REPORT

SYBR Safe™ DNA Gel Stain

Assessment of Mutagenicity and Environmental Safety

Compiled by Molecular Probes, Inc., from the results of two independent testing services:
Covance, Inc.
AMEC Earth & Environmental, Inc.

Executive Summary

SYBR Safe DNA gel stain does not induce transformations in primary cultures of Syrian hamster embryo (SHE) cells when compared with solvent alone, strongly indicating that the SYBR Safe stain is noncarcinogenic. In contrast, ethidium bromide tests positive in the SHE cell assay, consistent with its known activity as a strong mutagen.

SYBR Safe stain does not cause mutations in mouse lymphoma cells at the TK locus, nor does it induce chromosomal aberrations in cultured human peripheral blood lymphocytes, with or without S9 metabolic activation, using standardized tests against appropriate controls.

Compared to ethidium bromide, **SYBR Safe DNA gel stain causes fewer mutations in the standard Ames test**, as measured in several different strains of *Salmonella typhimurium*. Weakly positive results for SYBR Safe stain in this test occurred in four out of seven strains and only with activation by a mammalian S9 fraction.

A single oral administration of **SYBR Safe DNA gel stain produces no signs of mortality or toxicity** at a limit dose of 5000 mg/kg.

Based on extensive environmental safety testing, **SYBR Safe DNA gel stain is not classified as hazardous waste under U.S. Federal regulations** (Resource Conservation and Recovery Act (RCRA)). **SYBR Safe stain meets the requirements of the Clean Water Act** and the National Pollutant Discharge Elimination System (NPDES) regulations.

Covance Results

Northview Pacific Laboratories Results

AMEC Results

Columbia Analytical Services Results

Covance Results – Forward Mutation

The test article formed a transparent orange solution in DMSO at 62.5 mg/mL and formed an opaque suspension at higher concentrations. In treatment medium, SYBR Safe stain formed a precipitate at and above 78.5 µg/mL at treatment termination.

A preliminary dose rangefinding assay was initiated with ten treatments at 1.24, 2.47, 4.93, 9.85, 19.7, 39.3, 78.5, 157, 313 and 625 µg/mL. Cells were exposed to the test article for 4 hours in the presence and absence of rat liver S9 metabolic activation. Since cytotoxicity was observed on Day 1 with and without activation, the study was continued to determine if a good toxicity range could be obtained for a mutation assay using the cells from the dose rangefinding assay.

On Day 2, under nonactivation conditions, all treatments were terminated due to excessive cytotoxicity. Because a good range of cytotoxicity was not achieved for the nonactivation assay, the dose rangefinding assay was terminated prior to cloning and a mutation assay was initiated.

In the mutation assay without activation, eight treatments at 0.0313, 0.0625, 0.125, 0.250, 0.500, 0.750, 1.00 and 1.50 µg/mL were initiated, and three treatments at 0.125, 0.250 and 0.500 µg/mL were analyzed for mutant induction (Tables 9 and 10). SYBR Safe stain induced no cytotoxicity at 0.125 µg/mL and moderately high cytotoxicity at 0.250 µg/mL (80.3% and 26.5% relative total growth, respectively). A small increase in concentration from 0.250 to 0.500 µg/mL was excessively cytotoxic. The minimum criterion for a positive response in this nonactivation assay was 97.9×10^{-6}. No increases in the mutant frequency were observed that exceeded the minimum criterion.

Under activation conditions, treatments from 9.85 to 625 µg/mL were terminated due to excessive cytotoxicity. The remaining treatments were used for the mutation assay. Treatment at 1.24 µg/mL induced weak cytotoxicity, treatment at 2.47 µg/mL induced moderate cytotoxicity, and treatment at 4.93 µg/mL induced excessive cytotoxiciy (relative total growth of 65.1%, 47.1% and 7.6%, respectively). In the mutation assay, no increases in the mutant frequency were observed that exceeded the minimum criterion of 145.1×10^{-6}. Results of the mutation assay with SYBR Safe stain under activation conditions are shown in Tables 11 and 12.

SYBR Safe stain was evaluated as negative with and without metabolic activation in the L5178Y TK $^{+/-}$ Mouse Lymphoma Forward Mutation Screening Assay.

(See Tables 1-4.)

Covance Results – Transformation

At the request of Molecular Probes, Inc., Covance investigated the ability of SYBR Safe stain for inducing an increase in morphological transformation of cultured Syrian hamster embryo (SHE) cells, relative to vehicle control cultures, following a 7-day exposure period. Cryopreserved SHE cell stock prepared at Covance-Vienna from embryo cells obtained from time-pregnant Syrian golden hamsters at 13 to 13.5 days gestation was used for this assay.

Test Article Handling

The test article, SYBR Safe stain, was stored at room temperature with desiccant. Dimethylsulfoxide (DMSO, CAS No. 67-68-5, Acros Organics, Lot No. A017190501) was used as vehicle control. The test article formed a red, transparent solution in DMSO at 5 mg/mL. Orange, transparent solutions were obtained when the test article was dosed into media at concentrations of 3.33 and 10.0 µg/mL. At concentrations of 1.00 µg/mL and lower, the test article formed transparent solutions with normal media color. At a concentration of 10.0 µg/m, the test article did not have significant effect on pH and osmolality of the culture medium. Both pH and osmolality values were within acceptable range.

Dose Range-finding Study

A preliminary dose range-finding study was initiated with six treatments from 0.0333 µg/mL to 10.0 µg/mL (Table 1). Noncytotoxicity was observed at a test article concentration up to 0.100 µg/mL. The test article was moderately cytotoxic at 0.333 µg/mL and was excessively cytotoxic at higher doses. Based on the results, three doses ranging from 0.200 to 0.700 µg/mL were tested in the initial trial of the transformation assay.

Transformation Assay

The following three doses were tested in the initial trial of the transformation assay: 0.200, 0.400, and 0.700 µg/mL. However, this trial failed due to higher than expected cytotoxicity. A second trial was performed with the following concentrations: 0.0500, 0.150, and 0.300 µg/mL. Results of this trial of transformation was summarized by Table 2. SYBR Safe stain was essentially noncytotoxic at 0.0500 µg/mL (120% RPE), slightly cytotoxic at 0.150 µg/mL (88% RPE) and moderately cytotoxic at 0.300 µg/mL (59% RPE). None of the three treatment groups induced a significant increase in the frequency of morphological transformation compared to the concurrent vehicle control. In addition, a significant increase of the morphological transformation frequency was also obtained from the positive control treatment with benzyo[α]pyrene at 5.0 µg/mL. The test article was therefore evaluated as negative in the screening SHE cell transformation assay under 7-day exposure conditions of this study.

CONCLUSION

The test article, SYBR Safe stain, tested in the SHE cell cultures with a 7-day exposure, was evaluated as negative in the screening SHE cell transformation assay under 7-day exposure conditions of this study.

(See Tables 5-6.)

Covance Results – Chromosomal Aberration

At the request of Molecular Probes, Inc., Covance investigated the ability of SYBR Safe stain induce chromosomal aberrations in cultured human peripheral blood lymphocytes with and without exogenous metabolic activation. The assay was initiated both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor™-induced rat liver (S9).

Most known chemical clastogens (chromosome-breaking agents) require a period of DNA synthesis to convert initial DNA damage into chromosome alterations that become visible at mitosis. The lymphocytes in blood do not usually divide, but they were stimulated to divide in culture by exposure to phytohemagglutinin (PHA-M). At predetermined intervals after exposure to the test article, the lymphocytes were treated with a metaphase-arresting substance, Colcemid®, then were harvested and stained, and metaphase cells were analyzed microscopically for the presence of chromosomal aberrations.

Many mutagenic chemicals do not act directly on DNA but do so after being converted to active intermediates by enzymes found in liver. Human lymphocytes have only a limited capacity to metabolize some test articles, so an exogenous metabolic activation system (rat liver S9 homogenate) was included with a series of treatments to enhance the degree of conversion and the ability of the assay to detect clastogenic, metabolic intermediates.

This study evaluated structural chromosomal aberrations (defined as structural chromosome damage expressed as breakage, or breakage followed by reunion, of both sister chromatids at an identical site). Numerical aberrations (a change in the number of chromosomes from the modal number of 46 for the human cells) were not determined. However, the occurrence of polyploidy or endoreduplication, which was scored, may indicate that the test article has the potential to induce numerical aberrations.

The in vitro metabolic activation system (Maron and Ames, 1983) consisted of S9 and an energy-producing system (NADP plus isocitric acid). Various hepatic P450 isoenzyme levels were increased by treatment of the rats with Aroclor™ 1254 (single concentration of 500 mg/kg) and sacrificed 5 days later (Molecular Toxicology, Inc., Lot No. 1393). The S9 fraction, prepared in potassium chloride, was retained frozen at ≤-60°C until use. Aliquots of S9 were thawed immediately before use and added to the other components to form the activation system described as follows:

S9 Activation System

Component	Concentration in Cultures
NADP (sodium salt)	1.5 mg/mL (1.8 mM)
Isocitric acid	2.7 mg/mL (10.5 mM)
Homogenate (S9 fraction)	15.0 µL/mL* (1.5%)

* This concentration of rat S9, obtained from Molecular Toxicology Inc., Boone, NC, has consistently caused cyclophosphamide to be highly clastogenic.

Human venous blood from a healthy adult donor (nonsmoker without a history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections) was drawn into sterile, heparinized "vacutainers". Whole blood cultures were initiated in 15 mL centrifuge tubes by adding approximately 0.3 mL of fresh heparinized blood into a sufficient volume of culture medium so that the final volume is 5 mL in the assay without metabolic activation after the addition of the test article in its chosen vehicle or is 5 mL in the assay with metabolic activation after the addition of the test article in its chosen vehicle and the S9 mix.

Cultures were initiated in 15 mL tubes and were incubated with loose caps at 37°C ± 2°C in a humidified atmosphere of 5% ± 1.5% CO_2 in air. The medium was RPMI 1640 supplemented with approximately 20%

heat-inactivated fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and 2% phytohemagglutinin M (PHA-M). Single cultures were used for each dose of the test article.

