presented in Table 3. The tables containing the raw data from this study are available on the Xenometrix web site at <http://www.xeno.com> or can be obtained from P.G.

If a chemical was found to revert any of the strains in the Ames II test, the chemical was classified as mutagenic. A study design such as the one used here allows several different analyses of the data. A number of useful combinations of the Ames II and NTP test results have been evaluated for concordance, and are presented in Table 4.

3.1. Concordances

When the results from the individual TA7000 strains, the Mix, TA98 and TA1537, were compared to the summary NTP results for each of the 25 chemicals, the overall concordance was 88% in agreement (Table 4¹). This was calculated from a total of 22 chemicals (16 NTP mutagens and 6 NTP non-mutagens). There were two mutagens in the NTP (1-chloro-2-propanol and isobutyl nitrite) preincubation test that were not classified as mutagens, and one NTP non-mutagen (benzaldehyde) in the NTP procedure that was mutagenic in this study.

1-Chloro-2-propanol and isobutyl nitrite were not identified as mutagens by any of the strains in the Ames II test (Table 3). The overall NTP results classified equivocal (?) for TA98 in both the presence and absence of S9 fraction. 1-Chloro-2-pro-

Table 3
Reversion results

Chemical	± S9	AMAX									NTP preincubation test						
		7001	7002	7003	7004	7005	7006	Mix	1537	98	1535	1537	97	98	100	102/4	
9 Aminoacridine HCl · H ₂ O	NA	–	–	–	–	–	–	–	+	–	–	+	–	–			
	S9	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+ w	
2-Amino-5-nitrophenol	NA	–	–	–	–	–	–	–	–	–	+	–	+	–	–		
	S9	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+ w	
2-Amino-5-nitrophenol	NA	–	–	–	–	–	–	–	–	–	+	–	+	–	–		
	S9	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+ w	
5-Azacytidine	NA	–	–	–	+	–	+	+	–	+	+ w	–	–	–	?	+	
	S9	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	
Benzaldehyde	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	
Benzo[<i>a</i>]pyrene	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	+	+	–	+	+	+	+	+	+	?	+	–	–	+	+	
Benzyl chloride	NA	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+ w	
Benzyl chloride	NA	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+ w	
1-Chloro-2-propanol	NA	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–?	
	S9	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–?	
Coumarin	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	
Coumarin	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	
Crotonaldehyde	NA	–	+	–	+	+	+	+	–	+	–	–	–	–	–	+	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	
Cumene hydroperoxide	NA	–	+	–	+	+	+	+	+	+	–	–	–	–	–	–	
	S9	+	–	–	+	+	+	+	+	–	–	–	–	–	+	+ w	
Dicumyl peroxide	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Di(2-ethylhexyl)phthalate	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Dimethyl sulfoxide	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
1,2-Epoxybutane	NA	–	–	–	+	–	–	–	–	–	+	–	–	–	–	+	
	S9	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	
Ethylenediamine	NA	–	–	–	+	+	+	+	–	–	+	–	–	–	–	+ w	
	S9	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+ w	
Ethylenediamine	NA	–	–	–	+	+	–	+	–	–	+	–	–	–	–	+ w	
	S9	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+ w	
8-Hydroxyquinoline	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	+	–	–	+	–	+	+	+	–	–	–	–	+	–	
Isobutyl nitrite	NA	–	–	–	–	–	–	–	–	–	?	–	–	–	–	+	
	S9	–	–	–	–	–	–	–	–	–	–	+ w	–	–	–	+	
Isobutyl nitrite	NA	–	–	–	–	–	–	–	–	–	?	–	–	–	–	+	
	S9	–	–	–	–	–	–	–	–	–	–	+ w	–	–	–	+	
Nitrofurantoin	NA	–	+	–	+	+	+	+	–	+	–	–	–	–	+	+	
	S9	–	–	–	–	–	–	–	–	–	–	?	–	–	+	+	
4,4'-Oxydianiline	NA	–	–	–	–	+	–	+	–	–	–	–	–	–	–	+ w	
	S9	–	–	–	–	–	–	–	–	–	–	+ w	+	+	–	+	
Phenol	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

