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INITIAL SUBMISSION: FINAL REPORT, 2-HYDROXYPROPYL ACRYLATE CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS IN VITRO, WITH COVER LETTER DATED 4/12/2000		
Chemical Category		
2-PROPENOIC ACID, MONOESTER WITH 1,2-PROPANEDIOL		

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Degussa-Hüls Corporation

2 Turner Place, Piscataway, NJ 08855-0365  
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Document Processing Center (TS-790)  
Office of Toxic Substances  
U.S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460



8EHQ-00-14709

ATTN: Section 8 (e) Coordinator

RE: Product Name: 2-Hydroxypropyl acrylate  
CAS Registry No. 25584-83-2  
CAS Registry Name: 2-Propenoic acid, monoester with 1,2-propanediol

Dear Sir or Madam:

Degussa-Hüls Corporation received from Röhm GmbH, the enclosed report on "2-Hydroxypropyl acrylate, Chromosome Aberrations in Chinese Hamster Ovary Cells in vitro", which studies were conducted by Research Toxicology Center-Roma. This is a follow up to the TSCA 8(e) submission made January 10, 2000 on the subject product.

Pursuant to Section 8 (e) of the Toxic Substances Control Act, Degussa-Hüls Corporation provides this information to EPA.

Sincerely,

Kisha Pippins  
Product Safety Specialist

cc: S. Bearman

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**2-HYDROXYPROPYL ACRYLATE  
CHROMOSOME ABERRATIONS IN  
CHINESE HAMSTER OVARY CELLS IN VITRO**

FINAL REPORT

RTC Report no.: 7384-M-04899

Contain NO CBI

Seen and approved by:

A. Nunziata  
Responsible for Toxicological  
Experimentation as authorized  
by the Italian Ministry of Health

Sponsor  
RÖHM GmbH  
Chemische Fabrik  
Kirschenallee  
D-64293 Darmstadt  
Germany

**SEDE**

Via Tito Spino 12  
00040 Pomezia (Roma) - ITALY  
Tel. + 39 6 91095 1  
Fax + 39 6 910 5737  
C.P. 15301-00143 - Roma Eu. Laurentino  
E-mail: rtemk@tin.it

**UFFICI COMMERCIALI**

UFFICIO MILANO  
Via Corio 26  
20132 Milano  
ITALIA  
Tel + 39 2 2614 4686  
Fax + 39 2.2614 4778

UFFICIO PARIGI  
Rue du Port des Filles, 21 - Delta 109  
94536 Rungis Cedex (Paris)  
FRANCE  
Tel + 33.1 4560.9725  
Fax + 33.1.4687.9431

RTC S.p.A.  
Capitale sociale: 10.000.000.000  
C.C.I.A.A. n° 375376  
Reg. Soc. Trib. di Roma n° 2828/72  
Cod. Fisc. 00653120584  
Partita IVA: 00920611001

RTC Report no.: 7384-M-04899

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that the following report constitutes a true and faithful account of the procedures adopted, and the results obtained in the performance of the study. The aspects of the study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

- A. "Good Laboratory Practice Standards" of the U.S. Environmental Protection Agency Code of Federal Regulations 40, Part 792, U.S. Federal Register, Vol. 54, No. 158, 17<sup>th</sup> August 1989 and subsequent revisions.
- B. Decreto Legislativo 27 Gennaio 1992 n. 120 published in the Gazzetta Ufficiale della Repubblica Italiana 18 Febbraio 1992. (Adoption of the Commission Directive of 18 December 1989 adapting to technical progress the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (90/18/EEC)).



S. Cinelli, Biol.D.  
(Study Director) :

Date : 17-Jan-2000



J. Brightwell, Ph.D.  
(Scientific Director):

Date : 17.01.2000

RTC Report no.: 7384-M-04899

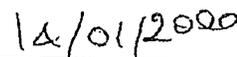
QUALITY ASSURANCE STATEMENT

(Relevant to the aspects of the study conducted by Research Toxicology Centre S.p.A.)

