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Integrated Laboratory Systems

STUDY TITLE
AS52/GPT MAMMALIAN MUTAGENESIS ASSAY

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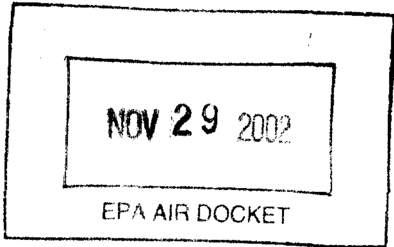
ILS Project No.
A073-003



Test Article
"DXP" Dessikarb

ILS Repository No.
94-48

Final Report Date
October 19, 1995

Sponsor
U.S. Army Environmental Hygiene Agency
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Aberdeen Proving Ground, MD 21005

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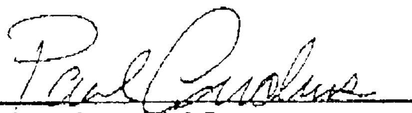
CERTIFICATION OF GOOD LABORATORY PRACTICES

ILS Project No.: A073-003
Test Article ID: "DXP" Dessikarb
ILS Repository No.: 94-48
Study Title: AS52/GPT Mammalian Mutagenesis Assay

This study was conducted in accordance with Good Laboratory Practice regulations as promulgated by the U. S. Environmental Protection Agency (40 CFR Part 792) except for the following: the positive/solvent control concentrations were not included in the protocol.



Paul Andrews, M.S.
Study Director

10/19/95
Date

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6.0 Primary Study Personnel:

Paul Andrews, M.S., Study Director
Raymond Tice, Ph.D., Scientific Director
Marie Vasquez, B.S., Research Assistant

7.0 Test Article:

7.1 Identification: "DXP" Dessikarb

7.2 Physical description and composition: White powder

7.3 Properties of the Test Article:

7.3.1 Compound Characterization: Determination of test article stability and test article characteristics is the responsibility of the sponsor. Information on the test article including its method of synthesis, analysis, physicochemical characteristics, and bulk stability is retained on file by the sponsor.

7.3.2 Storage Conditions: The test article was stored at room temperature. Stability under these conditions has been demonstrated by the Sponsor and documentation is on file with them. Normal safety precautions appropriate for potential clastogens were followed when handling the test article. A material safety data sheet was provided.

8.0 Test System:

8.1 Test System Justification: AS52 cells contain a single, functional, stably integrated copy of the *Escherichia coli* xanthine-guanine phosphoribosyl transferase (XPRT) gene (*gpt*). Mutations at the *gpt* locus can be detected as 6-thioguanine resistant (6-TG^r) colonies under conditions identical to those for detecting mutations at the *hprt* locus in CHO-K1-BH4 cells (1). However, in contrast to the *hprt* assay, the AS52/*gpt* assay is able to detect agents which induce primarily small and large deletion mutations in addition to point mutations (1,2). This ability to detect clastogens in addition to point mutagens results in an assay equal in sensitivity to the mouse lymphoma TK$^{+/-}$ assay while retaining the technical simplicity of the CHO-K1-BH4 *hgprt* assay (3). The AS52/*gpt* assay has been used to study a wide range of mutagens and clastogens, including radiation, a wide variety of chemicals, and complex mixtures.

8.2 AS52 Cell Line: The AS52 cell line, supplied by Dr. K.R. Tindall of the US National Institute of Environmental Health Sciences, is a proline auxotroph with a modal chromosome number of 20, a population doubling time of approximately 14 hours and a cloning efficiency (CE) normally greater than 80%. Cells were cultured in Ham's F-12 medium with 5% fetal bovine serum plus additives (xanthine, adenine, thymidine, mycophenolic acid, and aminopterin) at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air.

9.0 Experimental Design:

9.1 Preparation of the Dosage Formulation: As specified by the Sponsor, test article dosing solutions were freshly prepared in distilled water on the day of treatment. Alternately, phosphate buffered saline (PBS) was used as an aqueous solvent. The stock solutions were prepared at 100X the final concentration.

