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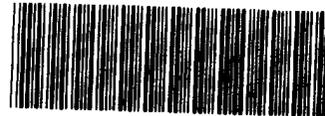
UNIROYAL CHEMICAL COMPANY, INC.

World Headquarters
Middlebury, Connecticut 06749

Health & Regulatory Compliance Department (HRC)

Contains No CBI

September 12, 1994



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Attn: Section 8(e) Coordinator
Office of Pollution Prevention and Toxics (TS-790)
US Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460

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94 SEP 16 PM 2:52

Re: Notification of Substantial Risk: DCQ CAS: 18671-97-1

*per Submitter
S.A.S.*

Dear Sir/Madam,

Uniroyal Chemical Company is submitting to the Agency the results of a study entitled "DCQ: Chromosome Aberration Test in Human Lymphocytes in Vitro" pursuant to the regulations set forth under §8(e) of the Toxic Substances Control Act. This study was performed in partial fulfillment of the requirements for non-domestic chemical notification.

Human lymphocytes treated with DCQ at three dose levels (497.5, 995, and 1990 mg/ml) showed a statistically significant ($p < 0.05$) increase in the frequency of cells with aberrations. Thus DCQ is considered to be clastogenic to human lymphocytes in vitro.

Respectfully submitted,

Mark A. Thomson, Ph.D.
Corporate Toxicologist

ORIGINAL

MAT146/cd

cc: H. S. Friedman

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~~CONFIDENTIAL~~

*Per submitter
EAB*

2

Contains No CBI

DCQ:

CHROMOSOME ABERRATION TEST IN HUMAN LYMPHOCYTES

IN VITRO

PROJECT NUMBER 445/71

Date Started: 12 April 1994

Experimental Procedures:

Date Completed: 10 August 1994

STUDY SPONSOR:

Uniroyal Chemical Company, Inc.
World Headquarters
Benson Road
Middlebury
CONNECTICUT 06749
UNITED STATES OF AMERICA

ISSUED BY:

Safeparm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: DERBY (0332) 792896

Facsimile: (0332) 799018

Telex: 377079 SAFPHM G

QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm Laboratories is carried out as a continuous process designed to encompass all major phases of each study type once per month. Dates of the most recently completed series of monthly inspections relevant to the study type(s) in this report are given below.

Date(s) of Inspection and Reporting:

05, 07, 11, 13, 15, 18 July 1994

This report has been audited by Safepharm Laboratories Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

Date of Report Audit:

31 August 1994

J.R. Pateman C. Biol., M.I. Biol.
FOR SAFEPHARM QUALITY ASSURANCE UNIT



DATE:

7/9/94

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated.

The work described was performed in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment Monograph No. 45 (OCDE/GD(92)32); and are in conformity with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

These international standards are acceptable to the Japanese Ministry of Agriculture, Forestry and Fisheries (59 NohSan, Notification No. 3850, Agricultural Production Bureau) - confirmed by an Arrangement between the Ministry and the UK Department of Health; and the Japanese Ministry of Health and Welfare (Notification No. 313, Pharmaceutical Affairs Bureau - as amended; and Kanpogyo No. 39 Environmental Agency, Yakuhatsu No. 229).

.....
Dr. P.C. Jenkinson B.Sc., Ph.D.
Associate Study Director
for Safepharm Laboratories

DATE: 7 September 1994

Study Director: Ms. N.P. Wright

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DCQ:

CHROMOSOME ABERRATION TEST IN HUMAN LYMPHOCYTES

IN VITRO

SUMMARY

1. Human lymphocytes, treated with DCQ were evaluated for chromosome aberrations at three dose levels, in duplicate, together with vehicle and positive controls. Four treatment conditions were used, i.e. 4 hours exposure with the addition of an induced rat liver homogenate metabolising system at 10% in standard co-factors with cell harvest after 16 and 40 hour expression periods and a 20 and 44 hour continuous exposure in the absence of activation. In Experiment 1 the dose range for evaluation was selected from a series of 8 dose levels on the basis of toxicity.

