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Via Courier

TSCA Confidential Business Information Center (7407M)
EPA East - Room 6428 Attn: Section 8(e)
U.S. Environmental Protection Agency
1201 Constitution Avenue, N.W.
Washington, DC 20004-3302

**Re: TSCA Section 8(e) submission for vanadium pentoxide;
preliminary results from mechanistic studies**

Dear Sir or Madam:

On behalf of its members, the Vanadium Producers and Reclaimers Association (VPRA) is advising the U.S. Environmental Protection Agency (EPA) concerning preliminary results from a nose-only, 16-day inhalation study on vanadium pentoxide sponsored by the Vanadium Technology Program, US Army, Grant No. W81XWH-09-2-0066.

This on-going study is designed to identify factors that might be useful in formulating a proposed mode of action for the pulmonary effects reported in the NTP study of vanadium pentoxide (NTP No. 507, 2002). The NTP in 2002 reported an increased incidence of pulmonary inflammation in B₆C₃F₁ mice and Fischer 344 rats exposed to vanadium pentoxide by whole body inhalation exposure for two years. In that study, pulmonary tumors were increased in male and female treated mice, but not increased in treated male rats. In female rats, the pulmonary tumor response was equivocal. The absence of a dose-related response in pulmonary tumor incidence was not attributable to mortality differences between groups and an explanation for the difference in response in rats and mice was not apparent. There were no signs of toxicity at any other site in mice or rats (NTP, 2002).

The current study was conducted in female B₆C₃F₁ mice exposed to atmospheres containing vanadium pentoxide at concentrations of 0.25, 1 or 4 mg/m³, for 6 hours a day for 16 days by nose-only exposure. Investigations included measurement of vanadium in the lungs, histopathology of the lung for microscopically observable tissue responses, comet assay of both pulmonary cells and of bronchio-alveolar lavage (BAL) cells to determine DNA strand breakage, measurement of several DNA adducts, measurement of cell proliferation (two methods) after 7 and 16 days of exposure, and measurement of biomarkers of

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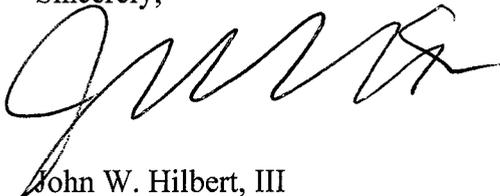
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biochemical response (α -tocopherol, reduced and total glutathione with computation of their ratio and F2-isoprostane measurements for lipid oxidation). The in-life phase has concluded and analyses of the results are being reported and/or are in progress.

A draft study report received September 15, 2009, while incomplete, did not appear to contain any new significant adverse effect information requiring reporting under section 8(e) of the Toxic Substances Control Act (TSCA). The draft report, which does not yet contain all of the written information that one would expect to see in the final study report, concludes that the lowest dose, 0.25 mg/m³, is a no effect level. For further background, the draft study report from the laboratory on September 15, 2009, is enclosed, for which the necessary quality control analyses have yet to be conducted.

A subsequent site visit report received on October 15, 2009, contains additional findings not available in the September 15, 2009, draft report. The findings of this site visit are being submitted under section 8(e) of TSCA. The purpose of the site visit was to review the results of the analyses of DNA from samples of lung taken from female B6C3F1 mice. The attached site visit report describes the preliminary observations being reported under section 8(e). Additional analyses were recommended to evaluate these findings that are underway at this time. The significance of the preliminary findings are unknown and they have not yet been incorporated into the draft study report.

Sincerely,

A handwritten signature in black ink, appearing to read "John W. Hilbert, III". The signature is fluid and cursive, with a large initial "J" and a long, sweeping underline.

John W. Hilbert, III

Enclosures:

(1) Site visit report, 13 October 2009, Laboratoire Lésions des Acides Nucléiques, Ravanat, Douki, McGregor

(2) Draft report, Vanadium Pentoxide, 16-Day Inhalation Toxicity Study in Female Mice, Harlan Laboratories Ltd. 2009

TSCA 8(e) Submission for:

Harlan Laboratories Ltd Study

Vanadium Pentoxide

16-Day Inhalation Toxicity Study in Female Mice

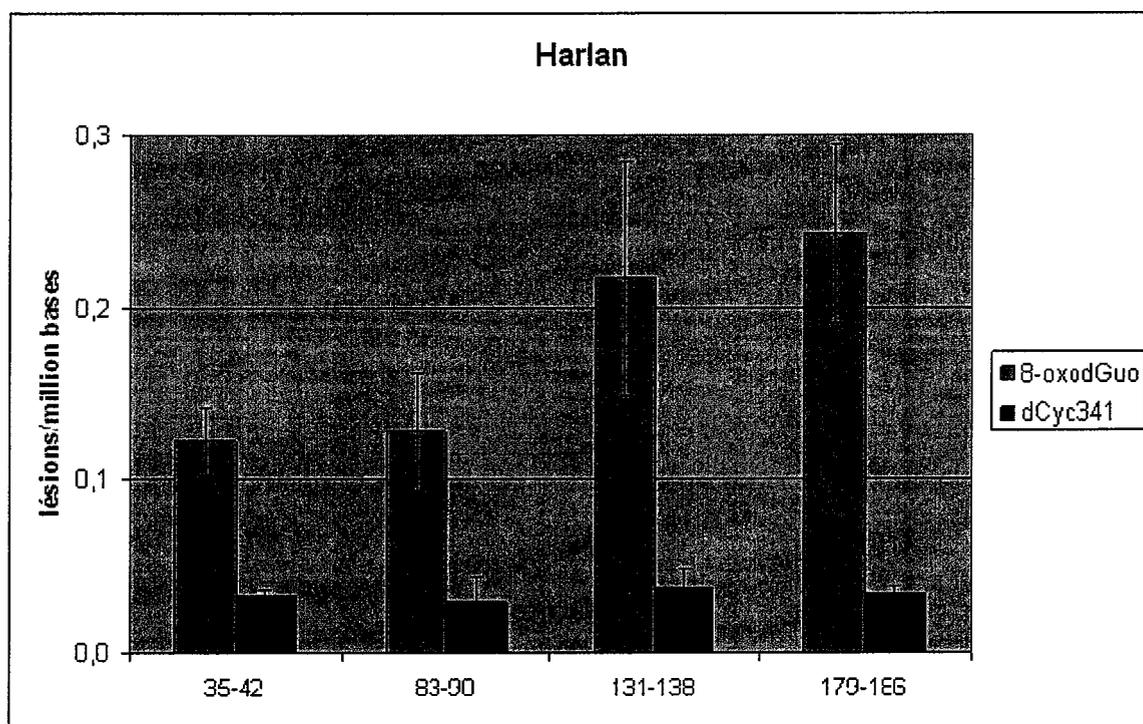
Visit report. 13 October 2009.

Laboratoire Lésions des Acides Nucléiques,
INAC/SCIB (UMR-E-3 CEA-UJF),
CEA Grenoble,
17 rue des Martyrs,
F38054 Grenoble cédex 9.

Jean-Luc Ravanat, Thierry Douki, Douglas McGregor.

The purpose of the visit was to review the results of the analyses of DNA from samples of lung taken from female B6C3F1 mice that had been exposed to V2O5 at atmospheric concentrations of 0, 0.25, 1 and 4 mg/m³ 6h/day for 16 days.

The samples had been frozen and transported in dry ice from Füllinsdorf, Switzerland to Grenoble, France for the analysis. Most lesions looked for were below the limits of quantification (these limits will be given in the report to be issued). Only two types of lesion were found within the range of quantification: a deoxycytidine residue, dCyd341, which should no statistically significant change with exposure, and a deoxyguanosine residue, 8-oxodGuo. For this residue there was no significant difference in concentration between the 0 and 0.25 mg/m³ exposure levels, but at 1 and 4 mg/mg³ there were increases of about two-fold that were clearly statistically significant. The charted means and error bars are shown below; the actual data are given in the Appendix.



[NB the scaling is wrong: it should be 0.0; 0.2; 0.4 and 0.6 lesions/10⁶ bases]

In addition, however, Dr. Ravanat said that he would like to re-run the remaining samples for thymidine oxy-adducts under different hplc-ms conditions of separation

and analysis. He had already analysed for such adducts (and did not find any), but the conditions were not ideal. There is practical reason of doing this and I agreed with the proposal. Fortunately we are working with a lab that is flexible and we do not need modifications to contracts, etc. in order to make these changes. It is hoped that the re-analyses will be done this week.

The issue is that 8-oxodGuo is so far the only DNA adduct found and this does not lead to DNA strand breakage. Almost every other lesion would produce strand breakage and, hence a significant result in the comet assay. In Ravanat's experience, the comet assay has been more sensitive than the chemical adduct assay in the detection of 8-oxodGuo lesions (although not specific, of course). The comet assay would yield negative results only if no adducts were formed in addition to 8-oxodGuo. This is the reason for modifying the assay conditions. If adducts in addition to 8-oxoGuo are formed then we will have to take another look at the comet assay and why it did not show any effect. On the other hand, if other adducts are not formed then there are three possible reasons for the outcome:

1. 8-oxodGuo was formed by *singlet oxygen ($^1\text{O}_2$);
2. 8-oxodGuo was formed by a one-electron oxidation;
3. the repair enzymes that remove the naturally occurring 8-oxodGuo every day from DNA are inhibited by V2O5 either oxidation of important sites on the enzymes or distortion of the enzyme (or of DNA itself) by particular species of V ions.

According to possibility 3, the 8-oxodGuo either may not have been formed by V2O5 at all, or a fraction of the daily yield of 8-oxodGuo may have been derived from V2O5.

For possibilities 1 and 2 it is unlikely that other bases would be oxidised, hence, there would be no DNA strand breakage and no significant comet results. For possibility 3 also there would be no significant comet result. This outcome (3) due to enzyme inhibition could be tested by *in vitro* experiments since the repair enzymes are commercially available.

I have communicated with John (Duffus) about this who has provided some papers that may be relevant.

A follow-up suggestion made was that V2O5 treated cells could be treated with Fpg protein before electrophoresis in the comet assay. The Fpg protein (a glycosylase) converts 8-oxodGuo to strand breaks that are then detectable in the comet assay. In effect, this is simply a different way of demonstrating the presence of 8-oxodGuo. It is a confirmatory assay.

Yet another follow-up experiment would be to see how long it took the 8-oxodGuo lesions to be repaired after the end of exposure. This might enable a better appreciation of the magnitude of the effect being produced and point to whether (apparently) oxygen-generated damage could account for the tumour incidence changes seen in mice.

My opinion with which the others agreed was that, at this stage, it will be more productive to pursue the comparative aspects of the pathology by repeating the study in rats. However, there is great curiosity as to the speciation of vanadium after it comes into contact with lung cells.

The meeting was, I believe, very productive and could - perhaps - be bringing us closer to an understanding of V2O5 carcinogenicity.

*The idea that singlet oxygen only produces 8-oxodGuo comes from experiments with chemicals that only produce $^1\text{O}_2$. E.g., the endoperoxide *N,N'*-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide (DHPNO₂), which releases $^1\text{O}_2$ upon thermal decomposition at 37°C. Ravanat et al. (2004) Singlet oxygen-mediated damage to cellular DNA determined by the comet assay associated with DNA repair enzymes. *Biol. Chem.*, **385**:17-20.

For

Appendix

Data from measurements of DNA lesions in female B6C3F1 mice exposed to V2O5 by inhalation.

	lesion/10 ⁶ bases	
	8-oxodGuo	dCyd341
harlan35	0,15	0,032
harlan36	0,11	0,036
harlan37	0,09	0,026
harlan38	0,11	0,035
harlan39	0,14	0,032
harlan40	0,12	0,032
harlan41	0,12	0,035
harlan42	0,13	0,038
harlan83	0,17	0,038
harlan84	0,16	0,043
harlan85	0,08	0,039
harlan86	0,09	0,025
harlan87	0,12	0,007
harlan88	0,13	0,042
harlan89	0,15	0,013
harlan90	0,11	0,037

	lesion/10 ⁶ bases	
	8-oxodGuo	dCyd341
harlan131	0,17	0,043
harlan132	0,14	0,035
harlan133	0,24	0,050
harlan134	0,18	0,044
harlan135	0,23	0,045
harlan136	0,34	0,015
harlan137	0,28	0,028
harlan138	0,16	0,041
harlan179	0,24	0,027
harlan180	0,30	0,030
harlan181	0,22	0,041
harlan182	0,20	0,035
harlan183	0,19	0,032
harlan184	n.d.	n.d.
harlan185	0,22	0,039
harlan186	0,33	0,034

REPORT



Vanadium Pentoxide

16-Day Inhalation Toxicity Study in Female Mice

Study Director: Dr. D. Schuler

Test Facility: **Harlan Laboratories Ltd.**
Wölferstrasse 4
4414 Füllinsdorf / Switzerland

Sponsor: **Advanced Technology Institute**
5300 International Blvd
N. Charleston, SC 29418 / USA

Study Identification: Harlan Laboratories Study **A94206**

Version: Draft 1

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BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 3 (MID DOSE)

Animal	118	119	120	121	122	123	124
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.6	-4.5	-7.9	-6.7	-2.2	-1.8
	16	-0.1	-1.6	-0.6	1.7	9.4	1.6
2.6							
Animal	125	126	127	128	129	130	131
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-2.2	-3.4	3.4	3.6	-8.7	4.5
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	125	126	127	128	129	130	131
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.5	-11.5	-6.5	-0.4	-2.4	-6.4
	16	1.1	-1.2	5.9	10.1	5.2	1.2
3.1							
3.1							
Animal	132	133	134	135	136	137	138
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.7	12.2	4.6	1.0	5.6	10.9
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	132	133	134	135	136	137	138
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-1.4	1.1	-6.0	-2.6	-3.0	-1.6
	16	-1.8	5.2	-2.1	4.1	-2.3	5.5
-2.2							
-3.0							
Animal	139	140	141	142	143	144	
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.4	7.0	-1.9	6.2	2.7	2.7
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	139	140	141	142	143	144	
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-4.0	-6.9	-2.4	-1.3	-0.3	1.2
	16	-0.7	-0.5	2.9	0.9	2.8	5.8

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 4 (HIGH DOSE)								
Animal	145	146	147	148	149	150	151	
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.7	5.4	-2.6	3.1	7.8	4.3	9.8
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	145	146	147	148	149	150	151	
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	4.6	-1.5	-1.1	0.8	1.4	1.5	-1.8
	16	-4.6	-6.6	-11.0	-4.1	-9.6	-5.7	---
Animal	152	153	154	155	156	157	158	
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	4.7	-1.8	7.9	-1.2	4.8	4.8	3.2
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	152	153	154	155	156	157	158	
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.1	-4.6	-9.1	-15.5	-3.4	0.8	-0.9
	16	---	---	---	---	---	-0.1	2.1
Animal	159	160	161	162	163	164	165	
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.8	1.4	6.7	2.1	3.7	0.6	-0.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	159	160	161	162	163	164	165	
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.0	2.3	2.0	2.7	-5.3	-8.5	-6.7
	16	6.2	3.2	8.4	1.6	-1.1	-0.6	3.5
Animal	166	167	168	169	170	171	172	
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	4.4	1.7	2.4	-1.9	0.7	0.3	2.7
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 3 (MID DOSE)

Animal		118	119	120	121	122	123	124
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.6	-4.5	-7.9	-6.7	-2.2	-1.8	-5.5
	16	-0.1	-1.6	-0.6	1.7	9.4	1.6	2.6
Animal		125	126	127	128	129	130	131
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-2.2	-3.4	3.4	3.6	-8.7	4.5	1.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		125	126	127	128	129	130	131
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.5	-11.5	-6.5	-0.4	-2.4	-6.4	3.1
	16	1.1	-1.2	5.9	10.1	5.2	1.2	3.1
Animal		132	133	134	135	136	137	138
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.7	12.2	4.6	1.0	5.6	10.9	2.7
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		132	133	134	135	136	137	138
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-1.4	1.1	-6.0	-2.6	-3.0	-1.6	-2.2
	16	-1.8	5.2	-2.1	4.1	-2.3	5.5	-3.0
Animal		139	140	141	142	143	144	
ACCLIMATISATION								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	
	8	0.4	7.0	-1.9	6.2	2.7	2.7	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal		139	140	141	142	143	144	
TREATMENT								
Day	1	0.0	0.0	0.0	0.0	0.0	0.0	
	8	-4.0	-6.9	-2.4	-1.3	-0.3	1.2	
	16	-0.7	-0.5	2.9	0.9	2.8	5.8	

BODY WEIGHT GAIN (%)

MAIN STUDY

FEMALES

Group 4 (HIGH DOSE)

Animal	145	146	147	148	149	150	151
--------	-----	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.7	5.4	-2.6	3.1	7.8	4.3	9.8
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal	145	146	147	148	149	150	151
--------	-----	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	4.6	-1.5	-1.1	0.8	1.4	1.5	-1.8
	16	-4.6	-6.6	-11.0	-4.1	-9.6	-5.7	---

Animal	152	153	154	155	156	157	158
--------	-----	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	4.7	-1.8	7.9	-1.2	4.8	4.8	3.2
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal	152	153	154	155	156	157	158
--------	-----	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.1	-4.6	-9.1	-15.5	-3.4	0.8	-0.9
	16	---	---	---	---	---	-0.1	2.1

Animal	159	160	161	162	163	164	165
--------	-----	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.8	1.4	6.7	2.1	3.7	0.6	-0.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal	159	160	161	162	163	164	165
--------	-----	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.0	2.3	2.0	2.7	-5.3	-8.5	-6.7
	16	6.2	3.2	8.4	1.6	-1.1	-0.6	3.5

Animal	166	167	168	169	170	171	172
--------	-----	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	4.4	1.7	2.4	-1.9	0.7	0.3	2.7
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 4 (HIGH DOSE)

Animal		166	167	168	169	170	171	172
--------	--	-----	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-5.2	-5.7	-4.0	-0.7	-0.5	-8.6	-4.1
	16	6.5	7.5	7.6	9.4	-0.6	-7.8	-5.7

Animal		173	174	175	176	177	178	179
--------	--	-----	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.3	0.2	9.5	7.5	-0.1	-3.4	-2.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal		173	174	175	176	177	178	179
--------	--	-----	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-5.0	-6.8	-4.9	1.2	-8.7	-4.3	0.1
	16	6.0	-10.8	-1.2	5.8	1.0	3.0	-2.8

Animal		180	181	182	183	184	185	186
--------	--	-----	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	10.7	0.9	4.9	7.9	3.7	1.6	0.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal		180	181	182	183	184	185	186
--------	--	-----	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.1	3.3	-1.3	0.7	2.6	-1.1	-1.0
	16	0.0	8.7	3.8	0.9	4.0	4.0	0.9

Animal		187	188	189	190	191	192
--------	--	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.4	5.2	-0.9	5.8	8.9	1.9
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---

Animal		187	188	189	190	191	192
--------	--	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.1	0.6	0.3	0.1	-3.3	-0.1
	16	6.6	0.7	12.1	0.5	4.2	2.9

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 5 (POS. CTRL.)

Animal	193	194	195	196	197	198
--------	-----	-----	-----	-----	-----	-----

ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.4	6.5	-0.7	12.4	6.8	-0.3
	12	9.9	12.0	7.0	11.9	8.3	-0.6
	19	21.0	19.5	12.5	15.4	17.4	3.1

Animal	193	194	195	196	197	198
--------	-----	-----	-----	-----	-----	-----

TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	---	---	---	---	---	---
	16	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 4 (HIGH DOSE)

Animal	166	167	168	169	170	171	172
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-5.2	-5.7	-4.0	-0.7	-0.5	-8.6
	16	6.5	7.5	7.6	9.4	-0.6	-7.8
							-5.7
Animal	173	174	175	176	177	178	179
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.3	0.2	9.5	7.5	-0.1	-3.4
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	173	174	175	176	177	178	179
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-5.0	-6.8	-4.9	1.2	-8.7	-4.3
	16	6.0	-10.8	-1.2	5.8	1.0	3.0
							-2.8
Animal	180	181	182	183	184	185	186
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	10.7	0.9	4.9	7.9	3.7	1.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	180	181	182	183	184	185	186
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.1	3.3	-1.3	0.7	2.6	-1.1
	16	0.0	8.7	3.8	0.9	4.0	4.0
							0.9
Animal	187	188	189	190	191	192	
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.4	5.2	-0.9	5.8	8.9	1.9
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	187	188	189	190	191	192	
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.1	0.6	0.3	0.1	-3.3	-0.1
	16	6.6	0.7	12.1	0.5	4.2	2.9

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 5 (POS. CTRL.)

Animal	193	194	195	196	197	198
ACCLIMATISATION						
Day	1	0.0	0.0	0.0	0.0	0.0
	8	8.4	6.5	-0.7	12.4	6.8
	12	9.9	12.0	7.0	11.9	8.3
	19	21.0	19.5	12.5	15.4	17.4
						3.1
Animal	193	194	195	196	197	198
TREATMENT						
Day	1	0.0	0.0	0.0	0.0	0.0
	8	---	---	---	---	---
	16	---	---	---	---	---

ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE

Date : 26-AUG-09 10:21:54

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 3 - VANADIUM

Satellite: A - ALLOCATION A

ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE

Comments

Exclusions

Not Reported

Selection of Organs

All Lu.Lob

Body W.

Further organs not reported

Animals without scheduled necropsy

ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE

Date : 26-AUG-09 10:21:54

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 3 - VANADIUM

Satellite: A - ALLOCATION A

ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE

Comments

Exclusions

Not Reported

Selection of Organs

All Lu.Lob

Body W.

