

TECHNICAL TASK GROUP ON VINYL CHLORIDE RESEARCH

Mr. Raymond J. Abramowitz
Technical Director
Ruco Division
Hooker Chemicals Corporation
P. O. Box 456
Burlington, N. J. 08016
(609) 499-2300 Ext. 70-221

Mr. Paul L. Armstrong
Technical Department Manager
Georgia-Pacific Corporation
P. O. Box 629
Plaquemine, Louisiana 70764
(504) 687-6321 Ext. 215

* Dr. Zeb G. Bell, Jr.
PPG Industries, Inc.
One Gateway Center
Pittsburgh, Pa. 15222
(412) 434-2585

Dr. Robert S. Brookman
Firestone Plastics Co.
Research Dev. & Tech. Ser.
P. O. Box 699
Pottstown, Pa. 19464
(215) 326-2000 Ext. 224

Mr. H. B. Carr
Tenneco Chemicals
P. O. Box 2
Piscataway, N. J. 08854
(201) 981-5176

B. W. Duck, M.D.
British Petroleum Co. Ltd.
Research Center-Chertsey Rd.
Sunbury-on-Thames
Middlesex, England
Tel. 85533 Ext. 451
Telex #266883

R. E. Eckardt, M.D.
Exxon Corporation
P. O. Box 45
Linden, New Jersey 07036
(201) 474-3801

Mr. J. E. Ertel
General Manager
ROBINTech Incorporated
P. O. Box 2342
Fort Worth, Texas 76101
(817) 332-7323 Ext. 23

Dr. A. W. Fuhrman
Vice President
Great American Chemical Corporation
650 Water Street
Fitchburg, Mass. 01420
(617) 343-6973 Ext. 55

Dr. Mac Roy Gasque
Olin Corporation
120 Long Ridge Road
Stamford, Conn. 06904
(203) 356-2561

Dr. R. L. Gibson
Gulf Oil Corporation
P. O. Box 1166
Pittsburgh, Pa. 15230
(412) 391-2400

* Dr. Walter D. Harris
UNIROYAL, Inc.
EMIC Building
Naugatuck, Conn. 06770
(203) 573-2000 Ext. 2139

Dr. C. A. Johnson
Goodyear Tire & Rubber Co.
1144 East Market St.
Akron, Ohio 44316
(216) 794-7676

* M. N. Johnson, M.D.
The B. F. Goodrich Company
Medical Center
500 South Main Street
Akron, Ohio 44318
(216) 379-3298

Dr. Flynt Kennedy
Continental Oil Company
1000 South Pine
Drawer 1267
Ponca City, Okla. 74601
(405) 762-3456 Ext. 4411

Dr. W. A. Knapp
Allied Chemical Corporation
P. O. Box 1057R
Morristown, N. J. 07960
(201) 455-2814

Mr. William R. Krause
Certain-Reed Products Corp.
Pipe & Plastics Group
P. O. Box 860
Valley Forge, Pa. 19482
(215) 687-5000 Ext. 7478

* Mr. Howard L. Kusnetz
Shell Oil Company
Box 2463
Houston, Texas 77001
(713) 220-2686

Mr. Robert W. Laundrie
Mgr., Ecology, Safety &
Health Engineering
The General Tire & Rubber Company
One General Street
Akron, Ohio 44329
(216) 798-3327 Ext. 59

Mr. S. K. Law
Process Engineer
Kaysor-Century Corp.
P. O. Box 308
Saugus, Calif. 91350
(805) 259-2360

Mr. A. B. Lindquist
Stauffer Chemical Co.
Product Regulations
Westport, Conn. 06880
(203) 226-1511

Mr. Avron B. Magram
Director of Marketing
Hatco Chemical Division
W. R. Grace & Co.
P. O. Box 27
Fords, N. J. 08863
(201) 738-1000 Ext. 301

Dr. R. W. McBurney
Diamond Shamrock Corporation
T. R. Evans Research Center
P. O. Box 348
Painesville, Ohio 44077
(216) 352-9311 Ext. 401