Study phases monitored by RTC's QAU according to current relevant Standard Operating Procedures	Quality Assurance Inspections (Day Month Year)		
	Inspection	Report to Study Director	Report to Company Management
<b>STUDY PROTOCOL</b>	06.10.99	06.10.99	06.10.99
<b>PROCEDURES ON THIS STUDY</b>			
Cell treatment	12.10.99	15.10.99	15.10.99
<b>PROCEDURES ON THIS TYPE OF STUDY</b>			
Dose preparation	07.09.99	-	17.09.99
Cell harvesting	03.09.99	-	08.09.99
Slide preparation	03.09.99	-	08.09.99
Slide staining	18.10.99	-	21.10.99
Slide coding	03.11.99	-	12.11.99
Slide scoring	06.10.99	-	07.10.99
Other Routine inspections of a procedural nature were carried out on activities not directly related to this type of study. The relevant documentation is kept on file although specific inspection dates are not reported here.			
<b>FINAL REPORT</b> Review of this report by RTC's QAU found the reported methods and procedures to describe those used and the results to constitute an accurate representation of the recorded raw data.	Review completed 14 Jan 2000		



M.M. Brunetti, Biol.D.  
Head of Quality Assurance



Date

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## 1. SUMMARY

- 1.1 The test substance 2-HYDROXYPROPYL ACRYLATE was assayed for the ability to cause chromosomal damage in Chinese hamster ovary cells, following *in vitro* treatment in the presence and absence of S9 metabolic activation.
- 1.2 Solutions of the test substance were prepared in culture medium (Ham's F10). In the first experiment, dose-levels of 5000, 2500, 1250, 625, 313, 156, 78.1 and 39.1 µg/ml were used both in the absence and presence of S9 metabolism. In the absence of S9 metabolism, treatments with the test substance showed severe toxicity at all dose-levels, while in the presence of S9 metabolism no dose-level showed an adequate toxicity for the scoring of chromosomal aberrations. In order to achieve an appropriate level of toxicity a second experiment was performed employing a greatly modified dose-range. In the absence of S9 metabolism, dose-levels of 120, 60.0, 45.0, 30.0, 15.0 and 7.50 µg/ml were used. Treatments in the presence of S9 metabolism were performed employing dose-levels of 240, 220, 200, 180, 160, 140, 120 and 100 µg/ml.

Both in the presence and absence of S9, the cells were treated for three hours and the harvest time of 20 hours, corresponding to approximately 1.5 cell cycle, was used. The experiment included appropriate negative and positive controls. Two cell cultures were prepared at each test point.

Dose-levels were selected for the scoring of chromosomal aberrations on the basis of the cytotoxicity of the test substance treatments as determined by the reduction of cell counts at the time of harvesting.

The treatment-levels selected for scoring were the following:

Assay No.:	S9	Treatment time (hours)	Dose-level (µg/ml)
2	-	3	45.0, 30.0 and 15.0
2	+	3	160, 140 and 100

One hundred metaphase spreads were scored for chromosomal aberrations from each culture, with the exception of one culture treated in the absence of S9 metabolism at 30.0 µg/ml when due to technical reasons 80 eligible metaphases were found. For cultures where, the number of aberrant metaphases excluding gaps was more than 50%, scoring was terminated at 50 metaphases.

- 1.3 Following treatment with 2-HYDROXYPROPYL ACRYLATE, statistically significant increases in the number of cells bearing aberrations (including and excluding gaps) were observed in the absence of S9 metabolism, at higher dose-levels selected for scoring.

Increases in aberrant cells (including gaps) were also observed at the intermediate dose-level (140 µg/ml) in the presence of S9 metabolism. As clear positive results were obtained, the additional experiment using modified experimental conditions was not performed.

Statistically significant increases in the number of cells bearing aberrations (including and excluding gaps) were observed following treatments with the positive controls Cyclophosphamide and Mitomycin-C, indicating the correct functioning of the test system.

- 1.4 It is concluded that 2-HYDROXYPROPYL ACRYLATE induces chromosomal aberrations in Chinese hamster ovary cells after *in vitro* treatment, under the reported experimental conditions.

**2. INTRODUCTION****2.1 Purpose**

This report describes the experiment performed to assess the clastogenic activity of 2-HYDROXYPROPYL ACRYLATE in Chinese hamster ovary cells following *in vitro* treatment in the presence and absence of S9 metabolic activation.

The study was performed to comply with the principles of Good Laboratory Practice standards of the U.S. EPA (CFR 40, Part 792) and European Directives 88/320 and 90/18. In addition, the study was designed to comply with the experimental methods indicated in:

- EEC Council Directive 92/69, Part B.
- OECD Guideline No. 473 (Adopted: 21<sup>st</sup> July 1997)

**2.2 Study organisation**Sponsor

RÖHM GmbH  
Chemische Fabrik  
Kirschenallee  
D-64293 Darmstadt  
Germany

Location of Study

Genetic and Cellular Toxicology Department  
Research Toxicology Centre S.p.A. (RTC)  
Via Tito Speri, 12  
00040 Pomezia (Roma)  
Italy