Table 3 (continued)
Reversion results

Chemical	± S9	AMAX									NTP preincubation test					
		7001	7002	7003	7004	7005	7006	Mix	1537	98	1535	1537	97	98	100	102/4
Proflavin HCl · 1/2 H ₂ O	NA	–	–	–	–	+	–	+	+	+	–	+		?	–	
	S9	–	–	–	–	–	–	–	–	–	–	+		+	+	
Quercetin	NA	–	+	–	+	+	–	+	+	+				+	+	
	S9	–	–	–	–	–	–	–	–	–				+	+	
Tetracycline HCl	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trichloroacetonitrile	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	?	
	S9	–	–	–	–	–	–	+	–	–	–	–	–	–	+	w
Tricresyl phosphate	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	S9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

NA, not tested with S9; –, negative; +, positive; + w, weakly positive; ?, equivocal.

panol was judged to be equivocal (?) in 3 tests and weakly positive (+w) in 2 tests out of the 5 experiments performed in the presence of 10% rat S9 fraction. Similarly, it was negative (–) in 2, equivocal (?) in 2 and weakly positive (+w) in 1 out of 5 experiments performed in the presence of 10% hamster S9 in TA100 [19]. However, it was clearly positive with and without 10% hamster and rat S9 in TA1535. There were no strains with comparable genetic backgrounds to TA1535 in this Ames II test.

Isobutyl nitrite, diluted with 95% ethanol, was tested as two independent unknown samples and the results were negative in both replicates. Positive results were observed in the NTP preincubation assay using TA100 in the presence of 10% hamster and 10% rat S9 when isobutyl nitrite was dissolved in 95% ethanol. Isobutyl nitrite, diluted in DMSO was also positive in the presence of 30% hamster and 30% rat S9 [17]. If the S9 fraction in the Ames II test was increased to 10%, it is possible that positive results might have been obtained.

Benzaldehyde was positive in this study, in disagreement with the negative results in TA1535, TA1537, TA98 and TA100 with and without 10% hamster and rat S9 [20]. Results of testing benzaldehyde in strains TA102, TA104 or TA97 were not available. This chemical reverted only TA7005, and was not detectable in the Mix culture, TA1537 or TA98. Strain TA7005 has a C:G base-pair sequence at the target site and reverts only to a A:T base pair to regain functional histidine biosynthesis. Croton-

aldehyde was also successful in reverting TA7005 in this study and we have obtained positive results with formaldehyde (unpublished data). There is a G:C base pair both 5' and 3' of the target mutation which is similar to the context of the 1 base pair deletion in TA1537 (*hisC3076*) which has the sequence, 'CGCGCG'. However TA1537 was not reverted by crotonaldehyde or benzaldehyde in this study, or by formaldehyde in a NTP study [20]. TA97 also has C:G base pair at its target site, but it has not been reported as being particularly responsive to these aldehydes.

3.2. Ames II test vs. NTP TA100 and TA98

The overall concordance is 88% (Table 4²) when the results of all of the strains (TA700x, Mix, TA1537 and TA98) in the Ames II test were compared to the NTP results obtained from just TA100 and TA98 in the preincubation assay. The discordances for isobutyl nitrite and benzaldehyde remain, however, there is a disagreement for 5-azacytidine, which is balanced by an agreement for 1-chloro-2-propanol. In this case, TA100 and TA98 classify 5-azacytidine as a non-mutagen in the NTP database (although it is mutagenic in TA102 and TA104). 5-Azacytidine was mutagenic in TA7004, TA7006, TA98 and the Mix in the Ames II test.

3.3. Ames II test vs. NTP (all strains)

The results from strains TA7001 to TA7006 in the Ames II test agreed with the NTP preincubation test

Table 4
Summary of concordances

	TA700x ^a	Mix	TA700x + Mix	TA98	TA1537	Mix + TA98 + TA1537	Mix + TA98	TA700x + Mix + TA98	Ames II all strains
NTP, all strains	19/25 (76%) ³	18/24 (75%) ⁶	20/25 (80%) ⁴			20/25 (80%) ⁵	19/24 (79%) ⁷	21/25 (84%)	22/25 (88%) ¹
TA98				21/25 (84%) ⁸					
TA1537					18/20 (94%) ⁹				
TA100 + TA98							19/24 (79%) ¹⁰		22/25 (88%) ²
TA100		17/24 (71%) ^{12 b}							
TA100 + TA98 + TA1537						20/24 (83%) ¹¹			

^a TA700x: Any one or more of TA7001, TA7002, TA7003, TA7004, TA7005 and TA7006.

