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Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)



**Re : Submission of TSCA 8(d) Health and Safety Study reports – BASF Corporation**

Dear 8(d) Coordinator:

In compliance with 40 CFR 716, BASF hereby submits requested Health and Safety Study reports for substances recently added to the list of subject chemicals (71 FR 47130).

This submission contains information for the following list of substances:

62-56-6	121-69-7	1738-25-6
83-41-0	127-68-4	3039-83-6
84-69-5	131-57-7	4316-73-8
96-22-0	137-20-2	61788-76-9
104-93-8	645-62-5	68188-18-1
110-18-9	939-97-9	68609-05-2
111-44-4	1111-78-0	68909-77-3

Should you have questions regarding this submission, please do not hesitate to contact me at 734-324-6593 or email [kara.sparks@basf.com](mailto:kara.sparks@basf.com).

Sincerely,  
BASF Corporation

Kara Sparks  
Manager, North America  
Product Regulatory Centre of Expertise

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302148

# I U C L I D

## D a t a S e t

**Existing Chemical** ID: 61788-76-9  
**CAS No.** 61788-76-9  
**EINECS Name** Alkanes, chloro  
**EC No.** 263-004-3  
**TSCA Name** Alkanes, chloro

**Producer Related Part**  
**Company:** BASF AG  
**Creation date:** 31-OCT-2006

**Substance Related Part**  
**Company:** BASF AG  
**Creation date:** 31-OCT-2006

**Memo:** master

**Printing date:** 17-JAN-2007  
**Revision date:**  
**Date of last Update:** 17-JAN-2007

**Number of Pages:** 18

**Chapter (profile):** Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10  
**Reliability (profile):** Reliability: without reliability, 1, 2, 3, 4  
**Flags (profile):** Flags: TSCA 8d



**1.0.1 Applicant and Company Information**

**1.0.2 Location of Production Site, Importer or Formulator**

**1.0.3 Identity of Recipients**

**1.0.4 Details on Category/Template**

**1.1.0 Substance Identification**

**1.1.1 General Substance Information**

**1.1.2 Spectra**

**1.2 Synonyms and Tradenames**

**1.3 Impurities**

**1.4 Additives**

**1.5 Total Quantity**

**1.6.1 Labelling**

**1.6.2 Classification**

**1.6.3 Packaging**

**1.7 Use Pattern**

**1.7.1 Detailed Use Pattern**

**1.7.2 Methods of Manufacture**

**1.8 Regulatory Measures**

**1.8.1 Occupational Exposure Limit Values**

**1.8.2 Acceptable Residues Levels**

**1.8.3 Water Pollution**

**1.8.4 Major Accident Hazards**

**1.8.5 Air Pollution**

**1.8.6 Listings e.g. Chemical Inventories**

**1.9.1 Degradation/Transformation Products**

**1.9.2 Components**

1. General Information

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Substance ID: 61788-76-9

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**1.10 Source of Exposure**

-

**1.11 Additional Remarks**

-

**1.12 Last Literature Search**

-

**1.13 Reviews**

-

**2.1 Melting Point**  
-

**2.2 Boiling Point**  
-

**2.3 Density**  
-

**2.3.1 Granulometry**  
-

**2.4 Vapour Pressure**  
-

**2.5 Partition Coefficient**  
-

**2.6.1 Solubility in different media**  
-

**2.6.2 Surface Tension**  
-

**2.7 Flash Point**  
-

**2.8 Auto Flammability**  
-

**2.9 Flammability**  
-

**2.10 Explosive Properties**  
-

**2.11 Oxidizing Properties**  
-

**2.12 Dissociation Constant**

**2.13 Viscosity**

**2.14 Additional Remarks**

**3.1.1 Photodegradation**

**3.1.2 Stability in Water**

**3.1.3 Stability in Soil**

**3.2.1 Monitoring Data (Environment)**

**3.2.2 Field Studies**

**3.3.1 Transport between Environmental Compartments**

**3.3.2 Distribution**

**3.4 Mode of Degradation in Actual Use**

**3.5 Biodegradation**

**3.6 BOD5, COD or BOD5/COD Ratio**

**3.7 Bioaccumulation**

**3.8 Additional Remarks**

**AQUATIC ORGANISMS**

**4.1 Acute/Prolonged Toxicity to Fish**

**4.2 Acute Toxicity to Aquatic Invertebrates**

**4.3 Toxicity to Aquatic Plants e.g. Algae**

**4.4 Toxicity to Microorganisms e.g. Bacteria**

**4.5 Chronic Toxicity to Aquatic Organisms**

**4.5.1 Chronic Toxicity to Fish**

**4.5.2 Chronic Toxicity to Aquatic Invertebrates**

**TERRESTRIAL ORGANISMS**

**4.6.1 Toxicity to Sediment Dwelling Organisms**

**4.6.2 Toxicity to Terrestrial Plants**

**4.6.3 Toxicity to Soil Dwelling Organisms**

**4.6.4 Toxicity to other Non-Mamm. Terrestrial Species**

**4.7 Biological Effects Monitoring**

**4.8 Biotransformation and Kinetics**

4. Ecotoxicity

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**4.9 Additional Remarks**

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**5.0 Toxicokinetics, Metabolism and Distribution**

**5.1 Acute Toxicity**

**5.1.1 Acute Oral Toxicity**

**5.1.2 Acute Inhalation Toxicity**

**5.1.3 Acute Dermal Toxicity**

**5.1.4 Acute Toxicity, other Routes**

**5.2 Corrosiveness and Irritation**

**5.2.1 Skin Irritation**

**5.2.2 Eye Irritation**

**5.3 Sensitization**

**5.4 Repeated Dose Toxicity**

**5.5 Genetic Toxicity 'in Vitro'**

**Type:** Ames test  
**System of testing:** TA 1535, TA 100, TA 1537, TA 98  
**Concentration:** 20, 100, 500, 2500, 5000 µg/plate  
**Cytotoxic Concentration:** not observed  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 471  
**Year:** 1987  
**GLP:** no  
**Test substance:** other TS

**Remark:** key study  
**Result:** An increase in the number of his+ revertants was not observed both in the standard plate test and in the preincubation test either without S-9 mix or after the addition of a metabolizing system. The test substance did not precipitate in the solvent at any concentration used.

**Test condition:** Standard plate test and preincubation test both with and without metabolic activation (S-9 mix) according to OECD test guideline 471. Tester strains: TA 1535, TA 100, TA 1537 and TA 98. The strains indicated were exposed (in triplicate) to 0, 20, 100, 500, 2500 and 5000 µg/plate Lipoderm Öl SK in ethanol (solvent). The substance was regarded as positive in the Ames test if there was a doubling of the spontaneous mutation rate (control), a dose-response relationship and reproducibility of the results.  
Positive controls were included by using 2-aminoanthracene in incubations without S9, and 4-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenyldiamine or 9-aminoacridine chloride monohydrate in incubations with S9.

**Test substance:** Lipoderm Öl SK, purity 100%  
**Conclusion:** According to the results of the present study, the test substance Lipoderm Öl SK is not mutagenic in the Ames test under the experimental conditions chosen.

**Reliability:** (2) valid with restrictions  
Guideline study (with the exception that E. coli WP2 uvrA was not tested), non-GLP

**Flag:** TSCA 8d, key study

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**Type:** Cytogenetic assay  
**System of testing:** Human lymphocytes  
**Concentration:** 5-5000 µg/mL culture medium  
**Cytotoxic Concentration:** not observed  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 473  
**Year:** 1989  
**GLP:** yes  
**Test substance:** other TS

**Remark:** key study  
**Result:** The test substance did not cause any biologically significant increase in the number of aberrant metaphases either without S-9 mix or after addition of a metabolizing system.

