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Salmonella Mutagenicity Test Results for 250 Chemicals

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INTRODUCTION

The Salmonella/mammalian microsome test developed by Ames and his associates [Ames et al, 1975] has become widely accepted as an initial test for the identification of chemicals with mutagenic activity. Substances that produce a positive mutagenic effect are considered to be potential animal mutagens and carcinogens and, by extension, potential human mutagens and carcinogens. This is because of the reported high correlations between mutagenicity in Salmonella, and genetic and carcinogenic effects in mammalian systems [McCann et al, 1975; Sugimura et al, 1976; Purchase et al, 1978; Rinkus and Legator, 1979; Bartsch et al, 1980]. However, chemicals that are not mutagenic in Salmonella cannot be considered benign. It has been well documented [McCann et al, 1975; Rinkus and Legator, 1979] that certain chemical classes, such as chlorinated hydrocarbons, contain a large number of carcinogens that are not mutagenic to Salmonella. Other chemicals are negative in the standard plate or preincubation protocol but are positive in protocols modified to achieve improved metabolism of the chemical to a mutagen or optimal exposure of the cells to the mutagen. Examples of these are the requirement for flavin mononucleotide (FMN) and reducing conditions or riboflavin for benzidine-based or other azo dyes [Prival and Mitchell, 1982; Hartman et al, 1978; Sugimura et al, 1977; Robertson et al, 1982], or the need for testing volatile liquids in a sealed chamber [Simmon et al, 1977].

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The testing of large numbers of chemicals for mutagenicity in Salmonella and other test systems is being performed within the National Toxicology Program (NTP) [Zeiger and Drake, 1980]. All chemicals selected for genetic toxicology screening are initially tested in Salmonella using a preincubation procedure [Yahagi et al, 1975], which is a modification of the Salmonella/mammalian microsome test of Ames et al [1975]. Additional tests—Drosophila melanogaster sex-linked recessive lethal and heritable translocation tests, induction of chromosome aberrations and sister chromatid exchanges (SCEs) in Chinese Hamster ovary (CHO) cells, and induction of mutation in L5178Y mouse lymphoma cells—are performed on selected chemicals based upon the needs of the NTP and results obtained from other mutagenicity test systems and carcinogenesis tests. The results obtained in these short-term mutagenicity tests will be used by the NTP in the prioritization of chemicals for subchronic or chronic toxicity testing, to assist in the evaluation of rodent test data, and to alert the cognizant government agencies and industries about potentially hazardous chemicals. A number of the chemicals were already reported by other workers as having been tested in Salmonella. Some appeared in the literature after the NTP program had begun; others were tested to develop a NTP data base for these chemicals or because they were members of a larger class of chemicals of interest.

The Salmonella tests were performed by three laboratories: Case Western Reserve University (CWR), Dr. William Speck; Microbiological Associates (formerly EG&G Mason Research Institute [EGG]), Dr. Steve Haworth; and SRI International (SRI), Dr. Kristien Mortelmans. Salmonella strains TA98, TA100, TA1535, and TA1537 were used in a modification of the preincubation test of Yahagi et al [1975]. The preincubation procedure was selected because of reports that it was no less sensitive than the plate test, and was more effective than the plate test for various chemicals such as aliphatic nitrosamines [Prival et al, 1979; Yahagi et al, 1975], pyrrolizidine alkaloids [Yamanaka, et al, 1979], and volatile chemicals [Rosenkranz et al, 1980]. Liver S-9 was prepared from male Sprague-Dawley rats (RLI) and Syrian hamsters (HLI) that were induced with Aroclor 1254 [Ames et al, 1975]. Hamster liver was used because of indications from a prior study (unpublished) and reports that the use of hamster S-9 would detect a number of chemicals undetected with rat S-9 [Prival and Mitchell, 1981; Bartsch et al, 1975; Sugimura, personal communication]. The protocol was standardized among the three laboratories, as discussed below. Each chemical was coded and tested as an unknown; the primary purpose of each test was to determine whether or not the chemical was mutagenic. This is why, in the case of a positive result, only the strains and activation systems that gave the positive results were repeated, not the entire series. The protocol was designed to allow the individual investigators the flexibility to change doses based on their interpretations of the results of the initial experiment. To monitor the performance of each laboratory, a set of positive and negative control chemicals was chosen. These chemicals were included, on a random basis, in batches of coded test chemicals sent to the testing laboratories. In addition to these controls, a small number of test chemicals selected, at random, were resubmitted to the same laboratory or sent to a second laboratory to determine interlaboratory reproducibility.

This publication is a presentation of Salmonella testing results on 250 coded chemicals, encompassing 370 tests (see Table I). The majority of these results were previously summarized in issues of the National Toxicology Program Technical Bulletin [1980a,b, 1981a,b, 1983]. However, some interpretations were changed since

publication in the NTP Bulletin, based upon a reevaluation of the data. The presentation here is designed both to summarize the results in the text and to present the data (Appendix 2) so that the reader has the opportunity of performing an independent evaluation of the data. Results from additional chemicals will be presented in future publications.

MATERIALS AND METHODS

Chemicals

The chemicals tested, their source, and purity (where known) are listed in Table I and their structures are given in Appendix 1. They were supplied to the testing laboratories by a chemical repository (Radian Corporation, Austin, TX), which was responsible for purchase and inventory of the chemicals, collection of physical, toxicological and safety data for each chemical, coding each test sample, shipment to the testing laboratories, and chemical analyses (when requested). Each sample sent out by the repository carried a unique, six-digit code number (Aliquot number) so that it could be tested under code as an unknown. The laboratories were also supplied with available information on the volatility, density, solubility, flammability, and stability of each chemical; Radian only performed solubility tests. Also sent, but in a sealed envelope coded with the Aliquot number, was the chemical name(s) along with the available information on its toxicological effects and decontamination procedures. The laboratories were instructed to open this envelope only in the event of a spill or exposure to the chemical and to treat all coded chemicals as potential mutagens and carcinogens. After completion of the testing, the unopened envelopes were returned to the Radian Corporation.