The dose rangefinding assay was conducted with a ~3-hour treatment in the presence of S9 and a ~22-hour treatment in the absence of S9. All cultures were harvested ~22 hours from the initiation of treatment. This harvest time corresponds to 1.5 times the cell cycle time of approximately 15 hours (Galloway *et al.*, 1994). If a dose level with adequate toxicity for a valid high dose was available from the dose-rangefinding assay, the chromosomal aberrations assay was not conducted and chromosomal aberrations were evaluated from the dose selected for analysis.

The chromosomal aberrations assay was conducted for those test articles and exposure conditions where a valid high dose was not available from the dose-rangefinding assay. This assay was also conducted with a ~3-hour treatment in the presence of S9 and a ~22-hour treatment in the absence of S9. All cultures were harvested ~22 hours from the initiation of treatment.

RESULTS

Test Article Handling

The dosing solutions were prepared in dimethylsulfoxide (DMSO; Acros Organics, Lot No. A017190501). The tes article was solubilized in DMSO at stock concentrations 100-fold higher than the dose in tissue culture medium. Lower doses were obtained by serial dilutions of the stocks with DMSO. A dose volume of 10.0 µL/mL was used. The 100 mg/mL stock of SYBR Safe stain was a dark, orangish-red, transparent solution.

A summary of the treatment times is given below.

Summary of Rangefinding/Chromosomal Aberrations Assay Treatment Schedule in Hours (Approximate)

Activation Condition	Test Article Added	Wash	Colcemid Added	Harvest Started
– S9	0	—	20	22
+ S9	0	3	20	22

In the dose rangefinding assay, concentrations of 7.81, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL were tested with and without S9.

In the assay without S9, excessive toxicity was observed at all doses tested (Table 1). Based on these data, a chromosomal aberrations assay was conducted testing concentrations of 0.500, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.0 µg/mL. In this trial, toxicity was observed at ≥2.00 µg/mL (Table 2). Structural chromosomal aberrations were evaluated at 1.00 µg/mL (Table 3). No significant increase in the number of cells with structural aberrations, polyploidy, or endoreduplication was observed.

In the assay with S9, excessive toxicity was observed at ≥15.6 µg/mL (Table 4). Structural chromosomal aberrations were evaluated at 7.81 µg/mL (Table 5). No significant increase in the number of cells with structural aberrations, polyploidy, or endoreduplication was observed.

CONCLUSION

SYBR Safe stain was considered negative for inducing structural chromosomal aberrations with and without metabolic activation.

REFERENCES

Evans, H.J., Chromosomal aberrations produced by ionizing radiation. International Review of Cytology, 13:221-321 (1962).

Evans, H.J., Cytological Methods for Detecting Chemical Mutagens. Chemical Mutagens, Principles and Methods for their Detection, Hollaender, A. (ed.), Vol. 4, pp. 1-29, Plenum Press: New York and London (1976).

Galloway, S.M., Aardema, M.J., Ishidate, M., Jr., Ivett, J.L., Kirkland, D.J., Morita, T., Mosesso, P., and Sofuni, T., Report from working group on in vitro tests for chromosomal aberrations. Mutation Research, 312(3):241-261 (1994) .

Maron, D.M., and Ames, B.N., Revised methods for the Salmonella mutagenicity test. Mutation Research, 113:173-215 (1983).

OECD Guideline 473, *In vitro* Mammalian Chromosomal Aberration Test, updated and adopted July 21, 1997.

Thakur, A.J., Berry, K.J., and Mielke, P.W., Jr., A FORTRAN program for testing trend and homogeneity in proportions. Computer Programs in Biomedicine, 19:229-233 (1985).

(See Tables 7-11.)

Covance Results – Ames Test

ABSTRACT

The objective of this study was to evaluate the test article, SYBR Safe stain, for the ability to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes at the histidine locus in the genome of several strains of *Salmonella typhimurium*.

The doses tested in the mutagenicity assay were selected based on the results of a previous study (Covance 24984-0-409SC). The tester strains used in the mutagenicity assay were *Salmonella typhimurium* tester strains TA97a, TA98, TA100, TA102, TA1535, TA1537 and TA1538. The assay was conducted with seven doses of test article in both the presence and absence of S9 mix, along with concurrent vehicle and positive controls using three plates per dose. The doses tested with all tester strains in the presence of S9 mix were 0.100, 0.333, 1.00, 3.33, 10.0, 25.0 and 50.0 µg per plate. The doses tested with all strains in the absence of S9 mix were 0.0100, 0.0333, 0.100, 0.333, 1.00, 3.33 and 10.0 µg per plate.

The results of the *Salmonella*/Mammalian-Microsome Reverse Mutation Assay indicate that under the conditions of this study, the test article, SYBR Safe stain, did cause positive increases in the mean number of revertants per plate with tester strains TA97a, TA98, TA102 and TA1538 in the presence of S9 mix. No positive increases were observed with any of the other tester strain/activation condition combinations.

Objective

The objective of this study was to evaluate the test article and/or its metabolites for their ability to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes at the histidine locus in the genome of several strains of *Salmonella typhimurium*. The assay design was based on OECD Guideline 471, updated and adopted 21 July 1997.

Study Timetable

Experimental Start Date	08 August 2003
Study Initiation Date	31 July 2003
Study Start Date	08 August 2003
Study End Date	12 September 2003
Experimental End Date	12 September 2003
Study Completion Date	At Finalization

Major Computer Systems

The major computer systems used on this study included the Material Tracking and Testing System, used for test article accessioning and dispensing and the Environmental Monitoring and Control System, used for the direct online capture of environmental control data. All version numbers of the applications are maintained by Information Technology at Covance.

Record Retention

All raw data, documentation, records, the protocol, and the final report generated as a result of this study will be archived in the storage facilities of Covance-Vienna, for at least 1 year following submission of the final report to the Sponsor. After the 1 year period, the Sponsor may elect to have the aforementioned materials retained in the storage facilities of Covance-Vienna, for an additional period of time or sent to a storage facility designated by the Sponsor.

TEST AND CONTROL ARTICLES

The test article was supplied by the Sponsor as a red flocculent solid on 19 March 2003 and was identified as follows:

Test Article	Lot Number	Storage	Purity (%)	Expiration Date
SYBR Safe stain	MPB031403	room temperature with desiccant	>95	Not Provided

Information on the identity, strength, purity, stability, uniformity or other characteristics that define the test and control articles is on file with the Sponsor or the respective manufacturer(s).

The vehicle control article was dimethylsulfoxide (DMSO, CAS #67-68-5). The vehicle was supplied as follows:

Control Article	Supplier	Lot Number	Storage	Purity (%)	Expiration Date	Reserve (Archive) Sample
DMSO	Acros Organics	A017777101	Room temperature	99.9	Not provided	No
DMSO	Acros Organics	A017190501	Room temperature	99.98	Not provided	No

Vehicle Controls

Vehicle controls were plated for all tester strains in the presence and absence of S9 mix. The vehicle control was plated, using a 50 µL aliquot of DMSO (equal to the maximum aliquot of test article dilution plated), along with a 100 µL aliquot of the appropriate tester strain and a 500 µL aliquot of S9 mix (when necessary), on selective agar.

Positive Controls

The combinations of positive controls, activation condition and tester strains plated concurrently with the assay are indicated in Table I.

Table I. Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA97a	+	2-aminoanthracene	2.5
TA97a	–	ICR-191	2.0
TA98	+	benzo[α]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA102	+	2-aminoanthracene	15.0
TA102	–	mitomycin (MMC)	1.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
TA1538	+	2-aminoanthracene	2.5
TA1538	–	2-nitrofluorene	1.0

The sources and grades of positive control articles are as follows:

benzo[α]pyrene (CAS #50-32-8), Sigma Chemical Co., purity ≥99.9%

2-aminoanthracene (CAS #613-13-8), Sigma Chemical Co., purity ≥97.4%

2-nitrofluorene (CAS #607-57-8), Aldrich Chemical Co., purity ≥99.2%

sodium azide (CAS #26628-22-8), Sigma Chemical Co., purity ≥99.8%

ICR-191 (CAS #17070-45-0), Sigma Chemical Co., purity ≥94.0%

mitomycin (MMC) (CAS #50-07-7), Sigma Chemical Co., purity not provided

Sterility Controls

The most concentrated test article dilution was checked for sterility by plating a 50 µL aliquot (the same volume used in the assay) on selective agar. The S9 mix was checked for sterility by plating 0.5 mL on selective agar.

S9 METABOLIC ACTIVATION SYSTEM

S9 Homogenate

Liver microsomal enzymes (S9 homogenate) were purchased from Molecular Toxicology, Inc., Lot 1547 (35.3 mg of protein per mL). The homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1254 (200 mg per mL in corn oil) at 500 mg/kg as described by Ames *et al.,* (1975).

S9 Mix

The S9 mix was prepared immediately prior to its use in any experimental procedure. The S9 mix contained the components indicated in Table II.

Table II. S9 Mix Components

Component	Amount
H_2O	0.70 mL
1M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 mL
0.25M Glucose-6-phosphate	0.02 mL
0.10M NADP	0.04 mL
0.825M KCl/0.2M $MgCl_2$	0.04 mL
S9 Homogenate	0.10 mL
	1.00 mL

TEST SYSTEM

Test System Rationale

The *Salmonella*/Mammalian-microsome reverse mutation assay detects point mutations, both frameshifts and/or base pair substitutions. The strains of *Salmonella typhimurium* used in this assay are histidine auxotrophs, by virtue of conditionally lethal mutations in the appropriate operons. When these histidine (*his-)* dependent cells are exposed to the test article and grown under selective conditions (minimal media with a trace amount of histidine), only those cells which revert to histidine (*his*+) independence are able to form colonies. The trace amount of histidine in the media allows all the plated bacteria to undergo a few cell divisions, which is essential for mutagenesis to be fully expressed. The *his*+ revertants are readily discernable as colonies against the limited background growth of the *his-* cells. By utilizing several different tester strains, both base pair substitution mutations and frameshift mutations can be detected. The bacterial reverse mutation assay has been shown to be a sensitive, rapid and accurate indicator of the mutagenic activity of many materials including a wide range of chemical classes.