Principal dates

Study commenced: 12-Oct-1999 (Experiment I - treatment)  
Study completed: 18-Nov-1999 (Experiment II - end of scoring)

Personnel involved in the study

Study Monitor:

Dr H. Müllerschön

Study Director:

S. Cinelli, Biol. D.

Scorer of slides:

J. Bates Dean (external consultant)

A. Büttner (external consultant)

Archiving:

The original data arising from this study, prepared microscope slides, and a copy of the final report consigned will be stored in the archives of Research Toxicology Centre S.p.A. for a period of five years from the date of consignment of the report. At the completion of this period the Sponsor will be contacted for despatch or disposal of the material, or further archiving. An aliquot of the test substance will be retained within the archives of the testing facility for a period of ten years after which it will be destroyed.

### 3. MATERIALS AND METHODS

#### 3.1 Test substance

A sample of 100 grams of HYDROXYPROPYL ACRYLATE was received from RÖHM GmbH on 14-Sept-1999.

The substance, a colourless liquid, was contained in a dark glass bottle and was stored at room temperature at RTC. Information received from the Sponsor indicated the following:

TEST SUBSTANCE	:	2-HYDROXYPROPYL ACRYLATE
CAS NUMBER	:	25584-83-2
BATCH NUMBER	:	790201167 (Invoice number: 29161290)
MOLECULAR WEIGHT	:	130.1 g/mol

On 07-Oct-1999, a subsample of 5.0 g of 2-HYDROXYPROPYL ACRYLATE was transferred from the Formulation Unit to the Department of Genetic and Cellular Toxicology and was stored in a dark glass bottle at room temperature.

Solutions of the test substance, as received, were prepared immediately before use in culture medium (HAM'S F10) on a weight/volume basis without correction for the displacement due to the volume of the test substance. Concentrations were expressed in terms of material as received. All test substance solutions were used within 30 minutes of the initial formulation. No assay of test substance stability, nor its concentration and homogeneity in solvent were undertaken. All dose-levels in this report are expressed to three significant figures.

#### 3.2 Control substances

Solutions of Mitomycin-C (batch 088/AFA, Kyowa Hakko Kogyo Co.Ltd., Japan;) and Cyclophosphamide "Endoxan", batch 806164D, produced by Asta Werke AG, Germany, were prepared in sterile distilled water immediately prior to use and served as positive controls. Injectable grade distilled water, batch 24741 was obtained from Laboratori Don Baxter S.p.A., Trieste, Italy.

**3.3 S9 tissue homogenate**

This study was performed using two batches of S9 homogenate (designated 99/8 and 99/10) and had the following characteristics:

S9 Batch	Protein content (mg/ml)	Aminopyrine demethylase activity ( $\mu$ M/g liver/5 min, formaldehyde production)
99/8	34.1 $\pm$ 2.17	3.25 $\pm$ 0.12
99/10	31.6 $\pm$ 2.13	4.12 $\pm$ 0.30

The S9 homogenate was prepared from the livers of five young male Sprague-Dawley rats which had received prior treatment with phenobarbital and betanaphthoflavone to induce high levels of xenobiotic metabolising enzymes. The efficacy of the tissue homogenate was checked in an Ames test and produced acceptable responses with the indirect mutagens 2-aminoanthracene and benzo(a)pyrene with *S. typhimurium* tester strain TA100.

**3.4 Methods**

The methods used were in compliance with the attached Study Protocol with the minor exception that cells were not checked at regular intervals for Mycoplasma contamination due to a problem receiving the kit for detection on time from the manufacturer. This is not considered to have affected the integrity of the study. The results presented for the treatment series in the presence of S9 metabolism were obtained in a repeat assay. In the original experiment, metaphase spreads in several slides were of insufficient quality to permit scoring. Data generated from the original experiment are not presented in this report, but are retained in the study file and archived as indicated in the study protocol.

## 4. RESULTS

### 4.1 Solubility test

The test substance was found to be directly soluble in culture medium (Ham's F10) at a concentration of 500 mg/ml. On the basis of this result a concentration of 5000 µg/ml was selected as the highest dose-level to be used in the cytogenetic assay.

### 4.2 Assay for chromosomal aberrations

Two assays for chromosomal damage was performed.

