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Pharma Development Central Toxicology

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IN V79 CHINESE HAMSTER CELLS

Pharma Development Central Toxicology D-6230 Frankfurt am Main 80

Laboratory Project ID: Study No. 92.0337

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Page 3 of 35 17-Nov-97 14:19



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Report No. 92,1024 Page 2 (34)

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Page 1 of 35 17-Nov-97 14:19

Hoechst

Pharma Development Central Toxicology

Report No. 92.1024 Page 3 (34)

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director

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of this document may therefore be prohibited?

Quality Assurance Statement

5 of 35 Page 17-Nov-97 14:19

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Report No. : 92.1024

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Hoechet Aktiengesellschaft quality Assurance (GLP)

14.12.1992

Title

Dodigen 4022

CHROMOSOMEN ABERRATIONS

IN VITRO

IN V79 CHINESE HAMSTER CELLS

STUDY NO.

92.0337

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This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below :

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PERNTOR Page 6 of 35 17-Nov-97 14:19



Pharma Development Central Toxicology

Report No. 92.1024 Page 5 (34)

CONTENTS

		PAGE	
	GLP - STATEMENT	, die 4	Se
1.	SUMMARY	8	<i>S</i> -
2.	INTRODUCTION		
3,	GENERAL	8	
4.	MATERIALS AND METHODS	- و , , , , ,	- 14
4.1	Test compound		
4.2	Preparation and storage of a liver homogenate fraction (S9).	10	
4,3	Preparation and storage of a liver homogenate fraction (S9). Preparation of S9-mix	10	
4.4	Mammalian Cells	,10	
4.5	Procedure of the assay	10	
4.6	Controls	12	
4.7	Experimental groups	12	
4.8	Analysis of metaphases	13	
4.9	Evaluation.	14	
4.10	RESULTS	14	
5.	RESULTS	15	
5.8	Solubility and toxicity	15	
5.2°	Mutagenicity test	.,15	
5,2.1	Experimental design	15	
5.2.2	Toxicity and mutation results	16	
5.2.3	Interpretation and discussion of the results	16	- 17
6.	APPENDIX	18	
6.1	Examples of aberrations	18	
7.	REFERENCES		
8.	TABLES		
9.	BIOMETRY (Tables)	31	- 34

TO: 0013146944028 FROM: TELECOH MSG: MO 451435

HONSONTO OPERATOR

7 of 35 Page 17-Nov-97 14:19

Hoechst

Pharma Development Central Toxicology

Report No. 92.1024 Page 6 (34)

SUMMARY

The study was performed to investigate the potential of Dodigen 4022 to induce chromosome aberrations in V 79 cells of the Chinoso hamster in vitro.

The assay was performed in two parallel_cultures, using identical procedures, both with and without rat liver microsomal activation.

The test article was tested with the following concentrations:

without S9-mix:

with S9-mix:

7 h: 6000* ug/ml 18 h: 6000*, 3000, 600 ug/ml 28 h: 6000* ug/ml

7 h: 6000* ug/ml 18 h: 6000*, 3000, 600 ug/ml 28 h: 6000* ug/ml

* = 10 mM

℮

According to the preliminary experiment for solubility and toxicity the concentration ranges were selected, 6000 ug/ml were chosen as the highest test concentration because of 10mm limitation

This concentration produced a distinct decrease of the mitotic index at the 7 hour fixation interval with and without metabolic activation. In contrast a dose-dependent increase of the mitotic index was observed at the 18 hour fixation interval. These effects can be explained as a consequence of a reversible block of the cell cycle.

This increase seems to be caused by some unspecific effects as indicated by the inhibition of the cell cycle Because of the absence of any increased chromosomal aberrations as compared to the controls after completion of the cell cycle (18h and 28h fixation intervals) this effect was considered as not sufficient to evaluate the compound as distinct genotoxic agents

Appropriate reference mutagens were used as positive controls and showed a dislinct increase in the chromosome aberrations indicating the sensitivity of the assay.