Further organs not reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	ALL LU.LOB
1	20.3	0.122
2	19.4	0.126
3	19.5	0.124
4	20.3	0.119
5	20.0	0.116
6	18.7	0.115

Group 2 (LOW DOSE)

Animal	BODY W.	ALL LU.LOB
49	19.2	0.124
50	19.6	0.133
51	18.3	0.120
52	20.0	0.127
53	19.1	0.117
54	19.6	0.125

Group 3 (MID DOSE)

Animal	BODY W.	ALL LU.LOB
97	19.4	0.152
98	22.3	0.150
99	19.2	0.142
100	19.0	0.136
101	20.7	0.152
102	20.7	0.149

ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W.	ALL LU.LOB
145	19.2	0.197
146	18.9	0.192
147	15.6	0.154
148	20.8	0.207
149	18.2	0.185
150	18.8	0.179

**ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	ALL LU.LOB
1	20.3	0.122
2	19.4	0.126
3	19.5	0.124
4	20.3	0.119
5	20.0	0.116
6	18.7	0.115

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Animal	BODY W.	ALL LU.LOB
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99	19.2	0.142
100	19.0	0.136
101	20.7	0.152
102	20.7	0.149

**ORGAN WEIGHTS (GRAM)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	ALL LU.LOB
145	19.2	0.197
146	18.9	0.192
147	15.6	0.154
148	20.8	0.207
149	18.2	0.185
150	18.8	0.179

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	ALL LU.LOB (%)
1	20.3	0.601
2	19.4	0.649
3	19.5	0.636
4	20.3	0.586
5	20.0	0.580
6	18.7	0.615

Group 2 (LOW DOSE)

Animal	BODY W. (GRAM)	ALL LU.LOB (%)
49	19.2	0.646
50	19.6	0.679
51	18.3	0.656
52	20.0	0.635
53	19.1	0.613
54	19.6	0.638

Group 3 (MID DOSE)

Animal	BODY W. (GRAM)	ALL LU.LOB (%)
97	19.4	0.784
98	22.3	0.673
99	19.2	0.740
100	19.0	0.716
101	20.7	0.734
102	20.7	0.720

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	ALL LU.LOB (%)
145	19.2	1.026
146	18.9	1.016
147	15.6	0.987
148	20.8	0.995
149	18.2	1.016
150	18.8	0.952

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	ALL LU.LOB (%)
1	20.3	0.601
2	19.4	0.649
3	19.5	0.636
4	20.3	0.586
5	20.0	0.580
6	18.7	0.615

Group 2 (LOW DOSE)

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54	19.6	0.638

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99	19.2	0.740
100	19.0	0.716
101	20.7	0.734
102	20.7	0.720

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	ALL LU.LOB (%)
145	19.2	1.026
146	18.9	1.016
147	15.6	0.987
148	20.8	0.995
149	18.2	1.016
150	18.8	0.952

**ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROFILARATION**

Date : 26-AUG-09 10:24:11

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 1 - 7 TAGE

Satellite: B - ALLOCATION B

ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROFILARATION

Comments

Exclusions

Not Reported

Selection of Organs

All organs reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROFILARATION**

Date : 26-AUG-09 10:24:11

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 1 - 7 TAGE

Satellite: B - ALLOCATION B

ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROFILARATION

Comments

Exclusions

Not Reported

Selection of Organs

All organs reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	LUNGS
7	19.9	0.156
8	18.6	0.151
9	20.6	0.179
10	19.2	0.151
11	17.9	0.160
12	18.5	0.153

Group 2 (LOW DOSE)

Animal	BODY W.	LUNGS
55	20.7	0.162
56	19.4	0.156
57	18.5	0.146
58	20.5	0.156
59	20.1	0.166
60	17.1	0.144

Group 3 (MID DOSE)

Animal	BODY W.	LUNGS
103	19.6	0.160
104	18.9	0.146
105	17.2	0.136
106	18.6	0.153
107	20.3	0.169
108	19.8	0.154

ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROLIFERATION
AFTER 7 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W.	LUNGS
151	20.1	0.177
152	20.6	0.195
153	18.8	0.166
154	18.5	0.169
155	17.8	0.178
156	19.1	0.175

**ORGAN WEIGHTS (GRAM)
ALLOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	LUNGS
7	19.9	0.156
8	18.6	0.151
9	20.6	0.179
10	19.2	0.151
11	17.9	0.160
12	18.5	0.153

Group 2 (LOW DOSE)

Animal	BODY W.	LUNGS
55	20.7	0.162
56	19.4	0.156
57	18.5	0.146
58	20.5	0.156
59	20.1	0.166
60	17.1	0.144

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103	19.6	0.160
104	18.9	0.146
105	17.2	0.136
106	18.6	0.153
107	20.3	0.169
108	19.8	0.154

**ORGAN WEIGHTS (GRAM)
ALOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	LUNGS
151	20.1	0.177
152	20.6	0.195
153	18.8	0.166
154	18.5	0.169
155	17.8	0.178
156	19.1	0.175

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. B / CELL PROLIFERATION
AFTER 7 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	LUNGS (%)
7	19.9	0.784
8	18.6	0.812
9	20.6	0.869
10	19.2	0.786
11	17.9	0.894
12	18.5	0.827

Group 2 (LOW DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
55	20.7	0.783
56	19.4	0.804
57	18.5	0.789
58	20.5	0.761
59	20.1	0.826
60	17.1	0.842

Group 3 (MID DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
103	19.6	0.816
104	18.9	0.772
105	17.2	0.791
106	18.6	0.823
107	20.3	0.833
108	19.8	0.778

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
151	20.1	0.881
152	20.6	0.947
153	18.8	0.883
154	18.5	0.914
155	17.8	1.000
156	19.1	0.916

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. B / CELL PROLIFERATION
AFTER 7 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	LUNGS (%)
7	19.9	0.784
8	18.6	0.812
9	20.6	0.869
10	19.2	0.786
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105	17.2	0.791
106	18.6	0.823
107	20.3	0.833
108	19.8	0.778

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. B / CELL PROLIFERATION
AFTER 7 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
151	20.1	0.881
152	20.6	0.947
153	18.8	0.883
154	18.5	0.914
155	17.8	1.000
156	19.1	0.916

**ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION**

Date : 26-AUG-09 10:26:06

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 2 - 16 Tage

Satellite: C - ALLOCATION C

ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION

Comments

Exclusions

Not Reported

Selection of Organs

All organs reported

Animals without scheduled necropsy

109

**ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION**

Date : 26-AUG-09 10:26:06

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 2 - 16 Tage

Satellite: C - ALLOCATION C

ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION

Comments

Exclusions

Not Reported

Selection of Organs

All organs reported

Animals without scheduled necropsy

109

**ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	LUNGS
13	18.9	0.132
14	17.9	0.129
15	18.1	0.137
16	21.1	0.149
17	19.0	0.141
18	18.3	0.137

Group 2 (LOW DOSE)

Animal	BODY W.	LUNGS
61	20.6	0.172
62	21.5	0.153
63	17.5	0.127
64	17.6	---
65	20.3	0.145
66	19.0	0.157

Group 3 (MID DOSE)

Animal	BODY W.	LUNGS
109	---	---
110	18.2	0.155
111	20.2	0.179
112	19.3	0.167
113	20.5	0.167
114	19.2	0.174

**ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	LUNGS
157	19.7	0.206
158	20.2	0.208
159	17.3	0.184
160	18.0	0.196
161	18.4	0.204
162	17.6	0.194

**ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	LUNGS
13	18.9	0.132
14	17.9	0.129
15	18.1	0.137
16	21.1	0.149
17	19.0	0.141
18	18.3	0.137

Group 2 (LOW DOSE)

Animal	BODY W.	LUNGS
61	20.6	0.172
62	21.5	0.153
63	17.5	0.127
64	17.6	---
65	20.3	0.145
66	19.0	0.157

Group 3 (MID DOSE)

Animal	BODY W.	LUNGS
109	---	---
110	18.2	0.155
111	20.2	0.179
112	19.3	0.167
113	20.5	0.167
114	19.2	0.174

**ORGAN WEIGHTS (GRAM)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	LUNGS
157	19.7	0.206
158	20.2	0.208
159	17.3	0.184
160	18.0	0.196
161	18.4	0.204
162	17.6	0.194

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	LUNGS (%)
13	18.9	0.698
14	17.9	0.721
15	18.1	0.757
16	21.1	0.706
17	19.0	0.742
18	18.3	0.749

Group 2 (LOW DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
61	20.6	0.835
62	21.5	0.712
63	17.5	0.726
64	17.6	---
65	20.3	0.714
66	19.0	0.826

Group 3 (MID DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
109	---	---
110	18.2	0.852
111	20.2	0.886
112	19.3	0.865
113	20.5	0.815
114	19.2	0.906

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. C / CELL PROLIFERATION
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
157	19.7	1.046
158	20.2	1.030
159	17.3	1.064
160	18.0	1.089
161	18.4	1.109
162	17.6	1.102

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	LUNGS (%)
13	18.9	0.698
14	17.9	0.721
15	18.1	0.757
16	21.1	0.706
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111	20.2	0.886
112	19.3	0.865
113	20.5	0.815
114	19.2	0.906

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
157	19.7	1.046
158	20.2	1.030
159	17.3	1.064
160	18.0	1.089
161	18.4	1.109
162	17.6	1.102

ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG

Date : 26-AUG-09 10:30:57

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 4 - 16 TAGE, ALL. D

Satellite: D - ALLOCATION D

ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG

Comments

Exclusions

Not Reported

Selection of Organs

Body W.

Left Lobe

Right Lungs

Further organs not reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG**

Date : 26-AUG-09 10:30:57

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 4 - 16 TAGE, ALL. D

Satellite: D - ALLOCATION D

ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG

Comments

Exclusions

Not Reported

Selection of Organs

Body W.

Left Lobe

Right Lungs

Further organs not reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
19	21.7	0.087	0.046
20	18.7	0.079	0.038
21	20.5	0.091	0.046
22	20.6	0.095	0.048
23	20.3	0.088	0.042
24	19.2	0.076	0.040
25	20.8	0.086	0.041
26	21.0	0.085	0.042

Group 2 (LOW DOSE)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
67	18.2	0.098	0.050
68	19.2	0.105	0.047
69	18.2	0.095	0.045
70	18.8	0.098	0.045
71	20.6	0.100	0.048
72	19.3	0.095	0.044
73	20.1	0.105	0.049
74	19.4	0.101	0.046

Group 3 (MID DOSE)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
115	19.8	0.115	0.056
116	17.4	0.102	0.049
117	19.9	0.111	0.055
118	19.4	0.114	0.054
119	17.8	0.093	0.045
120	18.7	0.098	0.050
121	18.8	0.120	0.053
122	23.0	0.104	0.053

**ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
163	19.6	0.119	0.061
164	18.5	0.130	0.063
165	19.3	0.125	0.061
166	18.7	0.125	0.059
167	19.4	0.139	0.070
168	19.8	0.131	0.063
169	20.6	0.130	0.062
170	17.7	0.113	0.053

**ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
19	21.7	0.087	0.046
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21	20.5	0.091	0.046
22	20.6	0.095	0.048
23	20.3	0.088	0.042
24	19.2	0.076	0.040
25	20.8	0.086	0.041
26	21.0	0.085	0.042

Group 2 (LOW DOSE)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
67	18.2	0.098	0.050
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69	18.2	0.095	0.045
70	18.8	0.098	0.045
71	20.6	0.100	0.048
72	19.3	0.095	0.044
73	20.1	0.105	0.049
74	19.4	0.101	0.046

Group 3 (MID DOSE)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
115	19.8	0.115	0.056
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118	19.4	0.114	0.054
119	17.8	0.093	0.045
120	18.7	0.098	0.050
121	18.8	0.120	0.053
122	23.0	0.104	0.053

**ORGAN WEIGHTS (GRAM)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	RIGHT LUNG	LEFT LOBE
163	19.6	0.119	0.061
164	18.5	0.130	0.063
165	19.3	0.125	0.061
166	18.7	0.125	0.059
167	19.4	0.139	0.070
168	19.8	0.131	0.063
169	20.6	0.130	0.062
170	17.7	0.113	0.053

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	RIGHT LUNG (%)	LEFT LOBE (%)
19	21.7	0.401	0.212
20	18.7	0.422	0.203
21	20.5	0.444	0.224
22	20.6	0.461	0.233
23	20.3	0.433	0.207
24	19.2	0.396	0.208
25	20.8	0.413	0.197
26	21.0	0.405	0.200

Group 2 (LOW DOSE)

Animal	BODY W. (GRAM)	RIGHT LUNG (%)	LEFT LOBE (%)
67	18.2	0.538	0.275
68	19.2	0.547	0.245
69	18.2	0.522	0.247
70	18.8	0.521	0.239
71	20.6	0.485	0.233
72	19.3	0.492	0.228
73	20.1	0.522	0.244
74	19.4	0.521	0.237

Group 3 (MID DOSE)

Animal	BODY W. (GRAM)	RIGHT LUNG (%)	LEFT LOBE (%)
115	19.8	0.581	0.283
116	17.4	0.586	0.282
117	19.9	0.558	0.276
118	19.4	0.588	0.278
119	17.8	0.522	0.253
120	18.7	0.524	0.267
121	18.8	0.638	0.282
122	23.0	0.452	0.230

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	RIGHT LUNG (%)	LEFT LOBE (%)
163	19.6	0.607	0.311
164	18.5	0.703	0.341
165	19.3	0.648	0.316
166	18.7	0.668	0.316
167	19.4	0.716	0.361
168	19.8	0.662	0.318
169	20.6	0.631	0.301
170	17.7	0.638	0.299

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 1 (AIR CONTROL)

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22	20.6	0.461	0.233
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24	19.2	0.396	0.208
25	20.8	0.413	0.197
26	21.0	0.405	0.200

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70	18.8	0.521	0.239
71	20.6	0.485	0.233
72	19.3	0.492	0.228
73	20.1	0.522	0.244
74	19.4	0.521	0.237

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116	17.4	0.586	0.282
117	19.9	0.558	0.276
118	19.4	0.588	0.278
119	17.8	0.522	0.253
120	18.7	0.524	0.267
121	18.8	0.638	0.282
122	23.0	0.452	0.230

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

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166	18.7	0.668	0.316
167	19.4	0.716	0.361
168	19.8	0.662	0.318
169	20.6	0.631	0.301
170	17.7	0.638	0.299

**ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG**

Date : 26-AUG-09 10:34:38

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 5 - 16 TAGE, ALL. E

Satellite: E - ALLOCATION E

ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG

Comments

Exclusions

Not Reported

Selection of Organs

All organs reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG**

Date : 26-AUG-09 10:34:38

User : HUH

Report Data

Types: Weights
 Body Ratios

Necropsies: 5 - 16 TAGE, ALL. E

Satellite: E - ALLOCATION E

ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG

Comments

Exclusions

Not Reported

Selection of Organs

All organs reported

Animals without scheduled necropsy

**ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	LUNGS
27	20.1	0.137
28	18.5	0.142
29	20.7	0.143
30	17.9	0.146
31	19.2	0.140
32	17.9	0.141
33	18.4	0.136
34	19.0	0.136

Group 2 (LOW DOSE)

Animal	BODY W.	LUNGS
75	20.2	0.154
76	17.0	0.124
77	17.0	0.118
78	18.2	0.131
79	18.9	0.140
80	21.7	0.154
81	17.5	0.132
82	18.4	0.138

Group 3 (MID DOSE)

Animal	BODY W.	LUNGS
123	19.2	0.167
124	19.2	0.160
125	20.7	0.143
126	18.8	0.141
127	19.9	0.163
128	19.3	0.168
129	17.6	0.159
130	17.8	0.149

**ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	LUNGS
171	18.2	0.192
172	17.8	0.189
173	19.6	0.183
174	17.3	0.178
175	19.8	0.198
176	19.7	0.236
177	19.1	0.208
178	18.2	0.189

**ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W.	LUNGS
27	20.1	0.137
28	18.5	0.142
29	20.7	0.143
30	17.9	0.146
31	19.2	0.140
32	17.9	0.141
33	18.4	0.136
34	19.0	0.136

Group 2 (LOW DOSE)

Animal	BODY W.	LUNGS
75	20.2	0.154
76	17.0	0.124
77	17.0	0.118
78	18.2	0.131
79	18.9	0.140
80	21.7	0.154
81	17.5	0.132
82	18.4	0.138

Group 3 (MID DOSE)

Animal	BODY W.	LUNGS
123	19.2	0.167
124	19.2	0.160
125	20.7	0.143
126	18.8	0.141
127	19.9	0.163
128	19.3	0.168
129	17.6	0.159
130	17.8	0.149

**ORGAN WEIGHTS (GRAM)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 4 (HIGH DOSE)

Animal	BODY W.	LUNGS
171	18.2	0.192
172	17.8	0.189
173	19.6	0.183
174	17.3	0.178
175	19.8	0.198
176	19.7	0.236
177	19.1	0.208
178	18.2	0.189

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	LUNGS (%)
27	20.1	0.682
28	18.5	0.768
29	20.7	0.691
30	17.9	0.816
31	19.2	0.729
32	17.9	0.788
33	18.4	0.739
34	19.0	0.716

Group 2 (LOW DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
75	20.2	0.762
76	17.0	0.729
77	17.0	0.694
78	18.2	0.720
79	18.9	0.741
80	21.7	0.710
81	17.5	0.754
82	18.4	0.750

Group 3 (MID DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
123	19.2	0.870
124	19.2	0.833
125	20.7	0.691
126	18.8	0.750
127	19.9	0.819
128	19.3	0.870
129	17.6	0.903
130	17.8	0.837

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
171	18.2	1.055
172	17.8	1.062
173	19.6	0.934
174	17.3	1.029
175	19.8	1.000
176	19.7	1.198
177	19.1	1.089
178	18.2	1.038

**ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

Group 1 (AIR CONTROL)

Animal	BODY W. (GRAM)	LUNGS (%)
27	20.1	0.682
28	18.5	0.768
29	20.7	0.691
30	17.9	0.816
31	19.2	0.729
32	17.9	0.788
33	18.4	0.739
34	19.0	0.716

Group 2 (LOW DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
75	20.2	0.762
76	17.0	0.729
77	17.0	0.694
78	18.2	0.720
79	18.9	0.741
80	21.7	0.710
81	17.5	0.754
82	18.4	0.750

Group 3 (MID DOSE)

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123	19.2	0.870
124	19.2	0.833
125	20.7	0.691
126	18.8	0.750
127	19.9	0.819
128	19.3	0.870
129	17.6	0.903
130	17.8	0.837

ORGAN/BODY WEIGHT RATIOS (%)
ALLOC. E / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

Group 4 (HIGH DOSE)

Animal	BODY W. (GRAM)	LUNGS (%)
171	18.2	1.055
172	17.8	1.062
173	19.6	0.934
174	17.3	1.029
175	19.8	1.000
176	19.7	1.198
177	19.1	1.089
178	18.2	1.038

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES

Date : 27-AUG-09 08:10:04

User : HUH

Parameters Definition

Language : Auswertung
Internal Messages: No
Death Status: All Necropsies
Satellite: A - ALLOCATION A

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES

Date : 27-AUG-09 08:10:04

User : HUH

Parameters Definition

Language : Auswertung

Internal Messages: No

Death Status: All Necropsies

Satellite: A - ALLOCATION A

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 1 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 2 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 3 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 4 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 5 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 6 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 49 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 50 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 51 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 52 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 53 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 54 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 1 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 2 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 3 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 4 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 5 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 6 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 49 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 50 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 51 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 52 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 53 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 54 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 97 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 98 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 99 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 100 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 101 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 102 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 145 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 146 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 147 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 148 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 149 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 150 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 97 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 98 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 99 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 100 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 101 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 102 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. A / VANADIUM PENTOXIDE
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 145 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 146 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 147 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 148 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 149 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

Animal 150 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 02-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES

Date : 27-AUG-09 08:13:13

User : HUH

Parameters Definition

Language : Auswertung

Internal Messages: No

Death Status: All Necropsies

Satellite: B - ALLOCATION B

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES

Date : 27-AUG-09 08:13:13

User : HUH

Parameters Definition

Language : Auswertung

Internal Messages: No

Death Status: All Necropsies

Satellite: B - ALLOCATION B

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 7 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 8 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 9 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 10 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 11 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 12 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 55 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 56 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 57 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 58 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 59 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 60 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 7 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 8 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 9 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 10 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 11 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 12 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 55 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 56 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 57 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 58 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 59 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 60 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 103 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 104 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 105 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 106 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 107 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 108 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROLIFERATION
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 151 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 152 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 153 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 154 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 155 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 156 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 103 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 104 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 105 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 106 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 107 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 108 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. B / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 151 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 152 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 153 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 154 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 155 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

Animal 156 PLANNED NECROPSY (AFTER 7 DAYS OF TREATMENT) , 25-MAY-2

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES

Date : 27-AUG-09 08:14:02

User : HUH

Parameters Definition

Language : Auswertung
Internal Messages: No
Death Status: All Necropsies
Satellite: C - ALLOCATION C

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES

Date : 27-AUG-09 08:14:02

User : HUH

Parameters Definition

Language : Auswertung
Internal Messages: No
Death Status: All Necropsies
Satellite: C - ALLOCATION C

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 13 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 14 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 15 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 16 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 17 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 18 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 61 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 62 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 63 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 64 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 65 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 66 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 13 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 14 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 15 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 16 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 17 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 18 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 61 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 62 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 63 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 64 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 65 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 66 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 109 SPONTAN. DEATH , 29-MAY-2009

LUNGS DISCOLORATION, REDDISH.

Animal 110 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 111 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 112 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 113 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 114 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 157 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 158 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 159 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 160 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 161 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 162 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 109 SPONTAN. DEATH , 29-MAY-2009

LUNGS DISCOLORATION, REDDISH.