9.2 Method of Administration and Justification: A standard dosing volume of 50 µl was added to each culture in all treatment groups, resulting in a solvent concentration of 1% in culture medium.

9.3 S9 Activation System: Immediately prior to use, freshly thawed aliquots of Aroclor 1254-induced rat liver homogenates (S9 fraction) (Molecular Toxicology, Rockville, MD) were mixed with a sterile cofactor pool. An isocitrate/NADP cofacter pool was initially used and later replaced with the cofacter pool specified in the protocol. The S9 reaction mixture was stored on ice until used.

9.4 Dose Levels: Selection of the dose levels in the mutagenesis assay was based upon toxicity as indicated by a decline in colony forming efficiency of the cells in the initial toxicity assay(s). The high dose for the mutagenesis assay was selected to give a cell survival of 10 to 30%. Precipitation of the test article in the culture medium was not observed up to the highest dose tested.

9.4.1 Negative Controls: The negative controls consisted of cultures treated with distilled water or PBS only.

9.4.2 Positive Control: Positive controls, both direct-acting and indirect-acting, were included to demonstrate the adequacy of the experimental conditions to detect known mutagens. Ethylmethanesulfonate (EMS) at 150 and 300 µg/ml was used as a direct-acting mutagen for the nonactivated portion, and dimethylnitrosamine (DMN) at 50 and 100 µg/ml was used as a promutagen that requires metabolic activation. Both positive control

substances were dissolved in distilled water and administered in a dosing volume of 50 µl per culture.

9.5 Identification: Using a permanent marking pen, all culturing and processing containers used in the study were uniquely identified with the ILS chemical number, S9 condition, dose, and date.

9.6 Type and Frequency of Tests:

9.6.1 Cytotoxicity Test: Cells seeded 18-24 hours earlier at 1.0×10^6 cells in 25 cm^2 flasks and in log phase when treated were exposed to solvent alone and 6 concentrations of the test article in duplicate for 5 hours in the presence and absence of S9. Based on information provided by the Sponsor, the concentrations evaluated in the initial toxicity assay were 156.25, 312.5, 625.0, 1250, 2500, and 5000 µg/ml "DXP" Dessikarb. The next day, the cells were trypsinized and plated in triplicate at a density of 200 cells per 60 mm dish. The relative and absolute CE were determined 10 days later. The cell survival of the treated groups is expressed relative to the solvent control group (relative cloning efficiency).

9.6.2 Mutagenesis Assay: Six concentrations of the test article with and without S9 mix (plus concurrent solvent and positive controls) were used in the mutagenicity assay. The concentrations selected were based on the findings from the initial toxicity assay. Briefly, the concentrations were selected as follows: the high dose was selected to give a cell survival of 10 to 30%. Five lower doses were selected, one which was known to be relatively non-toxic. Cells seeded 18-24 hours earlier were exposed to solvent alone and six concentrations of the test article in duplicate for 5 hours at 37 ± 1°C in the presence and absence of S9 (day 0). After treatment, the treatment medium was removed, the cells washed 2 times with Ca and Mg free Hanks Balanced Salt Solution (HBSS) and complete medium without additives was added for an additional 18-24 hours incubation.

9.6.2.1 Estimation of Cytotoxicity: Cytotoxicity determination was demonstrated by a lack of colony development. On day 1, 18-24 hours after the termination of treatment, flasks were subcultured, counted, and an aliquot of AS52 cells seeded in triplicate at a density of 200 cells/60 mm dish. After 7-10 days incubation at 37°C, colonies were fixed and stained, air dried, and counted. Cytotoxicity was expressed as relative CE (the ratio of the absolute CE of the treated cells to that of the solvent controls).