Table III. Tester Strain Genotypes

Tester Strain	*his* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA97a	*his*D6610	*uvr*B	*rfa*	pKM101
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA102	*his*G428	-	*rfa*	pKM101/pAQ1
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
TA1538	*his*D3052	*uvr*B	*rfa*	–

Tester Strains

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA97a, TA98, TA100, TA102, TA1535, TA1537 and TA1538 as described by Ames *et al.;* (1975) and Levin *et al.;* (1982). The specific genotypes of the strains are shown in Table III.

In addition to a mutation in the histidine operon, the tester strains contain two additional mutations which enhance their sensitivity to some mutagenic compounds. Mutation of either the *uvrB* gene (with the exception of TA102) results in a deficient DNA excision repair system that greatly enhances the sensitivity of these strains to some mutagens. Since the *uvr*B deletion extends through the *bio* gene, the *Salmonella typhimurium* tester strains containing this deletion also require the vitamin biotin for growth.

The *Salmonella typhimurium* tester strains also contain the *rfa* wall mutation, which results in the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals such as those containing large ring systems (i.e., benzo[α]pyrene) that would otherwise be excluded by a normal intact cell wall.

Strains TA97a, TA98, TA100 and TA102 also contain the pKM101 plasmid, which further increases the sensitivity of these strains to some mutagens. The suggested mechanism by which this plasmid increases sensitivity to mutagens has been suggested is by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

The mutational site of tester strain TA102 contains A-T base pairs unlike the other tester strains which contain G-C base pairs at the mutation site. Additionally, this strain is unique in that the *his*G428 ochre mutation has been introduced into a plasmid (pAQ1) so that under the appropriate experimental conditions, approximately 30 copies of the mutant gene are available for back mutation.

Tester strains TA97a, TA98, TA1537 and TA1538 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester strains TA100, TA1535 and TA102 are reverted from auxotrophy to prototrophy by base substitution mutagens. Tester strain TA102 is also reverted by DNA cross-linking agents.

Source of Tester Strains

The *Salmonella typhimurium* tester strains in use at Covance were received directly from Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley.

Frozen Permanent Stocks

Frozen permanent stocks were prepared by growing fresh overnight cultures, adding DMSO (0.09 mL/mL of culture) and freezing away appropriately vialed aliquots. Frozen permanent stocks of the tester strains were stored at -60°C to -80°C.

Master Plates

Master plates of the tester strains were prepared by streaking each tester strain from a frozen permanent stock onto minimal agar appropriately supplemented with either histidine and biotin, and for strains containing the pKM101 plasmid, ampicillin. For TA102, containing the pAQ1 plasmid, tetracycline was added to the master plate. Tester strain master plates were stored at >0°C to 10°C.

Preparation of Overnight Cultures

Inoculation

Overnight cultures for use in all testing procedures were inoculated by transferring a colony from the appropriate master plate to a flask containing culture medium. Inoculated flasks were placed in a shaker/incubator which was programmed to begin operation (shaking, 125 ± 25 rpm; incubation, 37 ± 2°C) so that the overnight cultures were in late log phase when turbidity monitoring began.

Harvest

To ensure that cultures were harvested in late log phase, the length of incubation was determined by spectrophotometric monitoring of culture density. Cultures were harvested once a predetermined density was reached which ensured that cultures had reached a density of at least 0.5 X 10^9 cells per mL and that the cultures had not overgrown. Overgrown (stationary) cultures may exhibit decreased sensitivity to some mutagens. Cultures were removed from incubation when the target density was reached and were held at >0°C to 10°C until used in the assay.

Confirmation of Tester Strain Genotype

Tester strain cultures were checked for the following genetic markers on the day of their use in the mutagenicity assay:

rfa Wall Mutation

For the *Salmonella* tester strains, the presence of the *rfa* wall mutation was confirmed by demonstration of the sensitivity of the culture to crystal violet. An aliquot of an overnight culture of each strain was overlaid onto plates containing selective media, and an antibiotic sensitivity disk containing 10 µg of crystal violet was added. Sensitivity was demonstrated by inhibition of bacterial growth in a zone immediately surrounding the disk.

pKM101 Plasmid

The presence of the pKM101 plasmid was confirmed for cultures of tester strains TA97a, TA98, TA100 and TA102 by demonstration of resistance to ampicillin.

pAQ1 Plasmid

The presence of the pAQ1 plasmid was confirmed for cultures of tester strain TA102 by demonstration of resistance to tetracycline.

Characteristic Number of Spontaneous Revertants

The mean number of spontaneous revertants per plate in the vehicle controls that is characteristic of the respective strains was demonstrated by plating 100 µL aliquots of each culture along with the appropriate vehicle on selective media.

Culturing Broth

The broth used to grow overnight cultures of the tester strains was Vogel-Bonner salt solution (Vogel and Bonner, 1956) supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2 (dry powder). For TA102, the culturing broth was supplemented with tetracycline (2µg/mL) to maintain the pAQ1 plasmid copy number.

Minimal Bottom Agar Plates

Bottom agar (25 mL per 15 x 100 mm petri dish) was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956), supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose.

Top Agar for Selection of Revertants

Top (overlay) agar was prepared with 0.7% agar (w/v) and 0.5% NaCl (w/v) and was supplemented with 10 mL of 0.5 mM histidine/biotin solution per 100 mL agar for selection of histidine revertants.

When S9 mix was required, 2.0 mL of the supplemented top agar was used in the overlay. However, when S9 mix was not required, water was added to the supplemented top agar (0.5 mL of water per 2 mL of supplemented top agar) and the resulting 2.5 mL of diluted supplemented top agar was used for the overlay. This dilution ensured that the final top agar and amino acid supplement concentrations remained the same both in the presence and absence of S9 mix.

Test Article Disposition

The remaining test article was appropriately discarded after issuance of the audited draft report. The disposal of the remaining test article was documented in the study file.

EXPERIMENTAL DESIGN

Mutagenicity Assay

Design

The assay was performed using tester strains TA97a, TA98, TA100, TA102, TA1535, TA1537, and TA1538 both in the presence and absence of S9 mix along with the appropriate vehicle and positive controls.

Frequency and Route of Administration

The tester strains were exposed to the test article via the plate incorporation methodology originally described by Ames *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the plate incorporation methodology, the test article, the tester strain, and the S9 mix (where appropriate) were combined in molten agar which was overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. All doses of the test article, the vehicle controls and the positive controls were plated in triplicate.

PROCEDURES

Plating Procedures

Each plate was labeled with a code which identified the test article, test phase, tester strain, activation condition and dose level. The S9 mix and dilutions of the test article were prepared immediately prior to their use.

When S9 mix was not required, 100 µL of tester strain and 50 µL of control or test article dilution were added to 2.5 mL of molten selective top agar (maintained at 45 ± 2°C). When S9 mix was required, 500 µL of S9 mix, 100 µL of tester strain and 50 µL of control or test article dilution were added to 2.0 mL of molten selective top agar. After the required components had been added, the mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar contained in a 15 x 100 mm petri dish. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C. Positive control articles were plated using a 50 µL plating aliquot.

Scoring the Plates

Plates which were not evaluated immediately following the incubation period were held at >0°C to 10°C until such time that colony counting and bacterial background lawn evaluation could take place.

Bacterial Background Lawn Evaluation

The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that dose level. Lawns were scored as normal (N), reduced (R), obscured by precipitate (O), macroscopic precipitate present (P), absent (A), or enhanced (E); contaminated plates (C) were also noted.

Counting Revertant Colonies

Revertant colonies were counted by automated colony counter or by hand.

Data Evaluation

Data Presentation

For all replicate platings, the mean revertants per plate and the standard deviation were calculated. The results of these calculations are presented in tabular form in the Data Tables section of this report. The historical control data are presented after the data tables.

Assay Acceptance Criteria

Before assay data were evaluated, the criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Tester Strain Integrity

rfa Wall Mutation

To demonstrate the presence of the *rfa* wall mutation, *Salmonella typhimurium* tester strain cultures exhibited sensitivity to crystal violet.

pKM101 Plasmid

To demonstrate the presence of the pKM101 plasmid, cultures of tester strains TA97a, TA98, TA100 and TA102 exhibited resistance to ampicillin.

pAQ1 Plasmid

The presence of the pAQ1 plasmid was confirmed for cultures of tester strain TA102 by demonstration of resistance to tetracycline.

Characteristic Number of Spontaneous Revertants

To demonstrate the requirement for histidine, the tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. The acceptable ranges for the mean vehicle controls were as follows:

TA97a	80		240
TA98	8	—	60
TA100	60	—	240
TA102	180		425
TA1535	4	—	45
TA1537	2	—	25
TA1538	3	—	35

Tester Strain Culture Density

To demonstrate that appropriate numbers of bacteria are plated, the density of tester strain cultures was greater than or equal to 0.5 x 10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 0.5 x 10^9 bacteria per mL.

Positive Control Values in the Absence of S9 Mix

To demonstrate that the tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.

Positive Control Values in the Presence of S9 Mix (S9 Mix Integrity)

To demonstrate that the S9 mix was capable of metabolizing a promutagen to its mutagenic form(s), the mean value of the positive control for a respective tester strain in the presence of the S9 mix exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.

An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both the integrity of the S9 mix and the ability of the tester strain to detect a mutagen.

Cytotoxicity

A minimum of three non-toxic doses was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. A thinning of the bacterial background lawn which was not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Assay Evaluation Criteria

Once the criteria for a valid assay had been met, responses observed in the assay were evaluated.

Tester Strains TA97a, TA98, TA100, and TA102

For a test article to be considered positive, it had to produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Tester Strains TA1535, TA1537, and TA1538

For a test article to be considered positive, it had to produce at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

RESULTS AND DISCUSSION

Test Article Handling

The test article, SYBR SAafe stain, was described at receipt as a flocculent red solid and was stored at room temperature with desiccant. In dimethylsulfoxide the test article was observed to form a non-viscous, transparent red solution at 1.00 mg/mL, which was the most concentrated stock dilution prepared. The test article remained in solution at all succeeding lower dilutions prepared for the mutagenicity assay.