Animal 110 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 111 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 112 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 113 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 114 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. C / CELL PROFILARATION
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 157 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 158 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 159 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 160 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 161 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

Animal 162 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 03-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES

Date : 27-AUG-09 08:14:33

User : HUH

Parameters Definition

Language : Auswertung

Internal Messages: No

Death Status: All Necropsies

Satellite: D - ALLOCATION D

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES

Date : 27-AUG-09 08:14:33

User : HUH

Parameters Definition

Language : Auswertung

Internal Messages: No

Death Status: All Necropsies

Satellite: D - ALLOCATION D

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 19 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 20 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 21 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 22 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 23 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 24 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 25 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 26 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 67 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 68 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 69 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 70 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 71 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 72 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 73 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 74 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 19 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 20 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 21 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 22 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 23 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 24 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 25 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 26 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES**

Group 2 (LOW DOSE)

Animal 67 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 68 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 69 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 70 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 71 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 72 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 73 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 74 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 115 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 116 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 117 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 118 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 119 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 120 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 121 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 122 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 163 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 164 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 165 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 166 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 167 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 168 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 169 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 170 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 115 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 116 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 117 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 118 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 119 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 120 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 121 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 122 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. D / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 163 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 164 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 165 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 166 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 167 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 168 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 169 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 170 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES

Date : 27-AUG-09 08:14:53

User : HUH

Parameters Definition

Language : Auswertung

Internal Messages: No

Death Status: All Necropsies

Satellite: E - ALLOCATION E

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES

Date : 27-AUG-09 08:14:53

User : HUH

Parameters Definition

Language : Auswertung
Internal Messages: No
Death Status: All Necropsies
Satellite: E - ALLOCATION E

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES

Animals without necropsy

Animals not recorded

Animals not completed

Animals with not translated finding

Not Reported

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 1 (AIR CONTROL)

Animal 27 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 28 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 29 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 30 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 31 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 32 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 33 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 34 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 75 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 76 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 77 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 78 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 79 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 80 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 81 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 82 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

**MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES**

Group 1 (AIR CONTROL)

Animal 27 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 28 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 29 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 30 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 31 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 32 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 33 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 34 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 2 (LOW DOSE)

Animal 75 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 76 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 77 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 78 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 79 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 80 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 81 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 82 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 123 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 124 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 125 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 126 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 127 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 128 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 129 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 130 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 171 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 172 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 173 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 174 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 175 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 176 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 177 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 178 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 3 (MID DOSE)

Animal 123 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 124 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 125 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 126 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 127 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 128 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 129 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 130 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
ALLOC. E / BIOMARKERS IN LUNG
ALL NECROPSIES
FEMALES

Group 4 (HIGH DOSE)

Animal 171 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 172 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 173 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 174 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 175 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 176 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 177 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

Animal 178 PLANNED NECROPSY (AFTER 16 DAYS OF TREATMENT) , 04-JUN-

NO FINDINGS NOTED

APPENDIX I - CHEMICAL ANALYSIS OF FEED



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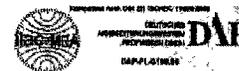
Date 11.12.2008
Customer no. 1209835
Page 1 of 2

TEST REPORT

Sample No. 596075

Order No. 565407 GLP Schadstoffuntersuchung
Sample Arrival 28.11.2008
Sample code M/R Haltung GLP pell. 10 mm eckig
Alleinfuttermittel für Mäuse und Ratten
Rezeptur 3433
GLP-Batch 76/08
Fabr.-Code: 0811011 - Hergestellt: 17.11.08 - MHD: 17.05.09
Sample packing plastic bag

	Unit	Result	limits acc. GV-SOLAS A-08-2001	Substance	Method
Trace-Elements/Heavy-Metals					
Copper	mg/kg	12,0		OM	VDLUFA VII 2.2.2.6
Selenium	mg/kg	0,29		OM	acc. to VDLUFA VII 2.2.2.5; HR-ICPMS
Cadmium	mg/kg	0,07	0,4	OM	acc. to VDLUFA VII 2.2.2.5; HR-ICPMS
Lead	mg/kg	<0,10	1,5	OM	acc. to VDLUFA VII 2.2.2.5; HR-ICPMS
Mercury	mg/kg	<0,02	0,1	OM	§64 LFGB L00.00-19
Arsenic	mg/kg	0,20	1	OM	acc. to VDLUFA VII 2.2.2.5; HR-ICPMS
Mycotoxins					
Aflatoxine B1	µg/kg	<1,00	10	OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine B2	µg/kg	<1,00	5	OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G1	µg/kg	<1,00	5	OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G2	µg/kg	<1,00	5	OM	HPLC-VDLUFA Bd. III, 16.1.4
Sum Aflatoxines	µg/kg	n.d.		OM	calculated
PCB					
PCB 28	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
PCB 52	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
PCB 101	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
PCB 118	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
PCB 138	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
PCB 153	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
PCB 180	mg/kg	<0,0020		OM	acc. to §64 LFGB L00.00-34
sum PCB	mg/kg	n.d.	0,05	OM	calculated
Organochlorous-Pesticides GC-Multiresidueanalysis					
Dieldrin	mg/kg	<0,002		OM	acc. to §64 LFGB L00.00-34
HCH-gamma (gammexane)	mg/kg	<0,002	0,1	OM	acc. to §64 LFGB L00.00-34
Heptachlor	mg/kg	<0,00200		OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxyde-cis	mg/kg	<0,00200		OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxyde-trans	mg/kg	<0,00200		OM	acc. to §64 LFGB L00.00-34
o,p-DDD	mg/kg	<0,00200		OM	acc. to §64 LFGB L00.00-34



APPENDIX I - CHEMICAL ANALYSIS OF FEED

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Date 11.12.2008
Customer no. 1209835
Page 1 of 2

TEST REPORT

Sample No. 596075

Order No. 565407 GLP Schadstoffuntersuchung
Sample Arrival 28.11.2008
Sample code M/R Haltung GLP pell. 10 mm eckig
Alleinfuttermittel für Mäuse und Ratten
Rezeptur 3433
GLP-Batch 76/08
Fabr.-Code: 0811011 - Hergestellt: 17.11.08 - MHD: 17.05.09
Sample packing plastic bag

	Unit	Result	limits acc GV-SOLAS A-08-2001	Substance	Method
Trace-Elements/Heavy-Metals					
Copper	mg/kg	12,0		OM	VDLUF A VII 2.2.2.6
Selenium	mg/kg	0,29		OM	acc to VDLUF A VII 2.2.2.5; HR-ICPMS
Cadmium	mg/kg	0,07	0,4	OM	acc to VDLUF A VII 2.2.2.5; HR-ICPMS
Lead	mg/kg	<0,10	1,5	OM	acc to VDLUF A VII 2.2.2.5; HR-ICPMS
Mercury	mg/kg	<0,02	0,1	OM	§64 LFGB L00.00-19
Arsenic	mg/kg	0,20	1	OM	acc to VDLUF A VII 2.2.2.5; HR-ICPMS
Mycotoxins					
Aflatoxine B1	µg/kg	<1,00	10	OM	HPLC-VDLUF A Bd. III, 16.1.4
Aflatoxine B2	µg/kg	<1,00	5	OM	HPLC-VDLUF A Bd. III, 16.1.4
Aflatoxine G1	µg/kg	<1,00	5	OM	HPLC-VDLUF A Bd. III, 16.1.4
Aflatoxine G2	µg/kg	<1,00	5	OM	HPLC-VDLUF A Bd. III, 16.1.4
Sum Aflatoxines	µg/kg	n.d.		OM	calculated
PCB					
PCB 28	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
PCB 52	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
PCB 101	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
PCB 118	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
PCB 138	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
PCB 153	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
PCB 180	mg/kg	<0,0020		OM	acc to §64 LFGB L00.00-34
sum PCB	mg/kg	n.d.	0,05	OM	calculated
Organochlorous-Pesticides GC-Multiresidueanalysis					
Dieldrin	mg/kg	<0,002		OM	acc to §64 LFGB L00.00-34
HCH-gamma (gammexane)	mg/kg	<0,002	0,1	OM	acc to §64 LFGB L00.00-34
Heptachlor	mg/kg	<0,00200		OM	acc to §64 LFGB L00.00-34
Heptachlorepoxide-cis	mg/kg	<0,00200		OM	acc to §64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	<0,00200		OM	acc to §64 LFGB L00.00-34
o,p-DDD	mg/kg	<0,00200		OM	acc to §64 LFGB L00.00-34



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Date 11.12.2008
Customer no. 1209835
Page 2 of 2

Sample No. 596075

	Unit	Result	limits acc. GV-SOLAS A-08-2001	Substance	Method
<i>o,p</i> -DDE	mg/kg	<0,00200		OM	acc to §84 LFGB L00.00-34
<i>o,p</i> -DDT	mg/kg	<0,002		OM	acc. to §84 LFGB L00.00-34
<i>p,p</i> -DDD	mg/kg	<0,00200		OM	acc. to §84 LFGB L00.00-34
<i>p,p</i> -DDE	mg/kg	<0,00200		OM	acc to §84 LFGB L00.00-34
<i>p,p</i> -DDT	mg/kg	<0,00200		OM	acc. to §84 LFGB L00.00-34
Sum DDTs	mg/kg	n.d.	0,05	OM	calculated
Sum Heptachlor	mg/kg	n.d.	0,01	OM	calculated

Organo-Phosphorous Pesticides GC-Multiresidueanalysis

Malathion	mg/kg	<0,010	1	OM	acc to §84 LFGB L00.00-34
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nitrosamines

<i>N</i> -Nitrosodibutylamin	µg/kg	<5,00		OM	GC-Inhousemethod
<i>N</i> -Nitrosodiethylamin	µg/kg	<5,00	10	OM	GC-Inhousemethod
<i>N</i> -Nitrosodisopropylamin	µg/kg	<5,00		OM	GC-Inhousemethod
<i>N</i> -Nitrosodimethylamin	µg/kg	<5,00	10	OM	GC-Inhousemethod
<i>N</i> -Nitrosodipropylamin	µg/kg	<5,00		OM	GC-Inhousemethod
<i>N</i> -Nitrosomethylethylamin	µg/kg	<5,00		OM	GC-Inhousemethod
<i>N</i> -Nitrosomorpholin	µg/kg	<5,00		OM	GC-Inhousemethod
<i>N</i> -Nitrosopiperidin	µg/kg	<5,00		OM	GC-Inhousemethod
<i>N</i> -Nitrosopyrrolidin	µg/kg	<5,00		OM	GC-Inhousemethod
Sum Nitrosamines	µg/kg	n.d.		OM	calculated

Estrogens

diestrol	µg/kg	<10,0		OM	no object
diethyl stäbestrol	µg/kg	<1,00		OM	no object
hexestrol	µg/kg	<2,00		OM	no object
Sum Estrogens	µg/kg	n.d.		OM	calculated

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

The actual limit of detection can be different to the standard value for a particular analysis due to matrix effects or insufficient sample volume.
Remark: OM=original matter, DM=dry matter

LUFA - ITL Dr. Wehage, Tel. 0431/1228-220

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PROVIMI KLIBA AG

External laboratory

TIERGESUNDHEITSDIENST, SENATOR-GERAUER STR 23, 85586 POING	
Parameter	Sum Estrogens
Zentrale Analytik - Organische Henkel KGaA, Henkelstrasse 67 ç Gebäude Z43, 40589 Düsseldorf	
Parameter	Sum Nitrosamines

The analytical results are valid for the delivered sample material only. The testing 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.



APPENDIX II - DRINKING WATER ANALYSIS

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Date 11.12.2008
Customer no. 1209835
Page 2 of 2

Sample No. 596075

Unit	Result	limits acc. GV-SOLAS A-08-2001	Substance	Method
o,p-DDE	mg/kg	<0,00200		OM acc. to §84 LFGB L00.00-34
o,p-DDT	mg/kg	<0,002		OM acc. to §84 LFGB L00.00-34
p,p-DDD	mg/kg	<0,00200		OM acc. to §84 LFGB L00.00-34
p,p-DDE	mg/kg	<0,00200		OM acc. to §84 LFGB L00.00-34
p,p-DDT	mg/kg	<0,00200		OM acc. to §84 LFGB L00.00-34
Sum DDTs	mg/kg	n.d.	0,05	OM calculated
Sum Heptachlor	mg/kg	n.d.	0,01	OM calculated

Organo-Phosphorous Pesticides GC-Multiresidueanalysis

Malathion	mg/kg	<0,010	1	OM	acc. to §84 LFGB L00.00-34
-----------	-------	--------	---	----	----------------------------

nitrosamines

N-Nitrosodibutylamin	µg/kg	<5,00		OM	GC-Inhousemethod
N-Nitrosodiethylamin	µg/kg	<5,00	10	OM	GC-Inhousemethod
N-Nitrosodiisopropylamin	µg/kg	<5,00		OM	GC-Inhousemethod
N-Nitrosodimethylamin	µg/kg	<5,00	10	OM	GC-Inhousemethod
N-Nitrosodipropylamin	µg/kg	<5,00		OM	GC-Inhousemethod
N-Nitrosomethylethylamin	µg/kg	<5,00		OM	GC-Inhousemethod
N-Nitrosomorpholin	µg/kg	<5,00		OM	GC-Inhousemethod
N-Nitrosopiperidin	µg/kg	<5,00		OM	GC-Inhousemethod
N-Nitrosopyrrolidin	µg/kg	<5,00		OM	GC-Inhousemethod
Sum Nitrosamines	µg/kg	n.d.		OM	calculated

Estrogens

dienestrol	µg/kg	<10,0		OM	no object
diethyl stilbestrol	µg/kg	<1,00		OM	no object
hexestrol	µg/kg	<2,00		OM	no object
Sum Estrogens	µg/kg	n.d.		OM	calculated

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

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

Remark: OM=original matter, DM=dry matter

LUFA - ITL Dr. Wehage, Tel. 0431/1228-220

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PROVIMI KLIBA AG

External laboratory

TIERGESUNDHEITSDIENST, SENATOR-GERAUER STR 23, 85586 POING

Parameter

Sum Estrogens

Zentrale Analytik - Organische Henkel KGaA, Henkelstrasse 67 2 Gebäude Z43, 40589 Düsseldorf

Parameter

Sum Nitrosamines

The analytical results are valid for the delivered sample material only. The testing 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



APPENDIX II - DRINKING WATER ANALYSIS



CHEMICAL WATER ANALYSIS, FÜLLINSDORF

Official Laboratory
Basel-Landschaft

Liestal, March 20, 2009
Ref. no. 200072802

Sampling point:

35.991.N, Net water
Harlan Ltd., Füllinsdorf, Bldg.2

Sampled on:

January 26, 2009

Time of sampling

07.30

Water temperature (°C)

9.5

CHEMICAL TEST:

Appearance			clear, colourless
Odor			not remarkable
Taste			not remarkable
UV-absorption at 254 nm/100 cm			1.43
pH value			7.4
Oxygen demand	(KMnO ₄ cons.)	mg/l	1.8
Turbidity	FNU		0.12
Chloride	Cl ⁻	mg/l	23.2
Nitrate	NO ₃ ⁻	mg/l	17.9
Sulphate	SO ₄ ⁻²	mg/l	63.3
Nitrite	NO ₂ ⁻	mg/l	<0.005
Total hardness		fr.H°	31.9
Alkaline hardness		fr.H°	25.1
Non carbonate hardness		fr.H°	6.8
Calcium	Ca ⁺⁺	mg/l	116.8
Magnesium	Mg ⁺⁺	mg/l	6.5

ASSESSMENT:

At the time of sampling, the tested chemical parameters met the requirements for drinking water according to article "Artikel 3 der Verordnung über Trink-, Quell-, und Mineralwasser (SR 817.022.102)

Official Laboratory
The Official Chemist



(signed by)



CHEMICAL WATER ANALYSIS, FÜLLINSDORF

Official Laboratory
Basel-Landschaft

Liestal, March 20, 2009
Ref. no. 200072802

Sampling point:

35.991.N, Net water
Harlan Ltd., Füllinsdorf, Bldg.2

Sampled on:

January 26, 2009

Time of sampling

07.30

Water temperature (°C)

9.5

CHEMICAL TEST:

Appearance			clear, colourless
Odor			not remarkable
Taste			not remarkable
UV-absorption at 254 nm/100 cm			1.43
pH value			7.4
Oxygen demand	(KMnO ₄ cons.)	mg/l	1.8
Turbidity	FNU		0.12
Chloride	Cl ⁻	mg/l	23.2
Nitrate	NO ₃ ⁻	mg/l	17.9
Sulphate	SO ₄ ⁻²	mg/l	63.3
Nitrite	NO ₂ ⁻	mg/l	<0.005
Total hardness		fr.H°	31.9
Alkaline hardness		fr.H°	25.1
Non carbonate hardness		fr.H°	6.8
Calcium	Ca ⁺⁺	mg/l	116.8
Magnesium	Mg ⁺⁺	mg/l	6.5

ASSESSMENT:

At the time of sampling, the tested chemical parameters met the requirements for drinking water according to article "Artikel 3 der Verordnung über Trink-, Quell-, und Mineralwasser (SR 817.022.102)

Official Laboratory
The Official Chemist



(signed)



CONTAMINANT ASSAY OF DRINKING WATER, FÜLLINSDORF

Harlan Laboratories Study.: C34957
Date of Sampling: January 26, 2009
Sample: H₂O, RCC Ltd, Füllinsdorf, Bldg. 2

PARAMETER	ASSAY LEVEL µg/l	LIMIT * µg/l
Lindane	< 0.05	0.1
Heptachlor	< 0.05	0.1
Malathion	< 0.05	0.1
DDT, total	< 0.05	0.1
Dieldrin	< 0.05	0.1
Cadmium	< 0.5	5
Arsenic	< 3	50
Lead	< 3	50
Mercury	< 1	1
Selenium	< 3	10
Copper	< 4	1500
PCBs (28, 52, 101, 138, 153, 180)	< 0.05	0.1
Nitrosamines, total (DMN, DEN, NPIP, NMORPH)	< 0.05	---

< 0.05 = less than 0.05 microgram per liter

* Schweizer Lebensmittelbuch

Issued by Dr. D. Flade

March 24, 2009

APPENDIX III - FORMULATION ANALYSIS



CONTAMINANT ASSAY OF DRINKING WATER, FÜLLINSDORF

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Heptachlor	< 0.05	0.1
Malathion	< 0.05	0.1
DDT, total	< 0.05	0.1
Dieldrin	< 0.05	0.1
Cadmium	< 0.5	5
Arsenic	< 3	50
Lead	< 3	50
Mercury	< 1	1
Selenium	< 3	10
Copper	< 4	1500
PCBs (28, 52, 101, 138, 153, 180)	< 0.05	0.1
Nitrosamines, total (DMN, DEN, NPIP, NMORPH)	< 0.05	----

< 0.05 = less than 0.05 microgram per liter

* Schweizer Lebensmittelbuch

Issued by Dr. D. Flade

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APPENDIX III - FORMULATION ANALYSIS

Determination of Vanadium Pentoxide on Filters, Particle Size Cascade Impactor Stages, in Mice Blood and Lungs

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1 MATERIALS AND METHODS

1.1 Analytical Standard

Data as provided by the supplier.

Identity:	Vanadium 1000 µg/mL AAS/ICP
Supplier:	J.T. Baker
Supplier Art. No.:	6945
Lot No.:	15.0090409
Expiry (Retest) Date:	September 2012
Content:	1000 µg/mL
Storage Conditions:	No particular precaution required – storage at Harlan Laboratories Ltd at room temperature

1.2 Study Samples and Storage

Filter and impactor stage samples were collected and dispatched under ambient conditions to the analytical laboratories where they were stored at room temperature until analysis. Blood and lung samples were sent on dry ice. Upon arrival these samples were kept on dry ice until analysis.

1.3 Reagents and Apparatus

Bidistilled water:	In-house prepared
Hydrogen fluoride (48.0 -51.0%):	Baker no. 9563
Low temperature ashing	Plasma system P200
Freeze dryer:	Christ Alpha 1-4
Balance:	Mettler PE 360

1.4 Analytical Procedure

1.4.1 Filter and Impactor Stage Samples

1.4.1.1 Preparation of Standard Solutions

A stock solution of vanadium analytical standard in 5% hydrogen fluoride with a concentration of 128 µg/mL was prepared by dissolving 12800 µL of the analytical standard (concentration: 1000 µg/mL) in 100 mL of 5% hydrogen fluoride. Standard solutions were prepared by

Determination of Vanadium Pentoxide on Filters, Particle Size Cascade Impactor Stages, in Mice Blood and Lungs

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1.4	Analytical Procedure	2
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Low temperature ashing	Plasma system P200
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A stock solution of vanadium analytical standard in 5% hydrogen fluoride with a concentration of 128 µg/mL was prepared by dissolving 12800 µL of the analytical standard (concentration: 1000 µg/mL) in 100 mL of 5% hydrogen fluoride. Standard solutions were prepared by

successive dilution of the stock solution with 5% hydrogen fluoride. The resulting concentrations ranged from 2.0 to 64.0 µg/mL. These standard solutions as well as the stock solution were used to calibrate the atomic absorption spectrometer.

1.4.1.2 Analysis of Samples

Each filter and impactor stage sample was dissolved in an appropriate volume of 5% hydrogen fluoride. Where necessary, the samples were further diluted with 5% hydrogen fluoride into the calibration range.

1.4.1.3 Atomic Absorption Spectrometry with Flame Assembly

Instrument: Perkin-Elmer model PE 2100 (software 4100)
Flame: Nitrogen (I) oxide / acetylene flame
Slit Width: 0.7 nm high
Wavelength: Vanadium: 318.4 nm

1.4.1.4 Evaluation of Results

Samples were quantified by atomic absorption spectroscopy (AAS) of vanadium with reference to the respective calibration curve (with zero intercept). The calibration curve (non-linear) and the vanadium concentration (in µg/mL) were calculated using the Perkin Elmer software. An example calibration is presented in Figure 1.