9.6.2.2 Phenotypic Expression: After mutation at the *gpt* locus, the mutant phenotype requires a period of time before it is completely expressed (expression requiring the loss of pre-existing enzyme activity). At the normal population doubling times of 12 to 16 hours for AS52 cells, an expression period of 6-7 days is required. On day 1, duplicate treatment flasks were trypsinized, counted, and an aliquot of AS52 cells seeded at a density of 1×10^6 cells per flask. Cells were subcultured on days 4 and 6 and selected for 6-TG resistance on day 6.

9.6.2.3 Mutant Selection: On day 6, plates from each treatment group were trypsinized, counted, and five replicate dishes plated at a density of 2×10^5 cells/100 mm dish in F-12 medium with 10 μM 6-TG. For cloning efficiency at the time of selection, 200 cells/60 mm dish were also plated in triplicate in F-12 medium without 6-TG. After 7-8 days of incubation at $37 \pm 1°C$, colonies were fixed, stained, and later counted for cloning efficiency and mutant selection.

9.7 Statistical Analysis: A decision to classify a mutagenic response as negative, equivocal, or positive must involve a consideration of the appropriateness of the concurrent control data, a formal statistical analysis of the experimental data, and interpretation as to the biological relevance of the response by an experienced scientific investigator. An alpha level of 0.05 was used to indicate statistical significance in all analyses. Due to the possibility of fluctuation, samples with less than 1×10^5 viable cells after treatment (i.e., $\leq 10\%$ survival) were not considered as valid data points. Exact statistical analysis is difficult because the distribution of the number of mutant colonies depends on the complex processes of cell growth and death after mutagen treatment. While other appropriate methods can be used, the commonly used method is to use a one-tailed trend test, based on the number of mutant cells per 10^6 clonable cells in duplicate cultures, to evaluate for a positive increase in mutant frequency with increasing dose followed by a comparison of each treatment group against the concurrent solvent control treatment group. This pairwise comparison was made using student's t test, based on the number of mutant cells per 10^6 clonable cells, in duplicate cultures.

10.0 Criteria for Determination of a Valid Test

10.1 Negative Control: The absolute CE of the solvent controls should not be less than 65% and the mutant frequency of the solvent controls in each experiment should fall within the range of 15 to 35 mutants per 10^6 clonable cells. Absolute CE

values lower than 65% could indicate suboptimal culturing conditions for the cells while a higher mutant frequency may preclude detection of weak mutagens.

10.2 Positive Control: The positive control must induce a statistically significant response over the concurrent solvent control.

10.3 Test Article: The highest test article concentration should, if possible, result in a significant cytotoxic response (e.g., 10% to 30% survival). This is particularly important if the response is negative.

11.0 Criteria for a Positive Response

The response to the test article will be deemed positive if a dose dependent increase in mutant frequency is observed with one or more of the six doses tested exhibiting a mutant frequency which is at least twice that of the solvent control and is increased above the solvent control by at least 10 mutants per million clonable cells.

If either, but not both, of the above conditions are met, the assay results will be evaluated by the study director and will be classified as positive, equivocal, or negative depending on the nature and magnitude of the response.

If neither of the above conditions are met, the test article is classified as negative for clastogenic activity in this *in vitro* test.

12.0 Records to be Maintained:

Data were recorded on loose work sheets adapted or prepared as necessary for the test results. All data, stained plates, an original copy of the final report, and all correspondence will be archived at ILS for a minimum of five years past the date of regulatory submission. This material will be made available to the sponsor upon request, and will not be discarded without written authorization from the sponsor.

13.0 Quality Assurance:

The protocol was reviewed by the ILS QAU before final approval. A quality assurance inspection of critical phases was conducted to assure the quality and integrity of the study results. An audit of the report was conducted to determine the consistency between the reported information and the raw data.

14.0 Test Article Disposition:

Any unused test article and a log accounting for all test article use will be returned to the sponsor upon completion of the study.