* Dr. W. E. Rinehart
Ethyl Corporation
Medical Department
451 Florida Avenue
Baton Rouge, La. 70801
(504) 387-0131

Dr. George Roush, Jr.
Monsanto Company
Medical Department
800 N. Lindbergh Blvd.
St. Louis, Mo. 63166
(314) 694-2188

Mr. Harry A. Russell
Executive Vice President
The Pantasote Company
Greenwich Office Park II
P. O. Box 1800
Greenwich, Conn. 06830
(203) 661-0405 or 0406

Mr. H. L. Schmidt, Jr.
Borden Chemical Div. of
Borden, Inc.
180 East Broad Street
Columbus, Ohio 43215
(614) 225-4882

* Dr. W. Mayo Smith
Air Products and Chemicals, Inc.
P. O. Box 538
Allentown, Pa. 18104
(215) 395-7215

* Dr. T. R. Torkelson
The Dow Chemical Company
Corporate Medical Department
2030 Dow Center
Midland, Mich. 48640
(517) 636-5197

* Mr. R. N. Wheeler
Union Carbide Corporation
Chemicals & Plastics Div.
P. O. Box 8004
S. Charleston, W. Va. 25303
(304) 747-2164

Research Coordinators

CYTOGENETIC STUDIES OF BONE MARROW CELLS FROM RATS
EXPOSED TO VINYL CHLORIDE

R. V. Johnston, D.V.M., D. J. Mensik, B.S.,
M. N. Pinkerton, B.S., E. B. Whorton, Jr., Ph.D.*

Dow Chemical U.S.A.
Texas Division - Freeport, Texas 77541
Industrial Health and Medicine Department
Biomedical and Comparative Toxicology Research Laboratory

*Whorton - Associate Professor and Director, Medical Education,
Computer Center, Preventive Medicine and Community
Health Biostatistics, Univ. of Texas Medical Branch,
Galveston, Texas 77550

CYTOGENETIC STUDIES OF BONE MARROW
CELLS FROM RATS EXPOSED TO VINYL CHLORIDE

R. V. Johnston, D.V.M., D. J. Mensik, B.S.,
M. N. Pinkerton, B.S., E. B. Whorton, Jr., Ph.D.

The Biomedical and Comparative Toxicology Research Laboratory of the Texas Division of Dow Chemical U.S.A. was asked by the Manufacturing Chemists Association to conduct cytogenetic studies on some of the rats which had been exposed to vinyl chloride at Industrial BIO-TEST Laboratories, Decatur, Illinois. The details of the exposure levels and pathological findings will be reported separately. Preliminary reports have appeared in Chemical Week (115:30, July 17, 1974) and in the Annals of the New York Academy of Sciences, Volume 246, page 219.

MATERIALS AND METHODS

Animals: The test animals were 20 male and 20 female Charles River CD outbred albino rats which were selected at random from larger groups of 100 each which are being used for carcinogenesis testing. At the time of harvest, the females weighed from 290 to 512 grams, and the males from 605 to 915 grams. The details are shown in Table 1.

Exposure: At the time of harvest, the rats had been exposed in chambers to vinyl chloride gas for 7 hours per day for 5 days per week for one year as follows:

<u>Group</u>	<u>Exposure Level</u>	<u>No. Males</u>	<u>No. Females</u>
C	None	5	5
T ₁	50 ppm	5	5
T ₂	200 ppm	5	5
T ₃	2500 ppm	5	5

Bone Marrow Collection Techniques:

On September 5, 1975, approximately 12 hours following the last exposure and beginning approximately 3 hours prior to harvest, the rats were injected intraperitoneally with 1 mg/kg of colchicine (CalBiochem, Brookhollow Station, Dallas, Texas 75247) using Hanks solution containing 1 mg/ml as shown in Table 1. Immediately prior to harvest, the rats were anesthetized in a carbon dioxide chamber and exsanguination was begun by severing the brachial artery. To obtain the bone marrow before it coagulated, the harvest was begun while the hemorrhage was in progress. The skin and muscles were removed from the right femur, and it was severed with a bone cutter. The bone marrow was aspirated into 6 ml of Hanks Media (Grand Island Biological Co., 3175 Staley Rd., Grand Island, New York 14072). The same technique was immediately followed on the left femur.