All chemicals were stored at the testing laboratories as recommended by the chemical repository. Each chemical was dissolved and diluted immediately prior to testing. The solvent of choice was distilled water; dimethyl sulfoxide (DMSO) was used if the chemical was insoluble or not sufficiently soluble in water. Ethanol (95%) or acetone was used if the chemical was not soluble or stable in DMSO.

As a rule, if a chemical was mutagenic or gave a questionable response, it was analyzed by Radian for identity and purity. Analyses had been performed previously by Midwest Research Institute (MRI) on selected other chemicals and on chemicals that had been tested in the National Cancer Institute's Carcinogenesis Bioassay Program. The results of these analyses are in Table I.

Bacterial Strains

Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 were obtained by the individual laboratories from Dr. Bruce Ames, University of California, Berkeley. Cultures of each tester strain were prepared for storage essentially as described in the Supplement to the Methods Paper [Ames et al, 1975] supplied with the tester strains by Dr. Ames. Frozen cultures were stored in liquid nitrogen (EGG) or in a −70°C freezer (CWR, SRI) in 0.2-ml aliquots (EGG), or in 1-ml aliquots (SRI) in sterile, screw-cap vials. To inoculate overnight cultures, CWR transferred a loopful of cells that were maintained on Columbia agar slants kept at 4°C into Columbia broth. EGG transferred a loopful of the thawed cultures into Oxoid Nutrient Broth #2 (CM 67) and discarded the unused portion of the thawed culture. SRI used all of the thawed 1-ml culture to inoculate minimal glucose medium [Vogel and

TABLE I. Sources, Purities, and Mutagenicities of 250 Chemicals in Salmonella (Continued)

	Chemical name	CAS number	Chemical source	Lot number
194	Pentachloroethane	76-01-7	Aldrich	CE 072487
195	Pentachloronitrobenzene	82-68-8	Aldrich	AC 071177
196	Pentachlorophenol	87-86-5	Aldrich	CC 022487
197	Phenol	108-95-2	Fluka; Textile Chem.	187130280; 79380
198	o-Phenyl phenol	90-43-7	Dow Chemical	MM09157
199	Phosphamidon	13171-21-6	Chevron	—
200	β-Picoline	108-99-6	MC/B	IIJ28
201	Picric acid	88-89-1	MC/B	8H09A
202	Piperazine	110-85-0	Eastman	A7C
203	Piperonal	120-57-0	Eastman	A8X
204	Piperonyl butoxide	51-03-6	FNC	—
205	Piperonyl sulfoxide	120-62-7	CPC International	291-N100-12
206	Prednisone	53-03-2	Upjohn	52836
207	β-Propiolactone	57-57-8	Tridom	196050 1178
208	1,2-Propylene glycol	57-55-6	MC/B	C6J13
209	Pyridine	110-86-1	Fisher	732113
210	Pyrimethamine	58-14-0	Burroughs-Wellcome	51416
211	Quinoline	91-22-5	Eastman	D6H
212	Resorcinol (1,3-benzenediol)	108-46-3	MC/B	C3J23
213	Ricinoleic acid, Na salt	5323-95-5	MC/B	D6F04
214	Semicarbazide HCl	563-41-7	Aldrich	EC 050287
215	Sodium fluoride	7681-49-4	Aldrich	DC 030887
216	Sodium phosphate, dibasic	7558-79-4	Fisher	783607
217	cis-Stilbene	645-49-8	Pfaltz & Bauer	—
218	trans-Stilbene	103-30-0	Aldrich	JC 072087
219	Streptomycin sulphate (2:3)	3810-74-0	Sigma	107C-0168
220	Sulfallate	95-06-7	Monsanto	H5215
221	1,2,3,4-Tetrachlorobenzene	634-66-2	Pfaltz & Bauer	—
222	1,2,3,5-Tetrachlorobenzene	634-90-2	Aldrich	101777
223	1,2,4,5-Tetrachlorobenzene	95-94-3	Pfaltz & Bauer	—
224	1,1,1,2-Tetrachloroethane	630-20-6	Aldrich	KE 7912KE
225	1,1,2,2,-Tetrachloroethane	79-34-5	Aldrich	060207
226	Tetrachloroethylene	127-18-4	Fisher	772783
227	1,2,3,4-Tetrachloronaphthalene	20020-02-4	Aldrich	DC 052347
228	2,3,4,5-Tetrachloronitrobenzene	879-39-0	Aldrich	090777
229	2,3,5,6-Tetrachloronitrobenzene	117-18-0	Aldrich	BB 120557
230	Tetramethyl lead	75-74-1	Ethyl Corporation	—
231	Thiocarbanilide	102-08-9	MC/B	11E22
232	Titanocene dichloride	1271-19-8	Pfaltz & Bauer	—
233	Toluene	108-88-3	Fisher	782825
234	Tributoxyethyl phosphate	78-51-3	Pfaltz & Bauer	—
235	Tributyl borate	688-74-4	MC/B	5738
236	1,2,3-Trichlorobenzene	87-61-6	Aldrich	HC 060787
237	1,2,4-Trichlorobenzene	120-82-1	MC/B	B3J08
238	1,3,5-Trichlorobenzene	108-70-3	Aldrich	KC 081087
239	1,1,1-Trichloroethane (chlorothene)	71-55-6	Fluka	31086 680
240	2,4,5-Trichlorophenol	95-95-4	Aldrich	JD 02197
241	2,4,6-Trichlorophenol	88-06-2	Eastman	B7X
242	1,2,3-Trichloropropane	96-18-4	Shell Chemical	JG 32449