In conclusion as described under the conditions above, Dodigen 4022 1s considered to be non-mutagenic in this chromosome aberration assay,



MONSANTO OPERATOR Page 8 of 35 FROM: TELECOM TO: 0013146944028 17-Nov-97 14:19 451435 HSG: HO

2. Introduction

The in vitro cytogenetic test is a mutagenicity test system for the detection of chromosomal aberrations in cultured mammalian cells (1). This system offers several advantages. When modern and adequate techniques are employed cell cultures show only minor variations between a series of passages with respect to the cell cycle, viability, plating efficiency, medium requirements and karyo-type. Furthermore the cells can be stored frozen as stock for many experiments

Chromosomal aberrations may be either structural or numerical. However, because cytogenetic assays are usually designed to analyse cells at their first posttreatment mitosis and numerical aberrations require at least one cell division to be visualized, this type of aberration is generally not observed in a routine cytogenetic assay. Structural aberrations may be of two types: chromosome or

Chromosome-type aberrations are induced when a compound acts in the Gi phase of the cell cycle. Chromatid-type aberrations are induced when a chemical acts in the 5 or G2 phase of the cell cycle.

- Chromosome-type aberrations are changes which result from damage expressed in both sister chromatids at the same locus
- Chromatid-type aberrations result from damage expressed as breakage of a single chromatid or breakage and/or reunion between chromatids
- Numerical aberrations are variations of the normal chromosome number characteristic of the cells used in the assay

The V79 cell line has been used successfully for many years in <u>in vitro</u> experiments. Especially the high proliferation rate (doubling time 12-16 hours in stock cultures) and a high plating efficiency of untreated cells, both necessary the high and a high place opriate performant, 4). for the appropriate performance of the study, recommend the use of this cell



FROM: IELECOM

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Page 9 of 35 17-Nov-97 14:19

Hoechst

Pharma Development Central Toxicology

Report No. 92.1024 Page 8 (34)

3. GENERAL

Study-No.

92.0337

Test compound

Dodigen 4022

Code

Hoe CG 0351 OE ZD82 0001

Ordered by

Hoachst AG, Abt. MTH-Entwicklung TVS, Harr

Test system

in vitro mammalian cytogenetic test

Test organism

Inder a redulation data brotection the owner of the owner owner of the owner of the owner ow cells of Chinese hamstor cell line V79

Initiation of the study :

July 22nd, 1992

Termination of the study :

November 3rd, 1992

Responsibility

Head of Genetic Toxicology:

Head of Toxicology

Quality assurance unit

Testing facilities and archives: Pharma Development Central Toxicology

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Page 10 of 35 17-Nov-97 14:19



Pharma Development Central Toxicology

Report No. 92,1024 Page 9 (34)

4. MATERIAL AND METHODS

4.1. Test compound

Name

: Dodigen 4022

Code

Hoe CG 0351 DE ZD 0001

Chemical nomenclature

Trimethyl-ethoxypolyoxpropylammoniumchloride

Purity

82 %

Stability

April 1993 guaranteed by the stable until

sponsor

Stability in solvent

: stable for four hours in water

Analylical Laboratory; Oekochemie/Analytik-E

dated December 4th, 1991

Appearance

viscous yellow liquid

certificate of analysis

No. 04701 dated August 20th, 1991

Boiling point

> 250 °C

Molecular weight

600 ca 600

Batch No.

Storage conditions

cell culture medium

dark at approx. 0 °C

MEM (Minima)

salts 🌮 MEM (Minimal essential medium) with Hanks-

salts and 25 mM Hepes-buffer

Concentration of stock solution

0.61 %

At the day of the experiment the test substance was dissolved as a solution in cell culture medium at appropriate concontrations. Two independent cell cultures (No. 1 and 2) were used.