Necropsy, gross pathology and collection of tissues for histopathology were performed by personnel of Industrial BIO-TEST Laboratories and will be reported separately.

Cytogenetic Techniques:

The bone marrow cells were processed at Industrial BIO-TEST Laboratories with hypotonic solution and fixative as outlined in Table 2. The fixed cell suspension was returned to the Texas Laboratories for slide preparation and evaluation. Ten slides were prepared from each animal (5 from each femur).

The scoring of the slides for aberrations was done by three experienced scorers (#03 of our laboratory and #25 and #26 under

contract at the Stanford Research Institute, Menlo Park, California). The techniques used are similar to those described in "Methods for the Analysis of Human Chromosome Aberrations" published by the World Health Organization, Geneva, 1973. The slides were coded so that the identity of the animal was unknown to the scorer. Ten metaphase spreads on each of 5 slides per animal were scored by scorer #03, whereas the other scorers each used a single slide to score 50 metaphase spreads. Only diploid or 2N-1 cells were scored.

Statistical Findings:

Since there were no multiple aberrations, miscellaneous aberrations, or chromosome aberrations, no analysis was necessary. Also since chromatid aberration rates were very nearly an image of the abnormal cell rates, the following analyses were done only for abnormal cells.

The analysis variable considered was $X = \frac{\text{number of abnormal cells}}{\text{number of cells scored}}$ on a per animal basis. Since the variable X is a proportion with an observed range of only .10, no transformation was used for analyses. Uniformly there were 50 cells scored for each animal by each of three scorers, or a total of 150 cells scored for each animal. There were 5 animals per Dose-Sex combination.

The data were summarized as shown in Table 5, and the summary of the analysis of variance is shown in Table 6.

Statistical Conclusions:

At the 5% level of significance, there are indeed no significant differences between sexes, among scorers, or among doses. There is some evidence (at the $p = .09$ significance level) that

sexes may be different. The overall rate for males was .013, while the female rate was .018. At the $p \leq .12$ level of significance there was slight evidence that dose effects are sex dependent. It should be noted that both these implications could be due only to chance and "appear" largely because of the results for Dose (T₃) females where the rate was .028.

SUMMARY

Studies by the Biomedical and Comparative Toxicology Research Laboratory of The Dow Chemical Company, Freeport, Texas, show that there was no significant increase in the chromosomal aberration rates of bone marrow cells of rats exposed to varying doses of vinyl chloride for one year at Industrial BIO-TEST Laboratories, Decatur, Illinois.

/mhi
6-9-75

IN VIVO COLCHICINE FOR RAT BONE MARROW CYTOGENETICS IN
ONE-YEAR VINYL CHLORIDE INHALATION STUDY

Decatur Research Laboratories
 Industrial BIO-TEST Laboratories, Inc.
 Decatur, Illinois
 Sponsored by Manufacturing Chemists Association