The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 473 "Genetic Toxicology: Chromosome Aberration Test" and Method B10 of Commission Directive 92/69/EEC.

2. All vehicle controls gave frequencies of cells with aberrations within the range expected for normal human lymphocytes.
3. All the positive control treatments gave statistically significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and of the activity of the metabolising system.
4. DCQ induced a statistically significant increase in the frequency of cells with aberrations. DCQ was shown to be clastogenic to human lymphocytes in vitro.

DCQ:

CHROMOSOME ABERRATION TEST IN HUMAN LYMPHOCYTES

IN VITRO

1. INTRODUCTION

This report describes the results of an in vitro study for the detection of structural chromosomal aberrations in cultured mammalian cells. It supplements microbial systems insofar as it identifies potential mutagens which produce chromosomal aberrations rather than gene mutations (Scott et al 1990).

The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 473 "Genetic Toxicology: Chromosome Aberration Test" and Method B10 of Commission Directive 92/69/EEC. The study design also meets the requirements of the U.K. Department of Health Committee on Mutagenicity Guidelines for the Mutagenicity Testing of Chemicals.

2. TEST MATERIAL

- | | | |
|-----------------------------|---|--|
| 1. Sponsor's identification | : | DCQ |
| 2. Chemical name | : | 2,6-Dichloroquinoxaline |
| 3. Lot number | : | 402062 |
| 4. Purity | : | 98.4% |
| 5. Major impurities | : | 6-Chloro-2-hydroxyquinoxaline = 0.5%
Moisture = 0.11% |
| 6. Date received | : | 30 March 1994 |
| 7. Description | : | light brown powder |
| 8. Container | : | brown glass bottle |
| 9. Storage conditions | : | 4°C in the dark over silica gel |

Data relating to the identity, purity and stability of the test material are the responsibility of the sponsor.

3. METHODS

3.1 Cells

Sufficient whole blood was drawn from the peripheral circulation of volunteers who had been previously screened for suitability. The volunteers had not been exposed to high levels of radiation, hazardous chemicals and had not knowingly recently suffered from a viral infection. The cell cycle time for the lymphocytes from the donor used in this study was determined using BrdU (bromodeoxyuridine) incorporation to assess the number of first, second and third division metaphase cells and so calculate the average generation time (AGT). Using the current batch of foetal calf serum the donors used in this study has an AGT of 14 hours.

3.2 Cell Culture

Cells were grown in minimal essential medium, (supplemented with sodium bicarbonate, L-glutamine, penicillin streptomycin, amphotericin B and 15% foetal calf serum) at 37°C with 5% CO₂ in air. The lymphocytes in fresh heparinised whole blood were stimulated to divide by the addition of phytohaemagglutinin (PHA) at 90 µg/ml final concentration.

3.3 Preparation of Test and Control Materials

DCQ was accurately weighed and suspended in THF (Fisons, Batch No. 35548463) and appropriate dilutions prepared. A 10mM concentration of DCQ was used as the maximum dose.

Analysis of the test material preparations for homogeneity, stability and concentration is not a requirement of the Test Guideline and was therefore not performed.

Vehicle and positive controls were used in parallel with the test material. The positive control materials were as follows:

Ethyl methanesulphonate (EMS) (Sigma, Batch No. 33H1262) 500 µg/ml for cultures in the absence of metabolising enzymes.

Cyclophosphamide (CP) (Sigma, Batch No. 72H0088) 25 µg/ml for cultures where S9 was included.

3. METHODS (contd)

3.4 Microsomal Enzyme Fraction

Lot No. Aro. S9/24/03/94, prepared on 24/03/94 and Lot No. Aro. S9/28/04/94, prepared on 28/04/94 were obtained from the British Industrial Biological Research Association on 12/04/94 and 17/05/94 and were used in Experiment 1 and Experiment 2 respectively.

They were prepared from the livers of male Sprague-Dawley rats weighing ~ 200g. These had received a single i.p. injection of Aroclor 1254 at 500 mg/kg, 5 days before S9 preparation. The S9 was stored at -196°C in a Statebourne liquid nitrogen freezer, model SXR 34.