TABLE I. Sources, Purities, and Mutagenicities of 250 Chemicals in Salmonella (Continued)

	Chemical name	CAS number	Chemical source	Lot number
243	Tricresyl phosphate (tritolyl phosphate)	1330-78-5	MC/B	C3J16
244	Tri-m-cresyl phosphate (TMCP)	563-04-2	Pfaltz & Bauer	T20605
245	Tris(2-chloroethyl)phosphate	115-96-8	Stauffer	0101F-1-3
246	Tris(2-chloroethyl)phosphite	140-08-9	Aldrich	KC 073187
247	m-Xylene	108-38-3	Eastman	B7B
248	o-Xylene	95-47-6	Aldrich	HD 061297
249	p-Xylene	106-42-3	Aldrich	CD 030197
250	Ziram	137-30-4	Uniroyal	—

[a]CWR, Case Western Reserve University; EGG, EG&G Mason Research Institute; SRI, SRI International.
[b]+, Positive, −, negative; ?, equivocal.
[c]None available.
[d]Purity based on Radian analysis.
[e]Purity based on MRI analysis.
[f]Two different lots tested.

Bonner, 1956] supplemented with biotin and an excess of histidine. All overnight cultures (late log phase) were obtained by incubation at 37°C on a shaker for 12–15 hr and were routinely checked for genetic integrity as recommended by Ames et al [1975].

Preparation of S-9 Fraction

Liver S-9 fractions were routinely prepared from male Sprague-Dawley rats and male Syrian hamsters that were injected, ip, with Aroclor 1254 (200 mg/ml in corn oil) at 500 mg/kg. Five days after injection, the animals were sacrificed by decapitation (EGG, SRI) or cervical dislocation (CWR) and the livers were removed aseptically. The animals were fasted for 12–24 hr immediately preceding sacrifice.

Liver homogenates were prepared aseptically at 0–4°C. Excised livers were rinsed with 0.15 M KCl, then minced and homogenized (3 ml of 0.15 M KCl/g wet tissue) in a Potter-Elvehjem apparatus with a teflon pestle (EGG, SRI) or in a Waring blender (CWR). The homogenate was centrifuged for 10 min at 9,000g at 4°C. The supernatant (S-9) was decanted and distributed into freezing ampules and stored at −70°C.

The microsomal enzyme reaction mix (S-9 mix) was prepared immediately prior to each assay. Unused S-9 mix was discarded and not refrozen. One milliliter of S-9 mix has the following composition: S-9, 0.10 ml; 0.04 M $MgCl_2$, 0.02 ml; 1.65 M KCl, 0.02 ml; 0.04 M β-nicotinamide adenine dinucleotide phosphate (NADP), 0.10 ml; 0.05 M glucose-6-phosphate, 0.10 ml; 1.0 M NaH_2PO_4 (pH 7.4), 0.10 ml; and distilled water, 0.56 ml.

Preincubation Methodology

All chemicals were tested using the preincubation procedure of the Salmonella assay [Ames et al, 1975] as described by Yahagi et al [1975]. Briefly, 0.5 ml of S-9

Label purity	Analyzed purity	Testing lab	Test result	
Technical		CWR,SRI	−,−	243
—		SRI	−	244
99.5%	98.2%[c]	SRI	−	245
—	87.8%[d]	CWR,SRI	+,+	246
Practical		EGG	−	247
97%		EGG	−	248
99%		EGG	−	249
—	86.2%[c]	SRI	+	250

mix or 0.1 M PO_4 buffer was dispensed into an appropriate number of 13 × 100 mm culture tubes maintained at 37°C in a dry-bath. Then, 0.05 ml of cells and 0.05 ml of solvent or chemical dilution were added to each tube. The mixture was vortexed and allowed to incubate with shaking in the early tests (CWR, EGG), or standing (SRI) for 20 min at 37°C. The protocol was later changed to eliminate the shaking procedure, because the commercial shakers available would not fit in the Class II, Type B hoods and, for the purposes of laboratory safety, it was inadvisable to incubate the chemicals at 37°C in the open laboratory. Following the preincubation period, 2.5 ml (EGG) or 2.0 ml (CWR, SRI) of molten top agar (45°C) supplemented with 0.5 mM L-histidine and 0.5 mM d-biotin was pipetted into the tubes, which were immediately vortexed, and their contents poured onto 25 ml of minimal glucose bottom agar [Vogel and Bonner, 1956] in a 15 × 100-mm plastic petri dish (Falcon Muta-Assay, 1028 [EGG, SRI] or Fisher Scientific petri dishes [CWR]). After the overlay solidified, the plates were inverted and incubated at 37°C for 48 h.

At least five doses of test chemical, in addition to the concurrent solvent and positive controls, were tested on each strain in the presence of S-9 mix or buffer. Three plates were used, and the experiment was repeated no less than 1 week after completion of the initial test.

Dose-Setting Experiment

To select the dose range for the mutagenesis assay, the test chemicals were checked for toxicity to TA100 up to a concentration of 10 mg/plate or the limit of solubility, both in the presence and absence of S-9 mix. One or more parameters were used as an indication of toxicity: viability on complete medium (EGG) and reduced numbers of revertant colonies per plate and/or thinning or absence of the bacterial lawn (CWR, EGG, SRI). If toxicity was not apparent in the preliminary toxicity determination, the highest dose tested was 10 mg/plate; otherwise the upper limit of solubility was used. If toxicity was observed, the doses of test chemical were chosen so that the high dose exhibited some degree of toxicity. Occasionally, in the earlier tests, the high dose was greater than 10 mg/plate.

Positive Controls

The positive control chemicals were tested concurrently with each test chemical. 2-Aminoanthracene (2-AA) was tested on all strains in the presence of rat and hamster S-9. 4-Nitro-o-phenylenediamine (NOPD) was tested on TA98 without S-9. Also without S-9, sodium azide (SA) was tested on TA100 and TA1535, and 9-aminoacridine (9-AAD) was tested on TA1537. The actual concentration for each positive control chemical used for each strain and activation condition was selected by the individual laboratory based on dose-response curves generated at the beginning of the testing program. The doses of the positive controls used by each laboratory are given in Table II.