^b If S9 specificity is ignored, then the concordance is 19/24 (79%).

N.B.: In comparisons where there were only 24 chemicals in total, benzyl chloride was not included because of conflicting results in the duplicate samples.

for 13 of the 18 NTP mutagens (72%) and 6 out of 7 NTP non-mutagens, which becomes a total of 19 out of the 25 chemicals (76%) tested (Table 4³). When the Mix was included, there was one additional chemical detected, to increase the agreement to 14 (78%) mutagens, which became a total of 20/25 (80%) (Table 4⁴). When the results from the Mix, TA98 and TA1537 were compared with all the strains used in the NTP database, the agreement between the AMAX test and the NTP test was 13 (72%) out of 18 (72%), NTP mutagens, and 7 (100%) out of 7 (100%) non-mutagens. This resulted in an overall agreement of 80% (20/25, Table 4⁵).

The results from the Mix alone, when compared to that of the NTP database, gave an overall agreement of 75% (18/24, Table 4⁶). Benzyl chloride was tested twice in this study as two independent chemicals, however the results for the Mix did not agree. Therefore, benzyl chloride results were not included in these calculations. The agreement increases by 1 chemical (2-amino-5-nitrophenol, Table 3) to 79% when the Ames II TA98 is added to the comparison (Table 4⁷).

3.4. Strains TA98 and TA1537

The tester strains, TA98 and TA1537, which detect small deletions, additions and suppressions of the frameshift mutations, have been included for routine use in the Ames II test. This allowed for a somewhat limited comparison of the NTP's preincubation method to the liquid format used in this study. Because the results for these two strains were reproducible among the 5 duplicated chemicals, these chemicals are considered here as single tests.

There was overall agreement in the TA98 results for 21 (84%) out of 25 (84%) chemicals (Table 4⁸). In this comparison, the Ames II test appeared to be more sensitive because there was only one chemical positive in the NTP test and negative in the Ames II test (4,4'-oxydianiline). By comparison, there were three positive (5-azacytidine, crotonaldehyde, 8-hydroxyquinoline, Table 3) in the Ames II test and negative in the NTP test. 4,4'-Oxydianiline (Table 3) was positive in the preincubation test only with S9, but negative in the Ames II test which was run only without S9. Because 4,4'-oxydianiline was positive in other strains, it was not tested with S9 in this

study. Similarly, proflavin was positive without S9 so it was not tested with S9 in the Ames II test. Proflavin was equivocal and therefore classified as negative, but was positive when tested with S9 in the NTP preincubation test. In contrast, cumene hydroperoxide was positive in the Ames II test without S9, and negative with S9, while S9 activation was required for mutagenicity in the NTP preincubation test. Another disagreement was with 8-hydroxyquinoline, which was negative in TA98 in the NTP study with and without S9, and positive in the Ames II test with S9. However, the sulfate salt of 8-hydroxyquinoline was mutagenic with S9 in the NTP protocol [22].

Of the 20 chemicals tested in TA1537 by the NTP, the test results agreed for 18 (94%, Table 4⁹); 3 were positive in both test systems, and 15 were negative. 4,4'-Oxydianiline was positive in the NTP tests only with S9. Its responses were considered to be concordant because it was non-mutagenic in the absence of S9 in both procedures. 2-Amino-5-nitrophenol was negative in TA1537 without S9 in the Ames II test, and positive in the NTP preincubation test. Cumene hydroperoxide was positive in the Ames II test with and without S9, and negative under these same conditions in the preincubation test. Benzaldehyde, dicumyl peroxide, 8-hydroxyquinoline and quercetin were not tested in TA1537 in the NTP preincubation protocol.