3.5 Culture Conditions - Experiment 1

Duplicate lymphocyte cultures (A and B) were established for each dose level by mixing the following components in bulk in a suitable sterile container giving, when dispensed into individual sterile plastic flasks, the following:

- 9.05 ml MEM, 15% (FCS)
- 0.1 ml Li-heparin
- 0.1 ml phytohaemagglutinin-M
- 0.75 ml heparinised whole blood

After 48 hours incubation at 37°C, 5% CO₂ in humidified air, the cells of the with-S9 cultures were centrifuged for approximately 5 minutes and all but 1 ml of the culture medium removed and reserved and replaced with 8.0 ml of MEM. 0.02 ml of the appropriate solution of vehicle, or DCQ was added to each culture. The final concentrations of DCQ were 1990, 995, 497.5, 248.75, 124.38, 62.19, 31.09 and 15.55 µg/ml in both the with and without-S9 cultures. 1 ml of 10% S9 in standard co-factors was added to the with-S9 cultures. All the cultures were then returned to the incubator.

3. METHODS (contd)

3.5 Culture Conditions - Experiment 1 (contd)

After 4 hours of treatment at 37°C the with-S9 cultures were centrifuged for 5 minutes, the treatment medium removed by suction and replaced with an 8 ml wash of MEM culture medium. After a further centrifugation the wash medium was removed by suction and replaced with the original culture medium. The cells were then re-incubated for a further 16 hours.

3.6 Culture Conditions - Experiment 2

These were as in 3.5 except that further cultures of lymphocytes were harvested 44 hours after the initiation of treatment as well as a 20-hour harvest as in Experiment 1. Positive controls were evaluated only in the 20-hour harvest cultures.

3.7 Cell Harvest

Mitosis was arrested by addition of demecolcine (0.1 ug/ml) two hours before the required harvest time. After incubation with demecolcine, the cells were centrifuged, the culture medium drawn off and discarded, and the cells resuspended in 0.075M hypotonic KCl. After fifteen minutes (including five minutes centrifugation), most of the hypotonic solution was drawn off and discarded. The cells were resuspended and then fixed by dropping the KCl cell suspension into fresh methanol/glacial acetic acid (3:1 v/v). The fixative was changed at least twice and the cells stored at 4°C for at least four hours to ensure complete fixation.

3.8 Preparation of Metaphase Spreads

The lymphocytes were resuspended in several mls of fresh fixative before centrifugation and resuspension in approximately 0.5 ml of fixative. Several drops of this suspension were dropped onto clean, wet microscope slides and left to air dry. Each slide was permanently labelled with the appropriate identification data.

3.9 Staining

When the slides were dry they were stained in Giemsa for 5 minutes, rinsed, dried and mounted with a coverslip using mounting medium.

3. METHODS (contd)

3.10 Coding

After checking that the slide preparations were of good quality, the slides were coded using a code from a computerised random number generator.

3.11 Scoring of Chromosome Damage

Where possible the first 100 consecutive well-spread metaphases from each culture were counted, and if the cell had 46 or more chromosomes, any gaps, breaks or rearrangements were noted according to the simplified system of Savage (1976) recommended in the 1983 UKEMS guidelines for mutagenicity testing. The details of the classification of chromosome aberrations and the evaluation criteria applied to test data are given in Appendix I. All chromosome aberrations were checked by a senior cytogeneticist prior to decoding the slides.

3.12 Mitotic Index

A total of 2000 lymphocyte cell nuclei were counted and the number of cells in metaphase recorded and expressed as the mitotic index and as a percentage of the vehicle control value.

3.13 Statistical Analysis

The frequency of cells with aberrations (both including and excluding gaps) and the frequency of polyploid cells was compared with the concurrent vehicle control value using Fisher's Exact test or Chi-squared test.