Page 11 of 35 17-Nov-97 14:19

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Pharma Development Central Toxicology

Report No. 92.1024 Page 10 (34)

4.2 Proparation and storage of a liver homogenate fraction (\$9)

Male Sprague Dawley rats (200-300 g) received a single intraperitoneal injection of Aroclor 1254 (500 mg/kg bodyweight) 5 days before sacrifice. Preparation is performed at 0 to 4 °C using cold sterile solutions and glassware. The livers from at least 5-6 animals are removed, pooled and washed in approx. 150 mM KCl (approx. 1 ml/g wet liver). The washed livers are cut into small pieces and homogenized in three volumes of KCl. The homogenate is centrifuged at 9000 g for 10 minutes. The supernatant is the S9 fraction. It is divided into small portions, rapidly frozen and stored at approx. -80 °C.

4.3 Preparation of \$9-mix

Sufficient S9 fraction is thawed immediately before each test at room temperature. One volume of S9 fraction is mixed with 9 volumes of the S9 cofactor solution and kept on icc until its use. This preparation is termed S9-mix. The concentrations of the different compounds in the 59-mix are:

B mM MgCl2 33 mM KČ1

, mr MADP+
100 mM phosphate buffer pH 7.4

4.4 Mammalian cells

Large stocks of the V79 cell line stored in liquid nitrogen in the Laboratory of Genetic Toxicology of Hoechst AG allow the repeated use of the same cell culture batch in experiments. Consequently, the callular parameters of the experiments remain similar because of the reproducible characteristics of the cells. The tawed stock cultures were propagated at approx. 37 °C in 175 cm² plastic flasks. Seeding was done with about 8-10 x 105 cells per flask in 30 ml of MEMmedium supplemented with approx. 10 % fctal calf serum (FCS). The cells were subcultured twice a week.

4.5 Procedure of the assay

Two days old, exponentially growing stock cultures which were over 50 % confluent were trypsinised and a single-cell suspension was prepared. The trypsin concentration was approx. 0.25 % in Ca-Mg-free salt solution. 1-3 x 106 ceils/flask were seeded into four 75 cm2 plastic flasks containing 15 ml MEM with approx. 10 % FCS (7 h preparation). 6.9 x 105 cells/flask were seeded into four 25 cm2 plastic flasks containing 5 ml MEM with approx. 10 % FCS (18 h preparation). 3-6 x 105 cells/flask were seeded into four 25 cm2 plastic flasks containing 5 mi MEM with approx. 10 % FCS (28 h preparation). After 24 h, the medium was replaced with medium containing approx. 5 % FCS and the test substance, both without S9-mix and with S9-mix. After 4 h this medium was replaced with normal medium after rinsing once with physiological saline solution.

Treatment was performed with three concentrations of the test substance.

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Report No. 92.1024 Page 11 (34)

The mitotic index was determined in samples of 1000 cells. The toxicity of the test substance was determined in a preliminary experiment by establishing the concentration-related plating efficiency. According to these data the concentration range was chosen.

4.5. 15.5 and 25.5 h after the start of the treatment colcemide was added (apprex. 0.04 ug/m] culture medium) to the cultures. 2.5 h later (7 h, 18 h and 28 h preparation) the cells were trypsinised.

For hypotonic treatment, approximately 5 ml of approx. 0.075 M potassium chloride solution at approx. 37 °C was quickly added and suspended. This suspension was then allowed to incubate for 10 minutes in a water bath at approx. 37 °C. Addition of 1.5 ml fixative and flow through with air.

After re-centrifuging for five minutes at 1000 rpm, all but one drop of the supernatant was drawn off by pipette. The sediment was carefully covered with a layer composed of 2.5 ml fixative (methanol : glacial acetic acid 3 + 1). After at least 20 minutes, the fixation was carefully removed with a pipette and suspended in 2.5 ml fixative. The mixture was centrifuged when needed after another 30 minutes, after which the liquid was removed by pipette and fixative added. The tubes were covered and kept for at least 12 hours (overnight) in a refrigerator at approx 4 °C.