**Test condition:** The substance Lipoderm Öl SK was tested for the ability to induce chromosomal aberrations in human lymphocytes following in vitro exposure in the presence and absence of a metabolizing system according to OECD test guideline 473. The concentrations selected were 500 µg/mL, 50 µg/mL and 5 µg/mL culture medium in the experiment without S-9 mix or 5000 µg/mL, 500 µg/mL and 50 µg/mL in the experiment with metabolic activation. This selection was based on the limited solubility of the test substance and not on the mitotic index because substance concentrations causing a reduction of the mitotic index are at dose levels which lead to a strong compound precipitation, thus no longer allowing evaluation. Ethanol was used as an emulsifier for the test substance. Negative controls were untreated and solvent control. Positive controls were mitomycin C in the absence of S-9 mix and cyclophosphamide in the presence of metabolic activation. Cultures were assayed in duplicate. Heparinized human venous blood was added to the culture medium (chromosome medium 1A with PHA). After mitogen stimulation of the lymphocytes using PHA and incubation at 37 °C, the cultures were treated with the test substance in the experiment without S-9 mix for 24 hours; in the experiment with S-9 mix test substance treatment lasted 2 hours followed by a reincubation for 22 hours using fresh culture medium without test substance. About 2-3 hours prior to harvesting the cells, Colcemid was added to arrest cells in a metaphase-like stage of mitosis (C-metaphase). After preparation of the lymphocyte chromosomes and staining with Giemsa, 100 metaphases of each culture in the case of the test substance, untreated control and solvent-control, or 50 cells of each culture in the case of the positive controls, were analyzed for chromosomal aberrations.

**Test substance:** Lipoderm Öl SK, purity 100%  
**Conclusion:** Under the experimental conditions chosen, Lipoderm Öl SK is considered to have no chromosome-damaging (clastogenic) effect in vitro using human lymphocytes.

**Reliability:** (1) valid without restriction  
Guideline study

**Flag:** TSCA 8d, key study

09-NOV-2006

(2)

**5.6 Genetic Toxicity 'in Vivo'**

-

**5.7 Carcinogenicity**

-

**5.8.1 Toxicity to Fertility**

-

**5.8.2 Developmental Toxicity/Teratogenicity**

-

**5.8.3 Toxicity to Reproduction, Other Studies**

-

**5.9 Specific Investigations**

-

**5.10 Exposure Experience**

-

5. Toxicity

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Substance ID: 61788-76-9

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**5.11 Additional Remarks**

-

**6.1 Analytical Methods**

**6.2 Detection and Identification**

**7.1 Function**

-

**7.2 Effects on Organisms to be Controlled**

-

**7.3 Organisms to be Protected**

-

**7.4 User**

-

**7.5 Resistance**

-

**8.1 Methods Handling and Storing**

**8.2 Fire Guidance**

**8.3 Emergency Measures**

**8.4 Possib. of Rendering Subst. Harmless**

**8.5 Waste Management**

**8.6 Side-effects Detection**

**8.7 Substance Registered as Dangerous for Ground Water**

**8.8 Reactivity Towards Container Material**

- (1) BASF AG (1987). Report on the study of Lipoderm Öl SK in the Ames test. Department of toxicology, unpublished data, report No. 40M0393/874102, 14 Oct 1987
- (2) BASF AG (1989). Report on in vitro cytogenetic investigations of Lipoderm Öl SK in human lymphocytes. Department of toxicology, unpublished data, report No. 30M0393/874173, 27 Dec 1989

**10.1 End Point Summary**

**10.2 Hazard Summary**

**10.3 Risk Assessment**



Tox an Ecotox Studie  
 (owned studies are highlighted in grey, "foreign" studies in white)

CAS Number	Product Name	Study Title	Report Date	Laboratory	Comment	Study Report attached?	Report No.
61788-76-9	Alkanes, chloro	Report on the study of Lipoderm OI SK in the ames test	14-Oct-87	BASF	BASF	yes	20.1
61788-76-9	Alkanes, chloro	In vitro cytogenetic investigation of Lipoderm OI SK in human lymphocytes	27-Dec-89	BASF	BASF	yes	20.2

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20.1  
BASF

Abteilung Toxikologie  
Department of Toxicology

6700 Ludwigshafen  
West Germany

en-db/415 OCT 14 1987

**REPORT**

on the Study of

**Lipoderm Öl SK**

(ZNT Test Substance No.: 87/393)

, in the

**AMES TEST**

(Standard Plate Test and Preincubation Test  
with *Salmonella typhimurium*)

Project No.: 40M0393/874102

Testing facility:

BASF Aktiengesellschaft  
Department of Toxicology, Z 470  
6700 Ludwigshafen/Rhein, FRG

This report consists of 14 pages and 8 tables.

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Annex: Tables 1 to 8	

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1. **SUMMARY**

The substance Lipoderm Öl SK was tested for mutagenicity in the Ames test.

Strains: TA 1535, TA 100, TA 1537, TA 98

Dose range: 20 µg - 5000 µg/plate

Test conditions: Standard plate test and preincubation test both with and without metabolic activation (S-9 mix).

Solubility: Complete solubility of the test substance in ethanol.

Toxicity: No bacteriotoxicity (reduced his<sup>-</sup> background growth) was observed.

**Mutagenicity:**

An increase in the number of his<sup>+</sup> revertants was not observed both in the standard plate test or in the preincubation test either without S-9 mix or after the addition of a metabolizing system.

**Assessment:**

According to the results of the present study, the test substance Lipoderm Öl SK is not mutagenic in the Ames test under the experimental conditions chosen here.

*Gelbke, Sept. 29, '87*  
Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke  
(Head of the Department of Toxicology)

*Engelhardt, Sept. 28, 1987*  
Dr.rer.nat. G. Engelhardt  
(Study director)

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## 2. INTRODUCTION

The Ames test is a short-term test in bacteria (1, 2) and is used as a screening method for detecting a point mutagenic effect of chemical substances.

Since most of the substances are not mutagenic or carcinogenic themselves, but only after metabolic transformation, and since the main part of all metabolic processes is catalyzed by the enzyme systems of the liver, the Ames test is carried out not only directly, but also in the presence of a metabolizing system obtained from rat livers. For this purpose, rats are pretreated with Aroclor 1254 for an activation of the enzymes which metabolize foreign substances.

The test is carried out in accordance with the OECD guideline for testing of chemicals "Genetic Toxicology: Salmonella typhimurium, Reverse Mutation Assay", No. 471'.

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Department of ToxicologyProject No.: 40M0393/874102

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**3. MATERIAL AND METHOD****3.1. Test substance:**

Name of test substance: Lipoderm Öl SK

Test substance No.: 87/393

Degree of purity: 100 %

Storage: +4°C

More detailed information about the test substance can be found in the raw data and may be requested from the sponsor (EPM/N; BASF Aktiengesellschaft).

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3.2. Tissue preparation

3.2.1. S-9 fraction

The S-9 fraction is prepared according to Ames et al. (2).

5 male Sprague-Dawley rats (200 - 300 g) receive a single intraperitoneal injection of 500 mg Aroclor 1254 (as a 20% solution in peanut oil - w/v) per kg body weight 5 days before sacrifice.

During this time the animals are housed in Makrolon cages in air-conditioned rooms. The day/night rhythm is 12 hours (light period from 6.00 - 18.00 hours and dark period from 18.00 - 6.00 hours).

Standardized pelleted feed and tap water from bottles are available ad libitum.

5 days after administration the rats are sacrificed, and the livers are prepared (all preparation steps for obtaining the liver microsome enzymes are carried out using sterile solvents and glassware at a temperature of +4°C). The livers are weighed and washed in an equivalent volume of a 150 mM KCl solution (1 ml  $\hat{=}$  1 g wet liver), then cut into small pieces and homogenized in three volumes of KCl solution. After centrifugation of the homogenate at 9000 x g for 10 minutes at +4°C, 5 ml portions of the supernatant (so-called S-9 fraction) are quickly deep-frozen in dry ice and stored at -70°C to -80°C for 2 months at the most.

Preparation of S-9 fraction: August 31, 1987 (1st experiment) and September 22, 1987 (2nd experiment).