4. RESULTS

4.1 Chromosome Aberration Study - Experiment 1

Treatment with DCQ induced a large, dose-related increase in the mitotic index (Table 1). Consequently, it was not possible to set the maximum dose level as that which gave approximately 50% mitotic inhibition. However, it was concluded that a dose-related

4. RESULTS (contd)

4.1 Chromosome Aberration Study - Experiment 1 (contd)

effect had occurred and therefore the maximum dose selected for scoring cells for chromosome aberrations was 1990 $\mu\text{g/ml}$ (10 mM); 995 and 497.5 $\mu\text{g/ml}$ were selected as the two lower doses. A precipitate of DCQ was observed in the culture flasks at all dose levels.

Both of the vehicle control cultures had frequencies of cells with chromosome aberrations within the expected range (Table 4). The highest frequency of cells with aberrations seen in the two vehicle control groups was 3.0% cells with aberrations including gaps in the absence of S9.

The positive control treatments gave significant increases in the frequency of cells with aberrations (Table 4), indicating that the metabolic activation system was satisfactory and that the test method itself was operating as expected.

DCQ was seen to induce a small, statistically significant increase in the frequency of cells with aberrations without gaps at 995 $\mu\text{g/ml}$ in the presence of S9 only (Table 4).

DCQ appeared to be acting as a spindle inhibitor by preventing cell division at metaphase. This was demonstrated by an increase in mitotic index (Table 1). This effect was observed even at the lowest dose of DCQ tested and the chromosomes were also observed to have a very condensed morphology.

DCQ did not induce a statistically significant increase in the numbers of polyploid cells at any dose-level in either of the treatment cases (Table 7).

4.2 Chromosome Aberration Study - Experiment 2

For the 20-hour treatments the dose selection was the same as that in Experiment 1, with 1990 $\mu\text{g/ml}$ (10mM) being the maximum dose selected for assessment of cells with aberrations. Additional doses were scored for the 44-hour treatment with S9 to ensure the response observed was dose-related. Treatment with DCQ increased the mitotic index in all cultures (Tables 2 and 3).

4. RESULTS (contd)

4.2 Chromosome Aberration Study - Experiment 2 (contd)

All of the vehicle control cultures had frequencies of cells with chromosome aberrations within the expected range (Tables 5 and 6).

The positive control treatments gave significant increases in the frequency of cells with aberrations (Table 5), indicating that the metabolic activation system was satisfactory and that the test material itself was operating as expected.

DCQ was seen to induce statistically significant increases in the frequency of cells with chromosome aberrations in the presence and absence of S9-mix in both the 20 and 44-hour treatment groups (Tables 5 and 6). The responses seen in the 20-hour treatment group were weak and there was a high degree of inter-culture variation. The response seen in the 44-hour treatment group with S9 was relatively large, however, it too was subject to inter-culture variation. The variation between cultures was considered to be due to poor morphology of the chromosomes as a result of the spindle inhibitor-like activity of DCQ.

DCQ induced a small but statistically significant increase in the numbers of polyploid cells at 995 $\mu\text{g/ml}$ in the 44-hour with-S9 group only, this was not dose-related and was considered to be of no toxicological significance (Table 7).

5. CONCLUSION

DCQ induced a statistically significant increase in the frequency of cells with chromosome aberrations in the presence and absence of a liver enzyme metabolising system. DCQ appeared to behave as a spindle inhibitor. DCQ was therefore considered to be clastogenic to human lymphocytes in vitro.

6. ARCHIVES

Unless instructed otherwise by the sponsor, all original data and a copy of the final report will be retained in the archives of Safepharm Laboratories for a period of ten years. After this period, the sponsor's instructions will be sought.

7. REFERENCES

Savage, J.R.K. (1976) Annotation: Classification and relationships of induced chromosomal structural changes. J. Med. Genet., 13, 103 - 122.

Scott, D., Dean, B.J., Danford, N.D., and Kirkland, D.J.: Metaphase chromosome aberration assays in vitro. In: Kirkland, D.J. Basic mutagenicity tests: UKEMS recommended procedures. Report. Part 1 revised. Cambridge: Cambridge University Press, 1990:62-84.