After re-centrifuging for 5 minutes at 1000 rpm, all but one drop of the liquid was removed by pipette and a new suspension formed with a small quantity of freshly prepared fixative. A few drops of this suspension were placed with a pasteur pipette onto clean microscopic slides which had been stored in distilled water at approx. 4 °C, the drops were then priefly passed through a Bunsen flame and air dried for 24 hours. Staining was performed as follows:

- staining for 10 minutes in approx. 2 % orcein solution rinsing 3 times in distilled water
- rinsing lwice in acetone
- bricf rinsing in acetone/xylene
- 2 minutes in acetone/xylens
- 5 minutes in xylene
- 10 minutes in xylene
- embedding in Entellan^R or Eukitt^R
- 2-5 slides were prepared from each flask.

In the same way both negative and positive controls were prepared 18 h after medium change or treatment respectively.



Pharma Development Central Toxicology

Report No. 92,1024 Page 12 (34)

4.6 Controls

Nagative controls:

Untreated cultures and cultures treated with the solvent (medium).

Positive controls:

EMS (Ethylmethanesulfonate) dissolved in nutrient medium in a concentration of 1750 ug/ml. The solution was prepared on the day of the experiment.

With metabolic activation:

CPA (Cyclophosphamide) - Endoxan final concentration in nutrient medium was 5.0 ug/ml. The solution was prepared on the day of the experiment.

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is proof of biological stability.

4.D

Negative control: untreated cells
Negative control: untreated cells
Solvent control: cells +
Solvent control: cells + Positive control: cells + EMS Positive control: cells treated with CPA + S9-mix Test group 1: cells + test substance Test group 1: cells + test substance Test group 2: cells + test substance 600 ug/m1 600 ug/ml cells + test substance + \$9-mix 3000 ug/m] 3000 ug/ml cells + test substance + 59-mix Test group 2: Cells + test substance 6000 ug/m] Test group 3: Test group 3: Cells + test substance + S9-mix 6000 ug/ml

Preparation time 7 and 28 h

Solvent control : cells + solvent Solvent control : cells + solvent + S9-mix cells + test substance 6000 ug/m? Test group 3: 6000 ug/ml cells + test substance + S9-mix Test group 3:

The concentrations were chosen from the data of the cytotoxicity assay in the preliminary experiment as follows:

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Report No. 92.1024 Page 13 (34)

The survival rate of cells treated with the test substance.

นq/กไ	without S9-mix	with S9-mix
Negative control	85.4 %	85.2 %
Solvent control	95.5 % = 100 %	82.2 % = 100 %
100	85.1 % 91.5 % 85.5 % 82.4 % 77.9 %	8. 1. 35. 19 % A. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.
250	85.1 % 91.5 % 85.5 % 82.4 % 77.9 %	74.7 %
500	85.5 % 82.4 % 77.9 % 62.4 %	79.5 %
1000	85.5 % 82.4 % 77.9 % 62.4 % 62.1 %	80.6 %
2000	77.9 %	76.3 %
3000	62,47	84,5 %
4000	62 A 7 8 B B B B B B B B B B B B B B B B B B	38.5 % .
5000	59.0 % O LIVE TO THE TO	76.9 %
6000	2 3 58,2 M	86.2 %

8

After the slides had been coded (Coding Scheme 552/92), 100 metaphases per experimental group were examined. The set of chromosomes was examined for completeness and the various chromosomal aberrations were assessed. The chromosomal aberrations were classified as shown in chapter 6.1. Only metaphases with 22 +/- 1 chromosomes are included in the analysis. The metaphases were examined for the following aberrations: gap (g), isogap (ig), break (b), isobreak (ib), fragment (f), isofragment (if), minute (m), isominute (im), deletion (d), isodaletion (id), exchanges including intrachanges (ex), dicentrics (di), chromosome disintegration (cd) and ring formation (ri). In addition, metaphases with 5 or more aberrations were classified separately as multiple aberrations (ma).⊘

After the metaphases had been evaluated, the code was lifted. The values for the Control group were compared with the results from the dose group and the positive control at each preparation time.