3.5. Ames II Mix + TA98 vs. NTP TA100 + TA98

The NTP test protocol uses TA98 and TA100, with and without S9 as an initial screen. If the chemical is mutagenic in one of these two strains, it is usually not tested in other strains. Chemicals not mutagenic in either of these two strains are tested in at least two additional strains [2,20,34]. This procedure was adopted because an analysis of the NTP database showed that 89% of the mutagenic chemicals would have been correctly classified if only TA98 and TA100 had been used [36]. This parallels the role proposed for the Mix + TA98 screen. Benzyl chloride was not included in this compilation because one of its duplicates yielded a positive response with the Mix, and the other sample was negative. When the Mix and TA98 were used, 11 (46%) of the chemicals were judged mutagenic, as

compared to the 15 (63%) chemicals which were classified as mutagens by TA100 and TA98 in the NTP preincubation test. There were 4 chemicals that were mutagenic in TA98 and/or TA100 in the preincubation test that were not detected by the Mix + TA98 strategy, and one chemical (5-azacytidine) that was mutagenic in the Mix and TA98, but not in TA98 or TA100 in the preincubation test. Overall, there was agreement in the test results for 19 (79%) out of the 24 chemicals considered (Table 4¹⁰).

These results demonstrate that the use of TA98 and TA100 detected a higher proportion of mutagens in the preincubation test than did TA98 and the Mix in the Ames II test. Of the 4 chemicals that were negative in the Ames II protocol, 9-aminoacridine was only weakly positive with S9 in the NTP preincubation assay, while 9-aminoacridine was not tested with S9 in the Ames II protocol because it was positive in TA1537 in the absence of S9. The addition of TA1537 to the Mix and TA98 in the Ames II test results would have allowed detection of 9-aminoacridine (Table 4¹¹).

3.6. Mix vs. individual strains

The Mix is designed to respond to treatments that mutate any one of the 6 strains, from TA7001 to TA7006. The Mix and one or more of the individual strains were positive for 20 (83%) out of 24 chemicals. Because the results with the Mix were discordant for the duplicate benzyl chloride samples, this chemical is not included in this compilation (Table 3). Both samples of benzyl chloride were mutagenic in TA7004 and TA7005, but only one was mutagenic in the Mix. Three chemicals (benzaldehyde, mutagenic in TA7005 with S9; coumarin, mutagenic in TA7002 with S9; 1,2-epoxybutane, mutagenic in TA7004 without S9) were detected by the individual strains, but not by the Mix, and one (trichloroacetonitrile) was detected by the Mix, but not by the individual strains.

3.7. Strain responsiveness

TA7004 and TA7005 were considered to be the most sensitive strains because they detected 12 and 14 out of the 18 mutagens, respectively (Table 3).

This was followed closely by TA98 which was positive for 10 mutagens. TA7002, TA7006 and TA1537 were reverted by 7 to 8 mutagens, and TA7001 was reverted by only two mutagens. TA7003 did not respond to any of the test chemicals. Streptonigrin, 5 ng/ml, was used as positive control chemical for strain, TA7003, in the liquid protocol. However, in the protocol used here, it never induced more than 5 ± 2 positive wells out of 48 possible wells in a total of 165 experiments that used streptonigrin in this study (data not shown). In these same experiments, the untreated controls were between 0 and 1 positive wells. In contrast, streptonigrin (0.1 μ g/plate) resulted in 63 ± 7 revertants per plate for TA7003 from a spontaneous response of 0.1 to 0.2 revertants per plate in the standard plate incorporation assay [9].

3.8. Reproducibility

There were 5 mutagens that were tested as duplicate samples unknown to the personnel performing the test. The overall conclusions from each of the duplicate tests were the same, although there were some differences with respect to the responding Salmonella strains. Four of the chemicals were reproducibly mutagenic and one, isobutyl nitrite, was non-mutagenic (Table 3). With respect to comparisons by strain and activation, two of the positive chemicals, 2-amino-5-nitrophenol and coumarin responded similarly in both tests. Benzyl chloride was mutagenic in TA7004 and TA7005 without S9 in both tests, but only one of the duplicate samples was mutagenic in the Mix. Ethylenediamine was mutagenic in TA7004, TA7005 and the Mix without S9 in both tests, but only one of the duplicate samples was positive in TA7006.

4. Discussion

4.1. Comparisons

This study evaluated the ability of the base-specific set of Salmonella tester strains (designated Ames II)TM, when tested in a high-throughput, fluctuation protocol, to duplicate the mutagenic responses of the standard Salmonella strains when tested in a

preincubation protocol. Thus, the two data sets generated differ in the *Salmonella* strains used, in the cell and chemical exposure regimen, and in the mutant expression conditions.