T A B L E 1

MITOTIC INDEX

EXPERIMENT 1 - 20-HOUR HARVEST

TEST MATERIAL: DCQ

DOSE LEVEL μg/ml	WITHOUT S9				WITH S9			
	A	B	MEAN	% OF CONTROL	A	B	MEAN	% OF CONTROL
0	5.10	4.10	4.60	100	12.10	9.85	10.98	100
15.55	15.95	16.10	16.03	348	8.70	8.45	8.58	78
31.09	25.80	27.10	26.45	575	12.75	11.95	12.35	112
62.19	9.70	26.85	18.28	397	6.60	18.95	12.78	116
124.38	27.85	25.35	26.60	578	31.75	34.80	33.28	303
248.75	22.65	26.15	24.40	530	32.0	24.70	28.35	258
497.5	32.15	24.50	28.33	616	11.25	12.55	11.90	108
995	22.90	13.60	18.25	397	34.85	37.20	36.03	328
1990	19.10	25.20	22.15	482	35.75	21.90	28.83	263
EMS 500	3.35	3.55	3.45	75	-	-	-	-
CP 25	-	-	-	-	4.80	4.25	4.53	41

T A B L E 2

MITOTIC INDEX

EXPERIMENT 2 - 20-HOUR HARVEST

TEST MATERIAL: DCQ

DOSE LEVEL μg/m ³	WITHOUT S9				WITH S9			
	A	B	MEAN	% OF CONTROL	A	B	MEAN	% OF CONTROL
0	6.10	4.90	5.50	100	6.05	7.20	6.63	100
497.5	20.15	17.80	18.98	345	14.4	8.55	11.48	173
995	20.70	19.45	20.08	365	13.0	12.25	12.63	190
1990	20.85	21.15	21.00	382	14.8	12.10	13.45	203
EMS 500	3.25	3.30	3.28	60	-	-	-	-
CP 25	-	-	-	-	0.9	1.00	0.95	14

T A B L E 3

MITOTIC INDEX

EXPERIMENT 2 - 44-HOUR HARVEST

TEST MATERIAL: DCQ

DOSE LEVEL $\mu\text{g}/\text{ml}$	WITHOUT S9				WITH S9			
	A	B	MEAN	% OF CONTROL	A	B	MEAN	% OF CONTROL
	0	4.90	3.70	4.30	100	3.95	6.45	5.20
497.5	-	-	-	-	7.90	6.90	7.40	142
995	-	-	-	-	8.90	7.10	8.00	154
1990	14.15	14.20	14.18	330	8.60	7.85	8.23	158

TABLE 6 (contd)

RESULTS OF CHROMOSOME ABERRATION TEST - EXPERIMENT 2

TEST MATERIAL: DCQ HARVEST TIME: 44 HOURS METABOLIC ACTIVATION: YES

TREATMENT GROUP IDENTIFICATION	REPLICATE	No. OF CELLS SCORED	TOTAL		CHROMATID		CHROMOSOME		OTHERS	TOTAL ABERRATIONS		ABERRANT CELLS
			GAPS	GAPS	BREAKS	EXCHANGES	BREAKS	EXCHANGES		X	(+Gaps)	
VEHICLE	A	100	0	1	0	0	0	0	0	1	1	1
	B	100	3	1	0	0	0	0	0	4	1	4
CONTROL	TOTAL	200	3	2	0	0	0	0	0	5	2	5
	%	100	(1.5)	(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(2.5)	(1.0)	(2.5)
497.5	A	100	3	2	0	0	0	0	0	5	2	5
	B	50	8	30	0	6	0	0	0	44	36	25
µg/ml	TOTAL	150	11	32	0	6	0	0	0	49	38	30***
	%	100	(7.3)	(21.3)	(0.0)	(4.0)	(0.0)	(0.0)	(0.0)	(32.7)	(25.3)	(20.0)
995	A	100	1	1	0	0	0	0	0	2	1	2
	B	100	18	46	1	2	0	0	0	67	49	37
µg/ml	TOTAL	200	19	47	1	2	0	0	0	69	50	39***
	%	100	(9.5)	(23.5)	(0.5)	(1.0)	(0.0)	(0.0)	(0.0)	(34.5)	(25.0)	(19.5)
1990	A	100	16	12	2	4	0	0	0	34	18	23
	B	50	18	22	2	15	0	0	0	57	39	27
µg/ml	TOTAL	150	34	34	4	19	0	0	0	91	57	50***
	%	100	(22.7)	(22.7)	(2.7)	(12.7)	(0.0)	(0.0)	(0.0)	(60.7)	(38.0)	(33.3)