15.0 Results:

15.1 Chemicals and Reagents: The chemicals and reagents used in this study (purity not provided) were obtained from the following commercial sources:

Chemical	Source	Lot No.
dimethylnitrosamine	Sigma	82H0365
ethylmethanesulfonate	Sigma	74H1107
fetal bovine serum	Irvine	300340224, 300240111,
giemsa	Gurr	4188220M
Ham's F-12	Irvine	905840623, 905841225
Hank's balanced salt solution	Irvine	922840222, 922841124, 922850425
Isocitrate	Sigma	69F-3776
methanol	Fisher	943188
NADP	Sigma	72H7823, 73H7879
phosphate buffered saline	Irvine	924021028
rat liver S9	Molecular Toxicology	0530
Thioguanine	Sigma	119F4024

15.2 Toxicity Tests: Based on information provided by the sponsor, the concentrations evaluated in the initial toxicity assay were 156.25, 312.5, 625, 1250, 2500, and 5000 µg/ml "DXP" Dessikarb.

15.2.1 Toxicity in Nonactivation Cultures: Individual culture data, absolute CE, and relative CE are presented in Table 1. A dose dependent decrease in the RCE was detected in treated cultures with a >30% RCE starting at a dose of 625 µg/ml. Greater than 10% toxicity was observed at doses of 2500 µg/ml and above. Based on these results, the maximum dose of the test article chosen to be tested in the absence of metabolic activation was selected to be 2500 µg/ml.

15.2.2 Toxicity in Activation Cultures: Individual culture data, absolute CE, and relative CE are presented in Table 2. A significant dose dependent decrease in the RCE was detected in treated cultures, with a RCE of >30% starting at a dose of 1250 µg/ml. Greater than 10% toxicity was observed at the highest dose only

(5000 µg/ml). Based on these results, the maximum dose of the test article to be tested in the presence of metabolic activation was selected to be 4000 µg/ml.

15.3 Mutagenic Activity in the Absence of Metabolic Activation: AS52 cells were initially exposed in the absence of metabolic activation to "DXP" Dessikarb at 250, 500, 1000, 1500, 2000, and 2500 µg/ml. Several trials had to be repeated due to no colony growth, poor solvent control cloning efficiency, or cell toxicity. The final doses tested for mutant selection were 250, 500, 750, 1000, 1250, and 1500 µg/ml. Individual and total plate counts as well as mutant frequency and induced mutant frequency data are presented in Table 4. The test article did not induce a significant increase in mutant frequency (based on one million clonable cells), as demonstrated by a nonsignificant one-tailed trend test ($p = 0.0657$) and the lack of a significant increase in mutant frequency at each dose group compared to the concurrent control ($p > 0.05$). The mean mutant frequency of the solvent controls was 13.4, 1.5 units below the lower level of 15 specified in the protocol. However, since 1 of the 2 cultures did exhibit a mutant frequency of 15, the mean was deemed acceptable by the study director. The positive control, EMS at 150 and 300 µg/ml, was mutagenic at both doses ($p < 0.001$) compared to the mutant frequency of the solvent controls.

Concurrent cytotoxicity data and cloning efficiency data are presented in Tables 3 and 5, respectively. A significant depression in the RCE immediately following dosing was observed among treated cultures, with a RCE of >30% observed at 750 µg/ml, the highest dose plated for mutant selection. The mean absolute cloning efficiency of the solvent controls at the time of selection was 94.8%.

15.4 Mutagenic Activity in the Presence of Metabolic Activation: AS52 cells were initially exposed in the presence of metabolic activation to "DXP" Dessikarb at 400, 800, 1600, 2400, 3200, and 4000 µg/ml. Several trials had to be repeated due to culture contamination and failure of the positive controls. The final doses tested for mutant selection were 400, 800, 1200, 1600, 2000, and 2400 µg/ml. Individual and total plate counts as well as mutant frequency and induced mutant frequency data are presented in Table 7. The test article did not induce a significant increase in mutant frequency (based on one million clonable cells), as demonstrated by both a nonsignificant one-tailed trend test ($p = 0.7920$) and the lack of a significant increase in mutant frequency at each dose group compared to the concurrent control ($p > 0.05$). The positive control, DMN, was significantly mutagenic at 50 µg/ml ($p = 0.0010$) but only marginally significant at 100 µg/ml ($p = 0.0615$).