Mutagenicity Assay

The mutagenicity assay results for SYBR Safe stain are presented in Tables 1 through 6. These data were generated in Trials 24984-B1 and 24984-B2. These data are presented as individual plate counts (Tables 1, 3, 5 and 6) and as mean revertants per plate ± standard deviation (Tables 2, 4, 5 and 6) for each treatment and control group.

In the mutagenicity assay, Trial 24984-B1 (Tables 1 through 4), positive increases in the mean number of revertants per plate were observed in the presence of S9 mix with tester strains TA97a (3.3-fold), TA98 (3.0-fold) and TA102 (3.8-fold). An increase was also observed with tester strain TA98 (2.3-fold) in the absence of S9 mix, however, this increase was not clearly dose-related and all observed values were within the acceptable vehicle control range for this strain. The observed increase appeared to be the result of a lower than routinely observed TA98 mean vehicle control value and was not considered to be biologically relevant. No positive increases were observed with any of the remaining tester strain/activation condition combinations.

In this trial, the mean vehicle control value for tester strain TA97a in the absence of S9 mix was not within the acceptable range specified in the protocol. For this reason, the data generated with TA97a in the absence of S9 mix in Trial 24984-B1 were not used to evaluate the test article. In addition, the mean positive control value for tester strain TA1538 in the absence of S9 mix did not exhibit at least a 3-fold increase over the mean vehicle control value. Also, indications of toxicity were observed with all tester strains in the presence of S9 mix in this trial except TA1538. For these reasons, the data generated with TA1538 in both the presence and absence of S9 mix in Trial 24984-B1 were not used to evaluate the test article. The test article was re-tested with tester strain TA97a in the absence of S9 mix and TA1538 in both the presence and absence of S9 mix in Trial 24984-B2.

In the repeat mutagenicity assay, Trial 24984-B2 (Tables 5 and 6), all data were acceptable and a 3.7-fold positive increase in the mean number of revertants per plate was observed with tester strain TA1538 in the presence of S9 mix. No positive increases were observed with tester strains TA97a and TA1538 in the absence of S9 mix.

All criteria for a valid study were met.

CONCLUSION

The results of the *Salmonella/*Mammalian-Microsome Reverse Mutation Assay indicate that under the conditions of this study, the test article, SYBR Safe stain, did cause positive increases in the mean number of revertants per plate with tester strains TA97a, TA98, TA102 and TA1538 in the presence of S9 mix. No positive increases were observed with any of the other tester strain/activation condition combinations.

REFERENCES

Ames, B.N., McCann, J., and Yamasaki, E., "Methods for detecting carcinogens and mutagens with the *Salmonella*/Mammalian-Microsome Mutagenicity Test." *Mutation Research, 31*:347-364 (1975).

Levin, D.M., Hollstein,M., Christian, M.F., Schwiers, E.A., and Ames B.N., "A new Salmonella tester strain (TA102) with A-T base pairs at the site of mutation detects oxidative mutagens." *Proc. Natl. Acad. Sci. USA* 79:7445-7449 (1982).

Maron, D.M. and Ames, B., "Revised Methods for the *Salmonella* Mutagenicity Test." *Mutation Research, 113*:173-215 (1983).

OECD Guideline 471, Bacterial Reverse Mutation Test, updated and adopted 21 July 1997.

Vogel, H.J. and Bonner, D.M., "Acetylornithinase of *E. coli*: Partial purification and some properties." *J. Biol. Chem., 218*:97-106 (1956).

(See Tables 12-17)

Northview Pacific Laboratories Results — Acute Oral Toxicity

SUMMARY

A single oral administration of SYBR Safe™ DNA gel stain in 0.5X TBE at a limit dose of 5000 mg/kg to three female rats produced no mortalities or toxic signs.

INTRODUCTION

This procedure is designed to determine the acute oral toxicity of the material under test.

A Limit Screen test was performed using three female Sprague Dawley rats, which received an oral Limit Dose of 5000 mg/kg of the test article. The animals were observed for mortality, weight change, and toxic signs for a two-week period.

Since all three rats survived for two weeks after the dose administration, the LD_{50} for the test article was considered to be greater than the Limit Dose and no additional testing was required.

TEST ARTICLE IDENTIFICATION

Name: SYBR Safe™ DNA gel stain in 0.5X TBE

Physical Description: pale pink liquid

Total Quantity Received for Testing: 1 L

Total Quantity Used for This Study: 10 g

Expiration Date: January-05-2005

Lot Number: 52E16-1

Sterility Status: non-sterile

Storage Condition: room temperature

PROTOCOL

This test was conducted according to Protocol Number X4H165G, which incorporates by reference Northview Standard Operating Procedure 16D-05 and is on file at Northview Pacific Laboratories, Inc. There were no amendments to or deviations from the protocol.

DATA DISPOSITION

Raw data and the final report from this study are archived at Northview Pacific Laboratories Inc., 551 Linus Pauling Drive, Hercules, CA 94547, under Northview Report Number X4H165G.

JUSTIFICATION FOR TEST SYSTEM

Rats are the species required by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Toxic Substances Control Act (TSCA), and Health Effects Guideline OPPTS 870.1100 — Acute Oral Toxicity, December 2002.

Study Timetable

Time		Procedure
Day	Hour	
-1		Food withheld overnight
0	0	Weighing and dosing
	0–4	Observation of animals
	4	Food restored
1–6		Daily observations
7		Observation and weighing
8–13		Daily observations
14		Observation and weighing

SAMPLE PREPARATION AND DOSING PROCEDURE

Sample Preparation — For each dose administration, 5 g of test article was dissolved in deionized water to a final volume of 10 mL. Test article solutions were used on the day they were prepared. The pH of the test solution was 8.29.

Animal Preparation— The animals were fasted beginning approximately 18 hours before dose administration. During the fasting they continued to receive water *ad libitum*. Food was withheld until four hours after dosing in order to facilitate gastrointestinal absorption of the test article.

One rat was dosed on the first day of dosing. Two days or more after the first rat, second and third rats were dosed. Because all three rats survived, no further testing was required.

Dosing Procedure — The animals were dosed with 10 mL/kg of the test article solution by means of a gavage needle attached to a hypodermic syringe. There were no control animals. The first rat was dosed on 8/25/04. The second and third rats were dosed on 8/31/04.

OBSERVATIONS

Clinical Observations — All of the animals were observed several times on the day of dosing and at least once each day for fourteen days. The animals were observed for clinical signs of toxicity such as unkempt appearance, altered feeding habits, weight loss, and other signs of distress or physical depression and for any signs of recovery from these conditions.

Weights — All of the animals were weighed on Day 0 (prior to test article administration), Day 7, and at the end of the study on Day 14.

Euthanasia — All animals were euthanized if they became moribund. All surviving animals were euthanized at the termination of the study. Euthanization was by IP injection of Beuthanasia-D (0.5 mL).

Necropsy — Gross necropsies were performed on all animals at the end of the study.

RESULTS AND DISCUSSION

Clinical Observations — All three rats remained healthy with no toxic signs throughout the duration of the study.

Weights — All animals gained weight during the test period.

Necropsy — No abnormalities were observed in any of the test animals.

CONCLUSION

A single oral administration of SYBR Safe™ DNA gel stain in 0.5X TBE at a limit dose of 5000 mg/kg to three female rats produced no mortalities or toxic signs.

AMEC Earth and Environmental Results

Ignitability: >212°F (not classified as ignitable)
Reactivity: Not Detected (for both Cyanide and Sulfide - mg/L)
Corrosivity: pH = 8.25 (not corrosive)
Corrositex Test Method: >60 minutes; classified as a Category 2 non-corrosive
Bioassay/Toxicity Test Results: LC_{50} > 500 mg/L; not classified as hazardous or toxic to aquatic life.

Sample and Bioassay Information:

Test Type: CCR Title 22 Acute Screening
Test Species: *Pimephales promelas*
Organism Supplier: Thomas Fish Co.
Mean Length (mm): 27.0
Range (mm): 24–31
Dilution Water: Charcoal-filtered tapwater adjusted
Sample Receipt Date: 9/3/03
Test Conditions: Static
Common Name: Fathead minnow
Number per Tank: 10
Mean Weight (g): 0.250
Loading Rate (g/L): 0.31
Test Solution Volume (liters): 8.0
Test Dates: 9/4/03 - 9/8/03

Summary Test Results:

Treatment	Rep.	Initial Count	Final Count	Percent Survival	Average Survival
Control	A	10	10	100	
	B	10	10	100	100
250 mg/L	A	10	10	100	
	B	10	10	100	100
500 mg/L	A	10	10	100	
	B	10	10	100	100
750 mg/L	A	10	10	100	
	B	10	10	100	100

Note: LC_{50} = the test concentration that produces a 50% lethal effect on the test organisms. LC_{50} value > 500 mg/L is classified as not hazardous under CCR Title 22 acute toxicity to aquatic life.

AMEC Earth and Environmental Results: Corrosivity (Corrositex)

Completion Date: September 10, 2003

EXECUTIVE SUMMARY

A single sample provided by AMEC Earth & Environmental, Inc. was evaluated with the Corrositex® test method to determine its corrosive potential and to designate its Packing Group classification. The results of this study may be summarized as follows:

Sample Description:	MPR71454
Mean Corrositex® Time (minutes):	>60
Packing Group:	NC

EVALUATION OF A SAMPLE PROVIDED BY AMEC EARTH & ENVIRONMENTAL, INC. UTILIZING THE CORROSITEX® TEST METHOD

Study Objective

A single sample provided by AMEC Earth & Environmental, Inc. was evaluated with the Corrositex® test method to determine its corrosive potential and to designate its Packing Group classification. To achieve this objective, the sample was subjected to a three-step testing process as described under Materials and Methods.

