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GENETICS ASSAY NO.: 6998

LBI SAFETY NO.: 8595

Berichts-Nr. 84/0191
Gewerbetoxykologie

MUTAGENICITY EVALUATION OF

PKOD-A038

IN THE
CHINESE HAMSTER BONE MARROW
CYTOGENETIC ASSAY

FINAL REPORT

SUBMITTED TO:

HOECHST AKTIENGESELLSCHAFT
D-6230 FRANKFURT AM MAIN 80
WEST GERMANY

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

LBI PROJECT NO.: 22202

REPORT DATE: MARCH, 1984



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PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-IX. Items I-IV provide Sponsor and test article identification information, type of assay, and the protocol reference number. Item V provides the initiation and completion dates of the study. Item VI identifies the supervisory personnel. Item VII indicates the tables and/or figures containing the test results. The interpretation of the results is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report describes the study design, which includes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices.

All test and control results presented in this report are supported by raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895.

Copies of the raw data will be supplied to the Sponsor upon request.

The described study was performed in accordance with Good Laboratory Practice regulations except if noted to the contrary. To the best of the signer's knowledge there were no significant deviations from the Good Laboratory Practice regulations which affected the quality or integrity of the study.



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CHROMOSOME ABERRATIONS IN CHINESE HAMSTER BONE MARROW CELLS

OBJECTIVE

The objective of this in vivo assay was to evaluate the ability of PKOD-A038 to induce chromosomal aberrations in Chinese hamster bone marrow cells from both male and female animals.

CONCLUSION

The compound tested, PKOD-A038, did not induce chromosomal aberrations under the conditions of the assay and is considered negative in the aberration test in Chinese hamster bone marrow cells.



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- I. SPONSOR: Hoechst Aktiengesellschaft
- II. MATERIAL (TEST ARTICLE):
- A. Client's Identification: PKOD-A038
 - B. Date Received: July 8, 1983
 - C. Physical Description: White powder
 - D. Genetics Assay No.: 6998
- III. TYPE OF ASSAY: CHINESE HAMSTER BONE MARROW CYTOGENETIC ASSAY
- IV. PROTOCOL NO.: 451, Ed. 8 Modified for Hoechst
- V. STUDY DATES:
- A. Initiation Date: August 11, 1983
 - B. Completion Date: January 6, 1984
- VI. SUPERVISORY PERSONNEL:
- A. Study Director: Michael C. Cimino, Ph.D. (until November 12, 1983)
Sheila M. Galloway, Ph.D. (from November 12, 1983)
James L. Ivett, Ph.D. (from December 26, 1983)
 - B. Laboratory Supervisor: Helen Lebowitz
- VII. RESULTS:
- The information on administration of the test article is in Tables 1A and 1B. The test results have been collected from raw data sheets and tabulated in summary form in Tables 2A, 2B and 2C, and a summary of pooled data appears in Table 3.
- VIII. INTERPRETATION OF RESULTS:
- Dose Selection
- The dose of PKOD-A038 at 5 g/kg body weight was suggested by the sponsor for use in the assay based upon maximum tolerated dose data. This dose proved not suitable due to animal lethality (100%, n=2) and a dose of 3.5 g/kg body weight was agreed on after consultation with the sponsor.
- Compound Preparation
- The compound was dissolved in 100% polyethylene glycol 400, (PEG) the solvent suggested by the sponsor. Due to the range in animal weights (28.5-44.4 g for the males, 23.8-32.1 for the females) the volume of test compound administered was weight adjusted for each animal. The 3.5 g/kg



VIII. INTERPRETATION OF RESULTS: (continued)

stock solution was prepared such that the animals received 0.015 ml/g body weight (4.1805 g of PKOD-A038 in 13.5 ml of PEG) using a 15 gauge gavage tube.

Two dosing trials were conducted in the testing of PKOD-A038. The first trial was not considered in the evaluation of the results since no positive results were obtained in the triethylenemelamine (TEM) treated positive control animals. The positive control dosage of 1 mg/kg TEM which was used in the first trial was increased to 3 mg/kg for the second dosing trial.

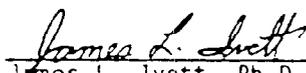
Information on the dosing volumes and numbers of animals per group in the second trial is summarized in Tables 1A and 1B. The cytogenetic data are shown in Table 2A, 2B and 2C and are summarized in Table 3. In both the male and female animals treated with PKOD-A038 there was no increase in the aberration frequency over the negative controls at any of the kill times (6, 24 or 48 hr). The negative control aberration frequency was in the normal background range for this laboratory (about 0-1.5% cells with aberrations with 0.3% or less about 50% of the time). The positive control compound, triethylenemelamine, induced a highly significant increase in aberrations in both sexes. Mitotic index which may reflect cytotoxicity, showed no clear suppression.