The amount of Vanadium pentoxide on filters and impactor stages was calculated according to equation 2:

$$A_{Actual} = \frac{C_s \cdot V \cdot D \cdot 100}{F} \quad (1)$$

where

A_{Actual} = Actual amount of test item on filter and impactor stage [µg/filter, µg/stage]
 C_s = Measured concentration of vanadium in sample [mg/L]
 V = Extraction volume [mL]
 D = Dilution factor
 F = 53.64% and 41.39% [content of vanadium in Vanadium pentoxide as determined within the present study]

Content of vanadium in the test item was calculated according to equation 2:

$$C_{Actual} = \frac{C_s \cdot V \cdot D}{1000} \quad (2)$$

where

C_{Actual} = Actual content of vanadium in test item sample [mg]
 V = Extraction volume [mL]
 D = Dilution factor

The sample recovery was determined as follows:

$$C\% = \frac{C_{Actual}}{C_{Nominal}} \cdot 100 \quad (3)$$

where

$C\%$ = Sample content [%]
 C_{Actual} = Actual sample content [mg]
 $C_{Nominal}$ = Nominal sample concentration [mg]

1.4.2 Blood and Lung Samples

1.4.2.1 Preparation of Standard Solutions

A stock solution of vanadium analytical standard in 5% hydrogen fluoride with a concentration of 10 µg/mL was prepared by dissolving 1000 µL of the analytical standard (concentration: 1000 µg/mL) in 100 mL of 5% hydrogen fluoride. Standard solutions were prepared by successive dilution of the stock solution with 5% hydrogen fluoride. The resulting concentrations ranged from 25 to 100 µg/L. These standard solutions as well as the stock solution were used to calibrate the atomic absorption spectrometer.

1.4.2.2 Analysis of Samples

Blood Samples:

A 100 µL aliquot of each blood sample and 400 µL of 5% hydrogen fluoride were homogenized immediately and again after 30 minutes using a vortex mixer.

Lung Samples:

Each of the delivered lung samples (about 0.1 g, exactly weighed to the third decimal place) was transferred into a tared quartz vessel. The samples were deep frozen and immediately freeze-

successive dilution of the stock solution with 5% hydrogen fluoride. The resulting concentrations ranged from 2.0 to 64.0 µg/mL. These standard solutions as well as the stock solution were used to calibrate the atomic absorption spectrometer.

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 F = 53.64% and 41.39% [content of vanadium in Vanadium pentoxide as determined within the present study]

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Blood Samples:

A 100 µL aliquot of each blood sample and 400 µL of 5% hydrogen fluoride were homogenized immediately and again after 30 minutes using a vortex mixer.

Lung Samples:

Each of the delivered lung samples (about 0.1 g, exactly weighed to the third decimal place) was transferred into a tared quartz vessel. The samples were deep frozen and immediately freeze-

dried for 24 hours. Afterwards, the weights were determined. The samples were digested using low temperature ashing (at 300 W; oxygen: 60 mL/min) for 22 hours. Finally, the residue and 1 mL of 5% hydrogen fluoride were homogenized immediately and again after 30 minutes using a vortex mixer.

1.4.2.3 Atomic Absorption Spectrometry with Graphite Furnace Assembly

Instrument: Perkin-Elmer model PE 5100 PC with graphite furnace
Sampler: AS-60
Slit Width: 0.7 nm
Wavelength: Vanadium: 318.4 nm

1.4.2.4 Evaluation of Results

Samples were quantified by atomic absorption spectroscopy (AAS) of vanadium with reference to the respective calibration curve (with zero intercept). The calibration curve (non-linear) and the vanadium concentration (in $\mu\text{g/L}$) were calculated using the Perkin Elmer software.

The concentrations of Vanadium pentoxide in blood samples were calculated according to equation 4:

$$C_{Actual} = C_s \cdot F \quad (4)$$

where

C_{Actual} = Actual concentration of Vanadium pentoxide in blood [$\mu\text{g/L}$]
 C_s = Measured concentration of vanadium in sample [$\mu\text{g/L}$]
 F = Conversion factor of 1.785 [Mw Vanadium pentoxide / Mw vanadium]

The amount of Vanadium pentoxide in lung samples were calculated according to equation 5:

$$A_{Actual} = \frac{C_s \cdot V \cdot F}{W \cdot 1000} \quad (5)$$

where

A_{Actual} = Actual amount of Vanadium pentoxide in lung [$\mu\text{g/g}$]
 C_s = Measured concentration of vanadium in sample [$\mu\text{g/L}$]
 V = Extraction volume [mL]
 W = Weight of sample [g]
 F = Conversion factor of 1.785 [Mw Vanadium pentoxide / Mw vanadium]

vanadium]

dried for 24 hours. Afterwards, the weights were determined. The samples were digested using low temperature ashing (at 300 W; oxygen: 60 mL/min) for 22 hours. Finally, the residue and 1 mL of 5% hydrogen fluoride were homogenized immediately and again after 30 minutes using a vortex mixer.

1.4.2.3 Atomic Absorption Spectrometry with Graphite Furnace Assembly

Instrument: Perkin-Elmer model PE 5100 PC with graphite furnace
Sampler: AS-60
Slit Width: 0.7 nm
Wavelength: Vanadium: 318.4 nm

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Samples were quantified by atomic absorption spectroscopy (AAS) of vanadium with reference to the respective calibration curve (with zero intercept). The calibration curve (non-linear) and the vanadium concentration (in µg/L) were calculated using the Perkin Elmer software.

The concentrations of Vanadium pentoxide in blood samples were calculated according to equation 4:

$$C_{Actual} = C_s \cdot F \quad (4)$$

where

C_{Actual} = Actual concentration of Vanadium pentoxide in blood [µg/L]
 C_s = Measured concentration of vanadium in sample [µg/L]
 F = Conversion factor of 1.785 [Mw Vanadium pentoxide / Mw vanadium]

The amount of Vanadium pentoxide in lung samples were calculated according to equation 5:

$$A_{Actual} = \frac{C_s \cdot V \cdot F}{W \cdot 1000} \quad (5)$$

where

A_{Actual} = Actual amount of Vanadium pentoxide in lung [µg/g]
 C_s = Measured concentration of vanadium in sample [µg/L]
 V = Extraction volume [mL]
 W = Weight of sample [g]
 F = Conversion factor of 1.785 [Mw Vanadium pentoxide / Mw

vanadium]

2 RESULTS

The test item contents of the samples were determined by analysis of the vanadium concentration using an atomic absorption spectrometer (AAS).

The results of Vanadium pentoxide on filters and impactor stages are presented in Table 1 and Table 2. The content of vanadium in the test item, the concentrations of the test item in blood and lung samples are shown in Table 3 to Table 5. An example of a calibration curve is presented in Figure 1.

Table 1 Detailed Results of Filters
(Rounded results presented are based on calculations with exact data)

Date of Sampling	Date of Analysis	Group No.	Filter No.	Amount Found [µg t.i./filter]
18-May-09	20-May-09	2	1	85.58
		3	1	356.4
		4	1	1351
19-May-09	20-May-09	2	1	86.38
		3	1	361.9
		4	1	1278
20-May-09	28-May-09	2	1	95.45
		3	1	410.8
		4	1	1646
21-May-09	28-May-09	2	1	69.71
		3	1	267.6
		4	1	1102
22-May-09	28-May-09	2	1	92.71
		3	1	381.5
		4	1	1571
23-May-09	28-May-09	2	1	102.6
		3	1	440.8
		4	1	1840
24-May-09	28-May-09	2	1	87.95
		3	1	372.8
		4	1	1504
25-May-09	05-Jun-09	2	1	84.99
		3	1	321.1
		4	1	1346
26-May-09	05-Jun-09	2	1	80.59
		3	1	317.0
		4	1	1277
27-May-09	05-Jun-09	2	1	104.3
		3	1	367.8
		4	1	1519
28-May-09	05-Jun-09	2	1	101.5
		3	1	356.5
		4	1	1443

t.i.: Vanadium pentoxide

2 RESULTS

The test item contents of the samples were determined by analysis of the vanadium concentration using an atomic absorption spectrometer (AAS).

The results of Vanadium pentoxide on filters and impactor stages are presented in Table 1 and Table 2. The content of vanadium in the test item, the concentrations of the test item in blood and lung samples are shown in Table 3 to Table 5. An example of a calibration curve is presented in Figure 1.

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		4	1	1277
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		3	1	367.8
		4	1	1519
28-May-09	05-Jun-09	2	1	101.5
		3	1	356.5
		4	1	1443

t.i.: Vanadium pentoxide

Table 1 Cont'd

Date of Sampling	Date of Analysis	Group No.	Filter No.	Amount Found [µg t.i./filter]
29-May-09	05-Jun-09	2	1	97.29
		3	1	336.9
		4	1	1372
30-May-09	05-Jun-09	2	1	79.68
		3	1	276.5
		4	1	1079
31-May-09	05-Jun-09	2	1	90.11
		3	1	337.7
		4	1	1353
01-Jun-09	05-Jun-09	2	1	88.69
		3	1	327.6
		4	1	1349
02-Jun-09	05-Jun-09	2	1	84.93
		3	1	318.46
		4	1	1349
03-Jun-09	05-Jun-09	2	1	92.29
		3	1	356.4
		4	1	1313
04-Jun-09	05-Jun-09	3	1	344.0
		4	1	1296
04-Jun-09	05-Jun-09	Blank HVLP	---	--- ¹

t.i.: Vanadium pentoxide
¹ below lowest calibration point

Table 2 Detailed Results of Particle Size Cascade Impactor Stages
(Rounded results presented are based on calculations with exact data)

Group No.	Date of Sampling	Date of Analysis	Stage No.	Amount Found [µg a.i./stage]
Blank 1	18-May-09	20-May-09	---	32.37
Blank 2			---	33.22
Blank 3			---	32.31
4	18-May-09	20-May-09	1	23.13 ¹
			2	22.31 ¹
			3	84.90 ¹
			4	357.1 ¹
			5	282.0 ¹
			6	195.3 ¹
			7	30.77 ¹
			8	31.44 ¹
Blank 1	19 to 22 - May-09	28-May-09	---	---
Blank 2			---	---
Blank 3			---	---
3	19 to 22 - May-09	28-May-09	1	33.63 ²
			2	32.82 ²
			3	108.7 ²
			4	365.3 ²
			5	431.8 ²
			6	237. ²
			7	43.28 ²
			8	36.75 ²
3	26 to 29-May -09	05-Jun-09	1	34.25
			2	36.31
			3	150.3
			4	277.7
			5	461.1
			6	214.6
			7	72.02
			8	60.46

t.i. Vanadium pentoxide

¹ Corrected for the mean vanadium value obtained for the blank impactor stage samples 1, 2 and 3

² Result of repeated analysis

Table 1 Cont'd

Date of Sampling	Date of Analysis	Group No.	Filter No.	Amount Found [µg t.i./filter]
29-May-09	05-Jun-09	2	1	97.29
		3	1	336.9
		4	1	1372
30-May-09	05-Jun-09	2	1	79.68
		3	1	276.5
		4	1	1079
31-May-09	05-Jun-09	2	1	90.11
		3	1	337.7
		4	1	1353
01-Jun-09	05-Jun-09	2	1	88.69
		3	1	327.6
		4	1	1349
02-Jun-09	05-Jun-09	2	1	84.93
		3	1	318.46
		4	1	1349
03-Jun-09	05-Jun-09	2	1	92.29
		3	1	356.4
		4	1	1313
04-Jun-09	05-Jun-09	3	1	344.0
		4	1	1296
04-Jun-09	05-Jun-09	Blank HVLP	---	--- ¹

t.i.: Vanadium pentoxide
¹ below lowest calibration point

Table 2 Detailed Results of Particle Size Cascade Impactor Stages
(Rounded results presented are based on calculations with exact data)

Group No.	Date of Sampling	Date of Analysis	Stage No.	Amount Found [µg a.i./stage]
Blank 1	18-May-09	20-May-09	---	32.37
Blank 2			---	33.22
Blank 3			---	32.31
4	18-May-09	20-May-09	1	23.13 ¹
			2	22.31 ¹
			3	84.90 ¹
			4	357.1 ¹
			5	282.0 ¹
			6	195.3 ¹
			7	30.77 ¹
			8	31.44 ¹
Blank 1	19 to 22 - May-09	28-May-09	---	---
Blank 2			---	---
Blank 3			---	---
3	19 to 22 - May-09	28-May-09	1	33.63 ²
			2	32.82 ²
			3	108.7 ²
			4	365.3 ²
			5	431.8 ²
			6	237. ²
			7	43.28 ²
			8	36.75 ²
3	26 to 29-May -09	05-Jun-09	1	34.25
			2	36.31
			3	150.3
			4	277.7
			5	461.1
			6	214.6
			7	72.02
			8	60.46

t.i. Vanadium pentoxide

¹ Corrected for the mean vanadium value obtained for the blank impactor stage samples 1, 2 and 3

² Result of repeated analysis

Table 2 Cont'd

Group No.	Date of Sampling	Date of Analysis	Stage No.	Amount Found [$\mu\text{g a.i./stage}$]
4	25-May-09	05-Jun-09	1	48.77
			2	60.80
			3	179.4
			4	542.3
			5	420.7
			6	232.8
			7	70.00
			8	45.08

t.i. Vanadium pentoxide

Table 3 Content of Vanadium in Test Item

Sample ID	Date of Analysis	Nominal [mg t.i.]	Actual [mg t.i.]	% of Nominal
1	20-May-09	251	134.6	53.64
2	28-May-09	251	103.8	41.39

t.i.: test item Vanadium pentoxide

Table 2 Cont'd

Group No.	Date of Sampling	Date of Analysis	Stage No.	Amount Found [µg a.i./stage]
4	25-May-09	05-Jun-09	1	48.77
			2	60.80
			3	179.4
			4	542.3
			5	420.7
			6	232.8
			7	70.00
			8	45.08

t.i. Vanadium pentoxide

Table 3 Content of Vanadium in Test Item

Sample ID	Date of Analysis	Nominal [mg t.i.]	Actual [mg t.i.]	% of Nominal
1	20-May-09	251	134.6	53.64
2	28-May-09	251	103.8	41.39

t.i.: test item Vanadium pentoxide

Table 4 Detailed Results of Blood Samples
(Rounded results presented are based on calculations with exact data)

Animal No.	Group	Date of Sampling	Date of Analysis	Vanadium pentoxide (µg/L)
1	1	02-Jun-09	09-Jul-09	---*
2	1	02-Jun-09	09-Jul-09	---*
3	1	02-Jun-09	09-Jul-09	---*
4	1	02-Jun-09	09-Jul-09	---*
5	1	02-Jun-09	09-Jul-09	---*
6	1	02-Jun-09	09-Jul-09	---*
49	2	02-Jun-09	09-Jul-09	---*
50	2	02-Jun-09	09-Jul-09	---*
51	2	02-Jun-09	09-Jul-09	---*
52	2	02-Jun-09	09-Jul-09	---*
53	2	02-Jun-09	09-Jul-09	---*
54	2	02-Jun-09	09-Jul-09	---*
97	3	02-Jun-09	09-Jul-09	111.2
98	3	02-Jun-09	09-Jul-09	84.16
99	3	02-Jun-09	09-Jul-09	89.13
100	3	02-Jun-09	09-Jul-09	95.32
101	3	02-Jun-09	09-Jul-09	82.57
102	3	02-Jun-09	09-Jul-09	85.23
145	4	02-Jun-09	09-Jul-09	335.8
146	4	02-Jun-09	09-Jul-09	252.9
147	4	02-Jun-09	09-Jul-09	282.0
148	4	02-Jun-09	09-Jul-09	302.2
149	4	02-Jun-09	09-Jul-09	266.9
150	4	02-Jun-09	09-Jul-09	275.2

* below lowest calibration point

Table 5 Detailed Results of Lung Samples
(Rounded results presented are based on calculations with exact data)

Animal No.	Group	Date of Sampling	Date of Analysis	Vanadium pentoxide (µg/g lung)
1	1	02-Jun-09	09-Jul-09	---*
2	1	02-Jun-09	09-Jul-09	---*
3	1	02-Jun-09	09-Jul-09	---*
4	1	02-Jun-09	09-Jul-09	---*
5	1	02-Jun-09	09-Jul-09	---*
6	1	02-Jun-09	09-Jul-09	---*
49	2	02-Jun-09	09-Jul-09	13.83
50	2	02-Jun-09	09-Jul-09	12.98
51	2	02-Jun-09	09-Jul-09	17.19
52	2	02-Jun-09	09-Jul-09	13.53
53	2	02-Jun-09	09-Jul-09	13.80
54	2	02-Jun-09	09-Jul-09	14.55
97	3	02-Jun-09	09-Jul-09	52.89
98	3	02-Jun-09	09-Jul-09	53.96
99	3	02-Jun-09	09-Jul-09	56.15
100	3	02-Jun-09	09-Jul-09	60.24
101	3	02-Jun-09	09-Jul-09	49.74
102	3	02-Jun-09	09-Jul-09	59.18
145	4	02-Jun-09	09-Jul-09	140.7
146	4	02-Jun-09	09-Jul-09	112.3
147	4	02-Jun-09	09-Jul-09	106.2
148	4	02-Jun-09	09-Jul-09	107.2
149	4	02-Jun-09	09-Jul-09	116.5
150	4	02-Jun-09	09-Jul-09	106.2

* below lowest calibration point

Table 4 Detailed Results of Blood Samples
(Rounded results presented are based on calculations with exact data)

Animal No.	Group	Date of Sampling	Date of Analysis	Vanadium pentoxide (µg/L)
1	1	02-Jun-09	09-Jul-09	---*
2	1	02-Jun-09	09-Jul-09	---*
3	1	02-Jun-09	09-Jul-09	---*
4	1	02-Jun-09	09-Jul-09	---*
5	1	02-Jun-09	09-Jul-09	---*
6	1	02-Jun-09	09-Jul-09	---*
49	2	02-Jun-09	09-Jul-09	---*
50	2	02-Jun-09	09-Jul-09	---*
51	2	02-Jun-09	09-Jul-09	---*
52	2	02-Jun-09	09-Jul-09	---*
53	2	02-Jun-09	09-Jul-09	---*
54	2	02-Jun-09	09-Jul-09	---*
97	3	02-Jun-09	09-Jul-09	111.2
98	3	02-Jun-09	09-Jul-09	84.16
99	3	02-Jun-09	09-Jul-09	89.13
100	3	02-Jun-09	09-Jul-09	95.32
101	3	02-Jun-09	09-Jul-09	82.57
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145	4	02-Jun-09	09-Jul-09	335.8
146	4	02-Jun-09	09-Jul-09	252.9
147	4	02-Jun-09	09-Jul-09	282.0
148	4	02-Jun-09	09-Jul-09	302.2
149	4	02-Jun-09	09-Jul-09	266.9
150	4	02-Jun-09	09-Jul-09	275.2

* below lowest calibration point

Table 5 Detailed Results of Lung Samples
(Rounded results presented are based on calculations with exact data)

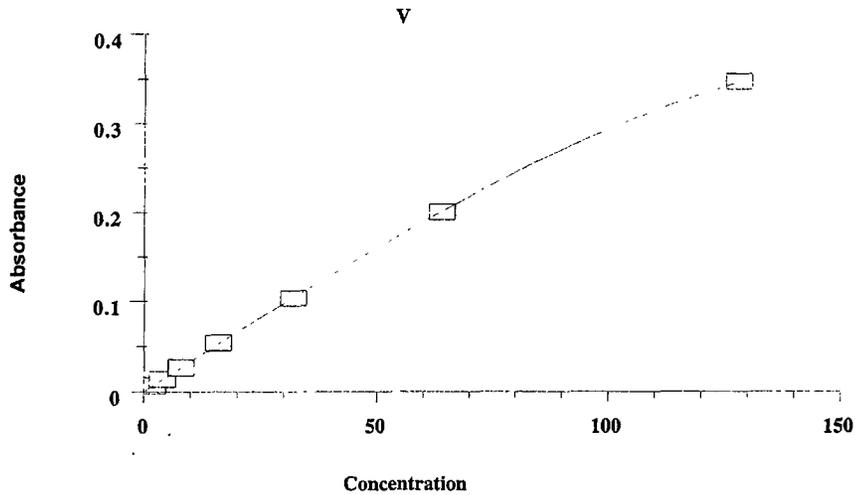
Animal No.	Group	Date of Sampling	Date of Analysis	Vanadium pentoxide (µg/g lung)
1	1	02-Jun-09	09-Jul-09	---*
2	1	02-Jun-09	09-Jul-09	---*
3	1	02-Jun-09	09-Jul-09	---*
4	1	02-Jun-09	09-Jul-09	---*
5	1	02-Jun-09	09-Jul-09	---*
6	1	02-Jun-09	09-Jul-09	---*
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50	2	02-Jun-09	09-Jul-09	12.98
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53	2	02-Jun-09	09-Jul-09	13.80
54	2	02-Jun-09	09-Jul-09	14.55
97	3	02-Jun-09	09-Jul-09	52.89
98	3	02-Jun-09	09-Jul-09	53.96
99	3	02-Jun-09	09-Jul-09	56.15
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146	4	02-Jun-09	09-Jul-09	112.3
147	4	02-Jun-09	09-Jul-09	106.2
148	4	02-Jun-09	09-Jul-09	107.2
149	4	02-Jun-09	09-Jul-09	116.5
150	4	02-Jun-09	09-Jul-09	106.2

* below lowest calibration point

Figure 1 Example of Calibration Curve

Calibration data for V

Standard ID	Mean Signal (Pk Height)	Entered Concentration (mg/L)	Calculated Concentration (mg/L)	Standard Deviation	%RSD
Calib Blank	0.7654	---	---	---	---
Stand. 2ug/ml	0.0066	2.000	1.976	0.0001	2.04
Stand. 4ug/ml	0.0133	4.000	4.006	0.0001	0.64
Stand. 8ug/ml	0.0265	8.000	8.003	0.0004	1.67
Stand. 16ug/ml	0.0538	16.000	16.32	0.0005	1.01
Stand. 32ug/ml	0.1034	32.000	31.73	0.0009	0.85
Stand. 64ug/ml	0.2004	64.000	63.66	0.0021	1.03
Stand. 128ug/ml	0.3475	128.000	128.5	0.0016	0.45
Correlation Coefficient: 0.99998		Slope: 0.00333		---	---

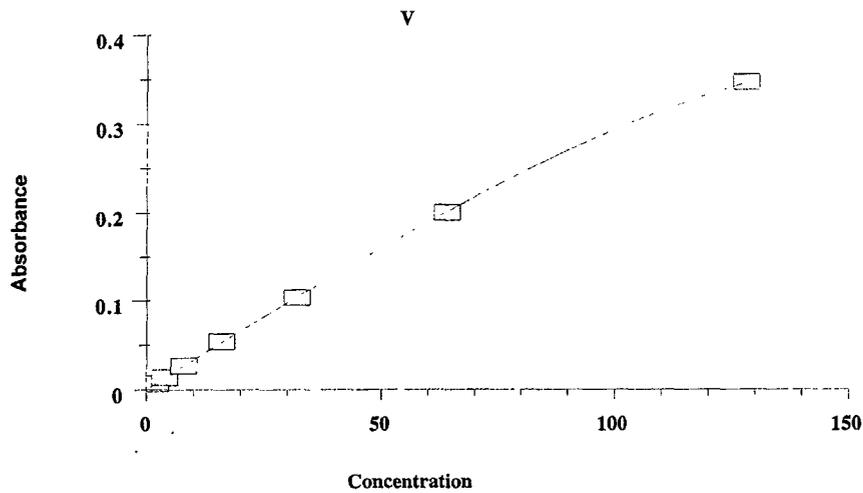


APPENDIX IV - PATHOLOGY AND CELL PROLIFERATION PHASE REPORT

Figure 1 Example of Calibration Curve

Calibration data for V

Standard ID	Mean Signal (Pk Height)	Entered Concentration (mg/L)	Calculated Concentration (mg/L)	Standard Deviation	%RSD
Calib Blank	0.7654	---	---	---	---
Stand.2ug/ml	0.0066	2.000	1.976	0.0001	2.04
Stand.4ug/ml	0.0133	4.000	4.006	0.0001	0.64
Stand.8ug/ml	0.0265	8.000	8.003	0.0004	1.67
Stand.16ug/ml	0.0538	16.000	16.32	0.0005	1.01
Stand.32ug/ml	0.1034	32.000	31.73	0.0009	0.85
Stand.64ug/ml	0.2004	64.000	63.66	0.0021	1.03
Stand.128ug/ml	0.3475	128.000	128.5	0.0016	0.45
Correlation Coefficient: 0.99998		Slope: 0.00333		---	---



**APPENDIX IV - PATHOLOGY AND CELL PROLIFERATION
PHASE REPORT**

PATHOLOGY PHASE REPORT

STUDY NUMBER A94206

AnaPath PHASE NUMBER 10225

Vanadium Pentoxide: 16-Day Inhalation Toxicity Study in Female Mice.

