



STUDY NUMBER 1305601

**CHROMOSOME ABERRATION ASSAY IN
BONE MARROW CELLS OF THE RAT**

WITH

cC6O4 AMMONIUM SALT

REPORT

Study Completion Date: July 13, 2010

1 COPY OF GLP CERTIFICATE



HESSEN



Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance (gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH
Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise (gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

- | | |
|--|--|
| 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften | 2 Toxicity studies |
| 3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo) | 3 Mutagenicity studies |
| 6 Prüfungen zur Bestimmung von Rückständen | 6 Residues |
| 8 Analytische Prüfungen an biologischen Materialien | 8 Analytical studies on biological materials |

15.08. und 27. – 29.10.2008

Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Referent, Wiesbaden, den 30. März 2009
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)



Hess. Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

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3 PREFACE

3.1 General

Title: Chromosome Aberration Assay in Bone Marrow Cells
of the Rat with cC6O4 AMMONIUM SALT

Sponsor: SOLVAY SOLEXIS S.p.A.
Viale Lombardia 20
20021 Bollate (MI)
Italy

Study Monitor:

[REDACTED]

Test Facility:

[REDACTED]

3.2 Responsibilities

Study Director:

[REDACTED]

Deputy Study Director:

[REDACTED]

Management:

[REDACTED]

Head of
Quality Assurance Unit:

[REDACTED]

3.3 Schedule

Experimental Starting Date: December 03, 2009

Experimental Completion Date: January 13, 2010

3.4 Project Staff Signatures

Study Director

[Redacted Signature]

[Redacted Signature]

Date: [Redacted Date]

Management

[Redacted Signature]

[Redacted Signature]

Date: [Redacted Date]

3.5 Good Laboratory Practice

The study will be performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002, and amended version dated July 02, 2008 ("BGBl.", p. 1146).

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

3.6 Guidelines

This study will follow the procedures indicated by the following internationally accepted guidelines and recommendations:

Ninth Addendum to the OECD Guidelines for Testing of Chemicals, Sektion 4, Nr. 475, accepted July 21, 1997, „Mammalian Bone Marrow Chromosome Aberration Test"

Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.5385 "Mammalian Bone Marrow Chromosome Aberration Test ", EPA 712-C-98-226, August 1998.

"European Union Method B.11, Mutagenicity: In Vivo Mammalian Bone Marrow Chromosome Aberration Test - Commission regulation (EC) No 440/2008, Official Journal of the European Union (EN), dated May 30, 2008"

3.7 Archiving

████████████████████ will be archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

Microscopic slides will be archived for at least 12 years.

No data will be discarded without the sponsor's written consent.

3.8 Deviations from the Study Plan

During the course of the study there were the following deviations from the study plan:

- Due to marriage the surname of the study director changed from ██████████ to ██████████
- In the first pre-experiment the 30 hour observation interval was missed out.
- During the acclimation phase of the animals for the first pre-experiment the relative humidity ranged between 33 – 65 % for a period of approximately 6 hours.

These deviations however do not affect the validity of the study.

4 STATEMENT OF COMPLIANCE

Study Number: 1305601
Test Item: cC6O4 AMMONIUM SALT
Study Director: [REDACTED]
Title: Chromosome Aberration Assay in Bone Marrow
Cells of the Rat with cC6O4 AMMONIUM SALT

This study performed in the test facility of [REDACTED] was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002, and amended version dated July 02, 2008 ("BGBl.", p. 1146).

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director [REDACTED]

[REDACTED]
Date: [REDACTED]

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1305601
Test Item: cC6O4 AMMONIUM SALT
Study Director: [REDACTED]
Title: Chromosome Aberration Assay in Bone Marrow Cells of the Rat with cC6O4 AMMONIUM SALT

The general facilities and activities of [REDACTED] are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	November 20, 2009 November 27, 2009	November 20, 2009 November 27, 2009
1 st Amendment to Study Plan:	May 14, 2010	May 14, 2010
2 nd Amendment to Study Plan:	July 09, 2010	July 09, 2010
<u>Study Inspection</u> Test Performance:	December 16, 2009	December 16, 2009
Report:	April 09 + 12, 2010	April 12, 2010

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit

[REDACTED]
[REDACTED]

Date: [REDACTED]

6 SUMMARY

This study was performed to investigate the potential of cC6O4 AMMONIUM SALT to induce chromosome aberrations in bone marrow cells of the rat.

The test item was formulated in sterile water. The volume administered orally was 10 ml/kg body weight (b.w.). The animals were treated once. 24 h and 48 h (only the high dose group) after the treatment the bone marrow cells were collected for chromosome aberration analysis. 7 males per test group were evaluated for the occurrence of cytogenetic damage. Per animal 100 well spread metaphases were scored for gaps, breaks, fragments, deletions, multiple aberrations, exchanges, and chromosomal disintegrations.

The test item was investigated in this cytogenetic assay at the following doses:

Males: 312.5, 625, and 1250 mg/kg b.w.

The maximum doses for the cytogenetic assay were determined in a pre-experiment for toxicity. 1250 mg/kg b.w. was estimated as to be close to the maximum tolerated doses. The animals treated with this dose showed clinical signs like a reduction in spontaneous activity, ruffled fur and hunchback posture indicating bioavailability of the test item.

No relevant reduction of the mitotic indices could be observed after treatment with the test item, indicating that the test item at the indicated concentrations was not cytotoxic in the bone marrow.

No statistically significant increase in the frequency of aberrant cells occurred after treatment with the test item as compared to the vehicle control.

An appropriate reference mutagen (cyclophosphamide) was used as positive control and showed a distinct and statistically significant increase of induced aberration frequency.

6.1 Conclusion

In conclusion, it can be stated that during the mutagenicity test described and under the experimental conditions reported, the test item did not induce chromosome mutations as determined by the chromosome aberration test with bone marrow cells of the rat.

Therefore, cC6O4 AMMONIUM SALT is considered to be non-mutagenic in this chromosome aberration assay *in vivo*.

7 OBJECTIVE

7.1 Aims of the Study

This in vivo experiment was performed to assess the mutagenic properties of the test item by means of the chromosome aberration test in bone marrow cells of the rat.

7.2 Reasons for the Study

The bone marrow cytogenetic test is an assay for the detection of structural chromosomal aberrations. These aberrations are frequently lethal to the damaged cells. However, cytogenetic damage in somatic cells is an indicator of a potential to induce more subtle chromosome damage that is compatible with cell division. Similar damage induced in germinal cells may lead to heritable cytogenetic abnormalities. Heritable cytogenetic abnormalities are known to have deleterious effects in man, e.g. induction of neoplastic events or birth defects.

Rats are exposed to the test item by an appropriate route and are sacrificed at sequential intervals. Chromosome preparations are made from bone marrow cells. The stained preparations are examined and metaphase cells are scored for chromosomal aberrations.