X = > 10 aberrations per cell (not included in total aberrations) Figures in brackets = aberrations per 100 cells
 *** = p<0.001

TABLE 7

INCIDENCE OF POLYPLOID CELLS (%) IN HUMAN LYMPHOCYTES

AFTER TREATMENT WITH DCQ

EXPERIMENT 1

DOSE LEVEL μg/ml	20 HOURS	
	WITHOUT S9	WITH S9
0	0.0	0.0
497.5	0.0	0.0
995	0.0	0.0
1990	0.5	0.0
EMS' 500	0.0	-
CP 25	-	0.0

EXPERIMENT 2

DOSE LEVEL μg/ml	20 HOURS		44 HOURS	
	WITHOUT S9	WITH S9	WITHOUT S9	WITH S9
0	0.0	0.0	0.0	0.5
497.5	0.0	0.0	-	0.7
995	0.0	0.0	-	4.0*
1990	0.0	0.0	1.0	2.0
EMS 500	0.0	-	-	-
CP 25	-	1.0	-	-

- = not applicable * represents $p \leq 0.05$

A P P E N D I X I

CHROMOSOME ABERRATIONS : CLASSIFICATION AND EVALUATION CRITERIA

A. CLASSIFICATION

1. Gaps

Gaps are small areas of the chromosome which are unstained. The chromatids remain aligned as normal and the gap does not extend along the chromatid for a distance greater than the width of a chromatid. If the gap occurs on one chromatid only it is a chromatid gap (g). If a gap appears in both chromatids at the same position it is a chromosome gap (G).

2. Chromatid Breaks

Chromatid breaks (ct) vary in appearance. The chromatid may remain aligned but show a gap which is too large to classify as a gap. Alternatively, the chromatid may be broken so that the broken fragment is displaced. In some cases, the fragment is not seen at all. A chromatid fragment (f) should be scored if the chromosome of origin cannot be identified. Very small fragments are scored as minutes (m).

3. Chromosome Breaks

Chromosome breaks (CS) are breaks in both chromatids of the chromosome. A fragment with two chromatids is formed and this may be displaced by varying degrees. Breaks are distinguished from gaps by the size of the unstained region. A chromosome break is scored if the fragment is associated with a chromosome from which it was probably derived. However, fragments are often seen in isolation and are then scored as chromosome fragments (F). Very small fragments are scored as minutes (M).

4. Exchanges

Exchanges are formed by faulty rejoining of broken chromosomes and may be of the chromosome or chromatid type. Chromatid exchanges (c/c) have numerous different forms but are generally not further classified. Where multiple exchanges have occurred each exchange point is counted as one chromatid exchange. Chromosome exchanges generally appear as either a dicentric (D) or a ring (R) form, either of which can be associated with a fragment, which if possible should be scored as part of the exchange.

A P P E N D I X I (contd)

CHROMOSOME ABERRATIONS : CLASSIFICATION AND EVALUATION CRITERIA

A. CLASSIFICATION (contd)

5. Multiple Aberrations

If many aberrations are present in one metaphase, the exact details may not be scorable. This is particularly the case when chromosome pulverisation occurs. If the number of aberrations is greater than 10 then the cell is classified as X.

6. Chromosome Number

If the chromosome (centromere) number was 46 then it was classified as a diploid cell and scored for aberrations. If less than 46 chromosomes were counted then the cell was ignored under the assumption that some chromosomes may have been lost for technical reasons. If greater than 46 chromosomes were counted then the cell was recorded and classified as an aneuploid cell. If multiple copies of the haploid chromosome number (other than diploid) were scored then the cell was recorded and classified as polyploid. If the chromosomes were arranged in closely apposed pairs, ie. 4 chromatids instead of 2, the cell was scored as endoreduplicated (E).