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Page 15 of 35 17-Nov-97 14:19



Pharma Development Central Toxicology

Report No. 92.1024 Page 14 (34)

4.9 Evaluation

The evaluation of the results was performed as follows:

- The test substance is classified as mutagenic if it induces a statistically significant increased aberration rate(without gaps) as compared with the solvent controls with one of the concentrations tested.

the test substance is classified as mutagenic if there is a reproducible concentration related increase in the aberration rate (without gaps).

the test substance is classified as non mutagenic when it tests negatively both with and without metabolic activation.



The Biometry of the results was performed with a one-sided Fisher - Exact test (see tables page 31 - 34).



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Report No. 92.1024 Page 15 (34)

RESULTS

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5.1 Solubility and toxicity

In a preliminary experiment Dodigen 4022 was solved in cell culture medium. At a concentration of 6000 ug/ml (= 10 mM, which is the highest dose level tolerated for the test system) no visible precipitation was observed.

Accordingly, the preliminary study was carried out using a maximum concentration of 6000 ug/ml and a wide range of lower dose levels. Results are presented on page 13.

In the cytotoxicity experiment Dodidon 4022 was slightly cytotoxic without S9-mix from 3000 ug/ml up to the concentration of 6000 ug/ml. In the presence of S9-mix no indication of cytotoxicity was observed

5.7 Mutagenicity

5.2.1 Experimental design

Preparation of chromosomes was carried out Th (high dose), 18 h (low, medium and high dose) and 28 h (high dose) after start of the treatment with the test article. The treatment interval was 4 h.

In each experimental group two parallel cultures were used. Per culture 100 metaphases were scored. The following dose levels were evaluated:

with \$9-mix:

7 h: 6000 ug/ml 18 h: 6000, 3000, 600 ug/ml

28 h: 6000 ug/ml



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Report No. 92.1024 Page 15 (34)

5.2.2 Toxicity and mutation results

In the main experiment the mitotic index was reduced after treatment with the highest dose levels at fixation intervals 7 h with and without S9-mix (page 20). After the 18h fixation interval a distinct increase of the mitatic index occured in the medium and high concentration with and without S9-mix. In contrast the mitotic index showed no remarkable changes compared to the controls after the 28 hour fixation interval.

Table 2 shows the number of polyploid cells. After treatment with the test article there was no relevant increase in the number of polyploid cells as compared with the solvent controls.

the test substance Dodigen 4022 was assessed for its mutagenic potential in vitro in the chromosome-aberration-test with two independent cell cultures without metabolic activation and two independent cell cultures with metabolic activation. The results of these experiments are presented in tables 1 - 8. The sensitivity of the test system was demonstrated by the enhanced mutation frequency in the cell cultures treated with the positive control substances.

There was an enhancement of the aberration rates 7 h after the start of the treatment with 6000 ug/ml with \$9-mix inclusive and exclusive gaps. Also at the same concentration at preparation time In without metabolic activation the number of cells with aberrations and the aberration rate were enhanced, both inclusive gaps.

These data were found significantly enhanced in the Fisher's exact-test. Although this statistical test did not indicate significance for other data.

5.2.3 Interpretation and discussion of the results

As mentianed above Dodigen 4022 produced a distinct decrease of the mitotic index after the 7h fixation interval as an expression of an inhibition of the cell cycle. This effect was reversible because af the dose-dependent increase of the mitotic index after the 18h fixation interval.

After removal of the Dodigen 4022 induced inhibition and synchronisation of the

cell cycle an enlarged number of cells went in mitosis.