<u>Animal</u>		<u>BIO-TEST</u>		<u>Biomed</u>	<u>Colchicine</u>		<u>Harvest</u>	<u>Code</u>
<u>Wt.gms</u>	<u>Sex</u>	<u>Animal</u>	<u>Treatment</u>	<u>Culture</u>	<u>Dose³</u>	<u>Time</u>		
		<u>No.</u>	<u>Group</u>	<u>No.</u>	<u>ml/kg</u>		<u>Time</u>	
735	M	54	C	VC#1 ¹ , 1A ²	.74	6:15AM	9:15AM	42
380	F	178	C	VC#2, 2A	.38	"	9:25AM	47
663	M	299	T1	VC#3, 3A	.66	"	9:40AM	52
329	F	349	T1	VC#4, 4A	.33	"	9:45AM	58
718	M	404	T2	VC#5, 5A	.72	"	10:05AM	66
402	F	594	T2	VC#6, 6A	.40	"	10:08AM	61
664	M	663	T3	VC#7, 7A	.66	"	10:23AM	69
381	F	742	T3	VC#8, 8A	.38	"	10:26AM	54
769	M	69	C	VC#9, 9A	.77	7:45AM	10:45AM	49
354	F	106	C	VC#10, 10A	.35	"	10:50AM	71
720	M	256	T1	VC#11, 11A	.72	"	11:02AM	73
356	F	381	T1	VC#12, 12A	.36	"	11:08AM	60
801	M	433	T2	VC#13, 13A	.80	"	11:23AM	76
457	F	524	T2	VC#14, 14A	.46	"	11:25AM	44
659	M	668	T3	VC#15, 15A	.66	"	11:35AM	80
327	F	735	T3	VD#16, 16A	.33	"	11:39AM	62
915	M	84	C	VC#17, 17A	.90	10:00AM	1:05PM	79
354	F	149	C	VC#18, 18A	.35	"	1:08PM	53
618	M	205	T1	VC#19, 19A	.62	"	1:10PM	74
366	F	397	T1	VC#20, 20A	.37	"	1:15PM	56
736	M	459	T2	VC#21, 21A	.74	"	1:30PM	78
341	F	550	T2	VC#22, 22A	.34	"	1:35PM	64
624	M	694	T3	VC#23, 23A	.62	"	1:45PM	45
507	F	732	T3	VC#24, 24A	.51	"	1:50PM	75
605	M	55	C	VC#25, 25A	0.61	12:00PM	3:10PM	68
355	F	200	C	VC#26, 26A	.36	"	3:14PM	65
739	M	264	T1	VC#27, 27A	.74	"	3:23PM	70
290	F	303	T1	VC#28, 28A	.29	"	3:25PM	55
679	M	469	T2	VC#29, 29A	.68	"	3:35PM	50
330	F	569	T2	VC#30, 30A	.33	"	3:40PM	41
720	M	609	T3	VC#31, 31A	.72	"	3:50PM	46
371	F	728	T3	VC#32, 32A	.37	"	3:53PM	63
649	M	39	C	VC#33, 33A	.65	2:00PM	4:45PM	72
398	F	160	C	VC#34, 34A	.40	"	4:50PM	57
696	M	279	T1	VC#35, 35A	.70	"	4:55PM	67
512	F	369	T1	VC#36, 36A	.50	"	5:03PM	77
694	M	489	T2	VC#37, 37A	.70	"	5:07PM	59
387	F	599	T2	VC#38, 38A	.39	"	5:10PM	51
757	M	639	T3	VC#39, 39A	.76	"	5:20PM	48
453	F	717	T3	VC#40, 40A	.45	"	5:25PM	43

¹ #1 right femur² #1A left femur³ Dose - Stock 1 mg Colchicine/ml

TABLE 2

IN VIVO RAT BONE MARROW CYTOGENETICS
BIOMEDICAL AND COMPARATIVE TOXICOLOGY LABORATORY
DOW CHEMICAL U.S.A.
TEXAS DIVISION, FREEPORT, TEXAS

I. MITOTIC ARREST

- A. Inject 1.0 mg/kg Colchicine 3 hours prior to sacrifice.
- B. Inject Colchicine intraperitoneally.
- C. Stock Colchicine: 1.0 mg/ml in Hanks Media.

II. SACRIFICE AND BONE MARROW CELL RECOVERY

- A. Sacrifice animal with CO₂.
- B. Remove all skin and most of the tissue from both femurs (try to avoid severing the femoral artery).
- C. Sever the femur at the distal end.
- D. Aspirate the bone marrow into a 10 cc syringe (with 20 gauge 1½" needle) containing 6 ml of Hanks Media.
- E. Place media and cells into a 15 ml centrifuge tube and mix cells vigorously making certain all cells are disassociated.

III. HARVEST OF BONE MARROW CELLS

- A. Centrifuge cells for 10 minutes at 1500 rpm.
- B. Discard all supernatant.
- C. Add 6 ml of 0.075 M KCl and mix thoroughly with siliconized dispopette.
- D. Incubate cells in 37°C water bath for 20 minutes.
- E. Centrifuge 5 minutes at 1500 rpm.
- F. Discard all supernatant.
- G. Add 5 ml of fresh fixative (3:1 methanol:acetic acid) and mix thoroughly.
- H. Refrigerate for minimum of 24 hours before making slides.