The bone marrow is the tissue of choice because it offers several advantages:

1. Low incidence of spontaneous chromosome aberrations.
2. Technical simplicity of the preparation.
3. High proportion of metaphase cells for analysis.

Chromosomal aberrations are generally evaluated in first post-treatment mitoses. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome-type aberrations also occur.

The time at which the aberration frequency is at a maximum varies from agent to agent. Because different chemicals have effects at different parts of the cell cycle and the marrow cell population is asynchronous, multiple post-treatment sample times are necessary to precisely define the response. Due to mitotic delay or metabolic and pharmacokinetic effects the appearance of the first post-treatment mitosis can be considerably delayed.

For the assessment of clastogenic activity the maximum tolerated dose or that producing some indication of cytotoxicity (partial inhibition of mitosis) was applied once and sampled at 24 h and 48 h after the treatment. To establish a dose response effect at least two additional dose levels were tested accordingly.

To demonstrate the sensitivity of the test system, a reference mutagen was tested in parallel to the test item.

8 MATERIALS AND METHODS

8.1 Test Item

Internal Test Item Number: S1069711

The test item and the information concerning the test item were provided by the sponsor.

Identity:	cC6O4 ammonium salt
Batch No.:	156/39
Purity:	97.5 %
Stability in solvent:	Not indicated by the sponsor
Storage:	In the refrigerator (+2 to +8 °C)
Expiration Date:	December 31, 2020

On the day of the experiment, the test item was formulated in sterile water. The vehicle was chosen to its relative non-toxicity for the animals. All animals received a single standard volume of 10 ml/kg b.w. orally.

8.2 Controls

8.2.1 Vehicle Control

The vehicle of the test item was used as vehicle control.

Name:	sterile water
Supplier:	B. Braun Melsungen AG
Catalogue no.:	6724092.00.00
Route and Frequency of Administration:	orally, once
Volume Administered:	10 ml/kg b.w.

8.2.2 Positive Control

Name:	CPA; Cyclophosphamide
Supplier:	Sigma-Aldrich Vertriebs-GmbH 82041 Deisenhofen, Germany
Catalogue no.:	C 0768
Dissolved in:	sterile water
Dosing:	15 mg/kg b.w.
Route and Frequency of Administration:	orally, once
Volume Administered:	10 ml/kg b.w.

Solution prepared on day of administration.

The stability of CPA at room temperature is good. At 25°C only 3.5 % of its potency is lost after 24 hours.

8.3 Test System

8.3.1 Reasons for the Choice of the Experimental Animal Species

The rat is an animal which has been used for many years as suitable experimental animal in cytogenetic investigations. In addition, the rat is an experimental animal used in many physiological, pharmacological and toxicological studies.

There are many data available from such investigations which may be helpful in the interpretation of results from the cytogenetic assay. Data from such experiments also may be useful for the design and the performance of the chromosome aberration test.

Strain: rat (Wistar)

Source:



Number of animals: 42 males

Acclimation: minimum 5 days

Age of the animals: 8 - 12 weeks

Initial body weight

at start of treatment: males: 180.2 ± 5.5 g

According to the supplier's assurance the animals were in healthy condition. The arriving animals underwent acclimation in the animal house of [REDACTED] for a minimum of five days. During this period the animals should not show signs of illness or altered behaviour.

The animals were distributed into the test groups at random and identified by their cage number and tail tags.

8.3.2 Husbandry

The animals were kept conventionally. The experiment was conducted under standard laboratory conditions.

Housing: group

Cage Type: Makrolon Type IV, with wire mesh top
(EHRET GmbH, 79302 Emmendingen, Germany)

Bedding: granulated soft wood bedding,
(Rettenmaier & Söhne GmbH + Co. KG,
73494 Rosenberg, Germany)

Feed: pelleted standard diet, ad libitum
(Harlan Laboratories GmbH, 33178 Borcheln, Germany)

Water: tap water, ad libitum
(Gemeindewerke, 64380 Roßdorf, Germany)

Environment: temperature $22 \pm 2^\circ\text{C}$
relative humidity 33 - 65 %
artificial light 6.00 a.m. - 6.00 p.m.

8.4 Experimental Performance

8.4.1 Pre-Experiments for Toxicity

A preliminary study on acute toxicity was performed with two animals per sex and test group each under identical conditions as in the mutagenicity study concerning: animal strain; vehicle; route, frequency, and volume of administration.

The animals were treated once orally with the test item and examined for acute toxic symptoms at intervals of 1 h, 2-4h, 6 h, 24h, 30 h (except for the first pre-experiment), and 48 h after each administration of the test item.

8.4.2 Dose Selection

It is generally recommended to use the maximum tolerated dose or the highest dose that can be formulated and administered reproducibly or 2000 mg/kg as the upper limit for non-toxic test items.

The maximum tolerated dose levels were determined to be the doses that caused toxic reactions without having effects on survival within 48 hours after the treatment.

Three adequately spaced dose levels, spaced by a factor of 2, were applied at the central sampling interval 24 h after treatment. For the highest dose level an additional sample was taken at 48 h after treatment.

8.4.3 Study Procedure

Test Groups:

Seven males are assigned to each test group.

The animals are identified by their cage number and tail tags as shown below in the table.

Test group	hours post-treatment	
	24	48
Negative control	1 – 7	36 – 42
Low dose	8 – 14	
Medium dose	15 – 21	
High dose	22 – 28	
Positive control	29 - 35	

8.4.4 Treatment:

At the beginning of the treatment the animals were weighed and the individual volume to be administered was adjusted to the animal's body weight. The animals received the test item once. Seven males were treated per dose group. The animals were examined for acute toxic symptoms around 1 h, 2-4 h, 6 h, 24 h and 48 h after treatment.

Prior (2.5 to 3 hours) to sacrifice, animals were injected intraperitoneally with the spindle inhibitor colcemid (2.0 mg/kg b.w.), to arrest cells in metaphase.

8.4.5 Preparation of the Animals:

The animals were sacrificed by cervical dislocation. The femora were removed, the epiphyses were cut off and the marrow was flushed out with approximately 5 ml hypotonic potassium chloride solution (0.56 % w/v, prewarmed to 37 °C). The hypotonic cell suspension was then incubated for 20 min at 37 °C. The cells were sedimented by a brief centrifugation (1000 rpm), the hypotonic supernatant was discarded and the cell pellet was fixed with 3+1 absolute methanol+glacial acetic acid fixative for 60 min. Then the cell pellet was gently resuspended with fixative and stored overnight at 4°C. Prior to making slides the fixative was changed and enough fixative was added to make a relatively thin cell suspension. The fixative-cell suspension was spread by flame-drying and stained with Giemsa. Cover slips were mounted with EUKITT (KINDLER, 79110 Freiburg, Germany). One or more slides were made from each bone marrow sample.