AnaPath PHASE NUMBER : 10225 **PATH NO.:** 10225 HJC

SPONSOR : Advance Technology Institute
5300 International Blvd
N. Charleston, SC 29418 / USA

TEST FACILITY : Harlan Laboratories Ltd.
Wölferstrasse 4
4414 Füllinsdorf/Switzerland

TEST SITE : AnaPath GmbH
Buchsweg 56
4625 Oberbuchsitzen
Switzerland

ARCHIVING : Harlan Laboratories Ltd.
STUDY DIRECTOR : Dr.D.Schuler

PRINCIPAL INVESTIGATOR : Dr.H.-J.Chevalier

TEST ITEM : Vanadium Pentoxide Details are described in Study Director's Report

TEST SYSTEM : Mouse Details are described in Study Director's Report

COMPLETION DATE OF PHASE REPORT :

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

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ANIMAL HEADING DATA: DOSE GROUP 4.....	52
TEXT OF GROSS AND MICROSCOPIC FINDINGS: DOSE GROUP 4.....	53
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¹ Animal organ finding table

PATHOLOGY PHASE REPORT

STUDY NUMBER A94206

AnaPath PHASE NUMBER 10225

Vanadium Pentoxide: 16-Day Inhalation Toxicity Study in Female Mice.

AnaPath PHASE NUMBER : 10225 **PATH NO.:** 10225 HJC

SPONSOR : Advance Technology Institute
5300 International Blvd
N. Charleston, SC 29418 / USA

TEST FACILITY : Harlan Laboratories Ltd.
Wölferstrasse 4
4414 Füllinsdorf/Switzerland

TEST SITE : AnaPath GmbH
Buchsweg 56
4625 Oberbuchsitzen
Switzerland

ARCHIVING : Harlan Laboratories Ltd.

STUDY DIRECTOR : Dr.D.Schuler

PRINCIPAL INVESTIGATOR

Dr.H.-J.Chevalier

TEST ITEM : Vanadium Pentoxide Details are described in Study Director's Report

TEST SYSTEM : Mouse Details are described in Study Director's Report

COMPLETION DATE OF PHASE REPORT :

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

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ANIMAL HEADING DATA: DOSE GROUP 4	52
TEXT OF GROSS AND MICROSCOPIC FINDINGS: DOSE GROUP 4.....	53
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¹ Animal organ finding table

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

STATEMENT OF COMPLIANCE

The undersigned hereby declares that the study phase Histopathology has been conducted in accordance with the agreed study plan and with the Standard Operating Procedures in use at AnaPath GmbH, Oberbuchsitzen, Switzerland. The Histopathology phase has also been performed in compliance with the

Swiss Ordinance relating to Good Laboratory Practice adopted May 18, 2005 [RS 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26, 1997 by decision of the OECD Council [C (97)186/Final].

Principal Investigator:

Dr. Hans-Jörg Chevalier
Veterinary Pathologist

.....

date:

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

QUALITY ASSURANCE GLP

Test Site Quality Assurance of AnaPath GmbH

c/o Harlan Laboratories Ltd., 4452 Itingen / Switzerland

STATEMENT

Harlan Laboratories Study:	B85318
Study Director:	Dr. D. Schuler
Anapath Phase Number:	10268
Test Item:	DA-3201
Principal Investigator	
Histopathology Phase:	Dr. Hans-Jörg Chevalier
Histopathology Phase to:	DA-3201: A 4-Week Inhalation Toxicity Study in the Rat

The general facilities and activities are audited periodically and the results are reported to the responsible person and the management.

The Quality Assurance audited this Histopathology phase report. The dates are given below.

Dates and Types of QA Inspection	Dates of Report to the Principal Investigator and to Test Site Management
..... Process Based (Histopathology)
.....Histopathology Phase Report

This statement also confirms that this final Histopathology phase report reflects the raw data.

Sections of the study plan and amendments related to the phase were reviewed and reported to the study director, lead QA, and the facility management on: Summary report of study related inspection was issued to the study director, Lead QA and Test Facility Management.

Quality Assurance

N. Häuselmann

Date:

PATHOLOGY PHASE REPORT NO. 10225

PAGE: 3

PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

STATEMENT OF COMPLIANCE

The undersigned hereby declares that the study phase Histopathology has been conducted in accordance with the agreed study plan and with the Standard Operating Procedures in use at AnaPath GmbH, Oberbuchsitzen, Switzerland. The Histopathology phase has also been performed in compliance with the

Swiss Ordinance relating to Good Laboratory Practice adopted May 18, 2005 [RS 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26, 1997 by decision of the OECD Council [C (97)186/Final].

Principal Investigator:

Dr. Hans-Jörg Chevalier
Veterinary Pathologist

.....

date:

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

QUALITY ASSURANCE GLP

Test Site Quality Assurance of AnaPath GmbH

c/o Harlan Laboratories Ltd., 4452 Itingen / Switzerland

STATEMENT

Harlan Laboratories Study: B85318
 Study Director: Dr. D. Schuler
 Anapath Phase Number: 10268
 Test Item: DA-3201
 Principal Investigator
 Histopathology Phase: Dr. Hans-Jörg Chevalier
 Histopathology Phase to: DA-3201: A 4-Week Inhalation Toxicity Study in the Rat

The general facilities and activities are audited periodically and the results are reported to the responsible person and the management.

The Quality Assurance audited this Histopathology phase report. The dates are given below.

Dates and Types of QA Inspection	Dates of Report to the Principal Investigator and to Test Site Management
..... Process Based (Histopathology)
.....Histopathology Phase Report

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Quality Assurance

N. Häuselmann

Date:

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

SUMMARY

The purpose of this 16-day inhalation toxicity study was to evaluate the range of toxicity on specific end-points in the lungs and to obtain data on the concentration in blood and lungs associated with the exposure of Vanadium Pentoxide to mice by nose-only inhalation (6 hours / day for 16 consecutive days).

The results will be used to provide a rational basis for the assessment of the toxicological risk to man.

Hundred ninety eight female mice were assigned to 5 groups of 48 mice each (1-4) and 6 mice in group 5. The animals were treated as described under 'Material and Methods'. At the end of the study periods, all animals were sacrificed, necropsied and examined *post mortem*. Histological examination was performed on allocation B and C mice as described under 'Material and Methods'.

Mortality:

One mouse of group 3 died after 12 days on test.

Gross Findings:

At necropsy, no gross findings of the lung were recorded that distinguished test item exposed mice from controls.

Microscopic Findings:

In the mice sacrificed after 16 days on test, the following findings were diagnosed:

multifocal/diffuse alveolar histiocytosis, dose-dependent in degree, in all 6 mice of groups 3 and 4;
multifocal subacute alveolitis, dose-dependent in degree, 5 mice of group 3 and all 6 mice of group 4.
multifocal granulocytic infiltration at similar severity in 4 mice of group 3 and 5 mice of group 4.

Immunohistochemistry:

An increased dose-dependent proliferation rate was noted in groups 3 and 4 after 7 and 16 days on test.

The NOEL in this study is considered to be 0.25 mg test item/m³ air.

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

MATERIAL AND METHODS

Allocation

Sub Groups:	Group 1 Air Control	Group 2 0.25 mg/m ³ Air	Group 3 1.0 mg/m ³ Air	Group 4 4.0 mg/m ³ Air	Group 5 Positive Control
A Vanadium Pentoxide	1 - 6	49 - 54	97 - 102	145 - 150	---
B Cell Proliferation (day 7)	7 - 12	55 - 60	103 - 108	151 - 156	---
C Cell Proliferation (day 16), Histopathology	13 - 18	61 - 66	109 - 114	157 - 162	---
D Biomarkers in Lung Tissue (Glutathione, α -tocopherol)	19 - 26	67 - 74	115 - 122	163 - 170	---
E Biomarkers in Lung Tissue (F2-isoprostanes)	27 - 34	75 - 82	123 - 130	171 - 178	---
F DNA lesions	35 - 42	83 - 90	131 - 138	179 - 186	---
G Comet Assay	43 - 48	91 - 96	139 - 144	187 - 192	193 - 198

Necropsy and Histopathology

Necropsies and histotechnique were performed at Harlan Laboratories Ltd., Itingen, Switzerland. Lung sections were stained with hematoxylin & eosin (Allocation C, groups 1 to 4) and for the proliferation markers PCNA and Ki67 (Allocation B and C, groups 1 to 4).

The determination of proliferation markers PCNA and Ki67 was done quantitatively by counting all marker-positive cells/microscope view area (1 cm²) at a magnification x400 examining 5 fields/animal. The results are presented in tables 1 to 4.

The following grading system was applied for the proliferation rate:

1-15 positive cells = grade minimal; 16-30 positive cells = grade slight; 31-45 positive cells = grade moderate; 46-60 positive cells = grade marked; >60 positive cells = grade severe.

Data Compilation

The animal data and necropsy findings were transferred via electronic diskette from Harlan Laboratories Ltd. to the PathData System.

The microscopic findings were recorded during histopathologic examination by the pathologist and directly entered into the PathData System. The slides were evaluated during August 2009.

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

SUMMARY

The purpose of this 16-day inhalation toxicity study was to evaluate the range of toxicity on specific end-points in the lungs and to obtain data on the concentration in blood and lungs associated with the exposure of Vanadium Pentoxide to mice by nose-only inhalation (6 hours / day for 16 consecutive days).

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Mortality:

One mouse of group 3 died after 12 days on test.

Gross Findings:

At necropsy, no gross findings of the lung were recorded that distinguished test item exposed mice from controls.

Microscopic Findings:

In the mice sacrificed after 16 days on test, the following findings were diagnosed:

multifocal/diffuse alveolar histiocytosis, dose-dependent in degree, in all 6 mice of groups 3 and 4;
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Immunohistochemistry:

An increased dose-dependent proliferation rate was noted in groups 3 and 4 after 7 and 16 days on test.

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PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

MATERIAL AND METHODS

Allocation

Sub Groups:	Group 1 Air Control	Group 2 0.25 mg/m ³ Air	Group 3 1.0 mg/m ³ Air	Group 4 4.0 mg/m ³ Air	Group 5 Positive Control
A Vanadium Pentoxide	1 - 6	49 - 54	97 - 102	145 - 150	---
B Cell Proliferation (day 7)	7 - 12	55 - 60	103 - 108	151 - 156	---
C Cell Proliferation (day 16), Histopathology	13 - 18	61 - 66	109 - 114	157 - 162	---
D Biomarkers in Lung Tissue (Glutathione, α -tocopherol)	19 - 26	67 - 74	115 - 122	163 - 170	---
E Biomarkers in Lung Tissue (F2-isoprostanes)	27 - 34	75 - 82	123 - 130	171 - 178	---
F DNA lesions	35 - 42	83 - 90	131 - 138	179 - 186	---
G Comet Assay	43 - 48	91 - 96	139 - 144	187 - 192	193 - 198

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PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Histologic changes were described, wherever possible, according to distribution, severity and morphologic character. Severity scores for H&E microscopy were assigned as given under "Explanation of Codes and Symbols".

All microscopic findings are listed in the "Table of Individual Microscopic Findings", along with an explanation of the codes and symbols used. Computer-generated incidence tables derived from these data are also present, as well as the complete narrative of the both macroscopic and microscopic findings.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

RESULTS

Mortality:

From the mice assigned to allocation C, animal #109 (group 3) died after 12 days on test.

Gross Findings:

At necropsy, no gross findings in the lungs were recorded that distinguished test item exposed mice from controls.

Microscopic Findings:

In the H&E stained lung sections from the mice sacrificed after 16 days on test, the following findings were diagnosed:

Alveolar histiocytosis was noted in 1 mouse of group 2 (focal, minimal), and all 6 mice of groups 3 and 4. This histiocytosis was multifocal to diffuse and minimal to slight in group 3, and diffuse and slight to moderate in group 4. The finding in the group 2 mouse is considered to be incidental.

Multifocal subacute alveolitis was noted in 5 mice of group 3 and all 6 mice of group 4 and was minimal to slight in group 3 and slight to moderate in group 4.

Multifocal granulocytic infiltration was noted in 4 mice of group 3 and 5 mice of group 4. The degree of severity was minimal to slight in both groups.

Immunohistochemistry:

Ki67: The mean numbers of marker-positive cells after 7 days were 10.1, 11.4, 18.1, and 24.7 in groups 1 to 4, respectively, and after 16 days 12.0, 9.2, 27.1, and 87.8 in groups 1 to 4, respectively.

PCNA: The mean numbers of marker-positive cells after 7 days were 25.1, 25.0, 41.4, and 47.8 in groups 1 to 4, respectively, and after 16 days 33.0, 26.3, 34.9, and 68.3 in groups 1 to 4, respectively.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Histologic changes were described, wherever possible, according to distribution, severity and morphologic character. Severity scores for H&E microscopy were assigned as given under "Explanation of Codes and Symbols".

All microscopic findings are listed in the "Table of Individual Microscopic Findings", along with an explanation of the codes and symbols used. Computer-generated incidence tables derived from these data are also present, as well as the complete narrative of the both macroscopic and microscopic findings.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

RESULTS

Mortality:

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Gross Findings:

At necropsy, no gross findings in the lungs were recorded that distinguished test item exposed mice from controls.

Microscopic Findings:

In the H&E stained lung sections from the mice sacrificed after 16 days on test, the following findings were diagnosed:

Alveolar histiocytosis was noted in 1 mouse of group 2 (focal, minimal), and all 6 mice of groups 3 and 4. This histiocytosis was multifocal to diffuse and minimal to slight in group 3, and diffuse and slight to moderate in group 4. The finding in the group 2 mouse is considered to be incidental.

Multifocal subacute alveolitis was noted in 5 mice of group 3 and all 6 mice of group 4 and was minimal to slight in group 3 and slight to moderate in group 4.

Multifocal granulocytic infiltration was noted in 4 mice of group 3 and 5 mice of group 4. The degree of severity was minimal to slight in both groups.

Immunohistochemistry:

Ki67: The mean numbers of marker-positive cells after 7 days were 10.1, 11.4, 18.1, and 24.7 in groups 1 to 4, respectively, and after 16 days 12.0, 9.2, 27.1, and 87.8 in groups 1 to 4, respectively.

PCNA: The mean numbers of marker-positive cells after 7 days were 25.1, 25.0, 41.4, and 47.8 in groups 1 to 4, respectively, and after 16 days 33.0, 26.3, 34.9, and 68.3 in groups 1 to 4, respectively.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Histologic changes were described, wherever possible, according to distribution, severity and morphologic character. Severity scores for H&E microscopy were assigned as given under "Explanation of Codes and Symbols".

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

RESULTS

Mortality:

From the mice assigned to allocation C, animal #109 (group 3) died after 12 days on test.

Gross Findings:

At necropsy, no gross findings in the lungs were recorded that distinguished test item exposed mice from controls.

Microscopic Findings:

In the H&E stained lung sections from the mice sacrificed after 16 days on test, the following findings were diagnosed:

Alveolar histiocytosis was noted in 1 mouse of group 2 (focal, minimal), and all 6 mice of groups 3 and 4. This histiocytosis was multifocal to diffuse and minimal to slight in group 3, and diffuse and slight to moderate in group 4. The finding in the group 2 mouse is considered to be incidental.

Multifocal subacute alveolitis was noted in 5 mice of group 3 and all 6 mice of group 4 and was minimal to slight in group 3 and slight to moderate in group 4.

Multifocal granulocytic infiltration was noted in 4 mice of group 3 and 5 mice of group 4. The degree of severity was minimal to slight in both groups.

Immunohistochemistry:

Ki67: The mean numbers of marker-positive cells after 7 days were 10.1, 11.4, 18.1, and 24.7 in groups 1 to 4, respectively, and after 16 days 12.0, 9.2, 27.1, and 87.8 in groups 1 to 4, respectively.

PCNA: The mean numbers of marker-positive cells after 7 days were 25.1, 25.0, 41.4, and 47.8 in groups 1 to 4, respectively, and after 16 days 33.0, 26.3, 34.9, and 68.3 in groups 1 to 4, respectively.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

CONCLUSIONS

In this 16-day inhalation toxicity study with Vanadium pentoxide in mice, inflammatory lesions (alveolar histiocytosis, alveolitis and granulocytic infiltration) were noted in groups 3 and 4 in a dose-dependent severity.

Immunohistochemically, an increased dose-dependent proliferation rate was noted in groups 3 and 4 after 7 and 16 days on test.

It is regarded as unlikely that these findings are related to oxidative stress. However, they may be due to an overload of the lungs with Vanadium Pentoxide particles resulting in incomplete lung clearance indicated by proliferation of histiocytes, inflammatory changes and increased proliferation rate demonstrated immunohistochemically.

The NOEL in this study is considered to be 0.25 mg test item/m³ air.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
 STUDY A94206**

TEST ITEM : Vanadium Pentoxide PATH. NO : 10225 HJC
 TEST SYSTEM : Mouse DATE :
 STUDY TYPE : 16-Day Inhalation SPONSOR : Advanced Technology Institute

Table 1 : Ki67-positive cells in animals sacrificed after 7 days on test (K1)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
7	9	7	10	5	10	41	8.2			
8	8	10	7	17	8	50	10.0			
9	18	10	9	8	8	53	10.6			
10	12	11	13	2	12	50	10.0			
11	10	17	17	5	9	58	11.6			
12	15	7	6	15	9	52	10.4			
								60.8	10.1	minimal (1)
55	15	12	5	12	11	55	11.0			
56	12	10	11	9	14	56	11.2			
57	14	10	18	9	5	56	11.2			
58	9	7	17	10	9	52	10.4			
59	20	16	15	21	12	84	16.8			
60	5	9	9	7	10	40	8.0			
								68.6	11.4	minimal (1)
103	22	34	19	24	33	132	26.4			
104	17	15	6	12	25	75	15.0			
105	15	14	10	11	10	60	12.0			
106	20	17	12	18	18	85	17.0			
107	23	11	12	12	16	74	14.8			
108	20	16	19	36	27	118	23.6			
								108.8	18.1	slight (2)
151	22	28	22	20	20	112	22.4			
152	19	17	13	34	21	104	20.8			
153	23	26	24	19	12	104	20.8			
154	30	22	21	53	21	147	29.4			
155	29	29	31	37	31	157	31.4			
156	20	31	16	29	21	117	23.4			
								148.2	24.7	slight(2)

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

CONCLUSIONS

In this 16-day inhalation toxicity study with Vanadium pentoxide in mice, inflammatory lesions (alveolar histiocytosis, alveolitis and granulocytic infiltration) were noted in groups 3 and 4 in a dose-dependent severity.

Immunohistochemically, an increased dose-dependent proliferation rate was noted in groups 3 and 4 after 7 and 16 days on test.

It is regarded as unlikely that these findings are related to oxidative stress. However, they may be due to an overload of the lungs with Vanadium Pentoxide particles resulting in incomplete lung clearance indicated by proliferation of histiocytes, inflammatory changes and increased proliferation rate demonstrated immunohistochemically.

The NOEL in this study is considered to be 0.25 mg test item/m³ air.