Concurrent cytotoxicity data and cloning efficiency data are presented in Tables 6 and 8, respectively. A significant depression in the RCE immediately following

dosing was observed among treated cultures, with a mean depression of 32.4% observed at 2400 µg/ml, the highest dose tested. The mean absolute cloning efficiency of the solvent controls at the time of selection was 61.5%, 3.5% below the lower level of 65% specified in the protocol. However, since 1 of the 2 cultures exhibited a cloning efficiency of 70.3%, the mean was deemed acceptable by the study director.

16.0 Conclusion

"DXP" Dessikarb (ILS # 94-48) in either the presence or absence of metabolic activation did not induce a significant increase in mutant frequency at the *gpt* locus in AS52 cells.

17.0 References

(1) Stankowski, L.F. Jr., and K.R. Tindall (1987) Characterization of the AS52 cell line for use in mammalian cell mutagenesis studies. In: Banbury Report 28: Mammalian Cell Mutagenesis (M.M. Moore, D.M. DeMarini, F.J. de Serres, and K.R. Tindall, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 71-79.

(2) Tindall, K.R., and L.F. Stankowski Jr. (1987) Deletion mutations are associated with the differential-induced mutant frequency response of the AS52 and CHO-K1-BH4 cell lines. In: Banbury Report 28: Mammalian Cell Mutagenesis (M.M. Moore, D.M. DeMarini, F.J. de Serres, and K.R. Tindall, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 283-292.

(3) Aaron, C.S., G. Bolesfoldi, H.-R. Glatt, M. Moore, Y. Nishi, L. Stankowski, J. Theiss and E. Thompson (1994) International workshop on standardization of genotoxicity test procedures: Mammalian cell gene mutation assays working group report. Mutation Res. 312: 235-240.

TABLE 1: INITIAL TOXICITY TEST COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	PLATE	S9	PLATE COUNTS 1	2	3	MEAN	ACE	RCE
Solvent	A	-	178	120	C	149.0	74.5	-
Solvent	B	-	149	156	144	149.7	74.8	-
	Mean					149.3	74.7	
156.25	A	-	48	62	46	52.0	26.0	34.8
156.25	B	-	67	60	57	61.3	30.7	41.1
	Mean					56.7	28.3	37.9
312.5	A	-	50	54	77	60.3	30.2	40.4
312.5	B	-	40	50	42	44.0	22.0	29.5
	Mean					52.2	26.1	34.9
625	A	-	29	30	39	32.7	16.3	21.9
625	B	-	22	14	16	17.3	8.7	11.6
	Mean					25.0	12.5	16.7
1250	A	-	28	31	27	28.7	14.3	19.2
1250	B	-	23	38	34	31.7	15.8	21.2
	Mean					30.2	15.1	20.2
2500	A	-	4	10	6	6.7	3.3	4.5
2500	B	-	19	18	17	18.0	9.0	12.1
	Mean					12.3	6.2	8.3
5000	A	-	7	4	2	4.3	2.2	2.9
5000	B	-	6	4	3	4.3	2.2	2.9
	Mean					4.3	2.2	2.9