In the first experiment dose-levels of 5000, 2500, 1250, 625, 313, 156, 78.1 and 39.1 µg/ml were used both in the absence and presence of S9 metabolism. Solutions of the test substance were prepared in culture medium (Ham's F10). In the absence of S9 metabolism, treatments with the test substance showed severe toxicity at all dose-levels. Results are presented in Table 1. In order to achieve an appropriate level of toxicity a second experiment was performed employing a greatly modified dose-range. Dose-levels of 120, 60.0, 45.0, 30.0, 15.0 and 7.50 µg/ml were used. In the presence of S9 metabolism severe toxicity was observed at higher dose-levels; marked toxicity was observed at 313 µg/ml reducing the number of viable cells to 27% of the negative control value. At this dose-level the mitotic index was reduced to 11% of the control and insufficient numbers of eligible metaphases were found. No relevant toxicity was observed over the remaining dose-range. In order to achieve an appropriate level of toxicity a second experiment was performed employing a greatly modified dose-range. Dose-levels of 240, 220, 200, 180, 160, 140, 120 and 100 µg/ml were used.

Appropriate negative and positive control cultures were included in each experiment. Positive control treated cultures received Mitomycin-C (0.30 µg/ml) in the absence of S9 metabolism or Cyclophosphamide (15.0 µg/ml) in the presence of S9.

Both in the absence and presence of S9 metabolic activation, the treatment time was 3 hours after which the cells were allowed to recover prior to harvesting. The harvest time of 20 hours, corresponding to approximately 1.5 cell cycle, was used.

As clear positive results were obtained, and in accordance with the Study Protocol the study was considered complete and the additional experiment, using modified experimental conditions, was not performed.

Two cultures were prepared at each test point. Air-dried slides were prepared from each culture and stained with 3% Giemsa.

Following treatment, the pH and osmolality values of the treatment media of cultures treated at the three higher dose-levels were determined. The test substance had no obvious effect on the treatment media compared with the control values for both parameters.

#### 4.3 Selection of dose-levels for scoring

Cell counts were performed at harvesting time for each culture of both treatment series, and the results are presented in Tables 3 and 4.

In the absence of S9 metabolism, marked toxicity was observed at the two higher dose-levels (120 and 60.0  $\mu\text{g/ml}$ ), reducing the number of the viable cells to 3 and 18% of the negative control value, respectively. At the next lower dose-level (45.0  $\mu\text{g/ml}$ ) moderate toxicity was observed and the number of viable cells was reduced to 45% of the negative control. Following treatment in the presence of S9 metabolism, viable cell count declined in a dose-related manner reaching 52% of the negative control value at 160  $\mu\text{g/ml}$ .

The highest dose-level selected for the scoring of aberrations should be a concentration causing moderate toxicity (ideally the reduction of cell count should be approximately 50%) and treatments reducing the cell count value to below 20% of the relevant control should not be scored. If no toxicity is observed then the highest practicable dose-level should be selected.

On the basis of the above results the treatment-levels selected for scoring were the following:

Assay No.:	S9	Treatment time (hours)	Dose-level ( $\mu\text{g/ml}$ )
2	-	3	45.0, 30.0 and 15.0
2	+	3	160, 140 and 100

#### 4.4 Assay results

One hundred metaphase spreads were scored for chromosomal aberrations from each culture with the exception of one culture treated in the absence of S9 at 30.0  $\mu\text{g/ml}$  where due to technical reasons 80 eligible metaphases were found. For cultures where the number of aberrant metaphases excluding gaps was more than 50% scoring was terminated at 50 metaphases. The results are presented in Tables 5 and 6.

In these tables the numbers and types of aberrations are presented, together with the total number of aberrations (chromatid type and chromosome type) including and excluding gaps. The total number of aberrant metaphases including and excluding gaps are also shown.

Following treatment with the test substance increases in the numbers of cells bearing chromosomal aberrations were observed both in the absence and presence of S9 metabolic activation. The observed aberrations consisted principally of chromatid deletions and exchanges and isolocus events.

These increases were more evident in the treatment series without S9 metabolic activation, where a dose-relationship was observed. In this case heavily damaged cells accompanied the increases in chromosomal damage. Following treatment in the presence of S9 metabolism the increase in the incidence of aberration-bearing cells was observed at the intermediate dose-level only. A large increase in the number of gaps was also observed at this dose-level.

Marked increases in the frequency of cells bearing aberrations (including and excluding gaps) were seen in the cultures treated with the positive control substances, indicating the correct functioning of the assay system.

The modal number of chromosomes observed in the cells of untreated cultures (in the absence of S9 metabolism) was 21 (90%) in agreement with our historical records for this cell line. The frequency of cells 19, 20 and 22 chromosomes was 3.5, 5.5 and 1% respectively.