Of the 30 coded substances tested, 5 of the mutagens were duplicates, a factor that was not divulged to the laboratory personnel until after the testing had been completed. This allowed an evaluation of the intra-laboratory reproducibility. Identical patterns of positive and negative responses were obtained for 3 of the 5 chemicals. Of the remaining two chemicals, one (ethylenediamine) differed in its response to strain TA7006; both samples showed increased responses, however there was no consistent dose response for all three cultures for one sample. The other chemical (benzyl chloride) differed in its response to the Mix. Both samples of benzyl chloride gave dose related increases but one sample was not significant ($\alpha = 0.008$) at $\alpha = 0.001$ to score positive in the Mix. Neither of these differences affected the overall evaluation of each of the test samples.

The concordances between the TA7000 series strains, and the NTP results must be measured within the limitations of the reproducibility of the NTP results. The intra-assay agreement of the *Salmonella* test, as performed by the NTP, is 84.5%, when measured as a strict, positive-versus-negative concordance, and 86.9% when measured as a pair-wise concordance [35]. These values reflect the reproducibility of the *Salmonella* test when tested in the same or different laboratories at different times, but with the same protocol. As such, they can be considered to provide an upper bound for the agreement to be expected from testing or test validation exercises.

4.2. Protocol effects

There are a number of differences between the Ames II test, as performed in this study, and the NTP preincubation test protocol, that could have contributed to the differences between the two sets of results. These differences are best seen in the TA98 and TA1537 results. These two strains were used in both procedures, and it may be assumed that the differences in the responses of these strains between the NTP and the Ames II tests resulted from the different testing procedures. The Ames II test identified mutagens in each strain that were not detected by the preincubation test; and visa versa.

However, the overall concordances were 84% and 94% for TA98 and TA1537, respectively, so the results from the two different protocols can be considered comparable.

The final concentration of Aroclor-induced rat liver S9 was 4.5% in the Ames II test. This was considerably lower than the 10% and 30% S9 used in the NTP protocol. For many of the NTP chemicals, the initial test used was 30% S9, based on observations that the higher S9 concentrations allowed more mutagens to be identified; therefore, it is not known if a positive chemical under these conditions would also have been positive if the S9 concentrations were decreased. Although, the NTP protocol used both rat and hamster liver-derived S9, all of the S9-dependent mutagenic chemicals chosen for this study were positive with rat S9.

Because the Ames II test is a colorimetric assay that is dependent on pH changes, raising the percentage of S9 fraction overcomes the buffering capacity of the colored Indicator media. Therefore it is technically difficult and costly to match the S9 concentrations used in the NTP preincubation tests. To further minimize the cost of testing, we tested only those chemicals that were negative in all strains in the presence of S9. For example, 4,4'-oxydianiline was not tested with S9 because it was positive in TA7005 and the Mix; however, it was positive in TA1537 and TA98 the NTP studies only in the presence of S9 [2].

The histidine concentration in the fluctuation test wells was optimized to 130 μM in the Ames II protocol such that there was a doubling in the optical density of the culture as measured at 600 nm in the Exposure medium during the 90-min exposure period for the solvent control. Since the cells were not washed after the exposure period, 130 μM histidine was estimated to be sufficient to support 1–2 cell divisions over the course of the assay. This in contrast to the preincubation test where the added histidine is sufficient for 6–8 cell divisions over the course of the assay (unpublished). This difference in the numbers of cell divisions in the presence of the mutagen is expected to have a significant effect on the sensitivity of the test. Therefore the sensitivity of the Ames II test might be improved by increasing both the histidine concentration and exposure time to allow fixing of more pre-mutagenic lesions.

The low spontaneous mutant response seen with some of the TA7000 series strains may increase the sensitivity of the strain to mutagenic damage, but could actually reduce the sensitivity of the test (particularly TA7003). It has been shown in the preincubation test using multiple well plates that maximum statistical sensitivity is obtained when the spontaneous response is 10% of the maximum number of wells [37]. When the solvent control response is 0, 1, or 2 positive wells, the increase in mutant wells cannot be accurately determined in practice. For example, a 3-fold increase of the spontaneous response could still leave the numbers of induced mutants too low to be adequately evaluated, or to provide confidence in a positive response.