B. EVALUATION CRITERIA

1. Historical Aberration Ranges for Vehicle and Untreated Control Cultures

Many experiments with human lymphocytes have established a range of aberration frequencies acceptable for control cultures in normal volunteer donors, these are currently in the range of 0 to 3.5% cells with structural aberrations including gaps, 0 to 2.5% cells excluding gaps and 0 to 1.5% polyploidy.

A positive response was recorded for a particular treatment if the % cells with aberrations markedly exceeded that seen in the concurrent control, either with or without a clear dose-response relationship. For modest increases in aberration frequency a dose-response relationship is generally required and appropriate statistical tests

APPENDIX I I



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Limited
P O Box No 45
Derby
DE1 2BT

DATE OF INSPECTION

31 January 1994

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

16/3/94 D. F. Moore
Director
UK GLP Monitoring Unit

19/04/1994



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Mark A. Thomson, Ph.D.
Corporate Toxicologist
Health and Regulatory Compliance Department
Uniroyal Chemical Company, Inc.
World Headquarters
Middlebury, Connecticut 06749

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

NOV 17 1994

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan
Risk Analysis Branch

Enclosure

13108 A



Recycled/Recyclable
Printed with Soy/Caros
contains at least 50%

Triage of 8(e) Submissions

Date sent to triage: JAN 11 1995 **NON-CAP** CAP

Submission number: 13108 A TSCA Inventory: Y N **D**

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

~~ECO~~ ~~AQUATO~~

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX **GTOX**
STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only	
entire document: 0 1 2 pages <u>1</u>	pages <u>1,6</u>
Notes:	
Contractor reviewer: <u>NEB</u>	Date: <u>11/14/94</u>

DECATS/IRIAGE TRACKING DBASE ENTRY FORM

DECATS DATA: Submission # REIIO 0994-13108 SEQ A

TYPE: INT SUPP FL WP

SUBMITTER NAME: Unroyal Chemical Company, Inc.

INFORMATION REQUESTED: FL WP DATE: _____
 0501 NO INFO REQUESTED
 0502 INFO REQUESTED (TECH)
 0503 INFO REQUESTED (VOL. ACTIONS)
 0504 INFO REQUESTED (REPORTING RATIONALE)
 DISPOSITION:
~~0639~~ REFER TO CHEMICAL SCREENING
 0678 CAP NOTICE

CELL/INITIARY ACTIONS:
 0401 NO ACTION REPORTED
 0402 STUDIES PLANNED/IN PROGRESS
 0403 NOTIFICATION OF WORK IN PROGRESS
 0404 LABEL/MSDS CHANGES
 0405 PROCESS/ANALYSIS CHANGES
 0406 APP/USE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

SUB DATE: 09/12/94 OTS DATE: 09/16/94 CSRAD DATE: 10/11/94

CHEMICAL NAME: 2,6-Dichloroguanoxaline CAS# 18671-77-1

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUR/REL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0299 OTHER	01 02 04
0211 CHR. TOX (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

TRIALS DATA: NON-CBI INVENTORY ONGOING REVIEW: YES (DROP/REFER) SPECIES: In VITRO TOXICOLOGICAL CONCERN: LOW USE: _____ PRODUCTION: _____

CAS SR: NO DETERMINED: NO (CONTINUE) REFER: _____ HIGH

COMMENTS: Non-Cap

6)

8EHQ-0994-13108: Rank - medium.

Chemical: 2,6-dichloroquinoxaline (DCQ: CAS# 18671-97-1).

DCQ: Chromosome aberration test in human lymphocytes in vitro, Safeparm Laboratories Ltd, Derby, UK, dated 10 August 1994: Positive for chromosome mutations (aberrations) in human lymphocytes in vitro both without and with metabolic activation.