Data Evaluation

Although procedures for the statistical analysis of Salmonella plate test data have been developed [Margolin et al, 1981], they were not incorporated into the initial data evaluations. The data were evaluated in an ad hoc manner by each testing laboratory and by NTP personnel. Prior to statistical analysis no formal rules were used; however, a positive response was indicated by a reproducible, dose-related increase, whether it be twofold over background or not. The matrix of test strains and activation systems used allowed the investigators to detect trends or patterns that might not be as evident if only one strain and activation system were examined. In addition to the standard "positive" and "negative" categories, there is also "questionable" (or "inconclusive"). This applied to low-level responses that were not reproducible within the laboratory or to results that showed a definite trend but with which the investigator did not feel comfortable in making a "+" or "−" decision. It also included tests in which an elevated revertant colony yield occurred at only a single dose level. After a decision on the mutagenicity of a sample was made, a request to decode the sample was sent to the repository, and the code was broken. The data were subsequently evaluated using an analysis based on the models presented by Margolin et al [1981]; this analysis will be described elsewhere [Risko et al, manuscript in preparation]. As a result of these statistical analyses, a number of calls were changed from the original "negative" to "equivocal." The statistical analysis did not result in any "positive" or "equivocal" calls being called "negative."

Because the criteria for "positive" or "questionable" decisions have evolved during the course of this study and because of the recent use of statistical analysis,

TABLE II. Concentrations of Positive Control Chemicals (μg/plate)

	TA98		TA100		TA1535		TA1537	
	−S-9 (NOPD)[a]	+S-9 (2-AA)	−S-9 (SA)	+S-9 (2-AA)	−S-9 (SA)	+S-9 (2-AA)	−S-9 (9AAD)	+S-9 (2-AA)
CWR	3.3	1.0	3.2	1.0	3.3	2.0	33.0	2.0
EGG	12.0	RLI 1.5[b] HLI 0.75	2.5	RLI 1.5 HLI 0.75	2.5	RLI 1.5 HLI 0.75	80.0	RLI 1.5 HLI 0.75
SRI	5.0	1.0	1.0	1.0	1.0	2.5	50.0	2.5

[a]NOPD, 4-nitro-o-phenylenediamine; 2-AA, 2-aminoanthracene; SA, sodium azide; 9AAD, 9-aminoacridine.

[b]Different concentrations for each S-9 source.

some of the results assigned in Table I differ from the initial evaluations published in the NTP Technical Bulletins [1980a,b, 1981a,b, 1982a,b, 1983]. The chemicals whose interpretations differ from those in the Bulletin are o-anisidine, boric acid (EGG Aliquot), 3-chloronitrobenzene, cyclohexanol, 2.4-dichlorophenol, 3,5-dichlorophenol, gallic acid (SRI Aliquot), glycerol (CWR Aliquot), and thiocarbanilide (SRI Aliquot).

RESULTS AND DISCUSSION

Summary results are presented in Table I and data in Appendix 2, Tables 1–250. The 250 chemicals tested encompass 370 separate samples (aliquots) tested under code using a standardized protocol. Fourteen of the chemicals were tested as coded, positive controls (AF-2, 2-aminoanthracene, 4-aminobiphenyl, benzo(a)pyrene, calcium chromate, cyclophosphamide, dimethylcarbamyl chloride, 3-methylcholanthrene, 4,4'-methylene-bis-2-chloroaniline, nitrofurantoin, N-nitrosodimethylamine, N-nitrosopiperidine, picric acid, and β-propiolactone) and five as coded, negative controls (choline chloride, glycerol, glycine, mannitol, and sodium phosphate). Streptomycin sulphate was originally chosen as a negative control, but was mutagenic in TA98 in two of the three laboratories testing it (see Appendix 2, Table 219.1,2,3). Among the 230 chemicals that were not originally selected as controls, 143 were clearly negative, 70 were clearly positive, and 17 were either questionable or did not show agreement between laboratories. These last 17 chemicals included five that were equivocal in one or both laboratories (o-anisidine, 3-chloronitrobenzene, cyclohexanol, 2,4-dichlorophenol, and 3,5-dichlorophenol). Of the remaining 12 chemicals, five were equivocal in one laboratory and positive or negative in the other(s). The final seven chemicals showed a definite disagreement between laboratories (p-anisidine, bromoform, 2,6-dimethylmorpholine, ethyl acrylate, ferrocene, isoproterenol hydrochloride, and 2-aminobiphenyl, which was negative in one laboratory the first time it was tested but positive the next).

It can be seen that, for the most part, there was good reproducibility between laboratories, even for relatively weak mutagens (Figs. 1–14). Occasionally, relatively large variations in the degrees of the response were seen, but it was difficult to determine to what extent these were a function of the laboratory or of the chemical/activation/Salmonella strain combination.

The negative control chemicals were all nonmutagenic in all tests with the exception of two positive responses from streptomycin sulphate and an equivocal response in one laboratory with glycerol. The positive controls were detected by all laboratories and were reproducible (Figs. 1–7).

The results presented in Appendix 2 are from the most definitive experiment conducted on each chemical. For the most part, the results from the confirmation (second) experiment are considered the most definitive. In a number of instances where the first or second experiment yielded weakly positive or questionably positive data, or the succeeding experiments were in disagreement with the first experiment, data from all of the experiments are presented. In all cases, however, if the reader wants data on a specific chemical in addition to that presented in Appendix 2, the specific testing laboratory should be contacted directly.