**5. ANALYSIS OF RESULTS**

**5.1 Statistical analysis**

For the statistical analysis, Fisher's Exact Test is used to compare the number of cells bearing aberrations (assumed to be Poisson distributed) in control and treated cultures. The analysis is performed using sets of data either including or excluding gaps. The results of the statistical analysis are presented in Tables 7 and 8.

**5.2 Criterion for outcome**

In this assay, the test substance is considered to have clastogenic properties if the following criteria are all fulfilled:

- (i) Statistically significant increases in the incidence of cells bearing aberrations are observed at any dose-level over the concurrent control.
- (ii) The increases must exceed the historical control values.
- (iii) The increases are reproduced in both replicate cultures.

The evaluation is based on the set of results, which excludes gaps. A more detailed explanation of the criteria for evaluation of the results is given in the Study Protocol.

**5.3 Evaluation**

The increases in the incidence of aberration-bearing cells observed following treatment with the test substance in the absence of S9 metabolism fulfilled all of the above criteria.

It must be concluded that 2-HYDROXYPROPYL ACRYLATE induces chromosomal aberrations in Chinese hamster ovary cells under the reported experimental conditions.

Statistically significant increases in the incidences of aberrant cells (both including and excluding gaps) compared with the relevant control values, were observed in the cultures treated with the positive controls Cyclophosphamide and Mitomycin-C, indicating the correct functioning of the assay system.

**6. CONCLUSIONS**

A summary of the results is presented in Table 9 giving the incidence of cells bearing aberrations (excluding gaps) and the relative cell count for each test point. The statistical significance of the recorded numbers of cells bearing aberrations is also shown.

On the basis of these results it is concluded that 2-HYDROXYPROPYL ACRYLATE induces chromosomal aberrations in Chinese hamster ovary cells after *in vitro* treatment, under the reported experimental conditions.

5

RTC Report no.: 7384-M-04899

**7. TABLES 1 - 9**

2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER  
OVARY CELLS

TABLE 1 - EXPERIMENT I - Cell count results - Without metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose-level ( $\mu\text{g/ml}$ )	Culture No.	Cell count ( $10^6/\text{ml}$ )	Mean	Relative cell count (%)																																																																				
Untreated		1	0.35	0.34	100																																																																				
		2	0.32			Test Substance	5000	3	0.01	0.01	1	4	0.00	Test Substance	2500	5	0.00	0.01	1	6	0.01	Test Substance	1250	7	0.00	0.00	0	8	0.00	Test Substance	625	9	0.02	0.02	4	10	0.01	Test Substance	313	11	0.00	0.00	0	12	0.00	Test Substance	156	13	0.03	0.02	6	14	0.01	Test Substance	78.1	15	0.05	0.06	18	16	0.07	Test Substance	39.1	17	0.25	0.22	66	18	0.19	Mitomycin-C	0.30	19	0.20
Test Substance	5000	3	0.01	0.01	1																																																																				
		4	0.00			Test Substance	2500	5	0.00	0.01	1	6	0.01	Test Substance	1250	7	0.00	0.00	0	8	0.00	Test Substance	625	9	0.02	0.02	4	10	0.01	Test Substance	313	11	0.00	0.00	0	12	0.00	Test Substance	156	13	0.03	0.02	6	14	0.01	Test Substance	78.1	15	0.05	0.06	18	16	0.07	Test Substance	39.1	17	0.25	0.22	66	18	0.19	Mitomycin-C	0.30	19	0.20	0.27	79	20	0.33				
Test Substance	2500	5	0.00	0.01	1																																																																				
		6	0.01			Test Substance	1250	7	0.00	0.00	0	8	0.00	Test Substance	625	9	0.02	0.02	4	10	0.01	Test Substance	313	11	0.00	0.00	0	12	0.00	Test Substance	156	13	0.03	0.02	6	14	0.01	Test Substance	78.1	15	0.05	0.06	18	16	0.07	Test Substance	39.1	17	0.25	0.22	66	18	0.19	Mitomycin-C	0.30	19	0.20	0.27	79	20	0.33												
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		8	0.00			Test Substance	625	9	0.02	0.02	4	10	0.01	Test Substance	313	11	0.00	0.00	0	12	0.00	Test Substance	156	13	0.03	0.02	6	14	0.01	Test Substance	78.1	15	0.05	0.06	18	16	0.07	Test Substance	39.1	17	0.25	0.22	66	18	0.19	Mitomycin-C	0.30	19	0.20	0.27	79	20	0.33																				
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		20	0.33																																																																						