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Table 1 : Ki67-positive cells in animals sacrificed after 7 days on test (K1)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
7	9	7	10	5	10	41	8.2			
8	8	10	7	17	8	50	10.0			
9	18	10	9	8	8	53	10.6			
10	12	11	13	2	12	50	10.0			
11	10	17	17	5	9	58	11.6			
12	15	7	6	15	9	52	10.4			
								60.8	10.1	minimal (1)
55	15	12	5	12	11	55	11.0			
56	12	10	11	9	14	56	11.2			
57	14	10	18	9	5	56	11.2			
58	9	7	17	10	9	52	10.4			
59	20	16	15	21	12	84	16.8			
60	5	9	9	7	10	40	8.0			
								68.6	11.4	minimal (1)
103	22	34	19	24	33	132	26.4			
104	17	15	6	12	25	75	15.0			
105	15	14	10	11	10	60	12.0			
106	20	17	12	18	18	85	17.0			
107	23	11	12	12	16	74	14.8			
108	20	16	19	36	27	118	23.6			
								108.8	18.1	slight (2)
151	22	28	22	20	20	112	22.4			
152	19	17	13	34	21	104	20.8			
153	23	26	24	19	12	104	20.8			
154	30	22	21	53	21	147	29.4			
155	29	29	31	37	31	157	31.4			
156	20	31	16	29	21	117	23.4			
								148.2	24.7	slight(2)

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Table 2 : Ki67-positive cells in animals sacrificed after 16 days on test (K0)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
13	9	14	10	17	11	61	12.2			
14	6	6	9	7	8	36	7.2			
15	13	13	21	14	18	79	15.8			
16	8	6	7	15	8	44	8.8			
17	9	8	6	12	13	48	9.6			
18	11	5	4	6	6	32	6.4			
								60.0	12.0	minimal (1)
61	18	10	23	13	11	75	15.0			
62	7	4	5	11	6	33	6.6			
63	12	9	11	7	11	50	10.0			
64	5	5	6	11	2	29	5.8			
65	11	8	7	13	5	44	8.8			
66	9	6	12	11	6	44	8.8			
								55.0	9.2	minimal (1)
109	16	21	6	25	14	82	16.4			
110	19	13	19	21	26	98	19.6			
111	20	18	14	33	20	105	21.0			
112	37	21	38	29	68	193	38.6			
113	38	32	42	49	37	198	39.6			
114	19	42	14	25	37	137	27.4			
								162.6	27.1	slight (2)
157	46	49	47	54	51	247	49.4			
158 *)	101	116	166	89	75	547	109.4			
159	97	104	62	75	57	395	79.0			
160	61	47	64	77	50	299	59.8			
161	116	165	188	182	147	798	159.6			
162	73	50	77	65	83	348	69.6			
								526.8	87.8	severe(5)

*) Lung collapsed

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Table 3 : PCNA-positive cells in animals sacrificed after 7 days on test (K1)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
7	35	47	39	31	26	178	35.6			
8	23	37	27	24	17	128	25.6			
9	36	30	29	26	35	156	31.2			
10	28	8	20	20	21	97	19.4			
11	16	26	15	28	17	102	20.4			
12	12	11	19	23	27	92	18.4			
								150.6	25.1	slight (2)
55	32	20	21	15	16	104	20.8			
56	21	17	20	26	24	108	21.6			
57	29	21	31	23	22	126	25.2			
58	18	35	28	20	29	130	26.0			
59	24	35	30	41	38	168	33.6			
60	20	16	27	24	28	115	23.0			
								150.2	25.0	slight (2)
103	32	33	32	34	26	157	31.4			
104	23	31	12	27	35	128	25.6			
105	39	23	18	32	33	145	29.0			
106	37	28	32	51	37	185	37.0			
107	44	54	66	56	66	286	57.2			
108	54	76	73	62	75	340	68.0			
								248.2	41.4	moderate (3)
151	37	32	43	38	29	179	35.8			
152	47	58	40	29	35	209	41.8			
153	55	64	34	56	60	269	53.8			
154	51	59	64	72	66	312	62.4			
155	54	55	74	71	63	317	63.4			
156	31	34	23	34	26	148	29.6			
								286.8	47.8	marked (3)

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
 STUDY A94206**

TEST ITEM : Vanadium Pentoxide PATH. NO : 10225 HJC
 TEST SYSTEM : Mouse DATE :
 STUDY TYPE : 16-Day Inhalation SPONSOR : Advanced Technology Institute

Table 2 : Ki67-positive cells in animals sacrificed after 16 days on test (K0)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
13	9	14	10	17	11	61	12.2			
14	6	6	9	7	8	36	7.2			
15	13	13	21	14	18	79	15.8			
16	8	6	7	15	8	44	8.8			
17	9	8	6	12	13	48	9.6			
18	11	5	4	6	6	32	6.4			
								60.0	12.0	minimal (1)
61	18	10	23	13	11	75	15.0			
62	7	4	5	11	6	33	6.6			
63	12	9	11	7	11	50	10.0			
64	5	5	6	11	2	29	5.8			
65	11	8	7	13	5	44	8.8			
66	9	6	12	11	6	44	8.8			
								55.0	9.2	minimal (1)
109	16	21	6	25	14	82	16.4			
110	19	13	19	21	26	98	19.6			
111	20	18	14	33	20	105	21.0			
112	37	21	38	29	68	193	38.6			
113	38	32	42	49	37	198	39.6			
114	19	42	14	25	37	137	27.4			
								162.6	27.1	slight (2)
157	46	49	47	54	51	247	49.4			
158 *)	101	116	166	89	75	547	109.4			
159	97	104	62	75	57	395	79.0			
160	61	47	64	77	50	299	59.8			
161	116	165	188	182	147	798	159.6			
162	73	50	77	65	83	348	69.6			
								526.8	87.8	severe(5)

*) Lung collapsed

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Table 3 : PCNA-positive cells in animals sacrificed after 7 days on test (K1)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
7	35	47	39	31	26	178	35.6			
8	23	37	27	24	17	128	25.6			
9	36	30	29	26	35	156	31.2			
10	28	8	20	20	21	97	19.4			
11	16	26	15	28	17	102	20.4			
12	12	11	19	23	27	92	18.4			
								150.6	25.1	slight (2)
55	32	20	21	15	16	104	20.8			
56	21	17	20	26	24	108	21.6			
57	29	21	31	23	22	126	25.2			
58	18	35	28	20	29	130	26.0			
59	24	35	30	41	38	168	33.6			
60	20	16	27	24	28	115	23.0			
								150.2	25.0	slight (2)
103	32	33	32	34	26	157	31.4			
104	23	31	12	27	35	128	25.6			
105	39	23	18	32	33	145	29.0			
106	37	28	32	51	37	185	37.0			
107	44	54	66	56	66	286	57.2			
108	54	76	73	62	75	340	68.0			
								248.2	41.4	moderate (3)
151	37	32	43	38	29	179	35.8			
152	47	58	40	29	35	209	41.8			
153	55	64	34	56	60	269	53.8			
154	51	59	64	72	66	312	62.4			
155	54	55	74	71	63	317	63.4			
156	31	34	23	34	26	148	29.6			
								286.8	47.8	marked (3)

PATHOLOGY PHASE REPORT NO. 10225
PRINCIPAL SECTION

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HARLAN LABORATORIES
STUDY A94206

TEST ITEM : Vanadium Pentoxide PATH. NO : 10225 HJC
TEST SYSTEM : Mouse DATE :
STUDY TYPE : 16-Day Inhalation SPONSOR : Advanced Technology Institute

Table 4 : PCNA-positive cells in animals sacrificed after 16 days on test (K0)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
13	30	37	36	25	34	162	32.4			
14	24	22	21	27	28	122	24.4			
15	54	52	48	41	60	255	51.0			
16	23	24	21	27	15	110	22.0			
17	43	38	22	33	28	164	37.2			
18	31	32	31	34	28	156	31.2			
								198.2	33.0	moderate (3)
61	35	39	39	28	33	174	34.8			
62	27	22	20	26	27	122	23.0			
63	50	40	41	31	35	197	39.4			
64	25	29	42	30	24	150	30.0			
65	11	10	11	9	7	48	9.6			
66	14	24	28	20	19	105	21.0			
								157.8	26.3	slight (2)
109	24	24	12	20	12	92	18.4			
110	35	30	31	31	17	144	28.8			
111	44	36	58	40	56	234	46.8			
112	43	36	50	51	41	221	44.2			
113	38	41	29	31	62	201	40.2			
114	27	26	22	29	50	154	30.8			
								209.2	34.9	moderate (3)
157	49	36	52	44	65	246	49.2			
158 *)	107	107	93	119	104	530	106.0			
159	43	57	56	56	74	286	57.2			
160	42	52	67	54	54	269	53.8			
161	79	76	68	57	72	352	70.4			
162	42	59	81	116	67	365	73.0			
								409.6	68.3	severe(5)

*) Lung collapsed

PATHOLOGY REPORT (DRAFT)

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Harlan Laboratories: A94206

TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

F = Female animal
K0 = Terminal sacrifice group
K1...K9 = Interim sacrifice groups 1...9
+ = Intercurrent death/sacrificed moribund
+1 = Found dead

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G = Gross observation checked off histologically
* = Comment in text of individual animal data
' = Histologic examination not required
+ = Organ examined, findings present
- = Organ examined, no pathologic findings noted (AOFT only)

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1 = Minimal / very few / very small
GRADE 2 = Slight / few / small
GRADE 3 = Moderate / moderate number / moderate size
P = Finding present, severity not scored

PATHOLOGY PHASE REPORT NO. 10225

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PRINCIPAL SECTION

**HARLAN LABORATORIES
STUDY A94206**

TEST ITEM	: Vanadium Pentoxide	PATH. NO	: 10225 HJC
TEST SYSTEM	: Mouse	DATE	:
STUDY TYPE	: 16-Day Inhalation	SPONSOR	: Advanced Technology Institute

Table 4 : PCNA-positive cells in animals sacrificed after 16 days on test (K0)

Animal #	Number of positive cells/area examined					Sum	Mean	Sum	Mean	Grade
13	30	37	36	25	34	162	32.4			
14	24	22	21	27	28	122	24.4			
15	54	52	48	41	60	255	51.0			
16	23	24	21	27	15	110	22.0			
17	43	38	22	33	28	164	37.2			
18	31	32	31	34	28	156	31.2			
								198.2	33.0	moderate (3)
61	35	39	39	28	33	174	34.8			
62	27	22	20	26	27	122	23.0			
63	50	40	41	31	35	197	39.4			
64	25	29	42	30	24	150	30.0			
65	11	10	11	9	7	48	9.6			
66	14	24	28	20	19	105	21.0			
								157.8	26.3	slight (2)
109	24	24	12	20	12	92	18.4			
110	35	30	31	31	17	144	28.8			
111	44	36	58	40	56	234	46.8			
112	43	36	50	51	41	221	44.2			
113	38	41	29	31	62	201	40.2			
114	27	26	22	29	50	154	30.8			
								209.2	34.9	moderate (3)
157	49	36	52	44	65	246	49.2			
158 *)	107	107	93	119	104	530	106.0			
159	43	57	56	56	74	286	57.2			
160	42	52	67	54	54	269	53.8			
161	79	76	68	57	72	352	70.4			
162	42	59	81	116	67	365	73.0			
								409.6	68.3	severe(5)

*) Lung collapsed

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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
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EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

F = Female animal
K0 = Terminal sacrifice group
K1...K9 = Interim sacrifice groups 1...9
+ = Intercurrent death/sacrificed moribund
+1 = Found dead

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G = Gross observation checked off histologically
* = Comment in text of individual animal data
' = Histologic examination not required
+ = Organ examined, findings present
- = Organ examined, no pathologic findings noted (AOFT only)

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1 = Minimal / very few / very small
GRADE 2 = Slight / few / small
GRADE 3 = Moderate / moderate number / moderate size
P = Finding present, severity not scored

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 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
 STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO. ANIMALS:	6	6	6	6	
LUNG H&E :	6	6	6	6	
- Alveol.Histiocytosis:	-	1	6	6	
- Alveolitis :	-	-	5	5	
- Granulocytic Infiltr:	-	-	4	5	
- Congestion :	-	-	1	-	
LUNG KI67 :	6	6	6	6	
- 1-15 positive cells :	5	6	-	-	
- 16-30 positive cells:	1	-	4	-	
- 31-45 positive cells:	-	-	2	-	
- 46-60 positive cells:	-	-	-	2	
- >60 positive cells :	-	-	-	4	
LUNG PCNA :	6	6	6	6	
- 1-15 positive cells :	-	1	-	-	
- 16-30 positive cells:	2	3	2	-	
- 31-45 positive cells:	3	2	3	-	
- 46-60 positive cells:	1	-	1	3	
- >60 positive cells :	-	-	-	3	

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TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K1

SEX :	FEMALE			
DOSE GROUP:	01	02	03	04
NO. ANIMALS:	6	6	6	6
LUNG KI67 :	6	6	6	6
- 1-15 positive cells :	6	5	3	-
- 16-30 positive cells:	-	1	3	5
- 31-45 positive cells:	-	-	-	1
LUNG PCNA :	6	6	6	6
- 16-30 positive cells:	4	5	2	1
- 31-45 positive cells:	2	1	2	2
- 46-60 positive cells:	-	-	1	1
- >60 positive cells :	-	-	1	2

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<i>TEST ITEM</i> : Vanadium Pentoxide	<i>PATHOL. NO.:</i> 10225 HJC
<i>TEST SYSTEM</i> : MOUSE, 16 Days, Inhalation	<i>DATE</i> : 27-AUG-09
<i>SPONSOR</i> : Advanced Technology Inst.	<i>PathData®System V6.2d2</i>

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
 STATUS AT NECROPSY: K0, INCL. DEATHS

	SEX :					
	DOSE GROUP:	01	02	03	04	FEMALE
	NO. ANIMALS:	6	6	6	6	
<hr/>						
LUNG H&E	:	6	6	6	6	
- Alveol.Histiocytosis:		-	1	6	6	
- Alveolitis	:	-	-	5	5	
- Granulocytic Infiltr:		-	-	4	5	
- Congestion	:	-	-	1	-	
<hr/>						
LUNG KI67	:	6	6	6	6	
- 1-15 positive cells :		5	6	-	-	
- 16-30 positive cells:		1	-	4	-	
- 31-45 positive cells:		-	-	2	-	
- 46-60 positive cells:		-	-	-	2	
- >60 positive cells :		-	-	-	4	
<hr/>						
LUNG PCNA	:	6	6	6	6	
- 1-15 positive cells :		-	1	-	-	
- 16-30 positive cells:		2	3	2	-	
- 31-45 positive cells:		3	2	3	-	
- 46-60 positive cells:		1	-	1	3	
- >60 positive cells :		-	-	-	3	

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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K1

SEX :	FEMALE			
DOSE GROUP:	01	02	03	04
NO. ANIMALS:	6	6	6	6
LUNG KI67 :	6	6	6	6
- 1-15 positive cells :	6	5	3	-
- 16-30 positive cells:	-	1	3	5
- 31-45 positive cells:	-	-	-	1
LUNG PCNA :	6	6	6	6
- 16-30 positive cells:	4	5	2	1
- 31-45 positive cells:	2	1	2	2
- 46-60 positive cells:	-	-	1	1
- >60 positive cells :	-	-	1	2

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 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
 STATUS AT NECROPSY: K0, INCL. DEATHS

	SEX :					FEMALE
	DOSE GROUP:	01	02	03	04	
	NO. ANIMALS:	6	6	6	6	
LUNG H&E	:	6	6	6	6	
- Alveol.Histiocytosis						
GRADE 1 :		-	1	4	-	
GRADE 2 :		-	-	2	2	
GRADE 3 :		-	-	-	4	
TOTAL AFFECTED :		-	1	6	6	
MEAN GRADE/TISS.AFF.:		-	1.0	1.3	2.7	
.....						
- Alveolitis						
GRADE 1 :		-	-	1	1	
GRADE 2 :		-	-	4	3	
GRADE 3 :		-	-	-	1	
TOTAL AFFECTED :		-	-	5	5	
MEAN GRADE/TISS.AFF.:		-	-	1.8	2.0	
.....						
- Granulocytic Infiltr						
GRADE 1 :		-	-	3	3	
GRADE 2 :		-	-	1	2	
TOTAL AFFECTED :		-	-	4	5	
MEAN GRADE/TISS.AFF.:		-	-	1.3	1.4	
.....						
- Congestion						
GRADE 3 :		-	-	1	-	
TOTAL AFFECTED :		-	-	1	-	
MEAN GRADE/TISS.AFF.:		-	-	3.0	-	

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 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 01, 0 mg/cbm Air

ANIMAL NUMBER :

	1	2	3	4	5	6	7	8	9	10
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1
LUNG KI67							+	+	+	+
- 1-15 positive cells							P.	P.	P.	P.
.....										
LUNG PCNA							+	+	+	+
- 16-30 positive cells							.	P.	.	P.
- 31-45 positive cells							P.	.	P.	.
.....										

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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

	SEX :					FEMALE
	DOSE GROUP:	01	02	03	04	
	NO. ANIMALS:	6	6	6	6	
LUNG H&E	:	6	6	6	6	
- Alveol.Histiocytosis						
GRADE 1 :		-	1	4	-	
GRADE 2 :		-	-	2	2	
GRADE 3 :		-	-	-	4	
TOTAL AFFECTED :		-	1	6	6	
MEAN GRADE/TISS.AFF.:		-	1.0	1.3	2.7	
.....						
- Alveolitis						
GRADE 1 :		-	-	1	1	
GRADE 2 :		-	-	4	3	
GRADE 3 :		-	-	-	1	
TOTAL AFFECTED :		-	-	5	5	
MEAN GRADE/TISS.AFF.:		-	-	1.8	2.0	
.....						
- Granulocytic Infiltr						
GRADE 1 :		-	-	3	3	
GRADE 2 :		-	-	1	2	
TOTAL AFFECTED :		-	-	4	5	
MEAN GRADE/TISS.AFF.:		-	-	1.3	1.4	
.....						
- Congestion						
GRADE 3 :		-	-	1	-	
TOTAL AFFECTED :		-	-	1	-	
MEAN GRADE/TISS.AFF.:		-	-	3.0	-	

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 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 01, 0 mg/cbm Air

ANIMAL NUMBER :

	1	2	3	4	5	6	7	8	9	10
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1
LUNG KI67							+	+	+	+
- 1-15 positive cells							P.	P.	P.	P.
.....										
LUNG PCNA							+	+	+	+
- 16-30 positive cells							.	P.	.	P.
- 31-45 positive cells							P.	.	P.	.
.....										

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 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 01, 0 mg/cbm Air

ANIMAL NUMBER :

	11	12	13	14	15	16	17	18	19	20
	FK1	FK1	FK0	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E	'	'	-	-	-	-	-	-	'	'
LUNG KI67	+	+	+	+	+	+	+	+	'	'
- 1-15 positive cells	P.	P.	P.	P.	.	P.	P.	P.		
- 16-30 positive cells	P.	.	.	.		
LUNG PCNA	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	P.	P.	.	P.	.	P.	.	.		
- 31-45 positive cells	.	.	P.	.	.	.	P.	P.		
- 46-60 positive cells	P.	.	.	.		

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 02, 0.25 mg/cbm Air

ANIMAL NUMBER :

	49	50	51	52	53	54	55	56	57	58
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1
LUNG KI67							+	+	+	+
- 1-15 positive cells							P.	P.	P.	P.
.....										
LUNG PCNA							+	+	+	+
- 16-30 positive cells							P.	P.	P.	P.
.....										

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 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 01, 0 mg/cbm Air

ANIMAL NUMBER :

	11	12	13	14	15	16	17	18	19	20
	FK1	FK1	FK0	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E	'	'	-	-	-	-	-	-	'	'
LUNG KI67	+	+	+	+	+	+	+	+	'	'
- 1-15 positive cells	P.	P.	P.	P.	.	P.	P.	P.		
- 16-30 positive cells	P.	.	.	.		
LUNG PCNA	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	P.	P.	.	P.	.	P.	.	.		
- 31-45 positive cells	.	.	P.	.	.	.	P.	P.		
- 46-60 positive cells	P.	.	.	.		

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TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 02, 0.25 mg/cbm Air

ANIMAL NUMBER :

	49	50	51	52	53	54	55	56	57	58
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1
LUNG KI67							+	+	+	+
- 1-15 positive cells							P.	P.	P.	P.
.....										
LUNG PCNA							+	+	+	+
- 16-30 positive cells							P.	P.	P.	P.
.....										

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 02, 0.25 mg/cbm Air

ANIMAL NUMBER :

	59	60	61	62	63	64	65	66	67	68
	FK1	FK1	FK0	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E			-	-	-	-	-	+		
- Alveol.Histiocytosis			1.		
.....										
LUNG KI67	+	+	+	+	+	+	+	+		
- 1-15 positive cells	.	P.								
- 16-30 positive cells	P.		
.....										
LUNG PCNA	+	+	+	+	+	+	+	+		
- 1-15 positive cells	P.	.		
- 16-30 positive cells	.	P.	.	P.	.	P.	.	P.		
- 31-45 positive cells	P.	.	P.	.	P.	.	.	.		
.....										

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 03, 1.0 mg/cbm Air

ANIMAL NUMBER :

	97	98	99	100	101	102	103	104	105	106
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1
LUNG KI67	'	'	'	'	'	'	+	+	+	+
- 1-15 positive cells							.	P.	P.	.
- 16-30 positive cells							P.	.	.	P.
.....										
LUNG PCNA	'	'	'	'	'	'	+	+	+	+
- 16-30 positive cells							.	P.	P.	.
- 31-45 positive cells							P.	.	.	P.
.....										

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 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFI)

DOSE GROUP : 02, 0.25 mg/cbm Air

ANIMAL NUMBER :

	59	60	61	62	63	64	65	66	67	68
	FK1	FK1	FK0	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E	'	'	-	-	-	-	-	+	'	'
- Alveol.Histiocytosis			1.		
.....										
LUNG KI67	+	+	+	+	+	+	+	+	'	'
- 1-15 positive cells	.	P.								
- 16-30 positive cells	P.		
.....										
LUNG PCNA	+	+	+	+	+	+	+	+	'	'
- 1-15 positive cells	P.	.	.
- 16-30 positive cells	.	P.	.	P.	.	P.	.	P.		
- 31-45 positive cells	P.	.	P.	.	P.	.	.	.		
.....										