The majority of chemicals judged positive or questionable induced a response in TA100 with or without additional positive responses in one or more of the other

strains. A number of chemicals produced higher responses with hamster S-9 (for example, see Figs. 6, 7, 15–19), and vice versa (Figs. 12, 20–22), but a few were positve only with rat or hamster S-9, and some only in the absence of S-9. Substituted nitrobenzenes generally exhibited their strongest mutagenic responses in the presence of hamster S-9. The chlorinated nitrobenzenes were generally quite toxic to the tester strains, which limited the concentrations of chemical that could be tested. Two of these chemicals (2- and 4-chloronitrobenzene) were also tested (EGG) using the plate incorporation method (data not shown). Under these test conditions, higher concentrations of the chemicals could be tested. As might be expected, greater mutagenic activity was observed using the plate incorporation method. These data would indicate that when testing very toxic chemicals, negative or equivocal results with the preincubation method may require confirmation with the plate incorporation method. The similarities in the dose responses of azobenzene and hydrazobenzene (Fig. 21) suggest that both may be mutagenic via the same metabolic product. No information is available on the comparative metabolism of these substances.

The results from this testing can also be used to determine the extent of interlaboratory variability and, with some chemicals, intralaboratory variability as well. Representative examples of the degree of agreement between and within laboratories can be seen in the results on AF-2 (Fig. 1), 2-aminoanthracene (Fig. 2), 2- and 4-aminobiphenyl (Fig. 3), calcium chromate (Fig. 4), 2- and 4-chloronitrobenzene (Fig. 8), chloropicrin (Fig. 9), 2,3-dichloronitrobenzene (Fig. 10), dimethoate (Fig.11), 3,3′-dimethoxybenzidine (Fig. 12), ethylenediamine (Fig. 13), formaldehyde (Fig. 14), nitrofurantoin (Fig. 5), N-nitrosodimethylamine (Fig. 6), and N-nitrosopiperidine (Fig. 7).

Although we have not attempted to measure the extent of agreement between tests mathematically, it can be seen to vary with the chemical and is not necessarily related to the magnitude of the mutagenic response. Also, as seen with 2-aminoanthracene (cf TA100 and TA1535, Fig. 2C, D vs E, F), the variability can also be derived from the particular Salmonella strain examined.

In many instances, disagreements between laboratories occurred because the chemicals were coded. These disagreements may be relatively subtle and the result of low levels of activity in one laboratory versus questionable or no activity in the other. Many of these differences might have disappeared if the laboratory with the lower or negative response knew of the other data and adjusted the protocol accordingly. Other chemicals exist that are clearly positive in one laboratory and negative in another; the reasons for these differences are not obvious and would have to be investigated on an individual chemical basis.

A large number of chemicals were positive in TA100 but not in TA1535; a few were positive in TA1535 but not TA100. The chemicals that were positive in both strains usually showed two types of responses. The first type is best exemplified by 1-aziridine ethanol (Appendix 2, Table 22; Fig. 23). In this response, the mutagen induces approximately the same absolute numbers of revertants in both TA1535 and TA100; the only difference is that in TA100, the revertants appear against the higher background reversion frequency. Chemicals of this type which induce only low levels of revertants may be detected more readily using TA1535, and may be considered negative in TA100 if the number of induced revertants falls within or close to the range of spontaneous revertants; for example, ethylenediamine (Appendix 2, Table 123; Fig. 13) and N-nitrosopiperidine (Appendix 2, Table 185; Fig. 7). The other

type of response is the one in which mutagenicity is observed only in TA100, such as with chloropicrin (Appendix 2, Table 63; Fig. 9), dimethoate (Appendix 2, Table 107; Fig. 11), chloronitrobenzenes (Appendix 2, Tables 53–55; Figs. 8, 10) and others, or a higher number of revertants is induced in TA100 than in TA1535 (1,2,3,-trichloropropane [Appendix 2, Table 242; Fig. 17] and 2-aminoanthracene [Appendix 2, Table 10; Fig. 2]). These responses are not unexpected and reflect the mechanisms of action of the mutagens and the degree to which the error-prone repair system coded for by the pKM101 plasmid recognizes the DNA adduct produced.

Limitations

In any study using coded chemicals with fixed protocols, one would expect to obtain negative results for chemicals that have been reported elsewhere as mutagenic. This is because, with a coded chemical, the testing laboratory does not have any preconceptions about the "expected" responses based upon knowledge of the chemical's structure or responses in other biological systems. Having this knowledge affords the researcher the ability to test at varying dose ranges with different levels and types of S-9 and in varied protocols if the anticipated result is not obtained in the standardized protocol. In a study of the type reported here,the testing laboratory does not have the luxury of knowing the "expected" response; therefore, some of the chemicals that are reported here as nonmutagenic may have been reported as positive in the literature.