IX. CONCLUSIONS:

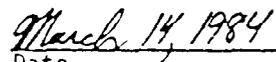
In conclusion, the compound tested, PKOD-A038, did not induce chromosomal aberrations under the conditions of the assay, and is considered negative in the aberration test in Chinese hamster bone marrow cells.

SUBMITTED BY:

Study Director

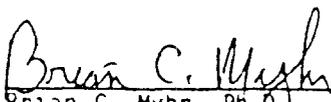


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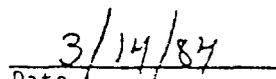


Date

REVIEWED BY:



Brian C. Myhr, Ph.D.
Director
Department of Molecular Toxicology



Date



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TABLE 1A

DOSING INFORMATION: CYTOGENETIC ASSAY

SPONSOR: Hoechst STUDY ID: Hamster Bone Marrow Aberrations DOSING INITIATION DATE: November 29, 1983
 PROJECT: 22202 STRAIN/SPECIES Chinese Hamster DOSING TERMINATION DATE: November 29, 1983
 TEST ARTICLE: PKOD-A038 BREEDER: Cambridge Diagnostics LOCATION: Rockville
 ASSAY NO.: 6998 (Repeat) PURCHASE ORDER NO.: 124058 ROOM NUMBERS: 120A

TREATMENT	VEHICLE	DOSAGE	ROUTE OF ADMINISTRATION	VOLUME/* ANIMAL (ml/g body wt)	NUMBER OF ADMINISTRATIONS	ANIMAL NUMBERS	DEAD OR REPLACED
<u>Positive Control</u> Triethylene- melamine (24 Hr Kill)	0.9% saline	M 3 mg/kg	I.P.	0.015	1	4290-4293 ^a	4289 (DEAD) not replaced
		F 3 mg/kg		0.015		4324-4328	
<u>6 Hr Kill</u>							
<u>Negative Control</u>	Polyethylene Glycol 400	M 16.9 g/kg	P.O.	0.015	1	4269-4273	
		F 16.9 g/kg		0.015		4304-4308	
PKOD-A038	Polyethylene Glycol 400	M 3.5 g/kg	P.O.	0.015	1	4274-4278 ^b	
		F 3.5 g/kg		0.015		4309-4313 ^b	

*Volume dosed was based on individual animal body weights.

Toxic signs: ^aAnimals lethargic with rear leg paralysis immediately after dosing - animal #4289 found dead on Day 2.

^bAnimals lethargic with labored breathing after colchicine.

TABLE 1B

DOSING INFORMATION: CYTOGENETIC ASSAY

SPONSOR: Hoechst STUDY ID: Hamster Bone Marrow Aberrations DOSING INITIATION DATE: November 29, 1983
 PROJECT: 22202 STRAIN/SPECIES Chinese Hamster DOSING TERMINATION DATE: November 29, 1983
 TEST ARTICLE: PKOD-A038 BREEDER: Cambridge Diagnostics LOCATION: Rockville
 ASSAY NO.: 6998 (Repeat) PURCHASE ORDER NO.: 124058 ROOM NUMBERS: 120A

<u>TREATMENT</u>	<u>VEHICLE</u>	<u>DOSAGE</u>	<u>ROUTE OF ADMINIS- TRATION</u>	<u>VOLUME/* ANIMAL (ml/g body wt)</u>	<u>NUMBER OF ADMINI- STRATIONS</u>	<u>ANIMAL NUMBERS</u>	<u>DEAD OR REPLACED</u>
<u>24 Hr K111</u>							
<u>Negative Control</u>							
	Polyethylene Glycol 400	M 16.9 g/kg F 16.9 g/kg	P.O.	0.015 0.015	1	4279-4283 4314-4318	
PKOD-A038	Polyethylene Glycol 400	M 3.5 g/kg F 3.5 g/kg	P.O.	0.015 0.015	1	4284-4288 ^a 4319-4323 ^a	
<u>48 Hr K111</u>							
<u>Negative Control</u>							
	Polyethylene Glycol 400	M 16.9 g/kg F 16.9 g/kg	P.O.	0.015 0.015	1	4294-4298 4329-4333	
PKOD-A038	Polyethylene Glycol 400	M 3.5 g/kg F 3.5 g/kg	P.O.	0.015 0.015	1	4299-4303 4334-4338 ^b	

*Volume dosed was based on individual animal body weights.