IV. SLIDE PREPARATION

- A. Centrifuge fixed cells for 5 minutes and discard all supernatant.
- B. Add 0.8-1.0 ml of fresh fixative and mix thoroughly.
- C. Drop cell suspension onto slides taken from 6% ethanol solution.
- D. Flame dry and then stain with 8% Giemsa at convenience.

TABLE 3
 MALE ATS SCORER 03
 BIOMEDICAL RESEARCH LABORATORY
 CHROMOSOME AND CHROMATID SUMMARY SHEET

CULTURE NO.	TREATMENT	NO. CELLS SCORED	MULTIPLE ABERRATIONS/CELLS SCORED (1)	MISCELLANEOUS ABERRATIONS/CELLS SCORED (2)	CHROMATID ABERRATIONS/CELLS SCORED (3)	CHROMOSOME ABERRATIONS/CELLS SCORED (4)	NO. ABNORMAL CELLS/CELLS SCORED (5)
VC #1	C	50	0	0	0	0	0
9	C	50	0	0	0	0	0
17	C	50	0	0	0	0	0
25	C	50	0	0	.02	0	.02
33	C	50	0	0	.02	0	.02
VC #3	T ₁	50	0	0	0	0	0
11	T ₁	50	0	0	0	0	0
19	T ₁	50	0	0	0	0	0
27	T ₁	50	0	0	.04	0	.04
35	T ₁	50	0	0	.04	0	.04
VC #5	T ₂	50	0	0	0	0	0
13	T ₂	50	0	0	.04	0	.04
21	T ₂	50	0	0	.02	0	.02
29	T ₂	50	0	0	0	0	0
37	T ₂	50	0	0	0	0	0
VC #7	T ₃	50	0	0	0	0	0
15	T ₃	50	0	0	0	0	0
23	T ₃	50	0	0	.04	0	.04
31	T ₃	50	0	0	0	0	0
39	T ₃	50	0	0	.02	0	.02

- (1) Multiple Aberrations -- a heavily damaged cell containing aberrations too numerous to count.
- (2) Miscellaneous Aberrations - a cell containing pulverizations, endoreduplications, etc.
- (3) Chromatid Aberrations -- includes breaks with fragment, breaks without fragments, intrachanges, and interchanges.
- (4) Chromosome Aberrations -- includes breaks with fragments, breaks without fragments, dicentrics, rings, translocations, and inversions.
- (5) Abnormal Cells -- scored as one if a cell contained any of the abnormalities listed in notes (1), (2), (3) or (4).

TABLE 3
MALL ATS SCORER 25
BIOMEDICAL RESEARCH LABORATORY
CHROMOSOME AND CHROMATID SUMMARY SHEET

CULTURE NO.	TREATMENT	NO. CELLS SCORED	MULTIPLE ABERRATIONS/CELLS SCORED (1)	MISCELLANEOUS ABERRATIONS/CELLS SCORED (2)	CHROMATID ABERRATIONS/CELLS SCORED (3)	CHROMOSOME ABERRATIONS/CELLS SCORED (4)	NO. ABNORMAL CELLS/CELLS SCORED (5)
VC #1	C	50	0	0	0	0	0
9	C	50	0	0	.02	0	.02
17	C	50	0	0	0	0	0
25	C	50	0	0	.02	0	.02
33	C	50	0	0	.04	0	.04
VC #3	T ₁	50	0	0	.02	0	.02
11	T ₁	50	0	0	0	0	0
19	T ₁	50	0	0	.04	0	.04
27	T ₁	50	0	0	.04	0	.04
35	T ₁	50	0	0	0	0	0
VC #5	T ₂	50	0	0	0	0	0
13	T ₂	50	0	0	0	0	0
21	T ₂	50	0	0	0	0	0
29	T ₂	50	0	0	0	0	0
37	T ₂	47	0	0	0	0	0
VC #7	T ₃	50	0	0	.04	0	.04
15	T ₃	50	0	0	0	0	0
23	T ₃	50	0	0	0	0	0
31	T ₃	50	0	0	0	0	0
39	T ₃	50	0	0	0	0	0