8.4.6 Analysis of Metaphase Cells:

Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. Gaps, breaks, fragments, deletions, exchanges and chromosomal disintegrations were recorded as structural chromosome aberrations. At least 100 well spread metaphases per animal were scored for cytogenetic damage on coded slides. The number of chromosome aberrations per metaphase was determined. Only metaphases with the characteristic chromosome number of 42 ± 2 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis; 1000 cells are scored) was determined.

All animals per test group were evaluated as described.

8.5 Data Recording

The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including experimental groups, vehicle, and positive control.

8.6 Acceptance Criteria

The test is acceptable since at least 5 evaluable animals per group were available, the positive control shows a statistically significant response and the aberration rate of the vehicle control (excl. gaps) is below 2 %.

8.7 Historical Data

Number of studies	Group	Dose mg/kg b.w.	Sex	Mean number of cells with aberrations (excl. gaps) $\pm$ standard deviation [%]
27	vehicles	0	males & females	0.6 ± 0.4 / range 0 - 2
1	CPA	25	males & females	30.2
7	CPA	20	males & females	17.4 ± 4.4
3	CPA	15	males	13.1 ± 7.4
6	CPA	15	females	14.3 ± 6.6
2	CPA	12.5	males	4.2 ± 0.3
10	CPA	12.5	females	9.3 ± 4.1
4	CPA	10	males	8.5 ± 2.3
1	CPA	10	females	9.6
8	CPA	7.5	males	5.1 ± 1.8
1	CPA	5	males & females	7.0

CPA = Cyclophosphamide

8.8 Evaluation of Results

A test item is classified as mutagenic if it induces either a dose-related increase in the number of structural chromosomal aberrations and a reproducible statistically significant positive response for at least one of the test points.

A test item producing neither a dose-related increase in the number of structural chromosomal aberration nor a statistically significant and reproducible positive response at any of the test points is considered non-mutagenic in this system.

This can be confirmed by means of the non-parametric Mann-Whitney test.

However, both biological and statistical significance should be considered together.

9 RESULTS

9.1 Pre-Experiments on Toxicity

In the first pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 1000 mg/kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The treated animals expressed toxic reactions as shown in the table below:

toxic reactions	hours post-treatment					
	male / female					
	1 h	2-4 h	6 h	24 h	30 h	48 h
ruffled fur	0/0	2/2	2/2	2/2	-/-	2/2

-/-: no observation made

In the second pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 2000 mg/kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The treated animals expressed toxic reactions as shown in the table below:

toxic reactions	hours post-treatment					
	male / female					
	1 h	2-4 h	6 h	24 h	30 h	48 h
reduction of spontaneous activity	0/0	0/0	2/2	2/2	1/2	-/1
ruffled fur	0/0	2/2	2/2	2/2	1/2	-/1
hunchback posture	0/0	0/0	0/0	2/0	0/0	-/0
death	0/0	0/0	0/0	0/0	1/0	1/1

In the third pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 1750 mg/kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The treated animals expressed toxic reactions as shown in the table below:

toxic reactions	hours post-treatment					
	male / female					
	1 h	2-4 h	6 h	24 h	30 h	48 h
reduction of spontaneous activity	2/2	2/2	2/2	2/2	1/2	1/2
ruffled fur	2/2	2/2	2/2	2/2	1/2	1/2
hunchback posture	0/0	0/0	2/2	2/2	1/2	0/0
salivation	0/0	0/0	0/0	0/0	0/1	0/0
death	0/0	0/0	0/0	0/0	1/0	0/0

In the fourth pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 1250 mg/kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The treated animals expressed toxic reactions as shown in the table below:

toxic reactions	hours post-treatment					
	male / female					
	1 h	2-4 h	6 h	24 h	30 h	48 h
reduction of spontaneous activity	0/0	2/2	2/2	2/2	2/2	2/2
ruffled fur	2/2	2/2	2/2	2/2	2/2	2/2
hunchback	0/0	0/0	1/2	0/0	0/0	1/0

On the basis of these data 1250 mg/kg b.w. was estimated to be suitable. No substantial differences between sexes in toxicity were observed, thus only male animals were used in the main experiment.

9.2 Toxic Symptoms in the Main Experiment

In the main experiment for the highest dose group 14 males received orally a single dose of 1250 mg /kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The animals treated with 1250 mg /kg b.w. expressed toxic reactions as shown in the table:

Toxic Reactions	hours post-treatment				
	1 h	2-4 h	6 h	24 h	48 h*
reduction of spontaneous activity	0	9	11	0	0
ruffled fur	14	14	14	14	7

*: data from 7 males only

For the mid dose group 7 males received orally a single dose of 625 mg /kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The animals treated with 625 mg /kg b.w. expressed toxic reactions as shown in the table:

toxic reactions	hours post-treatment			
	1 h	2-4 h	6 h	24 h
reduction of spontaneous activity	0	2	3	0
ruffled fur	4	4	4	7

For the low dose group 7 males received orally a single dose of 312.5 mg /kg b.w. cC6O4 AMMONIUM SALT formulated in sterile water. The volume administered was 10 ml/kg b.w..

The animals treated with 312.5 mg /kg b.w. expressed toxic reactions as shown in the table:

toxic reactions	hours post-treatment			
	1 h	2-4 h	6 h	24 h
ruffled fur	0	3	3	5

9.3 Mutagenicity Experiment

9.4 Summary of Results

Experimental group	dose mg/kg b.w.	preparation hours post administration	number of cells scored	% aberrant cells incl. gaps excl. gaps		mean mitotic index (%)
1: sterile water	0	24	1000	0.4	0.4	4.67
2: cC604 ammonium salt	312.5	24	1000	0.0	0.0	5.27
3: cC604 ammonium salt	625	24	1000	0.7	0.7	3.06
4: cC604 ammonium salt	1250	24	1000	0.7	0.7	3.94
5: Cyclophosphamide	15	24	1000	7.3	7.3	3.59
6: cC604 ammonium salt	1250	48	1000	1.1	1.1	5.19

9.4.1 Analysis of aberration types

group	gap	iso-gap	break	iso-break	frag-ment	iso-frag-ment	deletion	multiple aberra- tion ^a	ex- change	chrom. disinte- gration ^b
1	0	0	0	0	2	1	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	2	0	2	3	0	0	0	0
4	0	0	1	0	4	1	0	0	0	0
5	0	0	39	0	9	4	0	4	18	0
6	0	0	1	0	3	4	0	0	0	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

^b Pulverization

10 BIOMETRY

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

Vehicle control versus Test group (aberrant cells excl. Gaps)	Significance	p
cC6O4 AMMONIUM SALT, 312.5 mg/kg b.w., 24 h post treatment	n.t.	-
cC6O4 AMMONIUM SALT, 625 mg/kg b.w., 24 h post treatment	-	0.3899
cC6O4 AMMONIUM SALT, 1250 mg/kg b.w., 24 h post treatment	-	0.3899
Cyclophosphamide, 10 mg/kg b.w. 24 h post treatment	+	0.0003
cC6O4 AMMONIUM SALT, 1250 mg/kg b.w., 48 h post treatment	-	0.1655

n.t. = not tested, as the mean aberration rate was not above the corresponding control value.