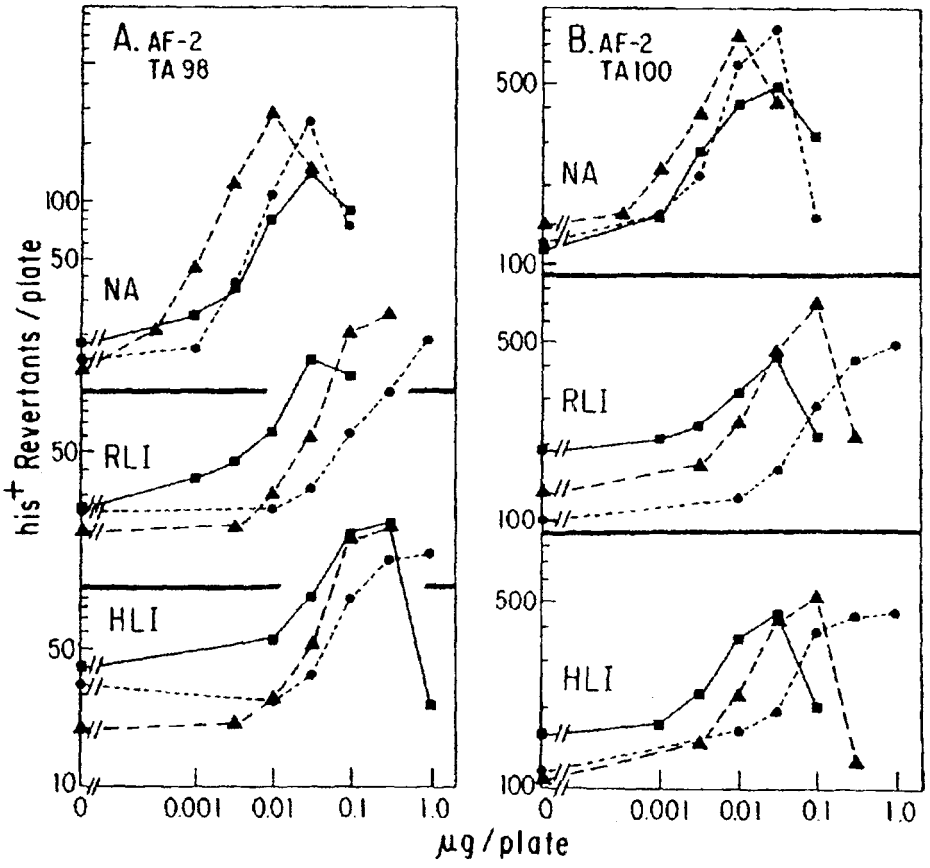

Fig. 1. Mutagenicity of AF-2 in S typhimurium TA98 (A) and TA100 (B) using no metabolic activation (NA), RLI, and HLI at CWR (■), EGG (▲), and SRI (●).

APPENDIX 2

Mutagenic responses of Salmonella tester strains TA100, TA1535, TA1537, and TA98 (mean ± SEM) to test chemicals. Chemicals are numbered as in Table I (pp. 6–17). Doses are in μg/plate; 0.0 dose is the solvent control.

Where only one test is reported for one chemical/laboratory combination (Aliquot), only the final test data are presented. If more than one Aliquot of the chemical was tested, the different aliquots are designated .1,.2, etc. Where more than one test from the same aliquot is reported, they are labeled with lower case letters, eg 1a . . . where a is the earliest data set.

Abbreviations are as follows: DMSO, Dimethyl sulfoxide (solvent); H_2O, Distilled water (solvent); 95% ETOH, 95% Ethanol (solvent); POS, Positive control (see Table II); CWR, Case Western Reserve University; EGG, EG&G Mason Research Corporation; SRI, SRI International; NA, not activated; RLI, rat liver S-9, Aroclor 1254 induced; HLI, hamster liver S-9, Aroclor 1254 induced; s, slight clearing of background lawn; t, complete clearing of background lawn; p, precipitate present in plates.

TABLE 1 ACETAMIDE [H2O] [EGG]

Dose	TA100 NA	TA100 RLI	TA100 HLI	TA1535 NA	TA1535 RLI	TA1535 HLI	TA1537 NA	TA1537 RLI	TA1537 HLI	TA98 NA	TA98 RLI	TA98 HLI
0.0	105± 1.8	123± 10.3	96± 5.7	17± 1.3	9± 2.1	11± 1.0	6± .9	8± 1.8	8± 2.5	20± 1.9	19± 3.5	18± 2.2
100.0	89± 5.0	119± 6.3	100± 4.3	17± 1.8	7± 1.5	12± 2.7	12± 3.4	4± .7	7± 2.0	19± 3.5	28± 1.3	25± 2.1
333.0	110± 16.1	100± 9.8	102± 2.7	17± 2.0	7± .6	8± 2.1	7± .9	8± 1.5	4± 1.2	22± .7	28± 2.2	20± 4.3
1000.0	96± 5.8	107± 8.2	105± 10.0	19± 1.7	10± 2.5	10± 1.5	6± 3.1	8± 2.8	8± 1.0	16± 2.3	27± 3.6	21± 2.0
3333.0	99± 4.9	94± 7.9	108± 8.5	21± 3.0	10± 1.3	10± 3.5	6± .3	9± 2.3	5± 1.9	23± 4.1	25± 4.3	19± 1.5
10000.0	102± 11.0	88± 5.8	93± 3.2	17± 2.6	9± 1.5	10± 1.5	9± 1.0	11± 2.1	5± 1.3	17± 1.7	27± 2.3	21± 3.1
POS	1432± 85.3	690± 82.6	1406± 64.9	1235± 75.6	50± 1.2	101± 2.3	178± 15.6	88± 4.1	154± 8.7	1605±193.3	802± 85.9	1083± 52.8

TABLE 2 ACETIN [H2O] [SRI]

Dose	TA100 NA	TA100 RLI	TA100 HLI	TA1535 NA	TA1535 RLI	TA1535 HLI	TA1537 NA	TA1537 RLI	TA1537 HLI	TA98 NA	TA98 RLI	TA98 HLI
0.0	97± 3.0	97± 3.5	108± 2.3	20± 3.1	10± 1.2	11± 3.1	5± .9	7± .7	6± .6	22± 3.2	35± 2.3	37± 3.4
100.0	82± 13.4	111± 1.8	116± 3.5				2± .3	6± .7	4± 1.0	31± 5.0	29± 5.3	48± 2.6
333.3	86± 7.2	98± 3.2	99± 7.8	17± 1.2	15± 3.2	22± 4.8	4± 1.2	6± 1.5	6± 1.3	24± 3.3	31± 1.2	35± 1.9
1000.0	89± 8.2	111± 10.3	119± 4.5	25± 2.0	20± 2.6	30± 1.5	3± .3	4± .6	7± 1.7	24± 2.7	31± 1.5	35± 3.3
3333.3	96± 5.3	97± 4.4	138± 10.5	42± .3	58± 3.5	68± 5.0	4± .6	6± .6	4± .9	25± 2.3	32± 5.7	25± 3.0
6666.7				79± 2.9	84± 6.0	117± 3.2						
10000.0	104± 6.4	133± 4.4	139± 9.8	106± 2.4	123± .3	149± 4.7	3± 1.2	4± 0.0	3± 0.0	20± 4.0	35± 2.1	31± 6.2
POS	268± 16.6	1492± 61.4	2368± 57.7	352± 17.7	211± 12.4	112± 14.0	278± 85.5	494± 38.2	522± 52.2	550± 97.1	1033± 57.8	1809±123.0