- (1) Multiple Aberrations -- a heavily damaged cell containing aberrations too numerous to count.
- (2) Miscellaneous Aberrations -- a cell containing pulverizations, endoreduplications, etc.
- (3) Chromatid Aberrations -- includes breaks with fragment, breaks without fragments, intrachanges, and interchanges.
- (4) Chromosome Aberrations -- includes breaks with fragments, breaks without fragments, dicentrics, rings, translocations, and inversions.
- (5) Abnormal Cells -- scored as one if a cell contained any of the abnormalities listed in notes (1), (2), (3), or (4).

TABLE 3
 MALE ATS SCORER 26
 BIOMEDICAL RESEARCH LABORATORY
 CHROMOSOME AND CHROMATID SUMMARY SHEET

CULTURE NO.	TREATMENT	NO. CELLS SCORED	MULTIPLE ABERRATIONS/CELLS SCORED (1)	MISCELLANEOUS ABERRATIONS/CELLS SCORED (2)	CHROMATID ABERRATIONS/CELLS SCORED (3)	CHROMOSOME ABERRATIONS/CELLS SCORED (4)	NO. ABNORMAL CELLS/CELLS SCORED (5)
VC #1	C	50	0	0	0	0	0
9	C	50	0	0	.02	0	.02
17	C	50	0	0	0	0	0
25	C	50	0	0	.02	0	.02
33	C	50	0	0	.02	0	.02
VC #3	T ₁	50	0	0	.02	0	.02
11	T ₁	50	0	0	0	0	0
19	T ₁	50	0	0	.04	0	.04
27	T ₁	50	0	0	0	0	0
35	T ₁	50	0	0	.04	0	.04
VC #5	T ₂	50	0	0	.02	0	.02
13	T ₂	50	0	0	0	0	0
21	T ₂	50	0	0	0	0	0
29	T ₂	50	0	0	.04	0	.02
37	T ₂	50	0	0	.02	0	.02
VC #7	T ₃	50	0	0	0	0	0
15	T ₃	50	0	0	0	0	0
23	T ₃	50	0	0	.04	0	.04
31	T ₃	50	0	0	.02	0	.02
39	T ₃	50	0	0	0	0	0

- (1) Multiple Aberrations -- a heavily damaged cell containing aberrations too numerous to count.
- (2) Miscellaneous Aberrations -- a cell containing pulverizations, endoreduplications, etc.
- (3) Chromatid Aberrations -- includes breaks with fragment, breaks without fragments, intrachanges, and interchanges.
- (4) Chromosome Aberrations -- includes breaks with fragments, breaks without fragments, dicentrics, rings, translocations, and inversions.
- (5) Abnormal Cells -- scored as one if a cell contained any of the abnormalities listed in notes (1), (2), (3) or (4).

TABLE 4
FEMALE RATS SCORER 26
BIOMEDICAL RESEARCH LABORATORY
CHROMOSOME AND CHROMATID SUMMARY SHEET

CULTURE NO.	TREATMENT	NO. CELLS SCORED	MULTIPLE ABERRATIONS/CELLS SCORED (1)	MISCELLANEOUS ABERRATIONS/CELLS SCORED (2)	CHROMATID ABERRATIONS/CELLS SCORED (3)	CHROMOSOME ABERRATIONS/CELLS SCORED (4)	NO. ABNORMAL CELLS/CELLS SCORED (5)
VC #2	C	50	0	0	0	0	0
10	C	50	0	0	0	0	0
18	C	50	0	0	.02	0	.02
26	C	50	0	0	.02	0	.02
34	C	50	0	0	.02	0	.02
VC #4	T ₁	50	0	0	.06	0	.06
12	T ₁	50	0	0	0	0	0
20	T ₁	50	0	0	0	0	0
28	T ₁	50	0	0	.04	0	.04
36	T ₁	50	0	0	.04	0	.02
VC #6	T ₂	50	0	0	0	0	0
14	T ₂	50	0	0	.06	0	.06
22	T ₂	50	0	0	.04	0	.04
30	T ₂	50	0	0	0	0	0
38	T ₂	50	0	0	.04	0	.04
VC #8	T ₃	50	0	0	.02	0	.02
16	T ₃	50	0	0	0	0	0
24	T ₃	50	0	0	.02	0	.02
32	T ₃	50	0	0	.02	0	.02
40	T ₃	50	0	0	.06	0	.06