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**3.2.2. S-9 mix**

The S-9 mix is prepared freshly prior to each experiment (1, 2). For this purpose, a sufficient amount of S-9 fraction is thawed at room temperature and 3 volumes of S-9 fraction are mixed with 7 volumes of S-9 supplement (cofactors). This preparation, the so-called S-9 mix, is kept on ice until used. The concentrations of the cofactors in the S-9 mix are:

MgCl <sub>2</sub>	8 mM
KCl	33 mM
glucose-6-phosphate	5 mM
NADP	4 mM
phosphate buffer (pH 7.4)	100 mM.

The phosphate buffer is prepared by mixing an Na<sub>2</sub>HPO<sub>4</sub> solution (25.42 g/l) with an NaH<sub>2</sub>PO<sub>4</sub> solution (22.28 g/l) in a ratio of about 4 : 1.

**3.3. Bacteria**

The rate of induced back mutations of several bacteria mutants from histidine auxotrophy to histidine prototrophy is determined (2, 3, 4). The indicator organisms TA 1535, TA 1537, TA 98 and TA 100 selected by Ames especially for this purpose are derivatives of *Salmonella typhimurium*. All strains have a defective excision repair system (uvrB), which prevents the repair of lesions which are induced in the DNA, and this deficiency results in greatly enhanced sensitivity of some mutagens. Furthermore, all strains show a considerably reduced hydrophilic polysaccharide layer (rfa), which leads to an increase in permeability to lipophilic substances.

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The strains TA 1535 and TA 100 are derived from histidine-prototrophic Salmonella strains by the substitution mutation his G 46 and are used to detect base pair substitutions. TA 1537 and TA 98 are strains for the detection of frameshift mutagens. These strains carry different frameshift markers, i.e. the +1 mutant his C 3076 in the case of TA 1537 and the +2 type his D 3052 in the case of TA 98.

The strains TA 98 and TA 100 carry an R factor plasmid pKM 101 (4) and, in addition to having genes resistant to antibiotics, they have a modified postreplication DNA repair system, which increases the mutation rate by inducing a defective repair in the DNA; this again leads to a considerable increase in sensitivity.

For testing, deep-frozen (-70°C to -80°C) bacterial cultures (1 ml in 15 ml glass tubes) are thawed at room temperature. 0.1 ml of this bacterial suspension is inoculated in nutrient broth solution (8 g Difco nutrient broth + 5 g NaCl/liter) and incubated in the shaking water bath at 37°C for 16 hours. As a rule, a germ density of  $\geq 10^8$  bacteria/ml is reached. These cultures grown overnight are kept in iced water from the beginning of the experiment until the end in order to prevent further growth.

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3.4. Mutagenicity tests

3.4.1. Standard plate test

The experimental procedure is based on the method of Ames et al. (1, 2).

Test tubes containing 2 ml portions of soft agar which consists of 100 ml agar (0.6% agar + 0.6% NaCl) and 10 ml amino acid solution (minimal amino acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin) are kept in a water bath at 45°C, and the remaining components are added in the following order:

0.1 ml test solution

0.1 ml bacterial suspension

0.5 ml S-9 mix (in tests with metabolic activation)

or

0.5 ml phosphate buffer (in tests without metabolic activation)

After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates) within approx. 30 seconds.

3.4.2. Preincubation test

The experimental procedure is based on the method described by Yahagi et al. (5) and Matsushima et al. (6).

0.1 ml test solution, 0.1 ml bacterial suspension and 0.5 ml S-9 mix are incubated at 37°C for the duration of 20 minutes. Subsequently, 2 ml of soft agar is added and, after mixing, the samples are poured onto the Vogel-Bonner agar plates within approx. 30 seconds.

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## Composition of the minimal glucose agar:

980 ml aqua dest.  
20 ml Vogel-Bonner E medium  
15 g Difco bacto agar  
20 g D-glucose, monohydrate.

After incubation at 37°C for 48 hours in the dark, the bacterial colonies (his<sup>+</sup> revertants) are counted.

## 3.5. Titer determination

In general, the titer is determined only in the experiments with S-9 mix both without test substance (solvent only) and after adding the two highest amounts of substance. For this purpose, 0.1 ml of the overnight cultures (see 3.3.) is diluted to 10<sup>-6</sup> in each case. Test tubes containing 2 ml portions of soft agar containing maximal amino acid solution (5 mM histidine + 0.5 mM biotin) are kept in a water bath at 45°C, and the remaining components are added in the following order:

0.1 ml solvent (without and with test substance)  
0.1 ml bacterial suspension (dilution: 10<sup>-6</sup>)  
0.5 ml S-9 mix

After mixing, the samples are poured onto the Vogel-Bonner agar plates within approx. 30 seconds. After incubation at 37° for 48 hours in the dark, the bacterial colonies are counted.

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3.6. Checking out the tester strains

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity ( $\Delta$  uvrB); ampicillin resistance (R factor plasmid).

Histidine auxotrophy is automatically checked in each experiment via the spontaneous rate.

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**3.7. Controls****3.7.1. Negative control**

Parallel with each experiment with and without S-9 mix, a negative control (solvent control, sterility control) is carried out for each tester strain in order to determine the spontaneous mutation rate.

**3.7.2. Positive controls**

The following positive control substances are used to check the mutability of the bacteria and the activity of the S-9 mix:

with S-9 mix                    10 µg 2-aminoanthracene (dissolved in DMSO) for the strains TA 100, TA 98, TA 1537 and TA 1535

without S-9 mix                5 µg N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) (dissolved in DMSO) for the strains TA 100 and TA 1535

10 µg 4-nitro-o-phenylendiamine (dissolved in DMSO) for the strain TA 98

100 µg 9-aminoacridine chloride monohydrate (dissolved in DMSO) for the strain TA 1537.

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3.8. Evaluation criteria

In general, a substance to be characterized as positive in the Ames test has to fulfill the following requirements:

- doubling of the spontaneous mutation rate (control)
- dose-response relationship
- reproducibility of the results.

3.9. Tester strains, doses, number of plates

1st Experiment:

Strains: TA 1535, TA 100, TA 1537, TA 98  
Doses: 0, 20, 100, 500, 2500 and 5000 µg/plate  
Solvent: ethanol  
Type of test, test condition: standard plate test with and without S-9 mix  
Number of plates: 3 test plates per dose or per control

2nd Experiment:

Strains: TA 1535, TA 100, TA 1537, TA 98  
Doses: 0, 20, 100, 500, 2500 and 5000 µg/plate  
Solvent: ethanol  
Type of test, test condition: preincubation test with and without S-9 mix  
Number of plates: 3 test plates per dose or per control

3.10. Retention of records

The raw data, protocol and the original of this report will be stored at BASF Aktiengesellschaft.

**BASF**Abteilung Toxikologie  
Department of ToxicologyProject No.: 40M0393/874102

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**4. RESULTS (Tables 1 - 8)**

The substance Lipoderm Öl SK was tested for mutagenicity in the Ames test (standard plate test and preincubation test) both in the presence and in the absence of a metabolizing system obtained from rat liver (S-9 mix) using the strains TA 1535, TA 100, TA 1537 and TA 98.

**4.1. Mutagenicity tests****4.1.1. Standard plate test  
(Tables 1 - 4)****4.1.1.1. Tests without S-9 mix**

TA 1535:

TA 100:

TA 1537:

TA 98:

No increase in the number of  
his<sup>+</sup> revertants**4.1.1.2. Tests with S-9 mix**

TA 1535:

TA 100:

TA 1537:

TA 98:

No increase in the number of  
his<sup>+</sup> revertants

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4.1.2. Preincubation test  
(Tables 5 - 8)

4.1.2.1. Tests without S-9 mix

TA 1535:  
TA 100:                   No increase in the number  
TA 1537:                  of his<sup>+</sup> revertants  
TA 98:

4.1.2.2. Tests with S-9 mix

TA 1535:  
TA 100:                   No increase in the number  
TA 1537:                  of his<sup>+</sup> revertants  
TA 98:

4.2. Toxicity

No bacteriotoxic effect (reduced his<sup>-</sup> background growth)  
was observed.

4.3. Solubility

Complete solubility of the test substance in ethanol.