ACE = absolute cloning efficiency, RCE = relative cloning efficiency

C = contaminated

TABLE 2: INITIAL TOXICITY TEST COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	PLATE	S9	PLATE COUNTS 1	2	3	MEAN	ACE	RCE
Solvent	A	+	223	164	131	172.7	86.3	-
Solvent	B	+	230	223	213	222.0	111.0	-
	Mean					197.3	98.7	
156.25	A	+	224	200	154	192.7	96.3	97.6
156.25	B	+	225	200	194	206.3	103.2	104.6
	Mean					199.5	99.8	101.1
312.5	A	+	177	176	180	177.7	88.8	90.0
312.5	B	+	176	183	165	174.7	87.3	88.5
	Mean					176.2	88.1	89.3
625	A	+	126	104	119	116.3	58.2	59.0
625	B	+	124	120	138	127.3	63.7	64.5
	Mean					121.8	60.9	61.7
1250	A	+	39	40	57	45.3	22.7	23.0
1250	B	+	64	63	54	60.3	30.2	30.6
	Mean					52.8	26.4	26.8
2500	A	+	32	24	26	27.3	13.7	13.9
2500	B	+	39	28	40	35.7	17.8	18.1
	Mean					31.5	15.8	16.0
5000	A	+	9	19	13	13.7	6.8	6.9
5000	B	+	10	19	18	15.7	7.8	7.9
	Mean					14.7	7.3	7.4

ACE = absolute cloning efficiency, RCE = relative cloning efficiency

TABLE 3: CONCURRENT CYTOTOXICITY COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE			PLATE COUNTS					
(ug/ml)	FLASK	S9	1	2	3	MEAN	ACE	RCE
Solvent	A	-	210	194	173	192.3	96.2	-
Solvent	B	-	210	191	186	195.7	97.8	-
	MEAN					194.0	97.0	-
250	A	-	189	200	173	187.3	93.7	96.6
250	B	-	228	240	204	224.0	112.0	115.5
	MEAN					205.7	102.8	106.0
500	A	-	126	119	150	131.7	65.8	67.9
500	B	-	107	94	112	104.3	52.2	53.8
	MEAN					118.0	59.0	60.8
750	A	-	34	42	33	36.3	18.2	18.7
750	B	-	55	54	32	47.0	23.5	24.2
	MEAN					41.7	20.8	21.5
1000	A	-				T		
1000	B	-				T		
	MEAN							
1250	A	-				T		
1250	B	-				T		
	MEAN							
1500	A	-				T		
1500	B	-				T		
	MEAN							
EMS 150	A	-	157	174	197	176.0	88.0	90.7
EMS 150	B	-	184	206	202	197.3	98.7	101.7
	MEAN					186.7	93.3	96.2
EMS 300	A	-	179	178	176	177.7	88.8	91.6
EMS 300	B	-	120	97	116	111.0	55.5	57.2
	MEAN					144.3	72.2	74.4

ACE = absolute cloning efficiency = (mean plate count/200 cells)*100

RCE = relative cloning efficiency = (ACE dose/mean ACE solvents)*100

T = Too Toxic To Clone

TABLE 4: MUTANT SELECTION COUNTS FOR AS52 CELLS TREATED WITH "DXF" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	FLASK	S9	PLATE COUNTS 1	2	3	4	5	TOTAL	MF	IMF	
Solvent	A	-	2	1	3	1	2	9	11.8	-	
Solvent	B	-	4	3	3	5	2	17	15.0	-	
	MEAN								13.4	-	
250	A	-	2	2	2	3	5	14	14.7	1.3	
250	B	-	0	1	2	1	1	5	6.0	-7.4	
	MEAN								10.3	-3.1	
500	A	-	2	0	3	3	1	9	19.3	5.9	
500	B	-	3	1	2	2	2	10	13.7	0.3	
	MEAN								16.5	3.1	
750	A	-	6	5	2	2	5	20	19.3	5.9	
750	B	-	2	5	2	2	2	13	17.7	4.3	
	MEAN								18.5	5.1	
1000	A	-						T			
1000	B	-						T			
	MEAN										
1250	A	-						T			
1250	B	-						T			
	MEAN										
1500	A	-						T			
1500	B	-						T			
	MEAN										
EMS 150	A	-	16	24	24	16	16	96	145.1	131.7	
EMS 150	B	-	12	11	14	6	7	50	152.3	138.9	
	MEAN								148.7	135.3	*
EMS 300	A	-	32	28	32	28	23	143	249.4	236.0	
EMS 300	B	-	20	28	28	25	21	122	269.1	255.7	
	MEAN								259.3	245.9	*