Background

The Corrositex test is a standardized and reproducible method that can be employed to determine the potential corrosivity and determine the Packing Group classification of specified categories of chemical compounds under the hazardous materials transportation regulations administered by the U.S. Department of Transportation (DOT) and international dangerous goods codes. The Corrositex test predicts the in vivo corrosive potential of a chemical compound or mixture by using as an endpoint the time it takes for the chemical to permeate through or destroy a synthetic biobarrier. When the chemical has passed through this biobarrier, a visual change is produced in a proprietary Chemical Detection System (CDS).

Materials/Methods

The Corrositex test is performed in three steps. First, a qualification test is done to insure that the test sample and the CDS reagent are compatible. This is achieved by placing either 150 uL of a liquid or 100 mg of a solid into an aliquot of the CDS reagent and observing it for the presence of any detectable change. If a physical or color change is observed, the sample is judged to be compatible with the detection solution and the remainder of the test is performed. The second step of the Corrositex test utilizes appropriate indicator solutions to permit categorization of the test sample as either a Category 1 or Category 2 material. Category 1 materials are typically strong acids/bases, while Category 2 materials are typically weak acids/bases. The third step in the test is performed by applying the test sample to the biobarrier. When the chemical permeates through or destroys the full thickness of this biobarrier, it comes into contact with the CDS which then undergoes a simple color change. This color change is visually observed and the time required for the color change to occur is recorded. As summarized in Table 2 below, the time required to destroy the biobarrier is recorded for four sample replicates and the mean of these replicates is utilized to designate the UN Packing Group classification as I (severe corrosivity), II (moderate corrosivity), III (mild corrosivity), or Noncorrosive (NC). Positive and negative controls are analyzed concurrently to confirm the test's validity.

Designation of UN Packing Groups

	Corrositex Time (minutes)			
Category I	0 to 3 min	>3 to 60 min	>60 to 240 min	>240 min
Category II	0 to 3 min	>3 to 30 min	>30 to 60 min	>60 min
	Packing Group I	Packing Group II	Packing Group III	Noncorrosive

Summary of Corrositex® Test Results

IVI #: C2623	Corrositex Time (minutes)
Sample: MPR71454	Replicate #1: >60
Conc. Tested: Neat	Replicate #2: >60
pH*: 8.6	Replicate #3: >60
Category: 2	Replicate #4: >60
Packing Group: NC	Mean ± SD: >60
* pH is taken at 10% aqueous solution	

DISCUSSION

A single sample obtained from AMEC Earth & Environmental, Inc. was analyzed by the Corrositex method to determine its corrosive potential and Packing Group designation. The results of this study indicated that the sample was compatible with the Corrositex system and was classified as a Category 2 material. The results obtained from the evaluation of four replicate samples were highly reproducible, demonstrating that a mean time of >60 minutes was required to destroy the synthetic biobarriers. These findings lead to the designation of this sample, MPR71454, as a non-corrosive.

Columbia Analytical Services Results — Pollutant Discharge

SUMMARY

SYBR Safe™ DNA gel stain meets the requirements of the Clean Water Act and the National Pollutant Discharge Elimination System (NPDES) regulations. No cyanide, phenolics, pollutant metals, organochlorine pesticides, PCBs, or semi-volatile /volatile organic compounds were detected in test samples.

PROTOCOL

Samples were received at CAS on 7/22/2004 and were stored at 4°C upon receipt. All analyses were performed consistent with the quality assurance program of Columbia Analytical Services, Inc. (CAS) according to National Environmental Laboratory Accreditation Conference (NELAC) standards.

Pollutant Discharge Test Results

Test (method, per CFR Title 40, Part 136) *	SYBR Safe Stain in 0.5X TBE †	0.5X TBE
pH (150.1)	8.45	8.48
Total cyanide (335.2)	None detected	None detected
Chemical oxygen demand (COD; 410.1)	7020	6840
Ammonia as nitrogen (350.1)	253	248
Total organic carbon (415.1)	2480	2360
Total phenolics (420.1)	None detected	None detected
Organochlorine pesticides and PCBs (608M)	None detected	None detected
Semi-volatile organic compounds (625)	None detected	None detected
Volatile organic compounds (624)	None detected	None detected
Metals (6010B, 7060A, 7421, 7470A, 7740, 7841)	None detected	None detected

* CFR = Code of Federal Regulations; † 1X SYBR Safe stain (Lot X40023) in 0.5X TBE.

TABLES

Table 1. Mutation Assay without Activation by SYBR Safe Stain. Test Article: Sybr Safe Stain; Treatment Date: 8/4/03; Genetics Assay No.: 24984-0-431sc; Cells Analyzed: 3 X 106; Vehicle: DMSO; Treatment Period: ~4 Hours; Selective Agent: Tft 3.0 µG/Ml; Expression Period: 2 Days

Test Condition	Daily Cell Counts (Cell/mL, x 10^5 Units)		Cumulative RSG [1]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency [2]		Relative Growth (%) [3]	Mutant Frequency (x 10-6 Units) [4]
	Day 1	Day 2		AVG VC				AVG VC		
Nonactivation Controls [5]										
Vehicle Control	11.9	10.8	14.3		139	702	116.9		120.0	39.5
Vehicle Control	12.1	9.3	12.5		141	579	96.6		86.7	48.6
Vehicle Control	12.0	9.4	12.5	13.1	185	631	105.1	106.2	94.7	58.8
MMS 13 µg/mL	10.3	6.5	7.4		460	327	54.5		29.2	281.3 [6]
MMS 13 µg/mL	10.0	6.0	6.7		531	311	51.8		24.8	341.8 [6]
Test Compound µg/mL			Relative to Vehicle Control (%) [5]				Relative to Vehicle Control (%) [5]			
0.125	12.0	7.1	72.2		195	708	111.1		80.3	55.2
0.250	13.3	4.4	49.6		115	340	53.4		26.5	67.3
0.500	Terminated due to excessive cytotoxicity									

1. RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured); **2.** Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100; **3.** Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency)/100; **4.** Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4}), Decimal is moved to express the frequency in units of 10^{-6}; **5.** Vehicle Control = 1% DMSO, Positive Control: MMS = Methyl methanesulfonate; **6.** Mutagenic. Exceeds Minimum Criterion of 97.9 x 10^{-6}.

Table 2. Sizing Data for Mutation Assay without Activation by SYBR Safe Stain; Test Article: SYBR Safe Stain; Genetics Assay No.: 24984-0-431sc; Vehicle: DMSO; Selective Agent: Tft 3.0 mg/mL; Treatment Date: 8/4/03.

		Cum. RSG (%) [1]		Cloning Efficiency [2]		Relative Growth (%) [3]	Mutant Frequency (x 10^{-6}) [4]		
Test Condition	Conc.	Day 1	Day 2	Abs %	Rel %		Total	Small	Large
Vehicle Control [5]									
	1%	99.2	109.0	116.9	110.1	120.0	39.5	19.0	20.5
	1%	100.8	95.4	96.6	90.9	86.7	48.6	22.2	26.4
	1%	100.0	95.6	105.1	99.0	94.7	58.8	26.6	32.2
MMS (µg/mL) [6]									
	13.000	85.8	56.8	54.5	51.4	29.2	281.3	166.0	115.3
	13.000	83.3	50.9	51.8	48.8	24.8	341.8	195.1	146.7
Test Article (µg/mL)									
	0.125	100.0	72.2	118.0	111.1	80.3	55.2	20.3	34.8
	0.250	110.8	49.6	56.7	53.4	26.5	67.3	41.7	25.6
	0.500	Terminated due to excessive cytotoxicity							

1. Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth; **2.** Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100; **3.** Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency)/100; **4.** Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4}), Decimal is moved to express the frequency in units of 10^{-6}, Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency; **5.** Vehicle Control = DMSO; **6.** Positive Control: MMS = Methyl methanesulfonate, Colony Counts increased by 9.099% to compensate for area of dish not scanned.

Table 3. Mutation Assay With Activation By SYBR Safe Stain. Test Article: SYBR Safe Stain; Treatment Date: 1/4/03; Genetics Assay No.: 24984-0-431sc; Cells Analyzed: 3 x 10^6; Vehicle: DMSO; Treatment Period: ~4 Hours; Selective Agent: Tft 3.0 mg/mL; Expression Period: 2 Days.

Test Condition	Daily Cell Counts (Cell/mL, x 10^5 Units)		Cumulative RSG [1]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency [2]		Relative Growth (%) [3]	Mutant Frequency (x 10-6 Units) [4]
	Day 1	Day 2								
S9-Activation Controls [5] S9 Batch Number: 1254				AVG VC				AVG VC		
Vehicle Control	10.5	8.6	10.0		309	815	135.8		102.9	75.8
Vehicle Control	8.1	14.9	13.4		216	535	89.1		90.2	80.8
Vehicle Control	9.2	11.5	11.8	11.7	208	682	113.6	112.9	100.9	61.1
MCA 2 µg/mL	7.2	8.9	7.1		872	391	65.1		35.0	446.4 [5]
MCA 4 µg/mL	5.3	7.6	4.5		726	303	50.5		17.1	478.4 [5]
Test Compound µg/mL			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
1.24	9.0	9.9	84.4		184	523	77.2		65.1	70.6
2.47	6.6	9.7	60.6		228	526	77.7		47.1	86.7
4.93	5.5	5.7	29.7		99	173	25.6		7.6	114.5
9.85	Terminated due to excessive cytotoxicity									

1. RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured); **2.** Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100; **3.** Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency)/100; **4.** Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4}), Decimal is moved to express the frequency in units of 10^{-6}; **5.** Vehicle Control = 1% DMSO, Positive Control: MCA = Methylcholanthrene; **6.** Mutagenic. Exceeds Minimum Criterion of 145.1 x 10^{-6}.