2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

TABLE 2 - EXPERIMENT I - Cell count results - With metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose-level (µg/ml)	Culture No.	Cell count (10 <sup>6</sup> /ml)	Mean	Relative cell count (%)
Untreated	-	21	0.40	0.40	100
		22	0.39		
Test Substance	5000	23	0.00	0.00	0
		24	0.00		
Test Substance	2500	25	0.00	0.00	0
		26	0.00		
Test Substance	1250	27	0.00	0.01	1
		28	0.01		
Test Substance	625	29	0.00	0.01	1
		30	0.01		
Test Substance	313	31	0.08	0.11	27
		32	0.13		
Test Substance	156	33	0.30	0.29	73
		34	0.28		
Test Substance	78.1	35	0.31	0.29	72
		36	0.26		
Test Substance	39.1	37	0.31	0.36	91
		38	0.41		
Cyclophosphamide	15.0	39	0.29	0.28	71
		40	0.27		

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2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER  
OVARY CELLS

TABLE 3 - EXPERIMENT II - Cell count results - Without metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose-level ( $\mu\text{g/ml}$ )	Culture No.	Cell count ( $10^6/\text{ml}$ )	Mean	Relative cell count (%)
Untreated		41	0.59	0.57	100
		42	0.55		
Test Substance	120	43	0.01	0.02	3
		44	0.02		
Test Substance	60.0	45	0.09	0.11	18
		46	0.12		
Test Substance	45.0	47	0.24	0.26	45
		48	0.27		
Test Substance	30.0	49	0.40	0.36	62
		50	0.31		
Test Substance	15.0	51	0.47	0.45	78
		52	0.42		
Test Substance	7.50	53	0.47	0.44	76
		54	0.40		
Mitomycin-C	0.30	55	0.41	0.43	75
		56	0.45		

RTC Report no.: 7384-M-04899

2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER  
OVARY CELLS

TABLE 4 - EXPERIMENT II - Cell count results - With metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose-level ( $\mu\text{g/ml}$ )	Culture No.	Cell count ( $10^6/\text{ml}$ )	Mean	Relative cell count (%)
Untreated	-	73	0.94	0.85	100
		74	0.76		
Test Substance	240	75	0.14	0.11	13
		76	0.08		
Test Substance	220	77	0.11	0.12	14
		78	0.13		
Test Substance	200	79	0.17	0.27	31
		80	0.36		
Test Substance	180	81	0.21	0.28	33
		82	0.35		
Test Substance	160	83	0.43	0.45	52
		84	0.46		
Test Substance	140	85	0.52	0.49	58
		86	0.46		
Test Substance	120	87	0.58	0.60	70
		88	0.61		
Test Substance	100	89	0.55	0.62	73
		90	0.69		
Cyclophosphamide	15.0	91	0.54	0.55	65
		92	0.56		

8. KEY TO TABLES 5 - 6

This table shows, for each test culture used in the main assay and by treatment group totals the types and numbers of aberrations, identified as follows:

- Gaps - This refers to either chromatid or chromosome gaps.
- Del - Interstitial or terminal deletions.
- Exch - Exchanges:
  - a) Chromatid exchanges  
Includes symmetrical and asymmetrical exchanges; intra- and inter-chromosome exchanges.
  - b) Chromosome exchanges  
Includes both dicentric and ring types.
- Other - These include
  - H: Heavily damaged cells  
(more than 5 aberrations/cell)
  - ER: Endoreduplicated cells
  - PP: Polyploid cells
- Isolocus - Includes isochromatid and isolocus breaks when these cannot be distinguished.
- Tot.abs - Total number of aberrations observed.
- Cells with abs. - Cells with aberrations

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2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

TABLE 5 - Scoring of Aberrations - Without metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose-level (µg/ml)	Culture No.	Cells Scored	Gaps	Chromatid Del	Exch	Del	Chromosome Exch	Isolocus	Other	Tot. abs (+gaps)	Tot. abs (-gaps)	Cells with abs (+gaps)	Cells with abs (-gaps)	
Untreated	-	41	100	0	1	0	0	0	2	0	3	3	3	3	
		42	100	1	2	0	0	0	0	0	0	3	2	3	2
			200	1	3	0	0	0	0	2	0	6	5	6	5
Test Substance	45.0	47	100	18	11	5	3	0	2	2H 2PP	39	21	29	18	
		48	100	7	13	11	0	1	1	1	2H	33	26	21	18
			200	25	24	16	3	1	3	3	4H 2PP	72	47	50	36
Test Substance	30.0	49	100	2	8	0	2	0	2	1H 1PP	14	12	14	12	
		50	80	9	3	0	1	0	0	1	1PP	14	5	13	5
			180	11	11	0	3	0	0	3	1H 2PP	28	17	27	17
Test Substance	15.0	51	100	4	0	0	0	0	2	0	6	2	4	2	
		52	100	5	1	0	0	0	1	0	7	2	6	2	
			200	9	1	0	0	0	0	3	0	13	4	10	4
Mitomycin-C	0.30	55	50	9	14	20	3	0	3	0	49	40	31	27	
		56	50	4	13	32	5	0	2	2H	56	52	36	35	
			100	13	27	52	8	0	5	2H	105	92	67	62	