- = not significant

+ = significant

11 DISCUSSION

This study was performed to assess the potential of cC6O4 AMMONIUM SALT to induce chromosome aberrations in bone marrow cells of the rat.

The test item was formulated in sterile water which was also used as the vehicle control. The volume administered orally was 10 ml/kg body weight (b.w.). 24 h and 48 h (only the high dose group) after a single administration of the test item the bone marrow cells were collected for the chromosome aberration analysis. Seven males per test item group, as well as vehicle and positive control were evaluated for the occurrence of cytogenetic damage. Per animal 100 well spread metaphases were scored for gaps, breaks, fragments, deletions, multiple aberrations, exchanges, and chromosomal disintegrations.

The study was acceptable as the positive control showed a statistically significant response (aberration rate excluding gaps was 7.3 %) and the aberration frequency exclusive gaps of the vehicle control was below 2 %.

The test item was investigated in this cytogenetic assay at the following doses: 312.5, 625, and 1250 mg/kg b.w.

The highest dose of 1250 mg/kg b.w. were determined in a pre-experiment for toxicity to be close to the maximum tolerated doses. The animals treated with this dose showed clinical signs like a reduction in spontaneous activity, ruffled fur and hunchback posture indicating bioavailability of the test item.

The mitotic indices were not relevantly reduced after treatment with cC6O4 AMMONIUM SALT, indicating that the test item did not have cytotoxic effects in the bone marrow.

No statistically significant or biologically relevant enhancement of the aberration frequencies occurred (aberration rates of the test item treated animals ranged between 0.0-1.1%) as compared to the vehicle control value (0.4%). The mean aberration frequencies observed after treatment with cC6O4 AMMONIUM SALT were consistently below 2 % aberrant cells exclusive gaps (given as the upper limit of a tolerable vehicle control value; see acceptance criteria). There were also no relevant dose dependent increases in the aberration rates of the treated animals.

Cyclophosphamide was used as positive control and showed statistically significant increases of induced aberration frequencies.

11.1 CONCLUSION

In conclusion, it can be stated that during the mutagenicity test described and under the experimental conditions reported, cC6O4 AMMONIUM SALT did not induce chromosome mutations as determined by the chromosome aberration test with bone marrow cells of the rat *in vivo*.

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13 DISTRIBUTION OF THE REPORT

Sponsor	1x copy of report 1x electronic copy
Study Director	1x original report

14 ANNEX 1

14.1 Tables of Results

Table 1: Chromosome aberrations in bone marrow cells of rats scoring 24 hours after treatment; Experimental group 1: Vehicle; Sterile water

animal no.	sex	number of cells scored	dose mg/kg b.w.	% aberrant cells	
				incl.gaps	excl. gaps
1	m	100	0	0	0
2	m	100		0	0
3	m	100		2	2
4	m	100		0	0
5	m	100		0	0
6	m	100		1	1
7	m	100		0	0
mean				0.4	0.4

Analysis of aberration types in each 100 cells

animal no	gap	iso-gap	break	iso-break	frag-ment	iso-frag-ment	dele-tion	multiple aberrat. ^a	ex-change	chrom. disinte-gration ^b
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	2	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	1	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	2	1	0	0	0	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

^b Pulverization

Table 2: Chromosome aberrations in bone marrow cells of rats scoring 24 hours after treatment; Experimental group 2: Test item; cC604 ammonium salt; 312.5 mg/kg b.w.

animal no.	sex	number of cells scored	dose mg/kg b.w.	% aberrant cells	
				incl. gaps	excl. gaps
8	m	100	312.5	0	0
9	m	100		0	0
10	m	100		0	0
11	m	100		0	0
12	m	100		0	0
13	m	100		0	0
14	m	100		0	0
mean				0	0

Analysis of aberration types in each 100 cells

animal no	gap	iso-gap	break	iso-break	fragment	iso-fragment	deletion	multiple aberrat. ^a	exchange	chrom. disintegration ^b
8	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
total	0	0	0	0	0	0	0	0	0	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

^b Pulverization

Table 3: Chromosome aberrations in bone marrow cells of rats scoring 24 hours after treatment; Experimental group 2: Test item; cC604 ammonium salt; 625 mg/kg b.w.

animal no.	sex	number of cells scored	dose mg/kg b.w.	% aberrant cells	
				incl. gaps	excl. gaps
15	m	100	625	2	2
16	m	100		2	2
17	m	100		0	0
18	m	100		1	1
19	m	100		0	0
20	m	100		0	0
21	m	100		0	0
mean				0.7	0.7

Analysis of aberration types in each 100 cells

animal no	gap	iso-gap	break	iso-break	fragment	iso-fragment	deletion	multiple aberrat. ^a	exchange	chrom. disintegration ^b
15	0	0	0	0	2	1	0	0	0	0
16	0	0	2	0	0	1	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	1	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
total	0	0	2	0	2	3	0	0	0	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

^b Pulverization

Table 4: Chromosome aberrations in bone marrow cells of rats scoring 24 hours after treatment; Experimental group 2: Test item; cC604 ammonium salt; 1250 mg/kg b.w.

animal no.	sex	number of cells scored	dose mg/kg b.w.	% aberrant cells	
				incl. gaps	excl. gaps
22	m	100	1250	0	0
23	m	100		2	2
24	m	100		0	0
25	m	100		2	2
26	m	100		0	0
27	m	100		0	0
28	m	100		1	1
mean				0.7	0.7

Analysis of aberration types in each 100 cells

animal no	gap	iso-gap	break	iso-break	frag-ment	iso-frag-ment	deletion	multiple aberrat. ^a	ex-change	chrom. disintegration ^b
22	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	2	1	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	2	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0
28	0	0	1	0	0	0	0	0	0	0
total	0	0	1	0	4	1	0	0	0	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

^b Pulverization

Table 5: Chromosome aberrations in bone marrow cells of rats scoring 24 hours after treatment; Experimental group 3: Positive control; cyclophosphamide

animal no.	sex	number of cells scored	dose mg/kg b.w.	% aberrant cells	
				incl. Gaps	excl. Gaps
29	m	100	15	7	7
30	m	100		5	5
31	m	100		13	13
32	m	100		7	7
33	m	100		6	6
34	m	100		7	7
35	m	100		6	6
mean				7.3	7.3