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**5. LITERATURE**

1. Ames, B.N.; Durston, W.W.; Yamasaki, E.; Lee, F.D.:  
Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection.  
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B. A. S. F. A. G.  
DEPARTMENT OF TOXICOLOGY

TABLE : 1

STUDY NUMBER: 874102  
STUDY DIREC. : ENG  
OPERATOR : SCHW  
DATE : 18. 09. 87

AMES TEST WITH : 87/393  
METHOD : STANDARD PLATE TEST

STRAIN: TA 1535

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	14 20 22	19	4	16 21 19	19	3	38 40 32	1.0	1.0
20	17 13 15	15	2	14 17 17	16	2		0.8	0.9
100	18 18 19	18	1	12 20 14	15	4		1.0	0.8
500	18 12 19	16	4	22 16 21	20	3		0.9	1.1
2500	16 19 19	18	2	18 17 17	17	1	32 34 30	1.0	0.9
5000	16 11 17	15	3	20 22 16	19	3	54 37 58	0.8	1.0
POSITIVE CONTROL Z-AA 10				406 404 385	398	12			21.3
POSITIVE CONTROL MNNG 5	2100 2100 2150	2117	29					113.4	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10

B. A. S. F. A. G.  
DEPARTMENT OF TOXICOLOGY

TABLE : 2

STUDY NUMBER: 874102  
STUDY DIREC.: ENG  
OPERATOR : SCHW  
DATE : 18. 09. 87

AMES TEST WITH : 87/393  
METHOD : STANDARD PLATE TEST

STRAIN: TA 100

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	123 124 106	118	10	109 113 118	113	5	43 32 34	1.0	1.0
20	108 112 113	111	3	104 126 117	116	11		0.9	1.0
100	115 116 103	111	7	120 130 143	131	12		0.9	1.2
500	99 104 85	96	10	105 107 132	115	15		0.8	1.0
2500	90 89 104	94	8	131 120 111	121	10	37 34 33	0.8	1.1
5000	110 92 87	96	12	119 118 122	120	2	46 39 45	0.8	1.1
POSITIVE CONTROL 2-AA 10				1940 1890 1720	1850	115			16.3
POSITIVE CONTROL MNG 5	2050 2200 2150	2133	76					18.1	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10

B. A. S. F. A. G.  
DEPARTMENT OF TOXICOLOGY

TABLE : 3

STUDY NUMBER: 874102  
STUDY DIREC. : ENG  
OPERATOR : SCHW  
DATE : 18. 09. 87

AMES TEST WITH : 87/393  
METHOD : STANDARD PLATE TEST

STRAIN: TA 1537

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	10 9 8	9	1	12 8 10	10	2	50 30 48	1.0	1.0
20	7 9 10	9	2	12 7 8	9	3		1.0	0.9
100	13 8 11	11	3	9 7 9	8	1		1.2	0.8
500	7 8 6	7	1	13 7 8	9	3		0.8	0.9
2500	6 8 9	8	2	6 - 12	9	4	26 33 40	0.9	0.9
5000	5 6 5	5	1	7 6 10	8	2	35 37 31	0.6	0.8
POSITIVE CONTROL 2-AA 10				137 132 181	150	27			15.0
POSITIVE CONTROL AAC 100	1090 1300 940	1110	181					123.3	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10  
-- CONTAMINATION

B. A. S. F. A. G.  
DEPARTMENT OF TOXICOLOGY

TABLE : 4

STUDY NUMBER: 874102  
STUDY DIREC.: ENG  
OPERATOR: SCHW  
DATE: 18. 09. '87

AMES TEST WITH : 87/393  
METHOD : STANDARD PLATE TEST

STRAIN: TA 98

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	20 23 24	22	2	31 32 32	32	1	37 36 42	1.0	1.0
20	23 22 21	22	1	35 34 37	35	2		1.0	1.1
100	22 21 24	22	2	39 39 33	37	3		1.0	1.2
500	21 21 12	18	5	31 35 27	31	4		0.8	1.0
2500	23 27 18	23	5	38 34 31	34	4	38 37 38	1.0	1.1
5000	16 13 11	13	3	34 42 29	35	7	31 37 49	0.6	1.1
POSITIVE CONTROL 2-AA 10				1270 1320 1200	1263	60			39.9
POSITIVE CONTROL NPD 10	672 734 757	721	44					32.3	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10

AMES TEST WITH 87/393  
METHOD PREINCUBATION TEST

STRAIN: TA 1535

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	17 12 13	14	3	17 20 19	19	2	37 37 24	1.0	1.0
20	19 13 13	15	3	18 18 15	17	2		1.1	0.9
100	15 14 13	14	1	16 16 19	17	2		1.0	0.9
500	11 15 14	13	2	16 17 17	17	1		1.0	0.9
2500	13 16 18	16	3	17 18 19	18	1	28 26 36	1.1	1.0
5000	13 22 16	17	5	15 19 13	16	3	29 23 35	1.2	0.8
POSITIVE CONTROL 2-AA 10				271 275 230	259	25			13.9
POSITIVE CONTROL MNNB 5	406 434 427	422	15					30.2	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP TO 10

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TABLE : 6

STUDY NUMBER: 87410  
STUDY DIREC. : ENI  
OPERATOR : SCHI  
DATE : 25. 09. 87

AMES TEST WITH : 87/393  
METHOD : PREINCUBATION TEST

STRAIN: TA 100

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL	108 114	110	3	106 109	107	2	26 25	1.0	1.0
ETHANOL	109			107			15		
20	104 122 119	115	10	113 111 112	112	1		1.0	1.0
100	106 101 99	102	4	118 121 113	117	4		0.9	1.1
500	103 110 106	106	4	116 115 112	114	2		1.0	1.1
2500	104 105 112	107	4	105 116 124	115	10	24 6 7	1.0	1.1
5000	122 123 114	120	5	117 138 138	131	12	6 6 10	1.1	1.2
POSITIVE CONTROL 2-AA 10				2450 2350 2500	2433	76			22.7
POSITIVE CONTROL MNNG 5	1460 1440 1480	1460	20					13.2	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10

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DEPARTMENT OF TOXICOLOGY

TABLE : 7

STUDY NUMBER: 874102  
STUDY DIREC. : ENG  
OPERATOR : SCHW  
DATE : 25. 09. 87

AMES TEST WITH : 87/393  
METHOD : PREINCUBATION TEST

STRAIN: TA 1537

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	13 10 9	11	2	9 7 10	9	2	71 67 71	1.0	1.0
20	8 8 9	8	1	8 10 12	10	2		0.8	1.2
100	8 7 8	8	1	8 10 9	9	1		0.7	1.0
500	8 8 6	7	1	10 11 8	10	2		0.7	1.1
2500	5 10 8	8	3	8 11 7	9	2	20 26 45	0.7	1.0
5000	11 7 9	9	2	9 6 7	7	2	5 16 18	0.8	0.8
POSITIVE CONTROL 2-AA 10				117 182 188	162	39			18.7
POSITIVE CONTROL AAC 100	1100 1250 1200	1183	76					110.9	

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10

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TABLE : 8

STUDY NUMBER: 874102  
STUDY DIREC. : ENG  
OPERATOR : SCHW  
DATE : 25.09.87

AMES TEST WITH : 87/393  
METHOD : PREINCUBATION TEST

STRAIN: TA 98

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL ETHANOL	22	25	3	36	38	2	25	1.0	1.0
	26			39			21		
	28			38			21		
20	23	25	3	42	39	5		1.0	1.0
	24			33					
	28			41					
) 100	28	24	3	45	45	1		1.0	1.2
	22			44					
	23			46					
500	23	23	5	31	33	2		0.9	0.9
	28			35					
	19			33					
2500	19	20	2	33	31	3	9	0.8	0.8
	18			27			9		
	22			33			10		
5000	19	23	3	31	32	2	14	0.9	0.8
	24			34			14		
	25			31			7		
) POSITIVE CONTROL 2-AA 10				1520	1483	100			39.4
				1370					
				1560					
POSITIVE CONTROL NPD 10	673	700	55					27.6	
	663								
	763								

\* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10

Confidential

20.2  
BASF  
Abteilung Toxikologie  
Department of Toxicology

DEC 27 1989  
6700 Ludwigshafen  
West Germany

en-db/1092

REPORT

In Vitro Cytogenetic Investigations of

**LIPODERM ÖL SK**

in Human Lymphocytes

Project No.: 30M0393/874173

Testing facility:

BASF Aktiengesellschaft  
Department of Toxicology, Z 470  
D-6700 Ludwigshafen/Rh., FRG

Head of Department of  
Toxicology:

Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

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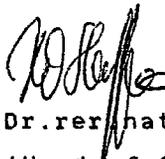
Project No.: 30M0393/874173

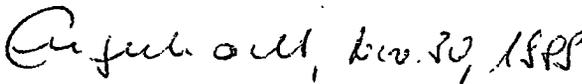
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**GLP STATEMENT**

**Title:** In Vitro Cytogenetic Investigations of  
Lipoderm Öl SK in Human Lymphocytes

This study was conducted in accordance with "OECD Principles of  
Good Laboratory Practice" (Paris, 1981).