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 03, 1.0 mg/cbm Air

ANIMAL NUMBER :

	97	98	99	100	101	102	103	104	105	106
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1
LUNG KI67							+	+	+	+
- 1-15 positive cells							.	P.	P.	.
- 16-30 positive cells							P.	.	.	P.
.....										
LUNG PCNA							+	+	+	+
- 16-30 positive cells							.	P.	P.	.
- 31-45 positive cells							P.	.	.	P.
.....										

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TEST ITEM : Vanadium Pentoxide
 TEST SYSTEM : MOUSE, 16 Days, Inhalation
 SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
 DATE : 27-AUG-09
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 03, 1.0 mg/cbm Air

ANIMAL NUMBER :

	107	108	109	110	111	112	113	114	115	116
	FK1	FK1	FK0+	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E	'	'	+G	+	+	+	+	+	'	'
- Alveol.Histiocytosis			1.	1.	1.	2.	1.	2.		
- Alveolitis			.	2.	2.	2.	1.	2.		
- Granulocytic Infiltr			.	.	1.	2.	1.	1.		
- Congestion			3.		
.....										
LUNG KI67	+	+	+	+	+	+	+	+	'	'
- 1-15 positive cells	P.		
- 16-30 positive cells	.	P.	P.	P.	P.	.	.	P.		
- 31-45 positive cells	P.	P.	.		
.....										
LUNG PCNA	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	.	.	P.	P.		
- 31-45 positive cells	P.	P.	P.		
- 46-60 positive cells	P.	.	.	.	P.	.	.	.		
- >60 positive cells	.	P.		
.....										

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 04, 4.0 mg/cbm Air

ANIMAL NUMBER :

	145	146	147	148	149	150	151	152	153	154
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1

LUNG KI67								+	+	+	+
- 16-30 positive cells								P.	P.	P.	P.
.....											
LUNG PCNA								+	+	+	+
- 31-45 positive cells								P.	P.	.	.
- 46-60 positive cells								.	.	P.	.
- >60 positive cells								.	.	.	P.
.....											

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 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

DOSE GROUP : 03, 1.0 mg/cbm Air

ANIMAL NUMBER :

	107	108	109	110	111	112	113	114	115	116
	FK1	FK1	FK0+	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E			+G	+	+	+	+	+		
- Alveol.Histiocytosis			1.	1.	1.	2.	1.	2.		
- Alveolitis			.	2.	2.	2.	1.	2.		
- Granulocytic Infiltr			.	.	1.	2.	1.	1.		
- Congestion			3.		
.....										
LUNG KI67	+	+	+	+	+	+	+	+		
- 1-15 positive cells	P.		
- 16-30 positive cells	.	P.	P.	P.	P.	.	.	P.		
- 31-45 positive cells	P.	P.	.		
.....										
LUNG PCNA	+	+	+	+	+	+	+	+		
- 16-30 positive cells	.	.	P.	P.		
- 31-45 positive cells	P.	P.	P.		
- 46-60 positive cells	P.	.	.	.	P.	.	.	.		
- >60 positive cells	.	P.		
.....										

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 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 04, 4.0 mg/cbm Air

ANIMAL NUMBER :

	145	146	147	148	149	150	151	152	153	154
	FK2	FK2	FK2	FK2	FK2	FK2	FK1	FK1	FK1	FK1

LUNG KI67							+	+	+	+
- 16-30 positive cells							P.	P.	P.	P.
.....										
LUNG PCNA							+	+	+	+
- 31-45 positive cells							P.	P.	.	.
- 46-60 positive cells							.	.	P.	.
- >60 positive cells							.	.	.	P.
.....										

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 04, 4.0 mg/cbm Air

ANIMAL NUMBER :

155 156 157 158 159 160 161 162 163 164
 FK1 FK1 FK0 FK0 FK0 FK0 FK0 FK0 FK3 FK3

	155	156	157	158	159	160	161	162	163	164
	FK1	FK1	FK0	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E	'	'	+	+	+	+	+	+	'	'
- Alveol.Histiocytosis			3.	2.	2.	3.	3.	3.		
- Alveolitis			1.	.	2.	3.	2.	2.		
- Granulocytic Infiltr			1.	.	1.	2.	1.	2.		
.....										
LUNG KI67	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	.	P.		
- 31-45 positive cells	P.		
- 46-60 positive cells	.	.	P.	.	.	P.	.	.		
- >60 positive cells	.	.	.	P.	P.	.	P.	P.		
.....										
LUNG PCNA	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	.	P.		
- 46-60 positive cells	.	.	P.	.	P.	P.	.	.		
- >60 positive cells	P.	.	.	P.	.	.	P.	P.		
.....										

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 26/ 57
 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 01, 0 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL OF NECROPSY	TEST DAYS	FIRST DAY UNDER TEST	AND LAST DAY UNDER TEST	DATE OF NECROPSY
1	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
2	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
3	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
4	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
5	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
6	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
7	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
8	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
9	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
10	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
11	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
12	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
13	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
14	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
15	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
16	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
17	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
18	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
19	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
20	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
21	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
22	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
23	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
24	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
25	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
26	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
27	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
28	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
29	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
30	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
31	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
32	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
33	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
34	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
 DOSE GROUP : 04, 4.0 mg/cbm Air

ANIMAL NUMBER :

155 156 157 158 159 160 161 162 163 164
 FK1 FK1 FK0 FK0 FK0 FK0 FK0 FK0 FK3 FK3

	155	156	157	158	159	160	161	162	163	164
	FK1	FK1	FK0	FK0	FK0	FK0	FK0	FK0	FK3	FK3
LUNG H&E	'	'	+	+	+	+	+	+	'	'
- Alveol.Histiocytosis			3.	2.	2.	3.	3.	3.		
- Alveolitis			1.	.	2.	3.	2.	2.		
- Granulocytic Infiltr			1.	.	1.	2.	1.	2.		
.....										
LUNG KI67	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	.	P.		
- 31-45 positive cells	P.		
- 46-60 positive cells	.	.	P.	.	.	P.	.	.		
- >60 positive cells	.	.	.	P.	P.	.	P.	P.		
.....										
LUNG PCNA	+	+	+	+	+	+	+	+	'	'
- 16-30 positive cells	.	P.		
- 46-60 positive cells	.	.	P.	.	P.	P.	.	.		
- >60 positive cells	P.	.	.	P.	.	.	P.	P.		
.....										

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 26/ 57
 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 01, 0 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL NECROPSY	TEST DAYS	FIRST DAY	AND LAST DAY UNDER TEST	DATE OF NECROPSY
1	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
2	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
3	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
4	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
5	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
6	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
7	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
8	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
9	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
10	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
11	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
12	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
13	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
14	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
15	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
16	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
17	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
18	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
19	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
20	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
21	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
22	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
23	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
24	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
25	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
26	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
27	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
28	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
29	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
30	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
31	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
32	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
33	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
34	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide
TEST SYSTEM : MOUSE, 16 Days, Inhalation
SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
DATE : 27-AUG-09
PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 9
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 10
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 30/ 57
Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide
TEST SYSTEM : MOUSE, 16 Days, Inhalation
SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
DATE : 27-AUG-09
PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 13
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 14
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 11
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 12
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 31/ 57
Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide
TEST SYSTEM : MOUSE, 16 Days, Inhalation
SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
DATE : 27-AUG-09
PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

* STATE AT NECROPSY: KO
DAYS ON TEST : 16 * ANIMAL NO. : 15
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-46-60 positive cells

* STATE AT NECROPSY: KO
DAYS ON TEST : 16 * ANIMAL NO. : 16
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 31/ 57
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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 15
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-46-60 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 16
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide
TEST SYSTEM : MOUSE, 16 Days, Inhalation
SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
DATE : 27-AUG-09
PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 17
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 18
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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<i>TEST ITEM</i>	: Vanadium Pentoxide	<i>PATHOL. NO.:</i>	10225 HJC
<i>TEST SYSTEM</i>	: MOUSE, 16 Days, Inhalation	<i>DATE</i>	: 27-AUG-09
<i>SPONSOR</i>	: Advanced Technology Inst.	<i>PathData®System</i>	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 34/ 57
 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 02, 0.25 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL NECROPSY	TEST DAYS	FIRST DAY	AND LAST DAY UNDER TEST	DATE OF NECROPSY
49	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
50	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
51	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
52	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
53	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
54	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
55	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
56	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
57	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
58	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
59	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
60	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
61	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
62	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
63	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
64	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
65	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
66	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
67	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
68	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
69	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
70	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
71	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
72	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
73	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
74	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
75	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
76	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
77	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
78	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
79	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
80	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
81	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
82	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, 0 mg/cbm Air FEMALE

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 34/ 57
Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 02, 0.25 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL OF NECROPSY	TEST DAYS	FIRST DAY	AND LAST DAY UNDER TEST	DATE OF NECROPSY
49	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
50	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
51	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
52	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
53	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
54	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
55	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
56	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
57	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
58	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
59	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
60	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
61	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
62	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
63	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
64	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
65	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
66	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
67	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
68	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
69	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
70	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
71	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
72	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
73	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
74	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
75	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
76	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
77	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
78	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
79	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
80	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
81	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
82	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 57
.....

* NECROPSY FINDINGS
NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 58
.....

* NECROPSY FINDINGS
NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

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Harlan Laboratories: A94206

TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 61
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 62
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 63
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 64
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 65
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-1-15 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 66
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, focal, margin of left lobe, grade 1
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 63
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 64
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 65

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
Organ examined, no pathologic findings noted
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-1-15 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 66

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, focal, margin of left lobe, grade 1
LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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<i>TEST ITEM</i>	: Vanadium Pentoxide	<i>PATHOL. NO.:</i>	10225 HJC
<i>TEST SYSTEM</i>	: MOUSE, 16 Days, Inhalation	<i>DATE</i>	: 27-AUG-09
<i>SPONSOR</i>	: Advanced Technology Inst.	<i>PathData®System</i>	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

— ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS —

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 03, 1.0 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL NECROPSY	TEST DAYS	FIRST DAY UNDER TEST	AND LAST DAY UNDER TEST	DATE OF NECROPSY
97	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
98	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
99	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
100	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
101	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
102	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
103	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
104	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
105	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
106	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
107	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
108	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
109	F	K0	+1	12	18-MAY-09	29-MAY-09	30-MAY-09
110	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
111	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
112	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
113	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
114	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
115	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
116	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
117	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
118	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
119	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
120	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
121	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
122	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
123	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
124	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
125	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
126	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
127	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
128	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
129	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
130	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 02, 0.25 mg/cbm Air FEMALE

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 03, 1.0 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL NECROPSY	TEST DAYS	FIRST DAY UNDER TEST	AND LAST DAY UNDER TEST	DATE OF NECROPSY
97	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
98	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
99	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
100	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
101	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
102	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
103	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
104	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
105	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
106	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
107	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
108	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
109	F	K0	+1	12	18-MAY-09	29-MAY-09	30-MAY-09
110	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
111	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
112	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
113	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
114	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
115	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
116	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
117	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
118	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
119	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
120	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
121	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
122	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
123	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
124	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
125	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
126	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
127	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
128	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
129	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
130	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

PATHOLOGY REPORT (DRAFT)
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Harlan Laboratories: A94206

TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 03, 1.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 105
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-16-30 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 106
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-31-45 positive cells

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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 03, 1.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 107

.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-1-15 positive cells
LUNG PCNA:
-46-60 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 108

.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-16-30 positive cells
LUNG PCNA:
->60 positive cells

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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 03, 1.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0/+1
DAYS ON TEST : 12 * ANIMAL NO. : 109
.....

* NECROPSY FINDINGS

LUNG H&E:
01: Discoloration, reddish.
NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, diffuse, grade 1
-Congestion, grade 3
This finding corresponds to necropsy observation no: 01.
LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-16-30 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 110
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

PATHOLOGY REPORT (DRAFT)
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TEST ITEM : Vanadium Pentoxide
TEST SYSTEM : MOUSE, 16 Days, Inhalation
SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
DATE : 27-AUG-09
PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 03, 1.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 112
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:

- Alveolar histiocytosis, diffuse, grade 2
- Alveolitis, multifocal, grade 2
- Granulocytic Infiltration, multifocal, grade 2

LUNG KI67:

- 31-45 positive cells

LUNG PCNA:

- 31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 113
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:

- Alveolar histiocytosis, multifocal, grade 1
- Alveolitis, multifocal, grade 1
- Granulocytic Infiltration, multifocal, grade 1

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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 03, 1.0 mg/cbm Air FEMALE

CONT./FF. ANIMAL NO. : 113

.....
LUNG KI67:
-31-45 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 114

.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, diffuse, grade 2
-Alveolitis, multifocal, grade 2
-Granulocytic Infiltration, multifocal, grade 1
LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-31-45 positive cells

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

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INDIVIDUAL ANIMAL DATA

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 Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
 TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
 SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA
 DOSE GROUP : 04, 4.0 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL OF NECROPSY	TEST DAYS	FIRST DAY	AND LAST DAY UNDER TEST	DATE OF NECROPSY
145	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
146	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
147	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
148	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
149	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
150	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
151	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
152	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
153	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
154	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
155	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
156	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
157	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
158	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
159	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
160	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
161	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
162	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
163	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
164	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
165	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
166	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
167	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
168	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
169	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
170	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
171	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
172	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
173	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
174	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
175	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
176	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
177	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
178	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

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INDIVIDUAL ANIMAL DATA

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Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

ANIMAL HEADING DATA

DOSE GROUP : 04, 4.0 mg/cbm Air

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL OF NECROPSY	TEST DAYS	FIRST DAY	AND LAST DAY UNDER TEST	DATE OF NECROPSY
145	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
146	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
147	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
148	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
149	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
150	F	K2	K2	16	18-MAY-09	02-JUN-09	02-JUN-09
151	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
152	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
153	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
154	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
155	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
156	F	K1	K1	7	18-MAY-09	24-MAY-09	25-MAY-09
157	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
158	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
159	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
160	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
161	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
162	F	K0	K0	16	18-MAY-09	02-JUN-09	03-JUN-09
163	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
164	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
165	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
166	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
167	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
168	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
169	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
170	F	K3	K3	16	19-MAY-09	03-JUN-09	04-JUN-09
171	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
172	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
173	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
174	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
175	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
176	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
177	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09
178	F	K4	K4	16	19-MAY-09	03-JUN-09	04-JUN-09

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 51/ 57
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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 4.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 151
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-31-45 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 152
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-31-45 positive cells

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INDIVIDUAL ANIMAL DATA

PAGE : 52/ 57
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TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 4.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 153
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-16-30 positive cells
LUNG PCNA:
-46-60 positive cells

* STATE AT NECROPSY: K1
DAYS ON TEST : 7 * ANIMAL NO. : 154
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG KI67:
-16-30 positive cells
LUNG PCNA:
->60 positive cells

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

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TEST ITEM	: Vanadium Pentoxide	PATHOL. NO.:	10225 HJC
TEST SYSTEM	: MOUSE, 16 Days, Inhalation	DATE	: 27-AUG-09
SPONSOR	: Advanced Technology Inst.	PathData®System	V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 4.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 16 * ANIMAL NO. : 160

.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:

- Alveolar histiocytosis, diffuse, grade 3
- Alveolitis, multifocal, grade 3
- Granulocytic Infiltration, multifocal, grade 2

LUNG KI67:

- 46-60 positive cells

LUNG PCNA:

- 46-60 positive cells
-

* STATE AT NECROPSY: K0

DAYS ON TEST : 16 * ANIMAL NO. : 161

.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:

- Alveolar histiocytosis, diffuse, grade 3
- Alveolitis, multifocal, grade 2
- Granulocytic Infiltration, multifocal, grade 1

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 56/ 57
Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide
TEST SYSTEM : MOUSE, 16 Days, Inhalation
SPONSOR : Advanced Technology Inst.

PATHOL. NO.: 10225 HJC
DATE : 27-AUG-09
PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 4.0 mg/cbm Air FEMALE

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 160
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, diffuse, grade 3
-Alveolitis, multifocal, grade 3
-Granulocytic Infiltration, multifocal, grade 2
LUNG KI67:
-46-60 positive cells
LUNG PCNA:
-46-60 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 161
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, diffuse, grade 3
-Alveolitis, multifocal, grade 2
-Granulocytic Infiltration, multifocal, grade 1

PATHOLOGY REPORT (DRAFT)
INDIVIDUAL ANIMAL DATA

PAGE : 57/ 57
Harlan Laboratories: A94206

TEST ITEM : Vanadium Pentoxide PATHOL. NO.: 10225 HJC
TEST SYSTEM : MOUSE, 16 Days, Inhalation DATE : 27-AUG-09
SPONSOR : Advanced Technology Inst. PathData®System V6.2d2

TEXT OF GROSS AND MICROSCOPIC FINDINGS
DOSE GROUP : 04, 4.0 mg/cbm Air FEMALE

CONT./FF. ANIMAL NO. : 161

.....

LUNG KI67:
->60 positive cells
LUNG PCNA:
->60 positive cells

* STATE AT NECROPSY: K0
DAYS ON TEST : 16 * ANIMAL NO. : 162

.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG H&E:
-Alveolar histiocytosis, diffuse, grade 3
-Alveolitis, multifocal, grade 2
-Granulocytic Infiltration, multifocal, grade 2
LUNG KI67:
->60 positive cells
LUNG PCNA:
->60 positive cells

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

APPENDIX V - BIOMARKER DETERMINATIONS

APPENDIX V - BIOMARKER DETERMINATIONS

Determination of α -Tocopherol, Glutathione and 8-Isoprostane F2 α

CONTENTS

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1 MATERIALS AND METHODS

1.1 Analytical Standards

Details of the analytical standards as provided by the supplier.

α -Tocopherol

Supplier / Art. No: Sigma / T3251
Batch Number: 078K1882
Expiration Date: 31-May-2014
Purity: 98%
Storage Conditions: 2-8 °C

Reduced Glutathione

Supplier / Art. No: Cayman / 703014
Batch Number: 0410577, 174571-174572
Expiration Date: 24-Mar-2010
Purity: Not applicable (100% used for calculations)
Storage Conditions: 4 °C

8-Isoprostane F2 α

Supplier / Art. No: Cayman / 10367
Batch Number: 0410257
Expiration Date: 02-Dec-2009
Purity: Not applicable (100% used for calculations)
Storage Conditions: -20 °C

[H³]Prostaglandine F2 α

Supplier / Art. No: Amersham / TRK464
Batch Number: B131
Expiration Date: Not applicable
Purity: 98.9%
Specific Activity: 7.8 TBq/mmol
Storage Conditions: -20 °C

Determination of α -Tocopherol, Glutathione and 8-Isoprostane F2 α

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1.2	Study Samples and Storage	3
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5.4.1	α -Tocopherol	3
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1.5	Statistical Analysis	4
2	RESULTS	5
2.1	α -Tocopherol	5
2.2	Reduced (GSH) and Oxidised (GSSG) Glutathione.....	5
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3	CONCLUSIONS	7
4	REFERENCES	8

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1 MATERIALS AND METHODS

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Purity: 98%
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Reduced Glutathione

Supplier / Art. No: Cayman / 703014
Batch Number: 0410577, 174571-174572
Expiration Date: 24-Mar-2010
Purity: Not applicable (100% used for calculations)
Storage Conditions: 4 °C

8-Isoprostane F2 α

Supplier / Art. No: Cayman / 10367
Batch Number: 0410257
Expiration Date: 02-Dec-2009
Purity: Not applicable (100% used for calculations)
Storage Conditions: -20 °C

[H³]Prostaglandine F2 α

Supplier / Art. No: Amersham / TRK464
Batch Number: B131
Expiration Date: Not applicable
Purity: 98.9%
Specific Activity: 7.8 TBq/mmol
Storage Conditions: -20 °C

1.2 Study Samples and Storage

Detailed information concerning the in-life phase is provided in the main study report. In brief, female B6C3F1 mice were treated daily for 16 days by inhalation with Vanadium Pentoxide at dose levels of 0 (group 1), 0.25 mg/m³ (group 2), 1.0 mg/m³ (group 3) and 4.0 mg/m³ (group 4).

Lung samples were collected at sacrifice, frozen in liquid nitrogen and shipped to Dr. P. Sagelsdorff, responsible for Biomarker Determinations. The samples were kept at -80 °C until analyses between 25-Jun-2009 and 17-Jul-2009.

1.3 Reagents and Materials

All further reagents (including supplier, grade) and materials used in this study are listed in the raw data. Quality control samples (QCs) and dilution samples were prepared in rat lung homogenate, respectively.

Reagent	Catalog No	Batch	Supplier
8-Isoprostane EIA Kit	516351	0411264	Cayman Chemical Company / USA
Glutathione Assay Kit	703002	0411593 0411594	Cayman Chemical Company / USA

1.4 Analytical Method

5.4.1 α -Tocopherol

Frozen lung samples were homogenized with 10 volumes 0.9% NaCl. Proteins were precipitated from 250 μ l of the homogenate with an equal volume of ethanol and α -tocopherol was extracted with 1 mL hexane. Following lyophilisation of exactly 0.5 ml of the hexane extract the residue was reconstituted in 100 μ l acetonitrile/methanol (85+15). An aliquot of 50 μ l was analysed for α -tocopherol by HPLC with fluorescence detection (excitation at 295 nm, emission at 330 nm) on a Nucleosil 5 μ m C18 column (125 x 4.6 mm) with acetonitrile/methanol (85+15) at a flow of 1.5 ml/min as eluent [see References (1)].

For a determination of the recovery control lung samples were spiked with α -tocopherol at a concentration of 57.3 μ g/g lung. A mean recovery of 79.5 \pm 7.6% (n=2) was obtained.