- (1) Multiple Aberrations -- a heavily damaged cell containing aberrations too numerous to count.
- (2) Miscellaneous Aberrations -- a cell containing pulverizations, endoreduplications, etc.
- (3) Chromatid Aberrations -- includes breaks with fragment, breaks without fragments, intrachanges, and interchanges.
- (4) Chromosome Aberrations -- includes breaks with fragments, breaks without fragments, dicentrics, rings, translocations, and inversions.
- (5) Abnormal Cells -- scored as one if a cell contained any of the abnormalities listed in notes (1), (2), (3) or (4).

TABLE 4
FEMAL CORER 25
BIOMEDICAL RESEARCH LABORATORY
CHROMOSOME AND CHROMATID SUMMARY SHEET

CULTURE NO.	TREATMENT	NO. CELLS SCORED	MULTIPLE ABERRATIONS/CELLS SCORED (1)	MISCELLANEOUS ABERRATIONS/CELLS SCORED (2)	CHROMATID ABERRATIONS/CELLS SCORED (3)	CHROMOSOME ABERRATIONS/CELLS SCORED (4)	NO. ABNORMAL CELLS/CELLS SCORED (5)
VC #2	C	50	0	0	0	0	0
10	C	50	0	0	0	0	0
18	C	50	0	0	.06	0	.06
26	C	50	0	0	.02	0	.02
34	C	50	0	0	.02	0	.02
VC #4	T ₁	50	0	0	0	0	0
12	T ₁	50	0	0	0	0	0
20	T ₁	50	0	0	0	0	0
28	T ₁	50	0	0	.02	0	.02
36	T ₁	50	0	0	.02	0	.02
VC #6	T ₂	50	0	0	.02	0	.02
14	T ₂	50	0	0	.02	0	.02
22	T ₂	50	0	0	0	0	0
30	T ₂	50	0	0	.02	0	.02
38	T ₂	50	0	0	.02	0	.02
VC #8	T ₃	50	0	0	.10	0	.10
16	T ₃	50	0	0	.02	0	.02
24	T ₃	50	0	0	0	0	0
32	T ₃	50	0	0	0	0	0
40	T ₃	50	0	0	.02	0	.02

- (1) Multiple Aberrations -- a heavily damaged cell containing aberrations too numerous to count.
- (2) Miscellaneous Aberrations -- a cell containing pulverizations, endoreduplications, etc.
- (3) Chromatid Aberrations -- includes breaks with fragment, breaks without fragments, intrachanges, and interchanges.
- (4) Chromosome Aberrations -- includes breaks with fragments, breaks without fragments, dicentrics, rings, translocations, and inversions.
- (5) Abnormal Cells -- scored as one if a cell contained any of the abnormalities listed in notes (1), (2), (3) or (4).