A slight increase of the aberration rates was observed only during the inhibition of the cell cycle. After the completion of the cell cycle (18 and 28 hour fixation intervals) no increase of chromosomal aberrations ocurred. The inhibition of the cell cycle indicates the same unspecific effect of Dodigen 4022 Induced an increased aberration rate after the 7 hour fixation interval.

However, these results should not be used for the definitive ranking of this Substance as a mutagenic compound because after competion of the cell cycle no signs of mutagenicity occured.

Page 18 of 35 17-Nov-97 14:19



Pharma Development Central Toxicology

Report No. 92,1024 Page 17 (34)

Therefore it is concluded that the test compaund is not mutagenic in the chromosome aberration test system in vitro with cells of the V79 Chinese hamster cell line as described under the conditions above.

Dr. IST/AB

Quality assurance unit B: 14,12.92

Pharma Development Central Toxicology

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SCHOOL SELECTOR

Head of Toxicology

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Page 19 of 35 17-Nov-97 14:19



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Report No. 92.1024 Page 18 (34)

6. APPENDIX

6.1 Examples of aberrations

1. Structural aberrations

Gap: Non-stained segment (achromatic gap) of chromatid without dislocation of the apparently separate part, irrespective of size of the non-stained area.

Break: A visible fracture of the chromatid structure where the broken piece is laterally dislocated or shifted in the longitudinal axis but can still be assigned to the corresponding contric part.

Fragment: Acentric part of a chromosome which may appear individually, regardless of its size.

Minute: Small chromatide body with a diameter smaller than the width of the

Delction: Terminal or interstitial loss of part of the chromatide.

Exchange: These are exchange aberrations, subdivided into intrachanges (the union of parts that can combine within a chromosome) and interchanges (the union of parts that can combine from two or more chromosomes). Dicentric chromosomes and ring chromosomes are included in this group.

The chromatide aborrations specified above can also occur as iso-chromatide aberrations (e.g. isochromatide break)

2. Numerical aberrations

Aneuploidy: A deviation from the typical number of individual chromosomes in a set of chromosomes; a decrease in the number is known as hypoploidy and an increase as hyperploidy.

Polyploidy: More than two sets of chromosomes.

Additional criterion:

Chromosomal disintegration: Where all or most of the chromosomes are irragular particles. If exchange figures occur in the metaphases, they are only included in this aberration group.

189

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Pharma Development Central Toxicology

Report No. 92.1024 Page 19 (34)

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 Genetic Toxicology:
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- EPA In Vitro Mammalian Cytogenetics HG-Chromo-In Vitro, August 1982 u.S. Environmental Pro Washington, D.C. 20460 Office of Toxic Substances Office of Pesticides and Toxic Substances U.S. Environmental Protection Agency

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Report No. 92.1024 Page 20 (34)

8. TABLES

Table 1: Mitotic index

Test group	Dose ug/ml	S9 mix	fixation interval (h)	mitoi sli l	tic index ide	(per cent ¹ abs.	rel.
Solvent control Test article	0 6000	-	7	8.2 3.6	9.1 2.5	8.7 3.1	100.0 35.6
Solvent control Test article	0 6000	+ +	7 ide	41.5 5.2	8.6 or 4.8	10.1	100.0 49.5
Solvent control Positive control EMS Test article Test article Tost article	0 1750 600 3000 6000	solling the state of the state	18 18 19 18 18	9.7 9.8 9.5 11.5 13.0	8.0 7.8 8.2 12.3 9.8	8.9 8.8 8.9 11.9	100.0 98.9 100.0 133.7 128.1
Solvent control Test article Solvent control Test article Solvent control Positive control Test article Solvent control Test article Solvent control Test article Solvent control Test article Solvent control Test article	0 5 600 3000 6000		19 18 18 18 18	8.9 4.3 10.2 12.5 12.9	9.6 6.0 7.6 10.9 15.3	9.3 5.2 8.9 11.7 14.1	100.0 55.9 95.7 125.8 151.6
Solvent control Test article	0 6000	•	28 28	12.5	11.4 7.0	12.0 7.7	100,0 64.2
Selvent control Test article	0 600D	+ +	28 28	11.0 13.1	12.0 11.3	11.5 12.2	100.0 106.1