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2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

TABLE 6 - Scoring of Aberrations - With metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose-level (µg/ml)	Culture No.	Cells Scored	Gaps	Chromatid Del	Exch	Chromosome Del	Exch	Isolocus	Other	Tot. abs (+gaps)	Tot. abs (-gaps)	Cells with abs (+gaps)	Cells with abs (-gaps)	
Untreated	-	73	100	1	0	0	0	0	0	1PP	1	0	1	0	
		74	100	1	2	0	0	0	0	0	0	3	2	3	2
			200	2	2	0	0	0	0	0	1PP	4	2	4	2
Test Substance	160	83	100	2	0	0	0	0	0	2PP 4ER	2	0	2	0	
		84	100	3	1	1	0	0	0	1PP	5	2	4	1	
			200	5	1	1	0	0	0	3PP 4ER	7	2	6	1	
		85	100	9	2	5	1	0	0	2PP 1ER	18	9	11	6	
		86	100	9	3	6	1	0	0	1PP	20	11	12	4	
	200	18	5	11	2	0	0	2 3PP 1ER	38	20	23	10			
Test Substance	100	89	100	2	1	0	0	0	0	1PP 1ER	3	1	3	1	
		90	100	1	1	0	0	0	0	3PP 2ER	2	1	2	1	
			200	3	2	0	0	0	0	4PP 3ER	5	2	5	2	
Cyclophosphamide	15.0	91	100	13	9	17	5	1	7	1PP	52	39	33	27	
		92	100	3	7	7	0	0	0	0	17	14	12	11	
			200	16	16	24	5	1	7	1PP	69	53	45	38	

**9. KEY TO TABLES 7 - 8**

This table summarises the statistical analyses for the individual treated cultures and for the treatment groups using the pooled data. The analyses are presented both including and excluding gaps from the data-sets. The following abbreviations are used:

**P. Value**                      The calculated probability value obtained from the comparison of the treated with the controls, using Fisher's Exact Test.

The test substance treatments are compared with the relevant solvent or untreated controls as appropriate (according to the vehicle used for the test material). Positive control treatments are compared with the untreated controls.

**Sig.**                              Significance level.

The significance level of the achieved P-value.

For the test substance treatments, correction is made for multiple comparisons (as indicated at the foot of each table).

For positive control treatments and for all dose-levels combined significance is indicated as:

\*      Statistically significant at  $P < 0.05$

\*\*     Statistically significant at  $P < 0.01$

\*\*\*   Statistically significant at  $P < 0.001$

**#**                                  No statistic was calculated since the proportion of cells bearing aberrations was identical for the treated and relevant negative control cultures.

**NS**                                Not significant.

2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

TABLE 7 - Statistical Analysis - Without metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Comparison with Negative control	Cells with aberrations					
	Dose µg/ml	CULT.	(+ gaps)		(- gaps)	
			P. values	Sig.	P. values	Sig.
Test Substance	45.0	47	0.0000	***	0.0000	***
		48	0.0000	***	0.0000	***
	Pooled		0.0000	***	0.0000	***
Test Substance	30.0	49	0.0006	**	0.0014	**
		50	0.0002	**	0.1228	N.S.
	Pooled		0.0000	***	0.0033	*
Test Substance	15.0	51	0.4408	N.S.	0.5707	N.S.
		52	0.1732	N.S.	0.5707	N.S.
	Pooled		0.2226	N.S.	0.5000	N.S.
All dose levels			0.0000	***	0.0003	***
Mitomycin-C	0.30	55	0.0000	***	0.0000	***
		56	0.0000	***	0.0000	***
	Pooled		0.0000	***	0.0000	***

In this table correction is made for the multiple comparisons of the Test substance treatments with the negative controls. For individual cultures, since six comparisons are made, the required "p" values for significance are 0.009(\*), 0.002(\*\*) and 0.0002(\*\*\*). For treatment levels, since three comparisons are made the required "p" values for significance are 0.017(\*), 0.003(\*\*) and 0.0003(\*\*\*).