Analysis of aberration types in each 100 cells

animal no	gap	iso-gap	break	iso-break	frag-ment	iso-frag-ment	dele-tion	multiple aberrat. ^a	ex-change	chrom. disinte-gration ^b
29	0	0	6	0	1	0	0	1	3	0
30	0	0	3	0	2	0	0	1	1	0
31	0	0	8	0	2	0	0	0	6	0
32	0	0	7	0	1	1	0	1	1	0
33	0	0	4	0	1	0	0	0	2	0
34	0	0	7	0	2	0	0	1	4	0
35	0	0	4	0	0	3	0	0	1	0
total	0	0	39	0	9	4	0	4	18	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded separately

^b Pulverization

Table 4: Chromosome aberrations in bone marrow cells of rats scoring 48 hours after treatment; Experimental group 4: Test item; cC604 ammonium salt; 1250 mg/kg

animal no.	sex	number of cells scored	dose mg/kg b.w.	% aberrant cells	
				incl. gaps	excl. gaps
36	m	100	1250	2	2
37	m	100		3	3
38	m	100		0	0
39	m	100		0	0
40	m	100		0	0
41	m	100		2	2
42	m	100		1	1
mean				1.1	1.1

Analysis of aberration types in each 100 cells

animal no	gap	iso-gap	break	iso-break	frag-ment	iso-frag-ment	deletion	multiple aberrat. ^a	ex-change	chrom. disintegration ^b
36	0	0	0	0	0	2	0	0	0	0
37	0	0	0	0	2	1	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0
41	0	0	1	0	1	0	0	0	0	0
42	0	0	0	0	0	1	0	0	0	0
total	0	0	1	0	3	4	0	0	0	0

^a More than 5 aberrations excluding gaps in one cell; exchanges (but no other aberrations) were recorded

^b separately
Pulverization

15 ANNEX 2

15.1 Certificate of Tap Water

Zentrallabor
Gräfenhäuser Straße 118
64293 Darmstadt
Tel.: 069/25490-5231, Fax: -5009



Hessenwasser GmbH & Co. KG · Taunusstraße 100 · 64521 Groß-Gerau

Gemeindewerke Roßdorf

Erbacher Straße 1
64380 Roßdorf

Analysenbericht

Analysen-Nr.: 200927278

Probe:

Kopie an das Gesundheitsamt Darmstadt

PNS-Nr. / Kürzel: 200401 KD: DA0015501
Adresse: 64380 Roßdorf, Erbacher Straße 1
Medium: Trinkwasser
Entnahmeanlass: RUV+TrinkwV
Probenahme: 07.10.2009 11:35 Uhr
durch: GWR /
Probenart: Ablaufprobe
Grenzwertliste: Trinkwasserverordnung 2001 (V)

Auftrag-Nummer: A-20050011
Kunden-Nr.: 145270
Probeneingang: 07.10.2009
Untersuchungsende: 06.11.2009
Befundausgabe: 06.11.2009

Parameter	Methode/Norm	Einheit	Ergebnis	Grenzwert
Geruch, qualitativ	DIN 38404 (B1.2)		ohne	
Geruchsschwellenwert	DIN 38404 (B1.2)	TON	1	3
Geschmack	DIN 38404 (B1.2)		neutral	
Trübung, qualitativ			klar	
Farbung, qualitativ	DIN EN ISO 7867 (C1-1)		farblos	
Temperatur	DIN 38404-C4	°C	14,8	
pH-Wert	DIN 38404-C5		7,10	6,5 / 9,5
Messtemperatur pH	DIN 38404-C4	°C	19,0	
Leitfähigkeit bei 25°C	DIN EN 27888 (C8)	µS/cm	951	
Leitfähigkeit bei 20°C	DIN EN 27888 (C8)	µS/cm	852	2500
Sauerstoff	DIN EN 25814 (G22)	mg/l	5,9	
Trübung	DIN EN ISO 7027 (C2-2)	FNU	0,11	
Koloniezahl (22°C)	TrinkwVef, Anl. 1.Nr.5	KBE/ml	0	100
Koloniezahl (36°C)	TrinkwVef, Anl. 1.Nr.5	KBE/ml	0	100
Coliforme Keime	Colien 18/ QuantiTray	MPN/100ml	0	0
Escherichia coli	Colien 18/ QuantiTray	MPN/100ml	0	0
Enterokokken	DIN EN ISO 7899-2:2000	KBE/100ml	0	0
Farbung (SAK 436nm)	DIN EN ISO 7867 (C1-2)	1/m	<0,1	0,5



DACH
DAC-PL-0070-99

Akkreditiert nach
DIN EN ISO/IEC 17025

Die Prüfergebnisse beziehen sich auf die untersuchten Proben.
Die Veröffentlichung und Vervielfältigung der Prüfberichte und Gutachten zu Werbezwecken sowie deren auszugswise Verwendung in sonstigen Fällen bedürfen unserer schriftlichen Genehmigung.



Analysen-Nr.: 200927278 MSB: 07.10.2009

Probe:

Parameter	Methode/Norm	Einheit	Ergebnis	Grenzwert
pHC: pH-Wert nach Calcit-Sättigung	DIN 38404-C10-R3		6,92	
Delta pH	DIN 38404-C10-R3		0,18	
Calcitlösekapazität	DIN 38404-C10-R3	mg/l	-44	10
Basekapazität (pH=8,2)	DIN 38409-H7	mmol/l	1,32	
Kohlendioxid, CO ₂ -frei	DEV-D8	mg/l	58,1	
Säurekapazität (pH=4,3)	DIN 38409-H7	mmol/l	7,60	
Karbonathärte	DEV-D8	°dH	21,2	
Gesamthärte	DIN EN ISO 11885 (E22)	°dH	27,0	
Gesamthärte	DIN EN ISO 11885 (E22)	mmol/l	4,8	
Härtebereich (WRMG 2007)			hart	
Hydrogencarbonat	DEV-D8	mg/l	460	
Fluorid	DIN 38405-D4-1	mg/l	0,16	1,5
Chlorid	DIN EN ISO 10304-1.2 (D19, D20)	mg/l	37,3	250
Nitrit	DIN EN ISO 13395 (D26)	mg/l	<0,030	0,5
Nitrat	DIN EN ISO 10304-1.2 (D19, D20)	mg/l	43,0	50
Sulfat	DIN EN ISO 10304-1.2 (D19, D20)	mg/l	64,9	240
Cyanid, gesamt	DIN EN ISO 14403 (D6)	mg/l	<0,005	0,05
Gesamtphosphor (P)	DIN EN ISO 11885 (E22)	mg/l	<0,050	
Gesamtphosphor (PO ₄)	DIN EN ISO 11885 (E22)	mg/l	<0,15	
Gesamtphosphor (HPO ₄)	DIN EN ISO 11885 (E22)	mg/l	<0,15	
Bor	DIN EN ISO 11885 (E22)	mg/l	0,016	1
Borat	DIN EN ISO 11885 (E22)	mg/l	0,087	
Ammonium	DIN EN ISO 11732 (E23)	mg/l	<0,026	0,5
Natrium	DIN EN ISO 11885 (E22)	mg/l	19,3	200
Kalium	DIN EN ISO 11885 (E22)	mg/l	1,45	
Magnesium	DIN EN ISO 11885 (E22)	mg/l	28,5	
Calcium	DIN EN ISO 11885 (E22)	mg/l	146	
Eisen, gesamt	DIN EN ISO 11885 (E22)	mg/l	<0,005	0,2
Mangan	DIN EN ISO 11885 (E22)	mg/l	<0,0010	0,05
Aluminium	DIN EN ISO 11885 (E22)	mg/l	<0,015	0,2
Antimon	DIN EN ISO 17294-2 (E28)	mg/l	<0,0001	0,005
Arsen	DIN EN ISO 17294-2 (E28)	mg/l	0,0019	0,01
Blei	DIN EN ISO 17294-2 (E28)	mg/l	0,0018	0,025
Cadmium	DIN EN ISO 17294-2 (E28)	mg/l	<0,0001	0,005
Chrom, gesamt	DIN EN ISO 11885 (E22)	mg/l	<0,005	0,05
Kupfer	DIN EN ISO 11885 (E22)	mg/l	0,041	2
Nickel	DIN EN ISO 17294-2 (E28)	mg/l	0,0007	0,02
Quecksilber	DIN EN 12338 (E31)	mg/l	<0,0002	0,001
Selen	DIN EN ISO 17294-2 (E28)	mg/l	0,0037	0,01

	Akkreditiert nach DIN EN ISO/IEC 17025	Die Prüfergebnisse beziehen sich auf die untersuchten Proben. Die Veröffentlichung und Vervielfältigung der Prüfberichte und Gutachten zu Werbezwecken sowie deren auszugsweise Verwendung in sonstigen Fällen bedürfen unserer schriftlichen Genehmigung.



Analysen-Nr.: 200927278

vom: 07.10.2009

Probe:

Parameter	Methode/Norm	Einheit	Ergebnis	Grenzwert
TOC	DIN EN 1484 (H3)	mg/l	0,91	
DOC, gelöster organischer Kohlenstoff	DIN EN 1484 (H3)	mg/l	0,90	
Oxidierbarkeit	DIN EN ISO 8467 (H5)	mg/l O ₂	0,7	5
AOX	DIN EN ISO 9562 (H14)	µg/l	<10,0	
POX	DIN 38407-F19	µg/l	<1,0	
1,2-Dichlorethan	DIN 38407-F19	µg/l	<0,1	3
Trichlorethan	DIN 38407-F19	µg/l	<0,1	
Tetrachlorethan	DIN 38407-F19	µg/l	<0,1	
Vinylchlorid	DIN 38407-F19	µg/l	<0,2	0,5
Bromdichlormethan	DIN 38407-F19	µg/l	<0,1	
Chlordibrommethan	DIN 38407-F19	µg/l	<0,1	
Tribrommethan	DIN 38407-F19	µg/l	<0,1	
Trichlormethan	DIN 38407-F19	µg/l	<0,1	
1,1,1-Trichlorethan	DIN 38407-F19	µg/l	<0,1	
Dichlormethan	DIN 38407-F19	µg/l	<0,5	
Tetrachlormethan	DIN 38407-F19	µg/l	<0,1	
cis-1,2-Dichlorethan	DIN 38407-F19	µg/l	<0,1	
Summe Trihalogenmethane	DIN 38407-F19	µg/l	n.b.	50
Benzol	DIN 38407-F19	µg/l	<0,1	1
Toluol	DIN 38407-F19	µg/l	<0,2	
Summe m/p-Xylol	DIN 38407-F19	µg/l	<0,2	
o-Xylol	DIN 38407-F19	µg/l	<0,1	
Ethylbenzol	DIN 38407-F19	µg/l	<0,1	
Summe BTEX	DIN 38407-F19	µg/l	n.b.	
Benzo-(a)-Pyren	DIN 38407-F8	µg/l	<0,002	0,01
Benzo-(b)-Fluoranthren	DIN 38407-F8	µg/l	<0,002	
Benzo-(ghi)-Perylen	DIN 38407-F8	µg/l	<0,002	
Benzo-(k)-Fluoranthren	DIN 38407-F8	µg/l	<0,002	
Indeno-(1,2,3-cd)-Pyren	DIN 38407-F8	µg/l	<0,003	
Summe 4 PAK (TV0, Anl. 2, 2001)	DIN 38407-F8	µg/l	n.b.	0,1
Atrazin	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Bentazon	Hausmethode (LC/MS)	µg/l	<0,02	0,1
Bromacil	Hausmethode (LC/MS)	µg/l	<0,02	0,1
Carbofuran	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Chlortoluron	DIN EN ISO 11369 (F12)	µg/l	<0,02	0,1
Desethylatrazin	DIN EN ISO 10695 (F8)	µg/l	0,02	0,1
Desisopropylatrazin	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Dichlorprop	Hausmethode (LC/MS)	µg/l	<0,03	0,1
Diuron	DIN EN ISO 11369 (F12)	µg/l	<0,02	0,1

	Akkreditiert nach DIN EN ISO/IEC 17025	Die Prüfergebnisse beziehen sich auf die untersuchten Proben. Die Veröffentlichung und Vervielfältigung der Prüfbescheide und Gutachten zu Werbezwecken sowie deren auszugsweise Verwendung in sonstigen Fällen bedürfen unserer schriftlichen Genehmigung.