Although TA7003 was shown to be responsive to a number of substances in the standard plate incorporation protocol ([9]; unpublished data), it did not respond to any of the chemicals used here. Whether because of its low spontaneous response, or as a function of the selection of test chemicals, it should be noted that none of the test chemicals produced an increase in this strain, whereas all other strains responded to at least one chemical. Because this strain was more responsive under plate test conditions [9], it is possible that a number of the chemicals tested here might revert TA7003 had the exposure been carried out under those conditions.

4.3. Genetic targets

Many factors affect the sensitivity or the ease with which a strain is reverted by potential mutagens. Some parameters such as consistent pipeting were addressed somewhat by robotics liquid transfers, and consistent dosing procedures; however, others depend on the reactivity of the target base and the context of the adjacent DNA sequence. The unresponsiveness of the TA7003 target site may be related to its context in the gene, and putting this base change in a different context may change its reactivity to mutagens.

Because TA100 has been shown to revert by 4 out of the 6 possible base substitutions, and by missense suppression [4], and it had been tested with all 25 chemicals, we compared it with the results from the Mix. We found that the Mix was only 71% in agreement with TA100; however, if the S9 speci-

ficity was ignored, that agreement was increased to 79% (Table 4¹²). Thus in spite of the two extra pathways of reversion offered by the Mix (TA7001 and TA7002) not found in TA100, it did not identify 25% of the mutagens tested in this study. T:A to A:T transversions and A:T to G:C transitions, which are detectable by the strains in the Mix, have not been detected in reversions of *hisG46* strains, including TA100.

While it was expected that the response from any culture of an individual strain would be more predominant than that of the Mix, trichloroacetonitrile was positive only when tested in the Mix. The significance levels of $\alpha = 0.007$, 0.016, and 0.003 for TA7004, TA7006 and TA98, respectively, did not reach the level ($\alpha = 0.001$) required to classify the responses as positive. Previous reports of the mutagenicity of trichloroacetonitrile were inconsistent. It was weakly positive in the NTP preincubation test [17], but was judged non-mutagenic in another laboratory using the same protocol [38]. A study using the plate incorporation assay found it to be non-mutagenic [39]. However, another study using only TA100 in the fluctuation test, which is most comparable to the Ames II test, found trichloroacetonitrile to be mutagenic [40].

The Ames II test failed to classify 1-chloro-2-propanol as a mutagen, although one experiment out of the triplicate gave a dose response up to 6-fold in TA7004, but this was not reproducible. Although responses were weak in TA100, 1-chloro-2-propanol was clearly mutagenic in TA1535. The strains TA1535 and TA100 both carry the *hisG46* mutation in very similar genetic backgrounds, except that TA1535 does not carry the R factor [41]. The genetic background of the TA7000 strains (Table 1) are thought to be comparable to that of TA100, although not isogenic with TA100. Others have reported positive results with this chemical using both the liquid preincubation and the plate incorporation protocols [42,43].

Isobutyl nitrite reverted TA1535 in a dose-related manner in the presence of 10% hamster S9, and there was an approximate 2-fold dose response in TA100 with 10% rat and hamster S9 up to 6.6 mg per plate, and slightly less than the response without S9 [17]. These results were consistent with others reported in the literature in which the standard plate incorpora-

tion, liquid preincubation and vapor exposure protocols were used [44,45].

Benzaldehyde has been reported to be non-mutagenic using both preincubation and plate incorporation protocols in the traditional strains, TA98, TA100, TA1535 and TA1537 [20,46,47]. We found that it caused C:G to A:T transversions as identified by a reproducible response in TA7005. Although the target codon in mutant allele (*his46*) in both TA100 and TA1535 has been known to revert by C:G to A:T transversions, the sequence context and/or the difference in genetic background probably accounts for the difference in the response to benzaldehyde.

4.4. Summary

The AMAX test, partial automation of a modified fluctuation assay using base-specific strains developed to determine mutagenicity in a *Salmonella*/microsomal reversion system, gave results comparable to those found in the NTP database for 25 chemicals. Some of the differences seen between the two procedures may result from differences in test protocol. The high concordance with the traditional *Salmonella* test, and the reproducibility among cultures and replicates, demonstrate that the Ames II test procedure using the Ames II tester strains with TA98 and TA1537, is an effective screen for identifying *Salmonella* mutagens.

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