MF = Mutant Frequency per million clonable cells (total counts/ACE*100)

IMF = Induced Mutant Frequency = MF dose - mean MF Solvent Controls

T = Too Toxic To Clone

* = significantly different at $p < 0.05$

TABLE 5: CLONING EFFICIENCY COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	FLASK	S9	PLATE COUNTS 1	2	3	MEAN	ACE	RCE
Solvent	A	-	151	145	160	152.0	76.0	-
Solvent	B	-	239	223	220	227.3	113.7	-
	MEAN					189.7	94.8	-
250	A	-	186	197	189	190.7	95.3	100.5
250	B	-	188	154	161	167.7	83.8	88.4
	MEAN					179.2	89.6	94.5
500	A	-	93	97	90	93.3	46.7	49.2
500	B	-	130	169	138	145.7	72.8	76.8
	MEAN					119.5	59.8	63.0
750	A	-	211	201	211	207.7	103.8	109.5
750	B	-	147	143	151	147.0	73.5	77.5
	MEAN					177.3	88.7	93.5
1000	A	-				T		
1000	B	-				T		
	MEAN							
1250	A	-				T		
1250	B	-				T		
	MEAN							
1500	A	-				T		
1500	B	-				T		
	MEAN							
EMS 150	A	-	145	144	108	132.3	66.2	69.8
EMS 150	B	-	59	81	57	65.7	32.8	34.6
	MEAN					99.0	49.5	52.2
EMS 300	A	-	119	118	107	114.7	57.3	60.5
EMS 300	B	-	93	92	87	90.7	45.3	47.8
	MEAN					102.7	51.3	54.1

ACE = absolute cloning efficiency = (mean plate count/200 cells)*100
RCE = relative cloning efficiency = (ACE dose/mean ACE solvents)*100
T = Too Toxic To Clone

TABLE 6: CONCURRENT CYTOTOXICITY COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	FLASK	S9	PLATE COUNTS 1	2	3	MEAN	ACE	RCE
Solvent	A	+	172	184	181	179.0	89.5	-
Solvent	B	+	174	184	177	178.3	89.2	-
	MEAN					178.7	89.3	-
400	A	+	159	176	137	157.3	78.7	88.1
400	B	+	159	181	165	168.3	84.2	94.2
	MEAN					162.8	81.4	91.1
800	A	+	173	194	184	183.7	91.8	102.8
800	B	+	151	171	163	161.7	80.8	90.5
	MEAN					172.7	86.3	96.6
1200	A	+	195	191	178	188.0	94.0	105.2
1200	B	+	163	178	166	169.0	84.5	94.6
	MEAN					178.5	89.3	99.9
1600	A	+	143	162	167	157.3	78.7	88.1
1600	B	+	183	198	193	191.3	95.7	107.1
	MEAN					174.3	87.2	97.6
2000	A	+	127	128	131	128.7	64.3	72.0
2000	B	+	163	146	143	150.7	75.3	84.3
	MEAN					139.7	69.8	78.2
2400	A	+	41	68	49	52.7	26.3	29.5
2400	B	+	56	72	61	63.0	31.5	35.3
	MEAN					57.8	28.9	32.4
DMN 50	A	+	102	115	125	114.0	57.0	63.8
DMN 50	B	+	107	111	104	107.3	53.7	60.1
	MEAN					110.7	55.3	61.9
DMN 100	A	+	78	84	68	76.7	38.3	42.9
DMN 100	B	+	82	79	73	78.0	39.0	43.7
	MEAN					77.3	38.7	43.3