Toxic signs: ^aOn Day 2 animal #4284 with lethargy, lack of coordination, labored breathing and bloody nose and animal #4323 with scruffy appearance and with labored breathing, all other animals fine.

^bOn Day 3 animal #4336 with scruffy appearance and with labored breathing all other animals fine.

TABLE 2A: CONTROLS

A SUMMARY OF THE CYTOGENETIC ANALYSIS IN BONE MARROW CELLS OF CHINESE HAMSTERS

SPONSOR: HoechstASSAY NO.: 6998

TREATMENT	SEX	KILL TIME (hrs) ^a	NO. OF ANI-MALS ^b	TOTAL NO. OF CELLS	STRUCTURAL ABERRATIONS ^d				NUMERICAL ^d ABERRATIONS		MITOTIC INDEX ^e
					TYPE ^c	ABERRATIONS PER CELL	CELLS WITH ABERRATIONS NO.	%	TYPE	FREQUENCY	
<u>Negative Control</u> Polyethylene Glycol 400 (16.9 g/kg)	MALE	6	4	200	5TG,1R	0.005	1	0.5%	2HR,2PP	0.020	9.0
		24	4	200	6TG,1TB	0.005	1	0.5%	3HR	0.015	7.6
		48	4	155	1TG	0.000	0	0.0	2HR,1PP	0.019	5.4
	FEMALE	6	5	250	1TG	0.000	0	0.0	1HR,1PP	0.008	6.2
		24	4	200	1TG,1IG	0.000	0	0.0	1HR,4PP	0.025	6.1
		48	5	250	7TG,4IG, 1TB	0.004	1	0.4%	2PP	0.008	8.3
<u>Positive Control</u> Triethylene-melamine (3.0 mg/kg)	MALE	24	4	200	29TG,4IG, 54TB,7SB, 14TF,9AF, 38TR,16QR, 1P+,9CR,3R, 2D,1AB, 1 PENTRAD,12>	>1.38*	74	37.0%	11HR,4PP	0.075	1.4
	FEMALE	24	5	250	29TG,13IG, 36TB,13SB, 8TF,5AF, 10TR,7QR, 1PU,25P+, 39PC,2D,1MT, 21D,2TD,25>	>1.60**	126	50.4%	3HR,1PP	0.016	1.7

^aTime after final exposure when bone marrow was harvested.^bIncludes only animals from which at least 5 scoreable metaphases were obtained.^cChromatid gaps (TG) and isochromatid gaps (IG) were not used in calculating the aberrations per cell or the percent cells with aberrations.^dKey attached.^e% of at least 500 cells per animal.*Significantly greater than negative control, $p < .05$, using Kruskal-Wallis test.**Significantly greater than negative control, $p < .01$, using Kruskal-Wallis test.

TABLE 2B: TREATED ANIMALS

A SUMMARY OF THE CYTOGENETIC ANALYSIS IN BONE MARROW CELLS OF MALE CHINESE HAMSTERS

SPONSOR: HoechstASSAY NO.: 6998

MALES	PKOD-A038 DOSE	KILL TIME (hrs) ^a	NO. OF ANI- MALS ^b	TOTAL NO. OF CELLS	STRUCTURAL ABERRATIONS ^d			NUMERICAL ^d ABERRATIONS		MITOTIC INDEX ^e
					TYPE ^c	ABERRATIONS PER CELL	CELLS WITH ABERRATIONS NO. %	TYPE	FREQUENCY	
	3.5 g/kg	6	5	250	2TG,2IG	0.000	0 0.0	2HR	0.008	5.3
	3.5 g/kg	24	4	200	3TG,1R, 1TF	0.010	2 1.0	3HR	0.015	4.9
	3.5 g/kg	48	4	200	2TG	0.000	0 0.0	3PP	0.015	6.8

^aTime after final exposure when bone marrow was harvested.^bIncludes only animals from which at least 5 scoreable metaphases were obtained.^cChromatid gaps (TG) and isochromatid gaps (IG) were not used in calculating the aberrations per cell or the percent cells with aberrations.^dKey attached.^e% of at least 500 cells per animal.