 Dec. 1, 1984  
Dr.rer.nat. H.D. Hoffmann  
(Head of Section)

 Dec. 30, 1985  
Dr.rer.nat. G. Engelhardt  
(Study Director)

Report; Project No.: 30M0393/874173

**STATEMENT  
OF THE QUALITY ASSURANCE UNIT**

Number of test substance: 87/393

Name of test substance: Lipoderm Öl SK

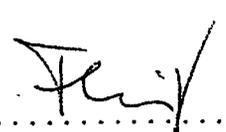
Title: In Vitro Cytogenetic Investigations of  
Lipoderm Öl SK in Human Lymphocytes

The Quality Assurance Unit inspected the study, audited the final report, and reported findings to the Study Director and to Management.

Phase of study/ inspection	Date of inspection	Report to Study Director and to Management
Protocol:	June 16, 1988	June 20, 1988
Conduct of study:	June 29, 1988	June 29, 1988
Audit of the report:	Nov. 21, 1989	Dec. 1, 1989

Remarks: The conduct of analytics was inspected independently by the Quality Assurance Unit of the analytical laboratory.

Ludwigshafen, Dec. 18, 1989

  
.....  
Dr.rer.nat. H. Fleig  
(Head of Quality Assurance Unit)

Project No.: 30M0393/874173

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1. **SUMMARY**

The substance Lipoderm Öl SK was tested for the ability to induce chromosomal aberrations in human lymphocytes following in vitro exposure in the presence and absence of a metabolizing system.

According to a pretest for the determination of the highest experimental dose and in consideration of the compound precipitation actually found in the present investigations, 500 µg/ml, 50 µg/ml and 5 µg/ml culture medium in the experiment without S-9 mix, or 5000 µg/ml, 500 µg/ml and 50 µg/ml culture medium in the experiment with metabolic activation, were selected. This selection was based on the limited solubility of the test substance and not on the mitotic index because the test substance concentrations causing a reduction of the mitotic index are at dose levels which lead to a strong compound precipitation, thus no longer allowing evaluation. The various doses were emulsified in ethanol.

For control purposes, negative controls (untreated and solvent) and positive controls both without S-9 mix (0.1 µg mitomycin C/ml culture medium) and with metabolic activation (6 µg cyclophosphamide/ml culture medium) were also tested.

Duplicate cultures were used for all experimental groups.

Heparinized human venous blood was added to the culture medium (chromosome medium 1A with PHA). After mitogen stimulation of the lymphocytes using PHA and incubation at 37°C for the cultures were treated with the test

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substance in the experiment without S-9 mix for 24 hours; in the experiment with S-9 mix test substance treatment lasted 2 hours followed by a reincubation for 22 hours using fresh culture medium without test substance. About 2 -3 hours prior to harvesting the cells, Colcemid was added to arrest cells in a metaphase-like stage of mitosis (C-metaphase). After preparation of the lymphocyte chromosomes and staining with Giemsa, 100 metaphases of each culture in the case of the test substance, untreated control and solvent control, or 50 cells of each culture in the case of the positive controls, were analyzed for chromosomal aberrations.

According to the results of the present study, the test substance did not cause any biological significant increase in the number of aberrant metaphases either without S-9 mix or after adding a metabolizing system.

Thus, under the experimental conditions chosen here Lipoderm Öl SK is considered to have no chromosome - damaging (clastogenic) effect in vitro using human lymphocytes.

*H.D. Hoffmann* Dec, 1, 1989  
Dr.rer.nat. H.D. Hoffmann  
(Head of Section)

*G. Engelhardt*, Nov 30, 1989  
Dr.rer.nat. G. Engelhardt  
(Study director)

Project No.: 30M0393/874173

---

2. **INTRODUCTION**

The aim of the present study was to establish whether the test substance Lipoderm Öl SK or its metabolite could interact with cells to induce chromosomal aberrations. For this purpose, an in vitro cytogenetic assay was carried out for measuring chromosome aberration frequencies in human lymphocytes. The study was carried out in accordance with the OECD guideline for testing of chemicals - "Genetic Toxicology: In vitro Mammalian Cytogenetic Test", No. 473. The test procedure was based on the method described by De JONG, B. and G.J.P.A. ANDERS (1).

The study was carried out in June/July 1988.

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**3. MATERIAL AND METHODS**

**3.1. Test substance**

Name of test substance: Lipoderm Öl SK  
Batch No.: Tank 100, abgefüllt am 29.06.87  
Test substance No.: 87/393  
Appearance, consistency: Yellowish liquid  
Degree of purity: 100%  
Storage: At room temperature in the  
darkness  
Solvent: Ethanol

The stability of the test substance throughout the study period will be verified analytically by reanalysis at a later date. The results of this analysis may be requested from the sponsor.

The stability of the test substance in the carrier ethanol and in aqua dest. was determined analytically.

The homogeneity of the test substance in the carrier was guaranteed by constant stirring during the removal and administration of the test substance formulation.

More detailed information about the test substance can be found in the raw data and may be requested from the sponsor (EPM; BASF Aktiengesellschaft).

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3.2: Culture method

"Chromosome medium 1A with PHA" (Gibco) was used as the culture medium; the total culture volume was 6.0 ml.

Human venous blood was drawn from volunteers aseptically into sterile syringes that contained heparin to prevent clotting. About 0.5 ml of heparinized blood was added to 6.0 ml of culture medium in each centrifuge tube, and the cultures were incubated with closed caps at 37°C.

3.3. Tissue preparation

3.3.1. S-9 fraction

The S-9 fraction was prepared according to Ames et al. (3).

5 male Sprague-Dawley rats (200 - 300 g) received a single intraperitoneal injection of 500 mg Aroclor 1254 (as a 20% solution in peanut oil - w/v) per kg body weight and were kept for 5 days.

During this time the animals were housed in Makrolon cages and were accommodated in fully air-conditioned rooms in which central air-conditioning guaranteed a range of temperature of 20 - 24°C and a relative humidity of 30 - 70%. The day/night rhythm was 12 hours (12 hours light from 6.00 - 18.00 hours and 12 hours darkness from 18.00 - 6.00 hours).

Standardized pelleted feed and tap water from bottles were available ad libitum.

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5 days after administration the rats were sacrificed and the livers were prepared (all preparation steps for obtaining the liver microsome enzymes were carried out using sterile solvents and glassware at a temperature of +4°C). The livers were weighed and washed in a weight equivalent volume of a 150 mM KCl solution (1 ml  $\hat{=}$  1 g wet liver), then cut into small pieces and homogenized in three volumes of KCl solution. After centrifugation of the homogenate at 9000  $\times$  g for 10 minutes at +4°C, 5-ml portions of the supernatant (so-called S-9 fraction) were quickly deep-frozen in dry ice and stored at -70°C to -80°C.

3.3.2. S-9 mix

The S-9 mix was prepared freshly prior to each experiment (2, 3). For this purpose, a sufficient amount of S-9 fraction was thawed at room temperature, and 1 volume of S-9 fraction was mixed with 1 volume of S-9 supplement (cofactors). This preparation, the so-called S-9 mix, was kept on ice until used. The concentrations of the cofactors in the S-9 mix were:

MgCl <sub>2</sub>	8 mM
KCl	33 mM
glucose-6-phosphate	5 mM
NADP	4 mM
phosphate buffer (pH 7.4)	100 mM.