2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

TABLE 8 - Statistical Analysis - With metabolic activation

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Comparison with Negative control	Cells with aberrations					
	Dose µg/ml	CULT.	(+ gaps)		(- gaps)	
			P. values	Sig.	P. values	Sig.
Test Substance	160	83	#	N.S.	0.4437	N.S.
		84	0.2564	N.S.	#	N.S.
	Pooled		0.3755	N.S.	0.5000	N.S.
Test Substance	140	85	0.0014	**	0.0183	N.S.
		86	0.0006	**	0.0979	N.S.
	Pooled		0.0001	***	0.0179	N.S.
Test Substance	100	89	0.4294	N.S.	#	N.S.
		90	#	N.S.	#	N.S.
	Pooled		0.5002	N.S.	#	N.S.
All dose levels			0.0212	*	0.2335	N.S.
Cyclophosphamide	15.0	91	0.0000	***	0.0000	***
		92	0.0006	***	0.0002	***
	Pooled		0.0000	***	0.0000	***

In this table correction is made for the multiple comparisons of the Test substance treatments with the negative controls. For individual cultures, since six comparisons are made, the required "p" values for significance are 0.009(\*), 0.002(\*\*) and 0.0002(\*\*\*). For treatment levels, since three comparisons are made the required "p" values for significance are 0.017(\*), 0.003(\*\*) and 0.0003(\*\*\*).

## 2-HYDROXYPROPYL ACRYLATE: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

TABLE 9 - Summary Table

STUDY NO.: 7384

SOLVENT: Ham's F10

TREATMENT TIME : 3 hours

SAMPLING TIME : 20 hours

Treatment	Dose µg/ml	Presence of S9 metabolism		Absence of S9 metabolism	
		%CA	(Rel. cell count)	%CA	(Rel. cell count)
Untreated	-	1.0	( 100)	2.5	( 100)
Test Substance	160	0.5	( 52)	-	( - )
Test Substance	140	5.0	( 58)	-	( - )
Test Substance	100	1.0	( 73)	-	( - )
Test Substance	45.0	-	( - )	18.0 ***	( 45)
Test Substance	30.0	-	( - )	9.4 **	( 62)
Test Substance	15.0	-	( - )	2.0	( 78)
Mitomycin-C	0.30	-	( - )	62.0 ***	( 75)
Cyclophosphamide	15.0	19.0 ***	( 65)	-	( - )

## Key:

% CA : Percentage of cells bearing aberrations (excluding gaps)  
 Rel. cell count: Relative to negative control (percent)  
 - : Not tested or not selected for the scoring of aberrations  
 \* : Statistically significant at P<0.05  
 \*\* : Statistically significant at P<0.01  
 \*\*\* : Statistically significant at P<0.001

**10 - APPENDIX I - Historical background incidences of aberrant cells**

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS  
Background incidences (%) of aberrant cells (Years 1990-1999)

	Absence of S9		Presence of S9 metabolism	
	24 hours sampling time		24 hour sampling time	
	+ gaps	- gaps	+ gaps	- gaps
<b>UNTREATED</b>				
Mean	1.4	0.8	1.5	1.0
SD ( $\sigma_{n-1}$ )	1.5	1.1	1.8	1.3
n	16	16	17	17
Minimum	0	0	0	0
Maximum	6	4	5.5	4
<b>SOLVENT</b>				
Mean	1.8	1.1	1.7	1.2
SD ( $\sigma_{n-1}$ )	1.7	1.2	2.0	1.4
n	9	9	9	9
Minimum	0	0	0	0
Maximum	5	3.5	6	4

	Absence of S9		Presence of S9 metabolism	
	20hours sampling time		20 hour sampling time	
	+ gaps	- gaps	+ gaps	- gaps
<b>UNTREATED</b>				
Mean	1.1	0.3	1.2	0.3
SD ( $\sigma_{n-1}$ )	0.9	0.4	1.5	0.4
n	12	12	10	10
Minimum	0	0	0	0
Maximum	2.5	1	4.5	1
<b>SOLVENT</b>				
Mean	2.1	0.8	1.9	0.8
SD ( $\sigma_{n-1}$ )	2.2	1.2	2.8	1.4
n	10	10	8	8
Minimum	0	0	0	0
Maximum	8	4	8	4

SD = standard deviation  
n = number of experiments

**11.- APPENDIX II - Study Protocol**