ACE = absolute cloning efficiency = (mean plate count/200 cells)*100

RCE = relative cloning efficiency = (ACE dose/mean ACE solvents)*100

TABLE 7: MUTANT SELECTION COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	FLASK	S9	PLATE COUNTS 1	2	3	4	5	TOTAL	MF	IMF
Solvent	A	+	7	1	4	2	7	21	39.9	-
Solvent	B	+	1	3	0	0	1	5	7.1	-
	MEAN								23.5	-
400	A	+	2	0	1	2	1	6	11.2	-12.3
400	B	+	2	1	1	1	1	6	20.8	-2.7
	MEAN								16.0	-7.5
800	A	+	1	2	5	2	0	10	22.4	-1.1
800	B	+	5	5	4	2	2	18	31.7	8.2
	MEAN								27.0	3.5
1200	A	+						C		
1200	B	+	4	4	5	5	3	21	34.3	10.8
	MEAN								34.3	10.8
1600	A	+	5	3	2	3	0	13	28.8	5.3
1600	B	+	1	5	1	4	5	16	39.0	15.5
	MEAN								33.9	10.4
2000	A	+	0	1	0	0	L	1	2.4	-21.1
2000	B	+	0	1	4	1	1	7	17.9	-5.5
	MEAN								10.2	-13.3
2400	A	+	0	1	3	5	1	10	19.2	-4.3
2400	B	+	0	1	0	1	0	2	3.6	-19.9
	MEAN								11.4	-12.1
DMN 50	A	+	C	16	25	13	C	54	558.6	535.1
DMN 50	B	+	C	15	14	20	14	63	525.0	501.5
	MEAN								541.8	518.3 *
DMN 100	A	+	16	30	C	C	32	78	531.8	508.3
DMN 100	B	+	9	C	C	18	C	27	249.2	225.7
	MEAN								390.5	367.0 *

MF = Mutant Frequency per million clonable cells (total counts/ACE*100)

IMF = Induced Mutant Frequency = MF dose - mean MF Solvent Controls

* = significantly different at $p < 0.05$

C = Contaminated, L = Plate Leaked

TABLE 8: CLONING EFFICIENCY COUNTS FOR AS52 CELLS TREATED WITH "DXP" DESSIKARB (ILS # 94-48)

DOSE (ug/ml)	FLASK	S9	PLATE COUNTS 1	2	3	MEAN	ACE	RCE
Solvent	A	+	106	101	109	105.3	52.7	-
Solvent	B	+	139	137	146	140.7	70.3	-
	MEAN					123.0	61.5	-
400	A	+	98	113	111	107.3	53.7	87.3
400	B	+	49	55	69	57.7	28.8	46.9
	MEAN					82.5	41.3	67.1
800	A	+	85	92	91	89.3	44.7	72.6
800	B	+	103	119	119	113.7	56.8	92.4
	MEAN					101.5	50.8	82.5
1200	A	+				C		
1200	B	+	149	102	116	122.3	61.2	99.5
	MEAN					61.2	61.2	99.5
1600	A	+	85	85	101	90.3	45.2	73.4
1600	B	+	83	88	75	82.0	41.0	66.7
	MEAN					86.2	43.1	70.1
2000	A	+	94	82	72	82.7	41.3	67.2
2000	B	+	56	86	92	78.0	39.0	63.4
	MEAN					80.3	40.2	65.3
2400	A	+	105	112	96	104.3	52.2	84.8
2400	B	+	109	117	111	112.3	56.2	91.3
	MEAN					108.3	54.2	88.1
DMN 50	A	+	12	22	24	19.3	9.7	15.7
DMN 50	B	+	31	19	22	24.0	12.0	19.5
	MEAN					21.7	10.8	17.6
DMN 100	A	+	24	26	38	29.3	14.7	23.8
DMN 100	B	+	20	22	23	21.7	10.8	17.6
	MEAN					25.5	12.8	20.7

ACE = absolute cloning efficiency = (mean plate count/200 cells)*100

RCE = relative cloning efficiency = (ACE dose/mean ACE solvents)*100

C = contaminated