The phosphate buffer was prepared by mixing an Na<sub>2</sub>HPO<sub>4</sub> solution with an NaH<sub>2</sub>PO<sub>4</sub> solution in a ratio of about 4 : 1.

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3.4. Conduct of the study

3.4.1. Selection of the doses

The doses were determined from an appropriate pretest with cultures exposed to a wide dose range of the test article, i.e. 0.05 µg/ml - 7500 µg/ml culture medium without S-9 mix and 0.1 µg/ml - 7500 µg/ml culture medium with S-9 mix. The highest doses (5000 µg/ml without S-9 mix and with S-9 mix) have to be selected with regard to the solubility of the test substance and not to the mitotic index as required in the OECD guideline method 473. The reason is that the required reduction of the mitotic index (about 50%) are at dose levels (> 500 µg - 5000 µg/ml) which lead to a strong compound precipitation, thus an evaluation of a sufficient number of metaphases was not possible.

500 µg/ml and 50 µg/ml with and without S-9 mix were chosen as further doses.

In addition, one higher dose and one lower dose were selected in the experiments both with and without S-9 mix i.e. 7500 µg/ml and 5 µg/ml. However, these additional dose groups were planned to be evaluated only if the results obtained regarding test substance precipitation deviated from those obtained in the pretest.

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## 3.4.2. Test groups and doses

The number of test groups evaluated can be seen from the following table. Duplicate cultures were used for all experimental groups.

Test group No.	S-9 mix	Dose ml and/or µg per ml culture medium	Metaphases
1	-	untreated control	200
2	-	solvent control 0.1 ml ethanol	200
3	-	500 µg	200
4	-	50 µg	200
5	-	5 µg*	200
6	-	0.1 µg mitomycin C	100
7	+	untreated control	200
8	+	solvent control 0.1 ml ethanol	200
9	+	5000 µg	200
10	+	500 µg	200
11	+	50 µg	200
12	+	6 µg cyclophosphamide	100

\* The evaluation of the microscopic slides indicated that a metaphase analysis of the actually selected highest dose of 5000 µg/ml was not possible due to a strong test substance precipitation. Therefore, the additionally selected lower dose, i.e. 5 µg/ml was evaluated (see item 3.4.1.)

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3.4.3. Control articles

Untreated controls:

Nothing was added to the negative controls with and without S-9 mix which contained cells and culture medium only.

Solvent controls:

The solvent controls with and without S-9 mix only contained the solvent for the test substance at the same concentration and volume used in the test culture.

Positive controls:

a) Without metabolic activation

0.1 µg mitomycin C/ml culture medium was added in a volume of 0.1 ml (dissolved in aqua dest.).

b) With metabolic activation (S-9 mix)

6 µg cyclophosphamide/ml culture medium was added in a volume of 0.1 ml (dissolved in aqua dest.).

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3.5. Experimental procedure

The test substance was emulsified in ethanol. By adding 0.1 ml of this formulation to the culture medium containing heparinized blood (see item 3.2.) the desired final concentrations were reached (see item 3.4.2.; table).

The formulations of the test substance and the solutions of the positive control substances were prepared immediately before use.

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3.6. Treatment of cultures

3.6.1. Assay without metabolic activation

Whole blood was added to the culture medium containing PHA as described before (see item 3.2.) and incubated at 37°C for about 48 hours. Solutions of the test substance, mitomycin C or the solvent and 0.2 ml phosphate buffer were then added to the cultures. After about 2 hours of incubation at 37°C, cells were washed twice with unsupplemented culture medium and then re-incubated in complete culture medium for further 22 hours (the whole duration of incubation was 72 hours). Colcemid was added 2 - 3 hours before harvesting the dividing lymphocytes.

3.6.2. Assay with metabolic activation

Whole blood was added to the culture medium containing PHA as described before (see item 3.2.) and incubated at 37°C for about 48 hours. Solutions of the test substance, cyclophosphamide or the solvent and the S-9 mix were then added to the cultures. After about 2 hours of incubation at 37°C, cells were washed twice with unsupplemented culture medium and then re-incubated in complete culture medium for further 22 hours. Colcemid was added 2 - 3 hours before fixation (the whole duration of incubation was 72 hours).

The volume of the blood culture was 6.8 ml consisting of the following components:

6.0 ml culture medium  
0.5 ml heparinized blood  
0.1 ml test substance solution or control substance  
0.2 ml S-9 mix or phosphate buffer

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3.7. Lymphocyte fixation and cell staining

The lymphocytes were prepared based on to the method described by De JONG, B. and G.J.A.P. ANDERS (1).

- 2 - 3 hours prior to harvesting the cells, 0.2 µg Colcemid/ml culture medium was added in order to arrest mitosis in the metaphase.
- After incubation at 37°C, the blood cultures were centrifuged at 1000 rpm for 10 minutes, the supernatant was removed except for a few drops, and the precipitate was resuspended.
- After hypotonic treatment with about 9 - 10 ml of a hypotonic solution (Hanks/aqua dest. 1 : 4) which was at 37°C, the suspension was kept in a water bath at 37°C for 20 minutes.
- After recentrifugation at 1000 rpm for 10 minutes, the supernatant was removed, and about 9 - 10 ml of fixative (methanol : glacial acetic acid/4 : 1) was slowly added to the sediment. After recentrifugation at 1000 rpm the fixative was replaced, the centrifuge tube was closed with Parafilm, and this suspension was kept at 4°C for 20 minutes.
- After recentrifugation at 1000 rpm, the supernatant was removed, and a suspension was prepared with a few drops of fresh fixative. 3 - 5 drops of this suspension were dropped onto clean, shortly iced microscopic slides using a Pasteur pipette.

The preparations were dried in the air and subsequently stained in a solution of Giemsa and Titrisol (10 ml Giemsa, 190 ml Titrisol pH 7.2) for 10 minutes.

- After being rinsed twice in aqua dest. and clarified in xylene, the preparations were embedded in Entellan.

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3.8. Cell scoring

3.8.1. Chromosome analysis

As a rule, 100 metaphases of each culture for the test substance, negative and solvent controls or 50 cells of each culture for the positive controls were analyzed for chromosome aberrations according to the following definitions (4, 5, 6):

a) Structural chromosome aberrations

- G' and G"            chromatid gap and isochromatid gap respectively  
  
                         unstained regions (so-called achromatic lesions) without dislocation of the segment which appears to be separated.
  
- B' and B"            chromatid break and chromosome break respectively  
  
                         visible discontinuity in chromatid or chromosome structure with lateral or longitudinal dislocation of the fragment.
  
- F' and F"            chromatid fragment and chromosome fragment respectively  
  
                         acentric chromosome segments which occur singly or in pairs.
  
- D' and D"            chromatid deletion and chromosome deletion respectively  
  
                         loss of a segment on the level of chromatids or chromosomes.
  
- m. A.                multiple aberrations  
  
                         metaphases with 5 or more aberrations excl. gaps.
  
- disintegration of chromosomal structure (pulverization)    The chromosomes are present as irregular particles, a chromosomal structure cannot be detected any longer.

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- Exchanges (translocations)

These exchange aberrations are divided into intrachanges and interchanges:

- Int' and Int"      intrachanges on the level of chromatids and chromosomes respectively
- the joining of broken ends capable of reuniting two or several chromatid regions within a chromosome (e.g., centric ring chromosomes, pericentric inversions).
- I' and I"      interchanges on the level of chromatids and chromosomes respectively
- the joining of broken ends capable of reuniting two or several chromosomes. They are classified according to:
- symmetric interchanges (e.g., reciprocal translocations between nonhomologous chromosomes, centric fusions, quadriradial structures)
  - asymmetric interchanges (e.g., dicentric and polycentric chromosomes, triradial and quadriradial structures).

b) Numerical chromosome aberrations (so-called heteroploidies)

- Aneuploidy      metaphases with absent-(hypoploid) or additional (hyperploid) chromosomes
- only hyperploid metaphases are registered.
- Euploidy      changes in the number of chromosomes by whole chromosome sets.  
(= polyploidy)

Preparations were analysed blinded. If only a few metaphases were found, a chromosome analysis was not carried out.