Analysen-Nr.: 200927278

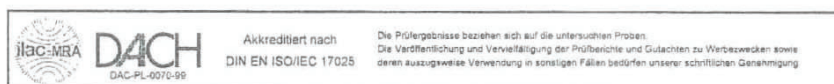
vom: 07.10.2009

Probe: [REDACTED]

Parameter	Methode/Norm	Einheit	Ergebnis	Grenzwert
gamma-Hexachlorcyclohexan	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Hexazinon	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Isoproturon	DIN EN ISO 11369 (F12)	µg/l	<0,02	0,1
MCPA	Hausmethode (LC/MS)	µg/l	<0,02	0,1
Mecoprop (MCP)	Hausmethode (LC/MS)	µg/l	<0,02	0,1
Metazachlor	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Methabenzthiazuron	DIN EN ISO 11369 (F12)	µg/l	<0,02	0,1
Metobromuron	DIN EN ISO 11369 (F12)	µg/l	<0,02	0,1
Monuron	DIN EN ISO 11369 (F12)	µg/l	<0,02	0,1
Parathion-Ethyl	DIN EN ISO 10695 (F8)	µg/l	<0,03	0,1
Propazin	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Sebuthylazin	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Simazin	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Terbuthylazin	DIN EN ISO 10695 (F8)	µg/l	<0,02	0,1
Summe PBSM	Verschiedene	µg/l	0,02	0,5

n.b. = nicht bestimmbar, d.h. Gehalt ist kleiner als die derzeitige Bestimmungsgrenze

Beurteilung: Die Beschaffenheit der Probe entspricht hinsichtlich der untersuchten Parameter den Anforderungen



15.2 Certificate of Animal Food

Teklad Certified Global 18% Protein Rodent Diet

2018C



Batch Number 2018C-012109MA
Certificate Number 09-N551
Date of Manufacture January 21, 2009
Expiry Date October 18, 2009

Certificate of Analysis

Action Limits						
Nutrients	Result		Min	Max	Status	DL
Moisture	13.0	%		13.0		0.1%
Protein (N x 6.25)	17.7	%	17.0	20.0		0.1%
Oil (Acid Hydrolysis)	6.9	%	4.0	7.0		0.01%
Crude Fibre	4.15	%	2.0	6.0		0.5%
Ash	5.3	%	4.0	7.0		0.1%
Calcium	0.93	%	0.7	1.3		100 mg/kg
Phosphorus	0.68	%	0.4	1.1		0.25 mg/kg
Magnesium	0.21	%	0.1	0.3		100 mg/kg
Sodium	0.2	%	0.15	0.35		0.06 %
Potassium	0.63	%	0.5	1.1		600 mg/kg
Chloride	0.41	%	0.25	0.65		0.07 %
Zinc	69.43	mg/kg	40.0	90.0		0.5 mg/kg
Manganese	80.1	mg/kg	80.0	160.0		0.25 mg/kg
Copper	13.03	mg/kg	8.0	20.0		0.25 mg/kg
Iron	177.1	mg/kg	100.0	350.0		0.75 mg/kg
Vitamin A	12.8	IU/g	8.0	20.0		0.8 IU/g
Vitamin E	83.0	IU/kg	50.0	150.0		0.2 IU/g
DL (mg/kg)						
Nitrate	nd	mg/kg		100.0		4.0
Nitrite	0.88	mg/kg		5.0		0.4
Selenium	0.26	mg/kg		0.5		0.02
Arsenic	0.15	mg/kg		1.0		0.025
Cadmium	0.07	mg/kg		0.4		0.006
Lead	0.09	mg/kg		3.0		0.025
Mercury	nd	mg/kg		0.1		0.03
Fluoride	15.0	mg/kg		40.0		15-30
Total DDT	nd	mg/kg		0.1		0.03
Dieldrin	nd	mg/kg		0.02		0.02
Lindane	nd	mg/kg		0.1		0.03
Heptachlor	nd	mg/kg		0.05		0.02
Malathion	nd	mg/kg		0.5		0.05
Phosphorus methyl	nd	mg/kg		5.0		0.05
Other residues*	nd	mg/kg		n/a		
Total PCBs	nd	mg/kg		0.25		0.01
Total Aflatoxins	nd	µg/kg		5.0		0.0005
Microbiology						
Total Viable Count	6,400.0	cfu/g		100,000.0		<10 cfu/g
Mesophilic Spores	3,480.0	cfu/g		10,000.0		<10 cfu/g
Salmonella	nd			0.0		nf in 25g
Coliforms	nd	cfu/g		5.0		10 cfu/g
E. coli	nd	cfu/g		0.0		ng in 1/g
Fungal Spores	30.0	cfu/g		200.0		nf in 10g
Antibiotic Activity	nd			0.0		no

Data entered by:

2009.02.13
11:44:46 -05'00'

harlan

Data checked by:

I have reviewed
this document
2009.02.14
10:11:48 -06'00'

nd - None detected (see the detection table, DL.)

DL - See substance related results, as this certificate is expected on a "no residual" analysis basis.

* See attached for the other residues listed.

Reformers for PCBs and Residues are 70-120%

Page 1 of 2

Teklad Certified Global 18% Protein Rodent Diet

2018C



Batch Number **2018C-012109MA**
Certificate Number 09-N551
Date of Manufacture **January 21, 2009**
Expiry Date **October 18, 2009**

Multi-Residue Screen

Level LOD		Level LOD		Level LOD	
Organophosphates (mg/kg)		Organochlorines (mg/kg)		Dicarbonylides (mg/kg)	
Acephate	nd 0.1	Aldrin	nd 0.02	Iprodione	nd 0.2
Azinphos Methyl	nd 0.2	Chlordane	nd 0.07	Procymidone	nd 0.1
Bromophos Ethyl	nd 0.08	DDT	nd 0.03	Vinclozolin	nd 0.05
Bromophos	nd 0.05	Dieldrin	nd 0.02		
Carbophenothion	nd 0.07	Endosulphan	nd 0.03		
Chlorfenvinphos	nd 0.1	Endrin	nd 0.05		
Chlorpyrifos	nd 0.04	Heptachlor	nd 0.02		
Chlorpyrifos Methyl	nd 0.05	Hexachlorobenzene (HCB)	nd 0.02		
Demeton-S-Methyl	nd 0.1	Hexachlorocyclohexane - a	nd 0.03		
Diazinon	nd 0.05	Hexachlorocyclohexane - b	nd 0.08		
Dichlorvos	nd 0.06	Hexachlorocyclohexane - g	nd 0.02		
Dimethoate	nd 0.1	PCA	nd 0.05		
Ethion	nd 0.07	Quintozene	nd 0.05		
Ethoxyphosphos	nd 0.05	Tecnazene	nd 0.05		
Etrifos	nd 0.05				
Fenchlorphos	nd 0.1				
Fenitrothion	nd 0.06				
Fenthion	nd 0.06				
Fonophos	nd 0.03				
Heptenophos	nd 0.1				
Malathion	nd 0.05				
Methamidophos	nd 0.2				
Methidathion	nd 0.1				
Mevinphos	nd 0.05				
Omethoate	nd 0.1				
Parathion	nd 0.03				
Parathion Methyl	nd 0.03				
Phosalone	nd 0.1				
Phosmet	nd 0.1				
Phosphamidon	nd 0.06				
Pirimiphos Methyl	nd 0.05				
Quinalphos	nd 0.05				
Tolclofos Methyl	nd 0.05				
Triazophos	nd 0.05				