TABLE 2C: TREATED ANIMALS

A SUMMARY OF THE CYTOGENETIC ANALYSIS IN BONE MARROW CELLS OF FEMALE CHINESE HAMSTERS

SPONSOR: HoechstASSAY NO.: 6998

FEMALES	PK00-A030 DOSE	KILL TIME (hrs) ^a	NO. OF ANI- MALS ^b	TOTAL NO. OF CELLS	STRUCTURAL ABERRATIONS ^c				NUMERICAL ^c ABERRATIONS		MITOTIC INDEX ^d
					TYPE	ABERRATIONS PER CELL	CELLS WITH ABERRATIONS NO.	%	TYPE	FREQUENCY	
	3.5 g/kg	6	5	217	2TB	0.009	2	0.9	5PP	0.023	6.7
	3.5 g/kg	24	5	250	8TG,6IG, 4TB,1SB	0.020	5	2.0	7HR,3PP	0.040	5.1
	3.5 g/kg	48	5	212	3TG,2IG	0.000	0	0.0	2HR,3PP	0.024	5.7

^aTime after final exposure when bone marrow was harvested.^bIncludes only animals from which at least 5 scoreable metaphases were obtained.^cChromatid gaps (TG) and isochromatid gaps (IG) were not used in calculating the aberrations per cell or the percent cells with aberrations.^dKey attached.^e% of at least 500 cells per animal.

TABLE 3

CYTOGENETIC ANALYSIS OF PKOD-A038 IN CHINESE HAMSTER BONE MARROW:
SUMMARY OF ALL HARVEST TIMES (6, 24, 48 HOURS) -
BOTH SEXES POOLED

SPONSOR: Hoechst ASSAY NO.: 6998

Dose Type	Treatment	Cells Scored	Cells with Structural Aberrations		Aberrations/100 Cells	
			No.	Per 100	Structural	Numerical
Positive Control Triethylene- melamine	3 mg/kg	450	200	44.44	>150.22**	4.22
Negative Control	Polyethylene Glycol 400	1255	3	0.24	0.24	1.51
PKOD-A038	3.5 g/kg	1329	9	0.68	0.68	2.11

**Significantly greater than negative control, $p < 0.01$ using Kruskal-Wallis test.



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STUDY DESIGN

1. PURPOSE

The purpose of this study was to determine the potential genetic activity of a chemical. The chemical was administered to Chinese hamsters whose bone marrow metaphase chromosomes were then examined for structural changes and rearrangements.

2. MATERIALS

A. Animals

Adult male and female Chinese hamsters, were purchased from Cambridge Diagnostics, Cambridge, MA.

B. Control Articles

Triethylenemelamine (TEM) at 3.0 mg/kg was used as the positive control article and was administered via a single intraperitoneal (IP) injection. The negative control article consisted of the solvent or vehicle used for the test article and was administered by the same route as, and concurrently with, the test article.

3. EXPERIMENTAL DESIGN

A. Animal Husbandry

Animals were housed individually. A commercial diet (Purina Laboratory Chow) and water was available ad libitum unless contraindicated by the particular experimental design.

Animals were assigned to study groups at random according to LBI Standard Operating Procedures (SOP). Prior to study initiation, animal were weighed to calculate dose levels according to SOP "Animal Weight Determination." The volume of test article administered per animal was established using this method unless there was significant variation among individuals, in which case individual calculations were made. Animals were uniquely identified by either ear targe or ear punch. Dose or treatment groups were identified by cage card.

Sanitary cages and bedding was used. Personnel handling animals or working within the animal facilities were required to wear suitable protective garments. When appropriate, individuals with respiratory or other overt infections were excluded from the animal facilities.



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3. EXPERIMENTAL DESIGN (continued)

B. Dose Selection

The dose level was selected by the Sponsor as 5 g/kg, based upon the maximum tolerated dose (MTD), however, due to animal lethality the highest dose achieved was 3.5 g/kg.

C. Route of Administration

The route of administration was per os.

4. METHODOLOGY

The basic design of the test is shown below. An acute (single dose) sequence was provided. A total of 70 animals was used in the test.

Number of Animals Used for Chinese Hamster Bone Marrow Cytogenetic Analysis^a

<u>Treatment</u>	<u>Acute Study</u> Number of Animals Killed After Dosing			<u>Total Animals</u>
	<u>6 Hr</u>	<u>24 Hr</u>	<u>48 Hr</u>	
Test Article	10	10	10	30
Positive control ^b	-	10	-	10
Negative control	10	10	10	30

^aHalf of the animals in each category are male; half are female.

^bPositive controls were administered on an acute, one-time basis only.