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3.8.2. Mitotic index

A mitotic index based on 1500 cells/culture was determined for at least the top two doses that yield metaphase cells, for the solvent and negative controls and for the positive controls.

3.9. Statistical evaluation

The exact FISHER test, which is applied to check for significant differences between control groups (untreated controls and solvent controls) and dose groups with regard to the rate of structural aberrant metaphases was used (7). A significant difference in the number of aberrant cells between treated samples and untreated controls or solvent controls at the 95% level is marked + and x respectively; a difference at the 99% level is marked ++ and xx respectively.

An appropriate computer program is used for all calculations.

3.10. Retention of records

The raw data, protocol, reserve sample and microscopic preparations as well as the original of this report are stored at BASF Aktiengesellschaft at least for the period of time specified in the GLP regulations. The microscopic preparations will be retained only as long as the quality of the material allows evaluation.

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#### 4. **RESULTS**

##### 4.1. Chromosome analysis (Tables 1 to 14)

Table 1: Summary table: results of all groups without S-9 mix

Table 2: Summary table: results of all groups with S-9 mix

Tables 3 - 8: Results of the individual cultures of each test group without S-9 mix

Tables 9 - 14: Results of the individual cultures of each test group with S-9 mix

##### 4.1.1. Assay without metabolic activation (Tables 1, 3 - 8)

###### **Untreated control:**

6 (3%) aberrant cells incl. gaps and 2 (1%) aberrant cells excl. gaps (1 x B'; 1 x F') were found.

###### **Solvent control:**

7 (3.5%) aberrant metaphases incl. gaps and 1 (0.5%) aberrant metaphases excl. gaps (1 x B'') were observed.

###### **500 µg/ml:**

22 (11%) chromosomally damaged cells incl. gaps and 3 (1.5%) aberrant cells excl. gaps (1 x B'; 1 x B''; 1 x F'') were detected.

###### **50 µg/ml:**

20 (10%) aberrant metaphases incl. gaps and 4 (2%) chromosomally damaged cells excl. gaps (3 x B'; 1 x F') were observed.

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**5 µg/ml:**

14 (7%) aberrant cells incl. gaps and 2 (1%) aberrant metaphases excl. gaps (1 x B"; 1 x 3 F") were analyzed.

**0.1 µg mitomycin C/ml:**

With 29 (29%) aberrant cells incl. gaps and 23 (23%) aberrant mitosis excl. gaps, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

No differences regarding aneuploidies (hyperploid metaphases) and polyploidies between the various dose groups and the negative controls were observed.

**4.1.2. Assay with metabolic activation**  
(Tables 2, 9 - 14)

**Untreated control:**

10 (5%) aberrant mitosis incl. gaps and 2 (1%) aberrant cells excl. gaps (2 x B') were analyzed.

**Solvent control:**

9 (4.5%) aberrant metaphases incl. gaps and 2 (1%) chromosomally damaged cells excl. gaps (1 x B'; 1 x B") were found.

**5000 µg/ml:**

15 (7.5%) chromosomally damaged cells incl. gaps and 7 (3.5%) aberrant cells excl. gaps (5 x B'; 1 x B"; 1 x F") were observed.

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**500 µg/ml:**

15 (7.5%) aberrant metaphases incl. gaps and 4 (2%) aberrant cells excl. gaps (1 x B'; 1 x B"; 1 x D"; 1 x F') were detected.

**50 µg/ml:**

17 (8.5%) chromosomally damaged cells incl. gaps and 3 (1.5%) aberrant cells excl. gaps (2 x B'; 1 x B") were found.

**6 µg cyclophosphamide/ml:**

With 35 (36%) aberrant cells incl. gaps and 23 (23%) aberrant metaphases excl. gaps, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

When compared to the negative control groups there were no differences regarding aneuploidies (hyperloid metaphases) and polyploidies in the dose groups.

**4.2. Mitotic index**

The mitotic index based on 1500 cells per culture for the different test groups without and with metabolic activation can be seen from the table on page 19. The numbers of mitotic cells in the samples scored are given as "absolute" values. The "relative" figures are related to the corresponding solvent controls which are set 100%.

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## Determination of the mitotic index (%)

Test groups	S-9 mix	1st culture % abs.	2nd culture % abs.	mean % abs.	% rel.
Untreated control	-	6.8	6.9	6.9	
Solvent control 0.1 ml ethanol	-	6.0	9.6	7.8	100
500 µg/ml c.m.	-	10.3	8.2	9.3	119.2
50 µg/ml c.m.	-	7.3	7.7	7.5	96.2
5 µg/ml c.m.	-	8.0	6.6	7.3	93.6
0.1 µg mitomycin C	-	4.3	5.8	5.1	65.4
Untreated control	+	8.5	8.5	8.5	
Solvent control 0.1 ml ethanol	+	8.1	7.5	7.8	100
5000 µg/ml c.m.	+	7.7	7.6	7.7	98.7
500 µg/ml c.m.	+	9.1	7.7	8.4	107.7
50 µg/ml c.m.	+	8.5	6.7	7.6	97.4
6 µg cyclophosphamide	+	7.4	6.1	6.8	87.2

c.m. = culture medium

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5. **CONCLUSIONS**

According to the results of the present study, the test substance Lipoderm Öl SK did not lead to an increase in the number of aberrant metaphases incl. and excl. gaps after the addition of a metabolizing system.

Without S-9 mix there was a slight and dose-dependent increase in the frequency of aberrant mitoses but only incl. gaps (statistical significance of 95%).

However, the genetic consequences of gaps are rather uncertain and the occurrence of this aberration type in isolation is no suitable criteria for the evaluation of a clastogenic event. Therefore, the test substance Lipoderm Öl SK is considered to have no chromosome-damaging (clastogenic) activity under in vitro conditions using human lymphocytes.

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30 M 0 3 8 3 / 8 7 4 1 7 3

CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK  
WITHOUT METABOLIC ACTIVITY

C H R O M O S O M E A N A L Y S I S

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

TABLE : 1

	GROUP01 UNTREATED C.	GROUP02 ETHANOL	GROUP03 500 UG/ML	GROUP04 50 UG/ML	GROUP05 5 UG/ML	GROUP06 MIT.0.1UG/ML
CULTURE TOTAL	2	2	2	2	2	2
ANALYZED METAPHASES	200	200	200	200	200	100
NUMBER OF CULTURES WITH ABERRANT METAPHASES	2	2	2	2	2	2
METAPHASES WITH ABERRATIONS INCLUDING GAPS / 100	6 / 3.00	7 / 3.50	22 / 11.00 X +	20 / 10.00 +	14 / 7.00	29 / 29.00 XX++
EXCLUDING GAPS	2 / 1.00	1 / 0.50	3 / 1.50	4 / 2.00	2 / 1.00	23 / 23.00 XX++
EXCHANGES	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0
MULT. ABERR. MET.	0	0	0	0	0	0
PULVERISATIONS	0	0	0	0	0	0
TEST AT FIXED TIME						
KEY	GROUP 01	SIGNIFICANCE 95% +	SIGNIFICANCE 99% ++	SIGNIFICANCE 99% ++		
TEST FISHER-YATES	02	X		XX		
ANEUPLOIDY MET.	0 / 0.0	0 / 0.0	2 / 1.00	1 / 0.50	0 / 0.0	0 / 0.0
POLYPLOIDY MET.	0 / 0.0	2 / 1.00	2 / 1.00	1 / 0.50	2 / 1.00	0 / 0.0

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CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK

WITH METABOLIC ACTIVITY

CHROMOSOME ANALYSIS

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

TABLE : 2

	GROUP07 UNTREATED C.	ETHANOL	GROUP08	GROUP09 5000 UG/ML	GROUP10 500 UG/ML	GROUP11 50 UG/ML	GROUP12 CPP 6 UG/ML
CULTURE TOTAL	2	2	2	2	2	2	2
ANALYZED METAPHASES	200	200	200	200	200	200	100
NUMBER OF CULTURES WITH ABERRANT METAPHASES	2	2	2	2	2	2	2
METAPHASES WITH ABERRATIONS INCLUDING GAPS / 100	10 / 5.00	9 / 4.50	15 / 7.50	15 / 7.50	15 / 7.50	17 / 8.50	36 / 36.00 XX++
EXCLUDING GAPS	2 / 1.00	2 / 1.00	7 / 3.50	4 / 2.00	3 / 1.50	23 / 23.00 XX++	
EXCHANGES	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0
MULT. ABERR. MET.	0	0	0	0	0	0	0
PULVERISATIONS	0	0	0	0	0	0	1
TEST AT FIXED TIME							
KEY	GROUP	SIGNIFICANCE 95%	SIGNIFICANCE 99%	SIGNIFICANCE 99%	SIGNIFICANCE 99%	SIGNIFICANCE 99%	SIGNIFICANCE 99%
TEST FISHER-YATES	07 08	+	+	+	+	+	+
		X	XX	XX	XX	XX	XX
AHEUPLOIDY MET.	0 / 0.0	0 / 0.0	0 / 0.0	0 / 0.0	1 / 0.50	1 / 0.50	0 / 0.0
POLYPLOIDY MET.	3 / 1.50	0 / 0.0	0 / 0.0	0 / 0.0	3 / 1.50	1 / 0.50	0 / 0.0

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CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK

WITHOUT METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

C H R O M O S O M E A N A L Y S I S

GROUP 01 UNTREATED C.

CULTURE 01 02 TOTAL

ANALYZED METAPHASES 100 100 200

ABERR. MET. INCL. GAPS 3 3 6

ABERR. MET. EXCL. GAPS 1 1 2

MET. WITH EXCHANGES

MULT. ABERR. MET.

PULVERISATIONS

ANEUPLOIDY MET.

POLYPLOIDY MET.

KEY

\* : ASSESSMENT NOT POSSIBLE

T : CULTURE NOT PREPARED

30 M 0 3 8 3 / 8 7 4 1 7 3

CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK

WITHOUT METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

C H R O M O S O M E A N A L Y S I S

GROUP 02 ETHANOL

CULTURE . . . . . 03 04 TOTAL

ANALYZED METAPHASES . . . . . 100 100 200

ABERR. MET. INCL. GAPS . . . . . 4 3 7

ABERR. MET. EXCL. GAPS . . . . . 1 1 1

MET. WITH EXCHANGES

MULT. ABERR. MET.

PULVERISATIONS

ANEUPLOIDY MET.

POLYPLOIDY MET.

2 2

KEY  
\* : ASSESSMENT NOT POSSIBLE  
T : CULTURE NOT PREPARED

TABLE : 4

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TABLE : 5

CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK

WITHOUT METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

C H R O M O S O M E A N A L Y S I S

GROUP 03 500 UG/ML

CULTURE 05 06 TOTAL

ANALYZED METAPHASES 100 100 200

ABERR. MET. INCL. GAPS 8 14 22

ABERR. MET. EXCL. GAPS 3 3 3

MET. WITH EXCHANGES

MULT. ABERR. MET.

PULVERISATIONS

ANEUPLOIDY MET.

POLYPLOIDY MET.

1 1 2

2 2 2

KEY \* : ASSESSMENT NOT POSSIBLE

T : CULTURE NOT PREPARED

30 M O 3 8 3 / 8 7 4 1 7 3

TABLE : 6

CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK  
WITHOUT METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

C H R O M O S O M E A N A L Y S I S

GROUP 04	50 UG/ML	07	08	TOTAL
CULTURE		100	100	200
ANALYZED METAPHASES		14	6	20
ABERR. MET. INCL. GAPS		3	1	4
ABERR. MET. EXCL. GAPS				
MET. WITH EXCHANGES				
MULT. ABERR. MET.				
PULVERISATIONS		1		1
ANEUPLOIDY MET.			1	1
POLYPLOIDY MET.				

KEY \* : ASSESSMENT NOT POSSIBLE  
T : CULTURE NOT PREPARED

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TABLE : 7

CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK  
WITHOUT METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

CHROMOSOME ANALYSIS

GROUP 05	5 UG/ML	09	10	TOTAL
CULTURE		100	100	200
ANALYZED METAPHASES		8	6	14
ABERR. MET. INCL. GAPS			2	2
ABERR. MET. EXCL. GAPS				
MET. WITH EXCHANGES				
MULT. ABERR. MET.				
PULVERISATIONS				
ANEUPLOIDY MET.				
POLYPLOIDY MET.		1	1	2

KEY  
\* : ASSESSMENT NOT POSSIBLE  
T : CULTURE NOT PREPARED

30 M 0 3 8 3 / 8 7 4 1 7 3

CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK  
WITHOUT METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

C H R O M O S O M E   A N A L Y S I S

GROUP 06	MIT. 0.1UG/ML	11	12	TOTAL
CULTURE		11	12	23
ANALYZED METAPHASES		50	50	100
ABERR. MET. INCL. GAPS		16	13	29
ABERR. MET. EXCL. GAPS		13	10	23
MET. WITH EXCHANGES				
MULT. ABERR. MET.				
PULVERISATIONS				
ANEUPLOIDY MET.				
POLYPLOIDY MET.				

KEY  
\* : ASSESSMENT NOT POSSIBLE  
T : CULTURE NOT PREPARED

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CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES  
SUBSTANCE: LIPODERM OEL SK  
WITH METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

CHROMOSOME ANALYSIS

GROUP 07 UNTREATED C.

CULTURE	13	14	TOTAL
ANALYZED METAPHASES	100	100	200
ABERR. MET. INCL. GAPS	7	3	10
ABERR. MET. EXCL. GAPS	2		2
MET. WITH EXCHANGES			
MULT. ABERR. MET.			
PULVERISATIONS			
ANEUPLOIDY MET.			
POLYPLOIDY MET.	2	1	3

KEY

\* : ASSESSMENT NOT POSSIBLE

T : CULTURE NOT PREPARED

TABLE : 9

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CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK  
WITH METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

CHROMOSOME ANALYSIS

GROUP 08	ETHANOL	15	16	TOTAL
ANALYZED METAPHASES		100	100	200
ABERR. MET. INCL. GAPS		2	7	9
ABERR. MET. EXCL. GAPS			2	2

MET. WITH EXCHANGES

MULT. ABERR. MET.

PULVERISATIONS

ANEUPLOIDY MET.

POLYPLOIDY MET.

KEY

\* : ASSESSMENT NOT POSSIBLE

T : CULTURE NOT PREPARED

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CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK  
WITH METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

CHROMOSOME ANALYSIS

GROUP 09 5000 UG/ML

CULTURE	17	18	TOTAL
ANALYZED METAPHASES	100	100	200
ABERR. MET. INCL. GAPS	9	6	15
ABERR. MET. EXCL. GAPS	3	4	7

MET. WITH EXCHANGES

MULT. ABERR. MET.

PULVERISATIONS

ANEUPLOIDY MET.

POLYPLOIDY MET.

KEY

\* : ASSESSMENT NOT POSSIBLE

T : CULTURE NOT PREPARED

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CHROMOSOME ANALYSIS ON HUMAN LYMPHOCYTES

SUBSTANCE: LIPODERM OEL SK

WITH METABOLIC ACTIVITY

PREPARATIONS 24H AFTER SUBSTANCE ADMINISTRATIONS

CHROMOSOME ANALYSIS

GROUP 10 500 UG/ML

CULTURE 19 20 TOTAL

ANALYZED METAPHASES 100 100 200

ABERR. MET. INCL. GAPS 5 10 15

ABERR. MET. EXCL. GAPS 1 3 4

MET. WITH EXCHANGES

MULT. ABERR. MET.

PULVERISATIONS

ANEUPLOIDY MET. 1 1

POLYPLOIDY MET. 3 3

KEY

\* : ASSESSMENT NOT POSSIBLE

T : CULTURE NOT PREPARED

TABLE : 12