Table 4. Sizing Data for Mutation Assay with Activation by SYBR Safe Stain. Test Article: SYBR Safe Stain; Genetics Assay No.: 24984-0-431sc; Vehicle: DMSO; Selective Agent: Tft 3.0 µg/ml; Treatment Date: 4/1/03

		Cum. RSG (%) [1]		Cloning Efficiency [2]		Relative Growth (%) [3]	Mutant Frequency (x 10^{-6}) [4]		
Test Condition	Conc.	Day 1	Day 2	Abs %	Rel %		Total	Small	Large
Vehicle Control [5]									
	1%	113.3	85.5	135.8	120.4	102.9	75.8	47.9	27.8
	1%	87.4	114.3	89.1	78.9	90.2	80.8	52.7	28.2
	1%	99.3	100.2	113.6	100.7	100.9	61.1	38.7	22.4
MCA[f] (µg/mL)									
	2.00	77.7	60.7	65.1	57.7	35.0	446.4	339.7	106.7
	4.00	57.2	38.1	50.5	44.8	17.1	478.4	363.3	115.1
Test Article (µg/mL)									
	1.24	97.1	84.4	87.1	77.2	65.1	70.6	37.6	33.0
	2.47	71.2	60.6	87.6	77.7	47.1	86.7	48.5	38.2
	4.93	59.4	29.7	28.9	25.6	7.6	114.5	88.1	26.4
	9.85	Terminated due to excessive cytotoxicity							

1. Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth; **2.** Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100; **3.** Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency)/100; **4.** Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4}), decimal is moved to express the frequency in units of 10^{-6}, expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency; **5.** Vehicle Control = DMSO; **6.** Positive Control: MCA = Methylcholanthrene. Colony counts increased by 9.099% to compensate for area of dish not scanned.

Table 5. Summary of Dose Range-finding Study. Test Article: MPR 71454; Genetic Toxicology Assay No.: 24984-0-485SC; Treatment Date: 4/2/03

	Colonies per Dish					Average Number Colonies/Dish	Average		RPE (%)[4]
Treatment Group	1	2	3	4	5		P.E.[2]	± S.D.(%)[3]	
Vehicle Control[1]	49	40	37	36	37	39.8	23.4	3.2	100
Test Article									
0.0333	39	40	40	39	43	40.2	23.6	1.0	101
0.100	48	38	42	49	44	44.2	26.0	2.6	111
0.333	33	32	32	32	26	31.0	18.2	1.7	78
1.000	8	5	4	8	7	6.4	3.8	1.1	16
3.330	0	0	0	0	0	0.0	0.0	0.0	0
10.000	0	0	0	0	0	0.0	0.0	0.0	0

1. Vehicle Control = 0.2% DMSO; **2**. PE = Plating efficiency = (Number of colonies per dish/number of target cells seeded) x 100%;
3. SD = Standard deviation; **4.** RPE = Relative plating efficiency = (Average PE of treatment group/vehicle control average PE) x 100%.

Table 6. Summary of Transformation Assay Results; Test Article: MPR 71454; Genetic Assay No.: 24985-0-485SC; Treatment Date: 6/11/03.

Treatment Group	MT Frequency[1] (%)	Total MT Colonies[2]	Total Colonies Scored[3]	Average Colonies per Dish[4]	Average		RPE (%)[7]
					PE[5]	± SD[6]	
DMSO (0.2%)	0.106	1	941	31.4	19.6	± 2.9	100
BaP 5.00	1.553 *	25	1610	35.8	22.4	± 3.4	114
Test Article							
	0.442	5	1132	37.7	23.6	± 3.0	120
[8]	0.315	4	1268	42.3	17.2	± 2.2	88
[8]	0.144	2	1393	46.4	11.6	± 1.6	59

1. MT Frequency = (Total MT colonies/Total colonies scored) x 100%; **2.** Total MT Colonies + Total number of morphologically transformed colonies; **3.** Total number of colonies from all dishes; **4.** Total colonies scored/total number of dishes;
5. PE = Plating efficiency = (Number of colonies per dish/number of target cells seeded per dish) x 100%; **6.** SD = Standard deviation; **7.** RPE = Relative plating efficiency = (Average PE of treatment group/vehicle control average PE) x 100%;
8. The number of cells seeded per dish increased to adjust for expected toxicity; * p 0.05 vs. DMSO (Fisher's Exact Test).

Table 7. Assessment of Toxicity for Chromosomal Aberrations Assay without Metabolic Activiation; ~22 Hour Treatment, ~22 Hour Harvest; Assay No.: 24984; Trial No.: A1; Date: 3/27/03; Lab No.: CY032903; Test Article: SYBR Safe stain.

Treatment		% Mitotic Index	% Mitotic Reduction
Negative Control: RPMI 1640		6.0	—
Vehicle Control: DMSO	10.00 µL/mL	6.3	0
Test Article	7.81 µg/mL	—[1]	—
	15.60 µg/mL	—[1]	—
	31.30 µg/mL	—[1]	—
	62.50 µg/mL	—[1]	—
	125.00 µg/mL	—[1]	—
	250.00 µg/mL	—[1]	—
	500.00 µg/mL	—[1]	—
	1000.00 µg/mL	—[1]	—

1. Only dead cells present on slide; RPMI 1640 = culture medium; DMSO = dimethylsulfoxide.

Table 8. Assessment of Toxicity for Chromosomal Aberrations Assay without Metabolic Activation; ~22 Hour Treatment, ~22 Hour Harvest; Assay No.: 24984; Trial No.: B1; Date: 4/16/03; Lab No.: CY041103; Test Article: SYBR Safe stain.

Treatment		% Mitotic Index	% Mitotic Reduction
Negative Control: RPMI 1640		8.9	—
Vehicle Control: DMSO	10.000 µL/mL	9.1	0
Test Article	0.500 µg/mL	7.3	20
	1.000 µg/mL	4.3	53
	2.000 µg/mL	2.3	75
	4.000 µg/mL	0.2	98
	6.000 µg/mL	0.0	100
	8.000 µg/mL	0.0	100
	10.000 µg/mL	0.0	100

RPMI 1640 = culture medium; DMSO = dimethylsulfoxide.

Table 9. Chromosomal Aberrations in Human Lymphocytes, without Metabolic Activation. ~22 Hour Treatment, ~22 Hour Harvest; Assay No.: 24984; Trial No.: B1; Date: 4/16/03; Lab No.: Cy041103; Test Article: SYBR Safe Stain.

							Numbers and Percentages (%) of Cells Showing Structural Chromosome Aberrations							
		Cells Scored	% Mitotic Index Reduction[1]	# Endo-reduplicated Cells	# Polyploid Cells	Judgement (+/-)[2]	Gaps	Simple Breaks	chte[3]	chre[4]	mab[5]	Totals[6] -g	Totals[6] +g	Judgement (+/-)[7]
Controls Negative: RPMI 1640[8]		A 100 Total 100 **Average %**	 -	0 0 **0.0**	0 0 **0.0**		1	 1 **1.0**				0 0 **0.0**	1 1 **1.0**	
Vehicle: DMSO[9]	10.000 µL/mL	A 100 Total 100 **Average %**	 **0**	0 0 **0.0**	0 0 **0.0**		5	 5 **5.0**				0 0 **0.0**	5 5 **5.0**	
Positive: MMC[10]	0.200 µg/mL	A 25 Total 25 **Average %**	0 -	0 0 **0.0**	 0 **0.0**	 -	6	 6 **24.0**	 9 **36.0**	8	 8 **32.0**	14 14 **56.0**	17 17 **68.0**	 +
Test Article	1.000 µg/mL	A 100 Total 100 **Average %**	0 **53**	0 0 **0.0**	 0 **0.0**	 -	8	 8 **8.0**	 5 **5.0**			5 5 **5.0**	13 13 **13.0**	 -

1. % Mitotic index reduction as compared to the vehicle control; **2.** Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$; **3.** chte: chromatid exchange; **4.** chre: chromosome exchange; **5.** mab: multiple aberrations, greater than 4 aberrations; **6.** -g = # or % of cells with chromosome aberrations, +g = # or % of cells with chromosome aberrations + # or % of cells with gaps; **7.** Significantly greater in -g than the vehicle control, $p \leq 0.01$; 8. RPMI 1640 = culture medium; 9. DMSO = Dimethylsulfoxide; **10.** MMC = Mitomycin C.

Table 10. Assessment of Toxicity for Chromosomal Aberrations Assay with Metabolic Activation; ~3 Hour Treatment, ~22 Hour Harvest; Assay No.: 24984; Trial No.: A1; Date: 3/27/03; Lab No.: CY032903; Test Article: SYBR Safe stain.

Treatment			% Mitotic Index	% Mitotic Reduction
Negative Control	RPMI 1640		5.5	—
Vehicle Control	DMSO	10.00 µL/mL	4.5	0
Test Article		7.81 µg/mL	1.7	62
		15.60 µg/mL	0.2	96
		31.30 µg/mL	0.0	100
		62.50 µg/mL	—[1]	—
		125.00 µg/mL	—[1]	—
		250.00 µg/mL	—[1]	—
		500.00 µg/mL	—[1]	—
		1000.00 µg/mL	—[1]	—

1. Only dead cells present on slide; RPMI 1640 = culture medium; DMSO = dimethylsulfoxide.

Table 11. Chromosomal Aberrations in Human Lymphocytes, with Metabolic Activation; ~3 Hour Treatment, ~22 Hour Harvest; Assay No.: 24984; Trial No.: A1; Date: 3/27/03; Lab No.: CY032903; Test Article: SYBR Safe stain.

		Cells Scored	% Mitotic Index Reduction [1]	# Endo-reduplicated cells	# Polyploid cells	Judgement (+/-) [2]	Numbers and Percentages (%) of Cells Showing Structural Chromosome Aberrations							Judgement (+/-) [4]
							Gaps	Simple Breaks	chte	chre	mab	Totals [3]		
												-g	+g	
Controls														
Negative: RPMI 1640		100		0	0							0	0	
		Average %	-	**0.0**	**0.0**							0.0	0.0	
Vehicle: DMSO	10.0 µL/mL	100		0	0		1	1	2			3	4	
		Average %	**0**	**0.0**	**0.0**		**1.0**	**1.0**	**2.0**			3.0	4.0	
Positive: CP	25.0 µg/mL	25		0	0		3	8	1		1	9	10	
		Average %	-	**0.0**	**0.0**	-	**12.0**	**32.0**	**4.0**		**4.0**	**36.0**	**40.0**	+
Test Article	7.81 µg/mL	A 100		0	0		5					0	5	
		Total 100		0	0		5					0	5	
		Average %	**62**	**0.0**	**0.0**	-	**5.0**					**0.0**	**5.0**	-

1. % Mitotic index reduction as compared to the vehicle control; **2.** Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$; **3.** -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps; **4.** Significantly greater in -g than the vehicle control, $p \leq 0.01$chte: chromatid exchange; chre: chromosome exchange; mab: multiple aberrations, greater than 4 aberrations; RPMI 1640 = culture medium; DMSO = Dimethylsulfoxide; MMC = Mitomycin C.