Three hours prior to kill, the animals were injected IP with 4.0 mg/kg of colchicine. The animals were killed with CO₂ at the times indicated. The adhering soft tissue and epiphyses of both tibiae and femora were removed according to the method of Legator *et al.*, (1969). The marrow was flushed from the bone and transferred to Hanks' balanced salt solution. The marrow button was collected by centrifugation and then resuspended in 0.075M KCl. The centrifugation was repeated and the pellet resuspended in fixative (methanol:acetic acid, 3:1). The fixative was changed once and cells left overnight at 4°C.

Cells in fixative were dropped onto glass slides and air-dried. Spreads were stained with 5% Giemsa at pH 6.8. After drying, slides were cover-slipped using Depex.



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4. METHODOLOGY (continued)

Slides were coded for control of bias, and then scored for chromosomal aberrations. Standard forms were used to score and record gaps, breaks, fragments and reunion figures. A list of aberrations scored includes:

chromatid gap	pulverized chromosome
chromatid break	pulverized chromosomes
chromosome gap	pulverized cells
chromosome break	ring chromosome
chromatid deletion	dicentric chromosome
fragment	minute chromosome
acentric fragment	double minute chromosome
translocation	abnormal metacentric chromosome
triradial	greater than 10 aberrations
quadriradial	polyploid
complex rearrangement	aneuploid (both hypo- and hyperploid)

Routinely, 50 spreads were read for each animal. The location of cells bearing aberrations was identified by the use of coordinates on the mechanical stage. A mitotic index based on at least 500 cells counted was also recorded. It was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

5. EVALUATION CRITERIA

A number of general guidelines was established to serve as an aid in determining the meaning of bone marrow chromosomal aberrations.

A. General

Basically, an attempt was made to establish whether a test article or its metabolites can interact with chromosomes to produce gross lesions or changes in chromosome numbers and whether these are of a type which can survive more than one mitotic cycle of the cell. All aberration figures detected by this assay resulted from breaks in the chromatin which either failed to repair or repaired in atypical combination. The cell transit time for bone marrow is normally 10 to 24 hours. The assay design was such that bone marrow samples were taken at 6, 24 and 48 hours after an acute administration of the test article to permit detection of chromosome aberrations in cells that were delayed in their progression through the mitotic cycle.

One would anticipate that many of the cells bearing breaks or reunion figures would be eliminated after their first mitotic division and, as a corollary, that those cells which survive the first anaphase would primarily bear balanced lesions. The detection of these lesions and hence a complete risk evaluation must usually rely on additional testing. In general, a cell bearing configurations such as small deletions or reciprocal translocations may be perpetuated and, therefore, constitute a greater risk to the individual than large deletions or complex rearrangements.



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5. EVALUATION CRITERIA (continued)

3. Data Interpretation

Data was summarized in tabular form and evaluated. Gaps were not counted as significant aberrations. Open breaks were considered as indicators of genetic damage as were configurations resulting from the repair of breaks. The latter included translocations, multiradials, rings, multicentrics, etc. Reunion figures such as these were weighted slightly higher than breaks since they usually resulted from more than one break.

The number of aberrations per cell was also considered significant; cells with more than one aberration were considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of mutagenic potential.

Frequently, one is unable to locate 50 suitable metaphase spreads for each animal even after preparing additional slices. Possible causes for this appear to be related to cytotoxic effects which may alter the duration of the cell cycle, kill the cell or cause clumping of the chromosomes. Additional information can be gained from the mitotic index which also appears to reflect cytotoxic effects.

In any event the type of aberration, its frequency and its correlation to dose in a given time period was considered in evaluating a test article as being mutagenically positive or negative.

Statistical analysis employed a Student t-test (Bancroft, 1957), or the Kruskal-Wallis test (Sokal and Rohlf, 1969), or other appropriate tests.

6. REFERENCES

Bancroft, H.: Introduction to Biostatistics, Hoeber-Harper, 1957.

Chromosome methodologies in mutagen testing: Report of the Ad Hoc Committee of the Environmental Mutagen Society and the Institute for Medical Research. Toxicol. Appl. Pharmacol., 22:259-275, 1972.

Dean, B.J.: Chemical-induced chromosome damage. Lab. Animal, 3:157-174, 1969.

Legator, M.S., Palmer, K.A., Green, S. and Peterson, K.W.: Cytogenetic studies in rats of cyclohexamine, a metabolite of cyciazate. Science, 165:1139-1140, 1969.