TABLE 3 P-ACETOPHENETIDIDE [DMSO] [CWR]

Dose	TA100 NA	TA100 RLI	TA100 HLI	TA1535 NA	TA1535 RLI	TA1535 HLI	TA1537 NA	TA1537 RLI	TA1537 HLI	TA98 NA	TA98 RLI	TA98 HLI
0.0	97± 6.8	121± 6.8	122± 11.6	6± .9	6± 2.1	6± 3.0	2± 1.2	6± 0.0	6± 1.2	15± 1.9	22± 1.2	19± .6
33.0	96± 3.8	121± 4.2	107± 6.9	3± .3	7± 1.2	8± 1.3	3± .7	5± .3	5± 1.2	9± 1.9	21± 3.3	20± 2.1
100.0	94± 6.8	103± 5.6	118± 9.3	3± 1.2	6± 1.2	7± .9	2± .9	5± 1.0	4± 1.5	14± 1.3	18± 2.4	21± 2.3
333.0	90± 2.8	110± 7.4	109± 4.8	4± .3	4± .9	7± .7	3± 1.2	5± 1.7	7± 1.5	18± 1.5	15± 4.3	17± 4.6
1000.0	87± 7.4	119± 2.7	124± 3.7	3± 1.3	4± .9	5± .9	3± .6	4± .3	7± 1.2	13± 4.3	18± 1.5	19± 4.7
2730.0	90± 5.9	105± 4.3	123± 11.6	3± .6	3± .9	7± .6	2± .6	8± 1.2	5± .6	15± 1.0	23± 2.9	25± 2.9
POS	459± 23.9	1057±174.8	1818±296.3	212± 33.8	38± 2.1	30± 3.7	398± 32.0	109± 2.3	107± 1.8	278± 25.4	626±139.2	984±100.2

TABLE 235.2 TRIBUTYL BORATE [ACETONE] [EGG]

Dose	TA100 NA	TA100 RLI	TA100 MLI	TA1535 NA	TA1535 RLI	TA1535 MLI	TA1537 NA	TA1537 RLI	TA1537 MLI	TA98 NA	TA98 RLI	TA98 MLI
0.0	111± 10.2	101± 3.7	99± 8.8	15± 2.3	9± 1.5	9± .9	7± 2.1	4± .7	6± 2.0	20± 5.2	17± .3	21± 1.5
77.0	118± 5.0	95± 8.2	102± .3	13± 1.7	9± 2.9	8± .6	6± 1.5	5± 1.3	6± 2.6	19± 2.8	15± 1.0	20± 2.9
256.7	106± 6.8	109± 7.5	97± 12.8	14± 2.1	7± .6	7± 1.2	5± .9	6± .3	8± 1.5	28± 3.3	18± 2.1	19± 2.8
770.0	112± 8.1	123± 8.8	114± 5.3	12± .9	7± .9	10± 1.5	8± .7	5± 1.8	5± .6	19± .6	19± 3.0	21± 1.2
2566.7	94± 9.8	106± 5.8	119± 1.8	10± 1.0	12± 2.6	7± .9	5± 2.0	7± 1.0	8± 1.5	15± 1.8	18± 3.3	23± 3.2
7700.0	81± 6.7	80± 5.9	103± 2.7	8± .9	8± .3	8± .3	3± .5	6± 1.3	t	7± 2.0	11± 2.0	11± 3.8a
POS	1338± 50.7	1862± 63.3	2536± 66.2	817± 19.0	149± 7.5	199± 6.9	393± 85.8	139± 10.2	81± 8.8	2515±139.9	1618± 62.3	2421± 74.8

TABLE 236 1,2,3-TRICHLOROBENZENE [DMSO] [SRI]

Dose	TA100 NA	TA100 RLI	TA100 MLI	TA1535 NA	TA1535 RLI	TA1535 MLI	TA1537 NA	TA1537 RLI	TA1537 MLI	TA98 NA	TA98 RLI	TA98 MLI
0.0	124± 9.9	138± 7.0	131± 7.3	25± 6.0	12± 2.9	18± .9	11± 2.7	20± .3	26± 3.5	25± 2.8	22± 5.0	35± 3.8
3.3	122± 13.2	122± 9.7	114± 4.1	17± 1.7	10± 2.2	16± 2.0	8± 2.1	15± 3.2	18± 3.0	20± 3.0	26± 2.2	32± 1.0
10.0	134± 18.0	131± 7.5	122± 7.2	20± 8.6	18± .3	13± .3	12± 1.3	18± 3.5	22± 3.0	16± 8.8	28± 1.7	31± 3.2
33.3	91± 3.8	131± 11.6	135± 10.6	17± 2.3	18± 2.9	16± 1.5	6± 1.3	13± 3.8	18± 1.5	20± .7	23± 3.7	32± 5.1
100.0	71± 6.9a	91± 6.8	124± 10.2	0± 0.0a	8± 1.5	14± 3.8	0± 0.0a	11± 1.8	21± 3.3	10± 2.9a	27± .6	31± 2.0
333.3	t	t	80± 2.8a	t	4± 2.2a	6± .7a	t	0± 0.0a	6± 1.0a	t	6± 3.2a	18± 6.2a
POS	550± 8.1	596± 29.5	556± 9.5	438± 5.3	338± 51.3	367± 6.2	163± 28.0	287± 3.6	467± 13.0	478± 16.3	388± 25.1	934± 19.9