Level LOD		Level LOD	
Organochlorines (mg/kg)		Organonitrogen (mg/kg)	
Aldrin	nd 0.02	Chlorpropham	nd 0.5
Chlordane	nd 0.07	Etridiazole	nd 0.1
DDT	nd 0.03	Fenarimol	nd 0.08
Dieldrin	nd 0.02	Metaxyl	nd 0.2
Endosulphan	nd 0.03	Metribuzin	nd 0.2
Endrin	nd 0.05	Pirimicarb	nd 0.2
Heptachlor	nd 0.02	Propachlor	nd 0.3
Hexachlorobenzene (HCB)	nd 0.02	Propham	nd 0.2
Hexachlorocyclohexane - a	nd 0.03	Propiconazole	nd 0.3
Hexachlorocyclohexane - b	nd 0.08	Propyzamide	nd 0.1
Hexachlorocyclohexane - g	nd 0.02	Thiabendazole	nd 1.0
PCA	nd 0.05	Tolyfluanid	nd 0.07
Quintozene	nd 0.05	Triadimefon	nd 0.2
Tecnazene	nd 0.05	Triadimenol	nd 0.1

Level LOD		Level LOD	
Pyrethroids (mg/kg)		Others (mg/kg)	
Bifenthrin	nd 0.1	Cadusafos	nd 0.05
Cyfluthrin	nd 0.1	Chlorothalonil	nd 0.2
Imada Cyhalothrin	nd 0.1	Chlorthal-dimethyl	nd 0.05
Cypermethrin	nd 0.1	Chlorthion	nd 0.05
Deltamethrin	nd 0.1	Dichlofluanid	nd 0.05
Fenvalerate	nd 0.1	Dicofol	nd 0.1
Flucythrinate	nd 0.1	Diphenylamine	nd 0.1
Permethrin	nd 0.5	Methoxychlor	nd 0.08
Tefluthrin	nd 0.05	Pendimethalin	nd 0.05

Level LOD		Level LOD	
Triazines (mg/kg)		Others (mg/kg)	
Atrazine	nd 0.1	Pentachlorobenzene (PCB)	nd 0.5
Cyanazine	nd 0.1	Tetradifon	nd 0.05
Prometryn	nd 0.1	Tebuconazole	nd 0.3
Simazine	nd 0.1		
Terbutylazine	nd 0.1		
Terbutryn	nd 0.1		

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15.3 Certificate of Animal Bedding

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J. RETTENMAIER & SÖHNE
28. Nov. 2008
EINGEGANGEN

Date 26.11.2008
Customer no. 21751
Page 1 of 3

TEST REPORT

Sample No. 584625

Order No.
Sample Arrival
Sample code

561279
14.11.2008
LIGNOCEL, TIERWOHL und Nageholz Sammelprobe aus Chargen-Nr.:
0510190101-0510190331
0510090101-0510090331
0210500101-0210500331
0300690101-0300690331
0210190101-0210190331
0210290101-0210290331
0330090101-0330090331
0301730101-0301730331
0510530101-0510530331
0090290101-0090290331
0510390101-0510390331
0300190101-0300190331
0512090101-0512090331
0320190101-0320190331
0510990101-0510990331
0211090101-0211090331
0301890101-0301890331
0302390101-0302390331
0511790101-0511790331
9720990101-9720990331
9715090101-9715090331
HF13490101-HF13490331
HF11590101-HF11590331
0211390101-0211390331
0211390101-0211390331

Sample packing

Kunststoffbeutel

Physico-chemical parameters	Unit	Result	Deviation	Substance	Method
Nitrite	mg/kg	<5,0		OM	1 analog §64 LFGB LD 1.00.78/1
Trace-Elements/Heavy-Metals					
Boron	mg/kg	2,17		OM	DIN EN ISO 11885
Fluor	mg/kg	<10		OM	CEN TC 327 W1 00327:2000
Selenium	mg/kg	<0,10		OM	acc. to VOLUFA VI 2.2.2.5. HR-ICPMS
Cadmium	mg/kg	0,10		OM	acc. to VOLUFA VI 2.2.2.5. HR-ICPMS
Lead	mg/kg	0,24		OM	acc. to VOLUFA VI 2.2.2.5. HR-ICPMS
Mercury	mg/kg	<0,02		OM	§64 LFGB LD 00.00-19
Arsenic	mg/kg	0,42		OM	acc. to VOLUFA VI 2.2.2.5. HR-ICPMS



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	Unit	Result	Declaration	Substance	Method
PCB					
PCB 28	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
PCB 52	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
PCB 101	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
PCB 118	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
PCB 126	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
PCB 153	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
PCB 180	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Organochlorous-Pesticides GC-Multiresidueanalysis					
Aldrin	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Dieldrin	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Endrin	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Chlordane alpha	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Chlordane gamma	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Chlordane-ox	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Endosulfan alpha	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Endosulfan beta	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Endosulfan sulfat	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
HCB (Hexachlorbenzene)	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
guthion-HCH	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
HCH-alpha	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
HCH-beta	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
HCH-delta	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
HCH-gamma (gammahexane)	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Heptachlor	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Heptachloropoxide-cis	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Heptachloropoxide-trans	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
o,p'-DDD	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
o,p'-DDE	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
o,p'-DDT	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
p,p'-DDD	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
p,p'-DDE	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
p,p'-DDT	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Methoxychlor	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Quintozene	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Tetrachloro	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Tetradifon	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Nitrofen	mg/kg	<0,005	OM	yes, to §64 LFGB L00-00-34	
Organo-Phosphorous Pesticides GC-Multiresidueanalysis					
Bromophos (-methyl)	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Bromophos-ethyl	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Chlorfenvinphos	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Chlorpyrifos	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Chlorpyrifos-methyl	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Clorhothion	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Diazinone	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Dichlorvos	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Dimethoate	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	
Ethion	mg/kg	<0,010	OM	yes, to §64 LFGB L00-00-34	



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Date 26.11.2006
Customer no. 21751
Page 3 of 3

Sample No. 584625

	Unit	Result	Declaration	Substance	Method
Fenitrothion	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Fenitron	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Malathion	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Mecarbate	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Methidathion	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Parathion-ethyl	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Parathion-methyl	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Phosphor-methyl	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Phosphor-methyl	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Profenofos	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	
Sulfotop	mg/kg	<0,010	OM	acc. to §64 LFGB L02.00-34	

Other Analyses

Nitrate mg/kg <50,0 OM according §64 LFGB L25.00-1

Explanation: "n.d." n.d., not detected, below limit of detection

This actual limit of detection can be different to the standard value for a particular analysis due to matrix effects or insufficient sample volume.

Remarks: OM=organic matter, OM-free matter

The analytical results are valid for mg delivered sample material only. The holding period is the time between the receipt of the sample and the reporting date. Validation of results is not possible for samples of unknown origin.