Sokal, R.R. and Rohlf, F.J. Biometry. Freeman, 1969.



7. RECORDS TO BE MAINTAINED

All raw data, protocol, modifications, test article weight and dispensation records and correspondence between LBI and the Sponsor are maintained in a central file within the Department of Molecular Toxicology. These records are filed under Departmental assay number and held up to 2 years following submission of the final report to the Sponsor. After 2 years they are transferred to the LBI Archives for permanent storage.



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DEFINITIONS OF ABERRATIONS

tg = Chromatid gap:	An achromatic region in one chromatid, the size of which is equal to or smaller than the width of the chromatid.
sg = Chromosome gap:	Same as tg only in both chromatids.
tb = Chromatid break:	An achromatic region in one chromatid larger than the width of the chromatid. It may either be aligned or unaligned.
sb = Chromosome break:	Same as tb only in both chromatids.
td = Chromatid deletion:	Deleted material at the end of one chromatid.
tf = Fragment:	A single chromatid without an evident centromere.
af = Acentric fragment:	Two aligned (parallel) chromatids without an evident centromere.
t = Translocation:	Obvious transfer of material between two or more chromosomes.
tr = Triradial:	An abnormal arrangement of paired chromatids resulting in a three-armed configuration.
qr = Quadriradial:	An abnormal arrangement of paired chromatids resulting in a four-armed configuration.
pu = Pulverized chromosome:	A spread containing one fragmented or pulverized chromosome.
pu+ = Pulverized chromosomes:	A spread containing two or more fragmented or pulverized chromosomes, but with some intact chromosomes still remaining.
puc = Pulverized cell:	A cell in which all the chromosomes are totally fragmented.
cr = Complex rearrangement:	An abnormal translocation figure which involves many chromosomes and is the result of several breaks and mispairing chromatids.
r = Ring:	A chromosome which is a result of telomeric deletions at both ends of the chromosome and the subsequent joining of the ends of the two chromosome arms.
d = Dicentric:	A chromosome containing two centromeres.

DEFINITIONS OF ABERRATIONS (continued)

mt = Minute:	A small chromosome which contains a centromere and does not belong in the normal karyotype.
dm = Double minute:	Small double dots, some of which are terminal deletions and some interstitial deletions; probably small rings.
ab = Abnormal monocentric:	A chromosome whose morphology is abnormal for the karyotype, the result of a translocation.
id = Interstitial deletion:	Length of chromatin excised from mid-region of a chromatid resulting in a small fragment or ring lying beside a shortened chromatid or gap in the chromatid.
> = Greater than 10 aberrations:	A cell which contains more than 10 structural aberrations.
pp = Polyploid:*	A numerical aberration in which the chromosome number of the cell is an even multiple of the haploid number, or n , and is greater than $2n$.
hr = Hyperploid:*	A numerical aberration in which the chromosome number of cell is greater than $2n$, but an even multiple of n .
e = Endoreduplication:	Cell with double chromosome number in which chromosome pairs have failed to separate and the homologues remain paired.

*In practice, cells in which the chromosome count is greater than $2n+10$ are tallied as polyploid (due to the possibility of cell breakage and chromosome loss because of preparative techniques); those with between $2n+1$ and $2n+10$ inclusive are tallied as hyperploid. The notation "TNTC" on the raw data sheet indicates that the cell is obviously polyploid but that all the chromosomes may not be clear enough to count accurately.



EXAMPLES OF CHROMOSOME ABERRATIONS

CHROMATID-TYPE

I BREAKS



Chromatid Gap



Chromatid Break

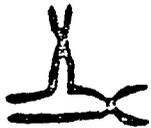


Chromatid Deletion



Fragment

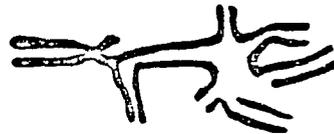
II EXCHANGES



Triradial



Quadriradial



Complex Rearrangement

I BREAKS

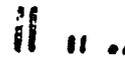
CHROMOSOME-TYPES



Chromosome Gap



Chromosome Break



Acentric Fragments



Minute Chromosomes

II EXCHANGES



Dicentric



Ring

Normal Abnormal



Translocation

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 22202

LBI Assay No. 6998

TYPE of STUDY Chinese Hamster Bone Marrow Cytogenetic Assay

This final study report was reviewed by the LBI Quality Assurance Unit on 3/13/84. A report of findings was submitted to the Study Director and to Management on 3/13/84.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Mitchell S. Elie
Auditor, Quality Assurance Unit



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