^{*}The mitotic index was determined in 1000 cells from each of two slides per test group

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Report No. 92.1024 Page 21 (34)

Number of polyploid cells Table 2:

Test group	na/wj	e2 mix	fixation interval (h)	polyploi slic	id cells* le 2	total	mean
Solvent control Test article	0 6000	-	7 7	6 D	See Stille	10.0 0.0	5,0 0.0
Solvent control Test article	0 6000	† +	7 7	of Boyles	Collings of Collings	4.0 54.0	2.0 0.5
Solvent control Positive control EMS Test article Test article Test article	0 1750 600 3000 6000	nd till of	18 18 18 18 18 18	2 4 8 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	4 6 0	8.0 3.0 6.0 10.0 1.0	4.0 1.5 3.0 5.0 0.5
Solvent control Test article Solvent control Test article Solvent control Positive control Test article Test article Test article Test article Test article Solvent control Positive control Positive control Test article Test article Test article Test article Solvent control Test article Test article Test article Test article	0 5 500 3000 6000		18 18 18 18 18 18	1 4 3 3	5 1 1 5 3	6.0 5.0 4.0 8.0 4.0	3.0 2.5 2.0 4.0 2.0
Solvent control Test article	6000		28 28	4 1	4	8.0 5.0	4.0 2.5
Solvent control Test article	6000	+	28 28	1	2	3.0 7.0	1.5

⁼ the number of polyploid cells was determined in 100 cells from each of the two slides per test group.

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Pharma Development Central Toxicology Table 4: Chromosome aberrat	Dose ug/ml	0	0	Total	6000	. 6090	Fota!	i ii un

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Figure Chronoscine aberrations in V79 cells Fable 5. Chronoscine aberrations in V79 cells Fable 5. Technology Fable 6. Technology Fable 6. Technology Fable 6. Technology Tech		900	90cnus	I	-1444	Ø											Te de	ert No	92.	1024
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Page 26 of 35 17-Nov-97 14:19

Report No. 92.1024 Page 25 (34)

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Chromosome aberrations in V79 cells Test substance: Dødigen 4022 Preparation: 18 h after administration (100 netaphases were analysed)

			4		5,5					•								
Dose ug/m]	Calture without S9 mix	No. with incl.	No. of phases th aberration cl. excl.	No. of phases with aberrations incl. excl.	incl.	chases No. of g ig b its f if d id ma ex cd rations abenrations excl.	6	†g	هـ	ð	.	if	.	jū	e e	ex ex	others	% ¥
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Table 6:	-orseller	P Tes	hromosome est substa reparation	Chromosome aberrations in V79 Test substance: Dodigen 4022 Preparation: 18 in after admin	cions in sargen 4	n V79 cells 4022 administration (100 metaphases were	11s ation	00K)	metap	hases	Were a	analysed)	(pag					
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le 6	<pre>Table 6 (cont.): Chromosome aberrations in V79 cells Test substance: Dodigen 4022 Preparation: 18 h after administration</pre>	Per	omosome et substa sparation	aberration nce: Dodi : 18 h al	ons in V igen 402 fter adm	n V79 cells 4022 administration (100 metaphases were analysed)	ls ation	001)	metap	hases	were	analy:	se d)	:			. All months	
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No. of phases aberrations twith aberrations aberrations excl.

without S9 mix **Culture**

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Total

Test substance; Dodigem 4022 Preparation: 28 b after administration (100 metaphases were analysed)

Chromosome aberrations in V79 cells

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Total

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S = solvent coutrol

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Report No. 92.1024 Page 23 (34)