TABLE 4
FEMAL RATS SCORER 03
BIOMEDICAL RESEARCH LABORATORY
CHROMOSOME AND CHROMATID SUMMARY SHEET

CULTURE NO.	TREATMENT	NO. CELLS SCORED	MULTIPLE ABERRATIONS/CELLS SCORED (1)	MISCELLANEOUS ABERRATIONS/CELLS SCORED (2)	CHROMATID ABERRATIONS/CELLS SCORED (3)	CHROMOSOME ABERRATIONS/CELLS SCORED (4)	NO. ABNORMAL CELLS/CELLS SCORED (5)
VC #2	C	50	0	0	0	0	0
10	C	50	0	0	.04	0	.04
18	C	50	0	0	0	0	0
26	C	50	0	0	.06	0	.06
34	C	50	0	0	0	0	0
VC #4	T ₁	50	0	0	0	0	0
12	T ₁	50	0	0	0	.02	.02
20	T ₁	50	0	0	0	0	0
28	T ₁	50	0	0	0	0	0
36	T ₁	50	0	0	0	0	0
VC #6	T ₂	50	0	0	.02	0	.02
14	T ₂	50	0	0	0	0	0
22	T ₂	50	0	0	0	0	0
30	T ₂	50	0	0	0	0	0
38	TC	50	0	0	0	0	0
VC #8	T ₃	50	0	0	.02	0	.02
16	T ₃	50	0	0	.06	.02	.06
24	T ₃	50	0	0	0	0	0
32	T ₃	50	0	0	.08	0	.08
40	T ₃	50	0	0	0	0	0

- (1) Multiple Aberrations -- a heavily damaged cell containing aberrations too numerous to count.
- (2) Miscellaneous Aberrations -- a cell containing pulverizations, endoreduplications, etc.
- (3) Chromatid Aberrations -- includes breaks with fragment, breaks without fragments, intrachanges, and interchanges.
- (4) Chromosome Aberrations -- includes breaks with fragments, breaks without fragments, dicentrics, rings, translocations, and inversions.
- (5) Abnormal Cells -- scored as one if a cell contained any of the abnormalities listed in notes (1), (2), (3) or (4).

TABLE 5
STATISTICAL SUMMARY

Sex of Animal	Scorer Number	Doses				Total
		C	T ₁	T ₂	T ₃	
Males	03	.008	.016	.012	.012	.012
	25	.016	.02	.00	.008	.014
	26	.012	.02	.012	.012	.014
	Total	.012	.019	.008	.011	.013
Females	03	.02	.004	.004	.032	.015
	25	.02	.008	.016	.028	.018
	26	.012	.024	.028	.024	.022
	Total	.017	.012	.016	.028	.019
TOTAL		.015	.016	.012	.020	.016

Scorer	Doses				Total
	C	T ₁	T ₂	T ₃	
03	.014	.010	.008	.022	.014
25	.018	.014	.008	.018	.015
26	.012	.022	.020	.018	.018
Total	.015	.016	.012	.020	.016

$p \doteq .60$

$p \doteq .50$

Sex of Animal	Dose				Total
	C	T ₁	T ₂	T ₃	
Male	.012	.019	.008	.011	.013
Female	.017	.012	.016	.028	.018
Total	.015	.015	.012	.019	.016

$p \doteq .09$

$p \doteq .50$

TABLE 6
ANALYSIS OF VARIANCE SUMMARY

<u>Source of Variation*</u>	<u>df</u>	<u>mean squares</u>	<u>F ratio</u>	<u>p-value</u>
Total	119			
Sex	1	.00108	3.176	.09
Dose	3	.00028	.810	ns
Sex by Dose	3	.00073	2.156	.12
Animals in Groups	32	.00034	--	--
Scorers	2	.00022	.490	ns
Sex by Scorer	2	.00007	.153	ns
Dose by Scorer	6	.00026	.568	ns
Sex by Dose by Scorer	6	.00026	.564	ns
Scorer by Animal in Groups	64	.00045	--	--

Hypotheses

- I. $H_0: \mu_m = \mu_f \quad p \doteq .09$
 $H_A: \mu_m \neq \mu_f$
- II. $H_0: \mu_{03} = \mu_{25} = \mu_{26} \quad p \doteq .60$
 $H_A: \text{At least one scorer is different}$
- III. $H_0: \mu_c = \mu_1 = \mu_2 = \mu_3 \quad p \doteq .50$
 $H_A: \text{At least one dose is different}$
- IV. $H_0: \text{Doses and Sexes are Independent} \quad p \doteq .12$
 $H_A: \text{Dose effect is sex dependent}$

- *
 1. Sex, Dose, Scorers considered Fixed Factors.
 2. Animals considered random.

H_0 -- Null Hypothesis
 H_A -- Alternate Hypothesis
 μ_m -- Mean for males
 μ_f -- Mean for females