TABLE 237 1,2,4-TRICHLOROBENZENE [DMSO] [SRI]

Dose	TA100 NA	TA100 RLI	TA100 MLI	TA1535 NA	TA1535 RLI	TA1535 MLI	TA1537 NA	TA1537 RLI	TA1537 MLI	TA98 NA	TA98 RLI	TA98 MLI
0.0	99± 5.2	113± 2.0	115± 8.7	20± 1.0	13± 3.0	13± .6	11± 1.5	18± 1.9	12± 2.7	37± 3.5	35± 2.3	37± 4.7
3.3	104± 4.0	107± 5.5	124± 2.0	16± 1.5	15± 2.5	13± 2.9	16± 3.8	10± 1.5	18± 5.2	38± 6.8	38± 2.3	33± .9
10.0	86± 6.7	115± 9.5	132± 5.8	18± 8.2	9± 0.0	10± 2.7	23± 2.7	11± 1.2	23± 3.7	28± 1.2	36± 4.8	35± 2.7
33.3	61± 10.1a	123± 2.2	124± 7.3	19± 1.2	13± 3.2	11± 1.7	11± 1.7	9± .3	17± .7	28± 1.7	29± .9	43± 2.3
100.0	t	112± 6.7	100± 6.9	6± 6.0a	9± 1.8	12± 2.7	9± 1.8	8± 1.7	17± 8.0	24± 1.3	34± 8.1	38± 6.4
333.3	t	114± 9.8	21± 21.5a	t	13± .7a	6± 1.5a	5± 2.3	7± 1.2	0± 0.0a	17± 2.5	34± 3.7	9± 8.5a
POS	625± 28.8	460± 15.3	963±104.3	488± 23.0	305± 3.2	266± 38.8	308± 8.0	158± 10.6	248± 5.8	850± 18.0	288± 3.0	1258±130.5

TABLE 238 1,3,5-TRICHLOROBENZENE [DMSO] [SRI]

Dose	TA100 NA	TA100 RLI	TA100 MLI	TA1535 NA	TA1535 RLI	TA1535 MLI	TA1537 NA	TA1537 RLI	TA1537 MLI	TA98 NA	TA98 RLI	TA98 MLI
0.0	124± 15.0	176± 1.2	108± 11.7	15± 2.8	18± 1.3	15± 1.7	24± 2.3	35± 5.2	26± 4.8	25± 4.8	41± 1.2	37± 5.8
33.3	99± 12.5	157± 1.8	126± 8.5	18± 3.2a	13± 2.0	8± .6	10± 1.2	30± 1.2	25± 3.5	18± .9	41± 1.2	36± 6.9
100.0	59± 1.8a	152± 2.8	117± 12.8	3± 1.3a	11± 2.0	9± 2.9	3± .6a	21± 3.3	25± 4.7	13± .6a	33± 4.8	39± 1.8
333.3	5± 5.3a	131± 2.3	101± 11.9	t	11± 2.8a	16± 1.5	0± 0.0a	10± 2.3a	13± 1.0	3± .3a	29± 2.0	31± 3.3
1000.0	28± 6.9a	115± 7.3a	81± 11.6a	0± 0.0a	7± 1.5a	13± 3.0	0± 0.0a	9± 1.7a	12± 1.2a	6± 1.3a	27± 4.5a	19± 1.5a
3333.3	62± 22.4a	121± 7.0a	70± 5.0a	6± 1.8a	6± 1.0a	11± 1.7	0± 0.0a	10± 1.3a	5± .6a	13± .3a	25± 4.3a	18± 1.0a
POS	617± 21.7	621± 5.1	442± 16.7	515± 8.9	197± 2.8	303± 8.8	136± 26.0	284± 7.2	322± 36.7	706± 18.6	538± 68.7	878± 31.9

TABLE 239.1 1,1,1-TRICHLOROETHANE (CHLOROTHENE) [DMSO] [CWR]

Dose	TA100 NA	TA100 RLI	TA100 MLI	TA1535 NA	TA1535 RLI	TA1535 MLI	TA1537 NA	TA1537 RLI	TA1537 MLI	TA98 NA	TA98 RLI	TA98 MLI
0.0	199± 8.8	240± 16.3	271± 5.9	6± .3	6± 1.3	7± 2.0	5± 1.2	13± 1.5	8± .9	22± 1.5	29± 1.2	23± 3.2
33.0												27± 2.2
100.0	176± 13.8	284± 6.6	323± 9.1	4± .7	7± 1.3	7± 1.9	4± .7	11± 2.7	7± 1.9	21± 1.8	28± .7	26± 8.7
333.0	193± 6.1	292± 15.6	331± 23.0	5± .3	7± 0.0	7± 1.2	6± .7	12± 2.7	9± 1.5	24± 3.6	31± 1.9	29± .9
1000.0	204± 15.2	290± 11.1	300± 12.9	4± .7	8± 1.3	7± 1.5	4± .9	11± 4.2	9± .8	20± 1.0	35± 2.8	28± 3.3
3333.0	203± 33.3	287± 15.9	292± 5.9	3± 0.0	5± 1.2	5± 1.5	5± .6	3± .3	8± .9	24± 1.3	26± 8.9	30± 1.8
10000.0	180± 13.8	293± 18.9	165± 30.8	1± 0.0	3± .6	4± 2.5	4± 0.0	4± 2.0	4± .9	22± 2.8	6± 3.3	
POS	867± 63.7	1126± 47.1	1905± 56.8	936± 79.2	99± 12.2	116± 16.2	83± 9.3	120± 1.2	163± 9.5	461± 49.0	645± 12.6	1946±183.3