Table 12. Mutagenicity Assay Results, Individual Plate Counts; Test Article ID: SYBR Safe stain; Assay No.: 24984-0-401; Trial No.: B1; Date Plated: 8/8/03; Vehicle: DMSO; Date Counted: 8/13/03, 8/18/03; Plating Aliquot: 50 µL.

		Revertants Per Plate												
	Dose/Plate	TA97a			TA98			TA100			TA102			Background Lawn[1]
		1	2	3	1	2	3	1	2	3	1	2	3	
Microsomes: Rat Liver														
Vehicle Control		127	150	148	25	24	20	80	87	112	321	288	332	N
Test Article	0.1000 µg	122	105	166	9	9	7	90	93	96	340	309	302	N
	0.3330 µg	136	152	144	9	5	12	105	97	102	289	324	273	N
	1.0000 µg	192	150	176	15	20	19	121	124	105	320	273	393	N
	3.3300 µg	268	142	235	35	44	32	155	160	163	543	610	503	N
	10.0000 µg	507	392	504	53	93	58	169	138	174	1564	1065	901	N/R[4]
	25.0000 µg	158	225	186	55	57	48	50	84	67	526	658	716	R
	50.0000 µg	80	53	30	0	0	0	8	9	0	347	342	242	R
Positive Control[2]		577	671	902	280	304	267	473	513	516	2541	2664	3088	N
Microsomes: None														
Vehicle Control		47	61	77	5	12	7	55	84	68	228	180	217	N
Test Article	0.0100 µg	60	54	62	8	23	17	65	63	63	177	162	187	N
	0.0333 µg	63	60	56	28	13	6	77	58	66	180	163	183	N
	0.1000 µg	92	32	56	11	25	C	84	60	75	134	150	146	N
	0.3330 µg	69	98	79	12	5	6	60	67	80	199	168	162	N
	1.0000 µg	143	121	112	10	8	16	121	75	54	209	200	172	N
	3.3300 µg	45	47	52	13	5	6	45	40	21	107	126	121	R
	10.0000 µg	7	4	6	0	0	0	5	0	14	22	29	32	R
Positive Control[3]		2175	2222	2529	232	212	217	865	1033	906	1216	1630	1607	N

1. Background Lawn Evaluation Codes: N = normal, R = reduced, O = obscured, A = absent, P = precipitate; **2.** TA97a, 2-aminoanthracene, 2.5 µg/plate; TA98, benzo[α]pyrene, 2.5 µg/plate; TA100, 2-aminoanthracene, 2.5 µg/plate; TA102, 2-aminoanthracene, 15.0 µg/plate; **3.** TA97a, ICR-191, 2.0 µg/plate; TA98, 2-nitrofluorene, 1.0 µg/plate; TA100, sodium azide, 2.0 µg/plate; TA102, mitomycin C, 1.0 µg/plate; C = No count due to contamination on the plate; **4.** The first entry is the lawn evaluation for tester strains TA97a and TA102. The second entry is the lawn evaluation for tester strains TA98 and TA100.

Table 13. Mutagenicity Assay Results, Summary. Test Article ID: SYBR Safe stain; Assay No.: 24984-0-401; Trial No.: B1; Date Plated: 8/8/03; Vehicle: DMSO; Date Counted: 8/8/03, 8/18/03; Plating Aliquot: 50 µL.

		Mean Revertants Per Plate with Standard Deviation								
	Dose/Plate	TA97a		TA98		TA100		TA102		Background Lawn [1]
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver										
Vehicle Control		142	13	23	3	93	17	314	23	N
Test Article	0.100 µg	131	31	8	1	93	3	317	20	N
	0.333 µg	144	8	9	4	101	4	295	26	N
	1.000 µg	173	21	18	3	117	10	329	60	N
	3.330 µg	215	65	37	6	159	4	552	54	N
	10.000 µg	468	66	68	22	160	20	1177	345	N/R [4]
	25.000 µg	190	34	53	5	67	17	633	97	R
	50.000 µg	54	25	0	0	6	5	310	59	R
Positive Control [2]		717	167	284	19	501	24	2764	287	N
Microsomes: None										
Vehicle Control		62	15	8	4	69	15	208	25	N
Test Article	0.0100 µg	59	4	16	8	64	1	175	13	N
	0.0333 µg	60	4	16	11	67	10	175	11	N
	0.100 µg	60	30	18	10	73	12	143	8	N
	0.333 µg	82	15	8	4	69	10	176	20	N
	1.000 µg	125	16	11	4	83	34	194	19	N
	3.330 µg	48	4	8	4	35	13	118	10	R
	10.000 µg	6	2	0	0	6	7	28	5	R
Positive Control [3]		2309	192	220	10	935	88	1484	233	N

1. Background Lawn Evaluation Codes: N = normal, R = reduced; O = obscured; A = absent; P = precipitate; **2.** TA97a, 2-amino-anthracene, 2.5 µg/plate; TA98, benzo, 2.5 µg/plate; TA100, 2-aminoanthracene, 2.5 µg/plate; TA102, 2-aminoanthracene, 15.0 µg/plate; **3.** TA97a, ICR-191, 2.0 µg/plate; TA98, 2-nitrofluorene, 1.0 µg/plate; TA100, sodium azide, 2.0 µg/plate; TA102, mitomycin C, 1.0 µg/plate; **4.** The first entry is the lawn evaluation for tester strains TA97a and TA102; The second entry is the lawn evaluation for tester strains TA98 and TA100.

Table 14. Mutagenicity Assay Results, Individual Plate Counts. Test Article ID: SYBR Safe Stain; Assay No.: 24984-0-401; Trial No.: B1; Date Plated: 8/8/03; Vehicle: DMSO; Date Counted: 8/13/03, 8/18/03; Plating Aliquot: 50 µL.

		Revertants Per Plate									
	Dose/Plate	TA1535			TA1537			TA1538			Background Lawn [1]
		1	2	3	1	2	3	1	2	3	
Microsomes: Rat Liver											
Vehicle Control		8	13	17	9	5	10	29	30	48	N
Test Article	0.100 µg	10	17	15	5	6	5	22	49	36	N
	0.333 µg	11	12	13	9	3	5	44	40	27	N
	1.000 µg	9	19	13	3	10	11	59	47	48	N
	3.330 µg	31	17	21	12	16	13	44	49	46	N
	10.000 µg	14	11	12	17	9	10	30	37	C [2]	N/R [4]
	25.000 µg	7	9	11	1	5	7	47	50	59	N/R [4]
	50.000 µg	4	0	0	0	0	0	56	49	47	N/R [4]
Positive Control [3]		59	79	82	111	108	80	126	143	59	N
Microsomes: None											
Vehicle Control		C [2]	12	17	3	5	0	18	19	21	N
Test Article	0.0100 µg	14	16	11	5	10	1	27	19	27	N
	0.0333 µg	8	7	9	5	C [2]	2	29	28	23	N
	0.100 µg	8	6	10	5	3	6	21	31	C [2]	N
	0.333 µg	18	13	11	4	2	8	21	20	39	N
	1.000 µg	17	5	14	2	1	2	29	31	29	N
	3.330 µg	7	8	C [2]	3	2	1	26	24	24	N/R [4]
	10.000 µg	3	6	0	0	0	0	0	0	0	R
Positive Control [5]		526	666	689	635	587	761	C [2]	0	32	N/R [6]

1. Background Lawn Evaluation Codes: N = normal, R = reduced, O = obscured, A = absent, P = precipitate; **2.** C = No count due to contamination on the plate; **3.** TA1535, 2-aminoanthracene, 2.5 µg/plate; TA1537, 2-aminoanthracene, 2.5 µg/plate; TA1538, 2-aminoanthracene, 2.5 µg/plate; **4.** The first entry is the lawn evaluation for tester strain TA1538, the second entry is the lawn evaluation for tester strains TA1535 and TA1537; **5.** TA1535, sodium azide, 2.0 µg/plate; TA1537, ICR-191, 2.0 µg/plate; TA1538, 2-nitrofluorene, 1.0 µg/plate; **6.** The first entry is the lawn evaluation for tester strains TA1535, TA1537 and one plate of tester strain TA1538, the second entry is the lawn evaluation for two plates of tester strain TA1538.

Table 15. Mutagenicity Assay Results, Summary. Test Article ID: SYBR Safe stain; Assay No.: 24984-0-401, Trial No.: B1; Date Plated: 8/8/03; Vehicle: DMSO; Date Counted: 8/13/03, 8/18/03; Plating Aliquot: 50 µL.