Pharma Development Central Toxicology

2/5

S/1

3/5

6000

Total

1/E

6000

Total

Calture with S9 mix

Table 8:

Hoechst 2

Pharma Development Central Toxicology

Report No. 92.1024 Page 30 (34)

<u>Table 9:</u> <u>Summary of results</u>

(1)

(i)

Test group	Number of cells analysed	Dose ug/ml	S9 mix	fixation interval (h)	incl.	nt aberra excl. gaps	exchanges
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Solvent control Test article Solvent control Test article Solvent control Test article Test article Solvent control Positive control Test article Test article Test article Solvent control Test article Test article Solvent control Test article Solvent control Test article	Z00 CPA 200 200 200 200	0 5 600 3000 6000	00 + 00 + 00 + 00 + 00 + 00 + 00 + 00	18 18 18 18 18	1.5 15.0 3.0 2.0 4.5	1.5 14.5 1.5 2.0 2.5	0.0 7.0 0.5 0.5 1.0
Solvent control Test article	200	6000	•	28 28	1.0	0,5 2.5	0.0
Solvent control Test article	200 200	6000	+ +	28 28	2.5 1.5	0.5 1.0	0.0 0.5

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Report No. 92.1024 Page 31 (34)

Pharma Development Central Toxicology

9. BIOMETRY

FISHER TEST

7 H -S9 No. of phases with aberrations inclusive gaps

92.0337	Res	onsa	P	5 Cilly	
Group	Yes	No	Prob	(f),	{2}
CONTROL 6000 ug/ml	9	199 191	0.0100		is of the

7 H -S9 No. of phases with aberrations exclusive gaps

5	2.0337	Resp	P = 0.05				
0	Group	Yes	No	Prob	{1}	{2}	
5 60 10	CONTROL 000 ug/ml		199 194	0.0609			







Page 32 (34)

FISHER TEST 7 H -S9 No of aberrations inclusive gaps

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92.0337	Res	onse	P :	- 0.	05
Group	Yes	No	Prob	(1)	{2
CONTROL SOGO ug/ml	1 9	199 191	0.0100	-65° ×	Hoch

7 H -S9 No. of aberrations exclusive gaps

	92.0337	Res	onse	P .	0.0)5
	Group	ASC (VIII)	Na		{1}	{2}
<u>.</u>	CONTROL 6000 ug/ml	Suj 110 6	€ 199 ,	0.0609		h
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Cousedneying,						



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Page 34 of 35



Pharma Development Central Toxicology

Report No. 92.1024 Page 33 (34)

FISHER TEST 7 H +S9 No. of phases with aberrations inclusive gaps

92.0337	Res	onse	P = 0.05		05
Group	Yes	No	Prob	(1)	(2)
CONTROL 6000 ug/ml	3	197 194	0.2514		illie.
		\ 	, of old	ion (Velcisy
		30	sight of	econ	ONL'HE

7 H +S9 No. of phases with aberrations exclusive gaps

92.0337	Resi	ognse	P = 0.05				
Graup	Yesucii	K No Kill	Prob	(1)	{2}		
CONTROL 6000 ug/ml	9) 18 30 CE	© 198 197	a.šooo				



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Page 35 of 35 17-Nov-97 14:19



Pharma Development Central Toxicology

Report No. 92.1024 Page 34 (34)

FISHER TEST 7 H +S9 No. of aberrations inclusive gaps

P = 0.05		
{1}	(2)	
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100	any in	

7 H +S9 No. of a	iberrations (exclusive	gans of	A Stille Se violage the ideas					
	92.0337	Resp	onse	P)5				
	Group	Yes	20 Noohill	Prob	(1)	(8)			
SELES SE	CONTROL 6000 ug/ml	18 20 10 18 9,00	198 191	0.0309	*	÷			
E Stobelty of Elyon	gion, goon	(2)							





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