		Mean Revertants Per Plate with Standard Deviation						
	Dose/Plate	TA1535		TA1537		TA1538		Background Lawn [1]
		Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver								
Vehicle Control		13	5	8	3	36	11	N
Test Article	0.100 µg	14	4	5	1	36	14	N
	0.333 µg	12	1	6	3	37	9	N
	1.000 µg	14	5	8	4	51	7	N
	3.330 µg	23	7	14	2	46	3	N
	10.000 µg	12	2	12	4	34	5	N/R [2]
	25.000 µg	9	2	4	3	52	6	N/R [2]
	50.000 µg	1	2	0	0	51	5	N/R [2]
Positive Control [3]		73	13	100	17	109	44	N
Microsomes: None								
Vehicle Control		15	4	3	3	19	2	N
Test Article	0.0100 µg	14	3	5	5	24	5	N
	0.0333 µg	8	1	4	2	27	3	N
	0.100 µg	8	2	5	2	26	7	N
	0.333 µg	14	4	5	3	27	11	N
	1.000 µg	12	6	2	1	30	1	N
	3.330 µg	8	1	2	1	25	1	N/R [2]
	10.000 µg	3	3	0	0	0	0	R
Positive Control [4]		627	88	661	90	16	23	N/R [5]

1. Background Lawn Evaluation Codes: N = normal, R = reduced, O = obscured, A = absent, P = precipitate; **2.** The first entry is the lawn evaluation for tester strain TA1538, the second entry is the lawn evaluation for tester strains TA1535 and TA1537; **3.** TA1535, 2-aminoanthracene, 2.5 µg/plate; TA1537, 2-aminoanthracene, 2.5 µg/plate; TA1538, 2-aminoanthracene, 2.5 µg/plate; **4.** TA1535, sodium azide, 2.0 µg/plate; TA1537, ICR-191, 2.0 µg/plate; TA1538, 2-nitrofluorene, 1.0 µg/plate; **5.** The first entry is the lawn evaluation for tester strains TA1535, TA1537 and one plate of tester strain TA1538. the second entry is the lawn evaluation for two plates of tester strain TA1538.

Table 16. Mutagenicity Assay Results, Individual Plate Counts and Summary. Test Article ID: SYBR Safe stain; Assay No.: 24984-0-401; Trial No.: B2; Date Plated: 9/5/03; Vehicle: DMSO; Date Counted: 9/10/03; Plating Aliquot: 50 µL.

	Dose/Plate	Revertants Per Plate			Mean Revertants Per Plate with Standard Deviation		Background Lawn [1]
		TA97a			TA97a		
		1	2	3	Mean	S.D.	
Microsomes: None							
Vehicle Control		70	82	91	81	11	N
Test Article	0.0100 µg	71	80	55	69	13	N
	0.0333 µg	60	75	68	68	8	N
	0.100 µg	93	89	102	95	7	N
	0.333 µg	96	82	91	90	7	N
	1.000 µg	77	75	86	79	6	N
	3.330 µg	64	64	49	59	9	R
	10.000 µg	0	0	0	0	0	R
Positive Control [2]		3240	3443	3048	3244	198	N

1. Background Lawn Evaluation Codes: N = normal, R = reduced, O = obscured, A = absent, P = precipitate; **2.** TA97a, ICR-191, 2.0 µg/plate.

Table 17. Mutagenicity Assay Results – Individual Plate Counts and Summary. Test Article ID: SYBR Safe stain; Assay No.: 24984-0-401; Trial No.: B2; Date Plated: 9/5/03; Vehicle: DMSO; Date Counted: 9/10/03; Plating Aliquot: 50 µL.

	Dose/Plate	Revertants Per Plate			Mean Revertants Per Plate with Standard Deviation		Background Lawn [1]
		TA1538			TA1538		
		1	2	3	Mean	S.D.	
Microsomes: Rat Liver							
Vehicle Control		14	15	14	14	1	N
Test Article	0.1000 µg	6	11	8	8	3	N
	0.3330 µg	17	14	21	17	4	N
	1.0000 µg	27	31	24	27	4	N
	3.3300 µg	46	61	50	52	8	N
	10.0000 µg	3	7	5	5	2	R
	25.0000 µg	3	5	4	4	1	R
	50.0000 µg	0	0	0	0	0	R
Positive Control [2]		880	1010	893	928	72	N
Microsomes: None							
Vehicle Control		15	6	8	10	5	N
Test Article	0.01000 µg	6	11	9	9	3	N
	0.0333 µg	8	C [3]	1	5	5	N
	0.1000 µg	7	14	7	9	4	N
	0.3330 µg	6	6	7	6	1	N
	1.0000 µg	3	5	11	6	4	R
	3.3300 µg	0	4	3	2	2	R
	10.0000 µg	0	0	0	0	0	R
Positive Control [4]		366	335	357	353	16	N

1. Background Lawn Evaluation Codes: N = normal, R = reduced, O = obscured, A = absent, P = precipitate; **2.** TA1538, 2-aminoanthracene, 2.5 µg/plate; **3.** C = No count due to contamination on the plate; **4.** TA1538, 2-nitrofluorene, 1.0 µg/plate.

Contact and Ordering Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, Oregon 97402
Tel: 1 541 465 8300
Fax: 1 541 335 0504

Customer Service
Tel: 1 541 335 0338
Fax: 1 541 335 0305
E-mail: order@probes.com

Technical Assistance
Tel: 1 800 438 2209
Fax: 1 541 335 0238
E-mail: tech@probes.com

Product List

Cat #	Product Name / Description	Unit Size
S33100	SYBR Safe™ DNA gel stain in 0.5X TBE	1 L
S33101	SYBR Safe™ DNA gel stain in 0.5X TBE	4 L
S33102	SYBR Safe™ DNA gel stain *10,000X concentrate in DMSO*	400 µL
S33110	SYBR Safe™ DNA Gel Stain Starter Kit *with 1 L of SYBR Safe™ DNA gel stain in 0.5X TBE (S33100) and one photographic filter (S37100)*	1 kit
S33111	SYBR Safe™ DNA gel stain in 1X TAE	1 L
S33112	SYBR Safe™ DNA gel stain in 1X TAE	4 L
S37100	SYBR Safe™ photographic filter	each

Molecular Probes
invitrogen detection technologies

MATERIAL SAFETY DATA SHEET

S33100, INGREDIENT A

Revision Number: 1.3
Revision Date: 03-Aug-2004

Product and Company Identification

Product Name: SYBR Safe™ DNA gel stain in 0.5X TBE
Catalog Number: S33100
Unit Size: 1 L
Manufacturer/Supplier:

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402-9132, USA
For US and Canada, Toll-Free Phone: 1-800-438-2209 · Fax: 1-800-438-0228
Phone: (541)465-8300 · Fax: (541)335-0305 · Web: http://www.probes.com
Technical Assistance: (541)335-0353 · E-mail: tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 · Fax: +31-71-5233419 · Web: http://www.probes.com
Technical Assistance: +31-71-5233378 · E-mail: eurotech@probes.nl

Composition / Information on Ingredients

- **INGREDIENT A:** SYBR Safe™ stain

Unit Size: <5 mg
Molecular Formula: N/A
Molecular Weight: 0.0
CAS Number/Name: N/A

- **Other Ingredients (See attached)**
  - **INGREDIENT B:** 0.5X Tris-Borate EDTA (0.5X TBE) buffer

Hazards Identification

Emergency Overview: We are not aware of any reported health hazards for this product. We recommend treating all chemicals with caution. Not classified as hazardous according to criteria of Worksafe Australia.

POTENTIAL HEALTH EFFECTS

Inhalation: See Emergency Overview above.
Ingestion: See Emergency Overview above.
Skin: See Emergency Overview above.
Eyes: See Emergency Overview above.
Chronic Exposures: See Emergency Overview above.
Target Organs: See Emergency Overview above.

First Aid Measures

Wash thoroughly after handling. If eye or skin contact occurs, wash affected area with water for 15 minutes and seek medical advice. If inhaled, move individual to fresh air and seek medical advice. If swallowed, seek medical advice.

Fire Fighting Measures

Not flammable

Accidental Release Measures

Use appropriate protective equipment and methods to clean up spilled substances promptly. Absorb spill onto an appropriate material. Collect and dispose of all waste in accordance with applicable laws.

S33100, INGREDIENT A (continued)

Handling and Storage

Desiccation recommended. Store at -20°C. Protect material from long-term exposure to light; may be exposed to light for short periods of time.

Exposure Controls / Personal Protection

Wear appropriate gloves, protective clothing and eyewear and follow safe laboratory practices.

ACGIH/OSHA Permissible Exposure Limit Data: Not determined

Physical and Chemical Properties

Form: Solid
Odor: Not determined
Solubility in Water: Low
Specific Gravity: Not determined
pH: Not determined
Boiling Point: Not determined
Melting Point: Not determined
Flash Point: Not determined
Vapor Pressure: Not determined

Stability and Reactivity

Thermal Decomposition: No decomposition if used according to specifications.

Dangerous Reactions: No dangerous reactions identified.

Dangerous Products of Decomposition: No dangerous decomposition products identified.

Toxicological Information

RTECS Number: Not in RTECS

Toxicity: Toxicity data: LC_{50}: > 750 mg/L, Acute Toxicity Test, fathead minnows, 96 hour exposure, 100% survival.

Health Hazards: Mutation Data: Cell transformation, Syrian hamster embryo (SHE) cells, negative; Forward gene mutation at the thymidine kinase (TK) locus, mouse lymphoma (L5178Y) cells, negative with and without metabolic activation; Chromosomal aberrations, human peripheral blood lymphocytes, negative with and without metabolic activation; Histidine reverse gene mutation, Ames assay, Salmonella typhimurium (TA97a, TA98, TA102, TA1538), positive with metabolic activation; Histidine reverse gene mutation, Ames assay, Salmonella typhimurium (TA97a, TA98, TA102, TA1538), negative without metabolic activation; Histidine reverse gene mutation, Ames assay, Salmonella typhimurium (TA100, TA1535, TA1537), negative with and without metabolic activation.

Potential Hazards: We are not aware of any reported health hazards for this product. We recommend treating all chemicals with caution.

Carcinogenicity: Not listed by NTP, IARC or OSHA.

Ecological Information

We are not aware of any ecological hazards presented by this material.

Disposal Considerations

Consult local, state or national regulations for applicable requirements.

Transport Information

Hazard Class: No physical hazards identified
Identification Number: Not listed
Packing Group: Not classified

Proper Shipping Name (Technical Name): Not classified

S33100, INGREDIENT A (continued)

Regulations

US Toxic Substances Control Act (TSCA): Not listed
US Other: Not applicable
EEC EINECS Number: Not Listed
EEC Risk Statements: NONE
Other Country Regulations: None identified

Other Information

This material is sold for research purposes only and is not required to appear on the TSCA inventory. It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. Molecular Probes shall not be held liable for any damage resulting from handling or contact with the above product.