5.4.2 Reduced (GSH) and Oxidised (GSSG) Glutathione

Frozen lung samples were homogenized in 10 volumes ice-cold 50 mM MES, pH 6-7, containing 1 mM EDTA. Proteins were precipitated by the addition of an equal volume of 10%

metaphosphoric acid and centrifugation at 2'000 g for 10 min. The supernatant was diluted 20 to 100-fold with MES buffer and total glutathione (GSH plus GSSG) concentration in liver homogenates was determined spectrophotometrically following reduction with glutathione reductase in the presence of NADPH and derivatisation with 5,5'-dithio-bis-(2-nitrobenzoic acid) at 405 nm according to the description of the glutathione assay kit (Cayman Chemical Company / USA). Oxidized glutathione (GSSG) was quantified similarly following inactivation of GSH with 2-vinylpyridine at dilutions of 2 to 10-fold with MES buffer [see References (2)].

For a determination of the recovery control lung samples were spiked with glutathione at concentrations of 1 and 250 $\mu\text{mol/g}$ lung. A mean recovery of $81.0 \pm 13.9\%$ (n=3) and $76.1 \pm 7.9\%$ (n=3), respectively, was obtained.

5.4.3 8-Isoprostane F2 α

Frozen lung samples were homogenised in 3 volumes 0.9% (w/v) NaCl. [H^3]Prostaglandin F2 α was added as internal standard and Isoprostanes were extracted with 2 volumes ethanol from the homogenate. Following centrifugation for 10 min at 1500g the supernatant was hydrolysed with an equal volume 15% (w/v) KOH for 1 h at 40°C for the release of esterified isoprostanes. The hydrolysate was diluted with 2 volumes water, acidified with 2 M HCl to pH 2.0-3.5 and loaded on Oasis HLB (30 mg) SPE column (Waters / USA), pre-equilibrated with 1 mL methanol and 1 mL 0.02 M HCl. The column was washed subsequently with 2 mL 0.02 M HCl and with 2 mL 40% methanol in 0.02 M HCl. The column was dried with air and rinsed with 2 mL heptane. Isoprostanes were eluted from the column with 2 mL ethylacetate. The final elute from the Oasis column was diluted with 2 mL heptane, loaded on a Sep-Pak Vac RC Silica (100 mg) column (Waters / USA), pre-equilibrated with 1 mL methanol and 1 mL ethylacetate/heptane (1+1 by volume), and eluted with 2 mL ethylacetate/methanol (1+1 by volume). The eluate was dried in a SpeedVac concentrator and reconstituted in 1 mL EIA-buffer. The recovery of the extraction was determined by scintillation counting of 100 μL of the reconstituted extract. Quantitative determination of 8-isoprostane F2 α was made by a commercially available ELISA (Cayman Chemical Company / USA) using different dilutions of the extract [see References (3)].

For a determination of the recovery control lung samples were spiked with 8-isoprostane F2 α at a concentration of 5 ng/g lung. A mean recovery of $94.1 \pm 27.9\%$ (n=4) was obtained.

1.5 Statistical Analysis

Dunnett's t-test following log transformation for a stabilization of the variances was applied to assess differences between treated groups and controls. Statistical significances are given in the tables with one asterisk (*: $p < 0.05$) and two asterices (**: $p < 0.01$).

1.2 Study Samples and Storage

Detailed information concerning the in-life phase is provided in the main study report. In brief, female B6C3F1 mice were treated daily for 16 days by inhalation with Vanadium Pentoxide at dose levels of 0 (group 1), 0.25 mg/m³ (group 2), 1.0 mg/m³ (group 3) and 4.0 mg/m³ (group 4).

Lung samples were collected at sacrifice, frozen in liquid nitrogen and shipped to Dr. P. Sagelsdorff, responsible for Biomarker Determinations. The samples were kept at -80 °C until analyses between 25-Jun-2009 and 17-Jul-2009.

1.3 Reagents and Materials

All further reagents (including supplier, grade) and materials used in this study are listed in the raw data. Quality control samples (QCs) and dilution samples were prepared in rat lung homogenate, respectively.

Reagent	Catalog No	Batch	Supplier
8-Isoprostane EIA Kit	516351	0411264	Cayman Chemical Company / USA
Glutathione Assay Kit	703002	0411593 0411594	Cayman Chemical Company / USA

1.4 Analytical Method

5.4.1 α -Tocopherol

Frozen lung samples were homogenized with 10 volumes 0.9% NaCl. Proteins were precipitated from 250 μ l of the homogenate with an equal volume of ethanol and α -tocopherol was extracted with 1 mL hexane. Following lyophilisation of exactly 0.5 ml of the hexane extract the residue was reconstituted in 100 μ l acetonitrile/methanol (85+15). An aliquot of 50 μ l was analysed for α -tocopherol by HPLC with fluorescence detection (excitation at 295 nm, emission at 330 nm) on a Nucleosil 5 μ m C18 column (125 x 4.6 mm) with acetonitrile/methanol (85+15) at a flow of 1.5 ml/min as eluent [see References (1)].

For a determination of the recovery control lung samples were spiked with α -tocopherol at a concentration of 57.3 μ g/g lung. A mean recovery of 79.5 \pm 7.6% (n=2) was obtained.

5.4.2 Reduced (GSH) and Oxidised (GSSG) Glutathione

Frozen lung samples were homogenized in 10 volumes ice-cold 50 mM MES, pH 6-7, containing 1 mM EDTA. Proteins were precipitated by the addition of an equal volume of 10%

metaphosphoric acid and centrifugation at 2'000 g for 10 min. The supernatant was diluted 20 to 100-fold with MES buffer and total glutathione (GSH plus GSSG) concentration in liver homogenates was determined spectrophotometrically following reduction with glutathione reductase in the presence of NADPH and derivatisation with 5,5'-dithio-bis-(2-nitrobenzoic acid) at 405 nm according to the description of the glutathione assay kit (Cayman Chemical Company / USA). Oxidized glutathione (GSSG) was quantified similarly following inactivation of GSH with 2-vinylpyridine at dilutions of 2 to 10-fold with MES buffer [see References (2)].

For a determination of the recovery control lung samples were spiked with glutathione at concentrations of 1 and 250 $\mu\text{mol/g}$ lung. A mean recovery of $81.0 \pm 13.9\%$ (n=3) and $76.1 \pm 7.9\%$ (n=3), respectively, was obtained.

5.4.3 8-Isoprostane F2 α

Frozen lung samples were homogenised in 3 volumes 0.9% (w/v) NaCl. [H^3]Prostaglandin F2 α was added as internal standard and Isoprostanes were extracted with 2 volumes ethanol from the homogenate. Following centrifugation for 10 min at 1500g the supernatant was hydrolysed with an equal volume 15% (w/v) KOH for 1 h at 40°C for the release of esterified isoprostanes. The hydrolysate was diluted with 2 volumes water, acidified with 2 M HCl to pH 2.0-3.5 and loaded on Oasis HLB (30 mg) SPE column (Waters / USA), pre-equilibrated with 1 mL methanol and 1 mL 0.02 M HCl. The column was washed subsequently with 2 mL 0.02 M HCl and with 2 mL 40% methanol in 0.02 M HCl. The column was dried with air and rinsed with 2 mL heptane. Isoprostanes were eluted from the column with 2 mL ethylacetate. The final elute from the Oasis column was diluted with 2 mL heptane, loaded on a Sep-Pak Vac RC Silica (100 mg) column (Waters / USA), pre-equilibrated with 1 mL methanol and 1 mL ethylacetate/heptane (1+1 by volume), and eluted with 2 mL ethylacetate/methanol (1+1 by volume). The eluate was dried in a SpeedVac concentrator and reconstituted in 1 mL EIA-buffer. The recovery of the extraction was determined by scintillation counting of 100 μL of the reconstituted extract. Quantitative determination of 8-isoprostane F2 α was made by a commercially available ELISA (Cayman Chemical Company / USA) using different dilutions of the extract [see References (3)].

For a determination of the recovery control lung samples were spiked with 8-isoprostane F2 α at a concentration of 5 ng/g lung. A mean recovery of $94.1 \pm 27.9\%$ (n=4) was obtained.

1.5 Statistical Analysis

Dunnett's t-test following log transformation for a stabilization of the variances was applied to assess differences between treated groups and controls. Statistical significances are given in the tables with one asterisk (*: $p < 0.05$) and two asterices (**: $p < 0.01$).

2 RESULTS

2.1 α -Tocopherol

(See Table 1 and Figure 1)

α -Tocopherol is a representative of lipid soluble cellular scavengers for reactive oxygen species. Induction of oxidative stress might result in a depletion of α -tocopherol. The respective tissue concentration, therefore, is a measure for intracellular oxidative stress [see References (4)].

In control animals mean α -tocopherol concentrations of 8.24 ± 1.60 $\mu\text{g/g}$ lung were obtained. Treatment at 0.25 mg/m^3 (group 2) slightly reduced mean α -tocopherol concentrations to 6.72 ± 1.24 $\mu\text{g/g}$ lung (81.6% of control, $p < 0.05$). Following treatment with the highest dose level (group 4, 4 mg/m^3) mean α -tocopherol concentrations increased slightly to 10.42 ± 0.74 $\mu\text{g/g}$ lung (126.5% of control, $p < 0.01$). Treatment at 1 mg/m^3 (group 2) had no effect on the mean α -tocopherol concentration in the lung.

2.2 Reduced (GSH) and Oxidised (GSSG) Glutathione

(See Table 2 to Table 4 and Figure 2 to Figure 4)

Reduced glutathione is the most abundant water soluble cellular scavenger for reactive oxygen species. Induction of oxidative stress might result in a depletion of GSH and concomittantly in an increase of oxidized glutathione, GSSG. The respective tissue concentrations, therefore, are measures for intracellular oxidative stress [see References (4)].

Mean GSH concentrations of 1.95 ± 0.30 $\mu\text{mol/g}$ lung were obtained in control animals. Treatment at the lowest dose of 0.25 mg/m^3 (group 2) slightly reduced mean GSH concentrations to 1.61 ± 0.31 $\mu\text{mol/g}$ lung (82.6% of control, $p < 0.05$). Treatment at higher doses had no significant effect on mean GSH concentrations in the lung.

Mean GSSG concentrations of 0.168 ± 0.036 $\mu\text{mol/g}$ lung were obtained in control animals. Treatment at 0.25 mg/m^3 , 1 mg/m^3 and 4 mg/m^3 all dose levels slightly increased mean GSSG concentrations to 0.268 ± 0.073 (158.9% of control, $p < 0.01$), 0.376 ± 0.161 (223.2% of control, $p < 0.01$) and 0.363 ± 0.103 $\mu\text{mol/g}$ lung (215.4% of control, $p < 0.01$), respectively.

As a result of the increased GSSG concentrations the ratio of reduced to oxidized glutathion (GSH/2xGSSG) decreased slightly from 5.95 ± 1.18 in control animals to 3.15 ± 0.79 (52.9% of control, $p < 0.01$), 3.29 ± 0.85 (55.4% of control, $p < 0.01$) and 3.12 ± 0.75 (52.5% of control, $p < 0.01$) in animals of group 2, group 3, and group 4, respectively.

2.3 8-Isoprostane F2 α

(See Table 5 and Figure 5)

8-Isoprostane F2 α is produced by the non-enzymatic oxidation of arachidonic acid by reactive oxygen species. Tissue concentrations of 8-isoprostane F2 α are known to be a sensitive parameter indicative for lipid peroxidation [see References (3)].

8-Isoprostane F2 α levels in the lungs of control animals of 39.8 ± 23.9 ng/g were found. Treatment at all dose levels had no effect on 8-isoprostane F2 α concentrations in the lung.

2 RESULTS

2.1 α -Tocopherol

(See Table 1 and Figure 1)

α -Tocopherol is a representative of lipid soluble cellular scavengers for reactive oxygen species. Induction of oxidative stress might result in a depletion of α -tocopherol. The respective tissue concentration, therefore, is a measure for intracellular oxidative stress [see References (4)].

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8-Isoprostane F2 α levels in the lungs of control animals of 39.8 ± 23.9 ng/g were found. Treatment at all dose levels had no effect on 8-isoprostane F2 α concentrations in the lung.

3 CONCLUSIONS

A role of oxidative stress has been proposed in the toxicity of numerous chemicals, in the process of ageing [see References (5)] as well as in the pathogenesis of many diseases and carcinogenesis [see References (6, 7)]. Oxidative stress is known to be followed by cellular and tissue damage whereby lipids, proteins and DNA are the major targets. These changes comprise altered cell function, such as decreased gap junctional communication, activation of transcription factors, changes in intracellular calcium and intracellular pH, or cell death [see References (4)].

Treatment of animals with hepatotoxic agents, known to induce oxidative stress (e.g. diquat, carbon tetrachloride, bromobenzene) clearly increased hepatic 8-oxoguanine, a marker for oxidative DNA damage [see References (7)], and malondialdehyde and 8-isoprostanes, markers for lipid peroxidation [see References (3)]. This increase was accompanied by a depletion of cellular antioxidants, e.g. ascorbic acid, α -tocopherol and reduced glutathione [see References (4)].

Following exposure of animals to inducers of oxidative stress by inhalation, similar effects can be expected in the lung. Exposure of mice to single-walled carbon nanotubes for example, induced an unusually robust pulmonary inflammatory response, which was accompanied by oxidative stress and antioxidant depletion [see References (8)]. In addition, treatment of lung cells with cerium oxide nanoparticles produced significant oxidative stress in the cells, as reflected by reduced glutathione and α -tocopherol levels and by an increase of malondialdehyde [see References (9)].

The result of the present investigation show a very slight increase in α -tocopherol and GSSG concentrations in the lung, and no effects on isoprostane concentrations following treatment by inhalation with vanadium pentoxide at dose levels up to 4 mg/m³.

The slight decrease in α -tocopherol in the lungs of group 2 was considered to be of no biological significance. The slight increase in group 4 might indicate an adaptive response to a weak but sustained oxidative stress and is probably not biologically significant. Furthermore, GSSG, as an oxidation product of GSH, was slightly increased, probably as a result of scavenging reactive oxygen by GSH. GSH is not affected since the increase of GSSG is negligible (0.2 to 0.4 μ mol/g) in comparison with the 10 fold excess of GSH (2 μ mol/g). In addition, the de novo synthesis of GSH as well as the glutathion reductase (which recycles GSSG to GSH) are known to be very fast inducible by oxidative stress, thereby compensating for a depletion of GSH.

Finally, the unchanged isoprostane levels confirm that the natural defense mechanisms of the lung against oxidative stress, are sufficient to protect the tissue against oxidative damage, which might be induced by vanadium pentoxide.

4 REFERENCES

1. Arnaud J, Fortis I, Blachier S, Kia D, Favier A:
Simultaneous determination of retinol, α -tocopherol and β -carotene in serum by high-performance liquid chromatography.
J Chromatogr 572, 103-116 (1991).
2. Griffith OW:
Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine.
Anal Biochem 106, 207-212 (1980).
3. Morrow JD, Awad JA, Kato T, Takahashi K, Badr KF, Roberts LJ 2nd, Burk RF:
Formation of novel non-cyclooxygenase-derived prostanoids in carbon tetrachloride hepatotoxicity.
J Clin Invest 90, 2502-2507 (1992).
4. Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J, Stevenson DE, Walborg EF Jr:
The role of oxidative stress in chemical carcinogenesis.
Environ Health Perspect 106, Supl 1, 289-295 (1998).
5. Stadtman ER:
Protein oxidation and aging.
Science 257, 1220-1224 (1992).
6. Cerutti PA:
Prooxidant states and tumor promotion.
Science 227, 375-381 (1985).
7. Frenkel K, Gleichauf C:
Hydrogen peroxide formation by cells treated with a tumor promoter.
Free Rad Res Comms 12-13, 783-794 (1991).
8. Shvedovaa AA, Kisina ER, Murraya AR, Gorelikb O, Arepallib S, Castranova V, Younga SH, Gaoc F, Tyurinad YY, Ouryc TD, Kagan VE:
Vitamin E deficiency enhances pulmonary inflammatory response and oxidative stress induced by single-walled carbon nanotubes in C57BL/6 mice.
Tox Appl Pharmacol 221, 339-348 (2007).
9. Lin W, Huang Y, Zhou XD, Ma Y:
Toxicity of cerium oxide nanoparticles in human lung cancer cells.
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Finally, the unchanged isoprostane levels confirm that the natural defense mechanisms of the lung against oxidative stress, are sufficient to protect the tissue against oxidative damage, which might be induced by vanadium pentoxide.

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Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine.
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5. Stadtman ER:
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Table 1 α -Tocopherol Concentrations in the Lung

Group	Animal	α -Tocopherol		
		[μ g/g Lung]	Mean [μ g/g Lung]	Std.Dev. [μ g/g Lung]
1	19	6.43	8.24	1.60
	20	6.97		
	21	8.76		
	22	8.96		
	23	6.93		
	24	11.50		
	25	8.08		
	26	8.26		
2	67	5.98	6.72	1.24*
	68	6.91		
	69	6.02		
	70	9.40		
	71	6.58		
	72	6.67		
	73	5.19		
	74	7.04		
3	115	9.43	8.37	0.97
	116	7.91		
	117	7.77		
	118	7.93		
	119	9.15		
	120	6.80		
	121	9.64		
	122	8.32		
4	163	10.12	10.42	0.74**
	164	9.78		
	165	10.71		
	166	9.57		
	167	9.71		
	168	11.65		
	169	11.05		
	170	10.74		

Table 2 GSH Concentrations in the Lung

Group	Animal	GSH		
		[$\mu\text{mol/g}$ Lung]	Mean [$\mu\text{mol/g}$ Lung]	Std.Dev. [$\mu\text{mol/g}$ Lung]
1	19	1.75	1.95	0.30
	20	1.86		
	21	2.10		
	22	2.64		
	23	1.82		
	24	1.78		
	25	1.91		
2	26	1.75	1.61	0.31*
	67	1.65		
	68	1.19		
	69	1.95		
	70	1.87		
	71	1.87		
	72	1.81		
3	73	1.24	2.26	0.27
	74	1.32		
	115	2.62		
	116	2.04		
	117	2.44		
	118	2.33		
	119	2.19		
4	120	1.91	2.21	0.28
	121	2.57		
	122	1.98		
	163	2.25		
	164	2.23		
	165	2.54		
	166	2.25		
167	2.35			
168	n.d.			
169	2.23			
170	1.64			

n.d. not determined

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	22	8.96		
	23	6.93		
	24	11.50		
	25	8.08		
	26	8.26		
2	67	5.98	6.72	1.24*
	68	6.91		
	69	6.02		
	70	9.40		
	71	6.58		
	72	6.67		
	73	5.19		
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	117	2.44		
	118	2.33		
	119	2.19		
	120	1.91		
	121	2.57		
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	163	2.25		
	164	2.23		
	165	2.54		
	166	2.25		
	167	2.35		
	168	n.d.		
	169	2.23		
170	1.64			

n.d. not determined

Table 3 GSSG Concentrations in the Lung

Group	Animal	GSSG		
		[$\mu\text{mol/g}$ Lung]	Mean [$\mu\text{mol/g}$ Lung]	Std.Dev. [$\mu\text{mol/g}$ Lung]
1	19	0.131	0.168	0.036
	20	0.157		
	21	0.243		
	22	0.166		
	23	0.139		
	24	0.143		
	25	0.176		
	26	0.191		
2	67	0.376	0.268	0.073**
	68	0.245		
	69	0.225		
	70	0.355		
	71	0.242		
	72	0.309		
	73	0.158		
	74	0.232		
3	115	0.418	0.376	0.161**
	116	0.235		
	117	0.747		
	118	0.283		
	119	0.370		
	120	0.326		
	121	0.354		
	122	0.274		
4	163	0.296	0.363	0.103**
	164	0.595		
	165	0.303		
	166	0.394		
	167	0.356		
	168	0.297		
	169	0.379		
	170	0.283		

Table 4 GSH/GSSG Ratios in the Lung

Group	Animal	GSH/2xGSSG		
		Ratio	Mean	Std.Dev.
1	19	6.65	5.95	1.18
	20	5.92		
	21	4.32		
	22	7.94		
	23	6.54		
	24	6.21		
	25	5.43		
	26	4.58		
2	67	2.20	3.15	0.79**
	68	2.43		
	69	4.34		
	70	2.64		
	71	3.86		
	72	2.93		
	73	3.94		
	74	2.84		
3	115	3.13	3.29	0.85**
	116	4.34		
	117	1.63		
	118	4.11		
	119	2.96		
	120	2.93		
	121	3.63		
	122	3.61		
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	165	4.20		
	166	2.85		
	167	3.31		
	168	n.d.		
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	117	1.63		
	118	4.11		
	119	2.96		
	120	2.93		
	121	3.63		
	122	3.61		
4	163	3.80	3.12	0.75**
	164	1.87		
	165	4.20		
	166	2.85		
	167	3.31		
	168	n.d.		
	169	2.94		
	170	2.90		

n.d. not determined

Table 5 8-Isoprostane F2 α Concentrations in the Lung

Group	Animal	8-isoprostane F2 α		
		[ng/g Lung]	Mean [ng/g Lung]	Std.Dev. [ng/g Lung]
1	27	85.66	39.8	23.9
	28	55.28		
	29	n.d.		
	30	30.89		
	31	31.97		
	32	24.60		
	33	13.15		
	34	36.81		
2	75	48.17	24.7	11.1
	76	29.80		
	77	20.98		
	78	24.82		
	79	10.12		
	80	17.55		
	81	22.30		
	82	23.76		
3	123	68.46	31.5	25.5
	124	74.94		
	125	16.49		
	126	20.91		
	127	10.43		
	128	11.13		
	129	27.48		
	130	22.24		
4	171	37.39	30.5	7.2
	172	28.67		
	173	42.90		
	174	25.55		
	175	20.98		
	176	29.28		
	177	25.18		
	178	34.13		

Figure 1 α -Tocopherol Concentrations in the Lung

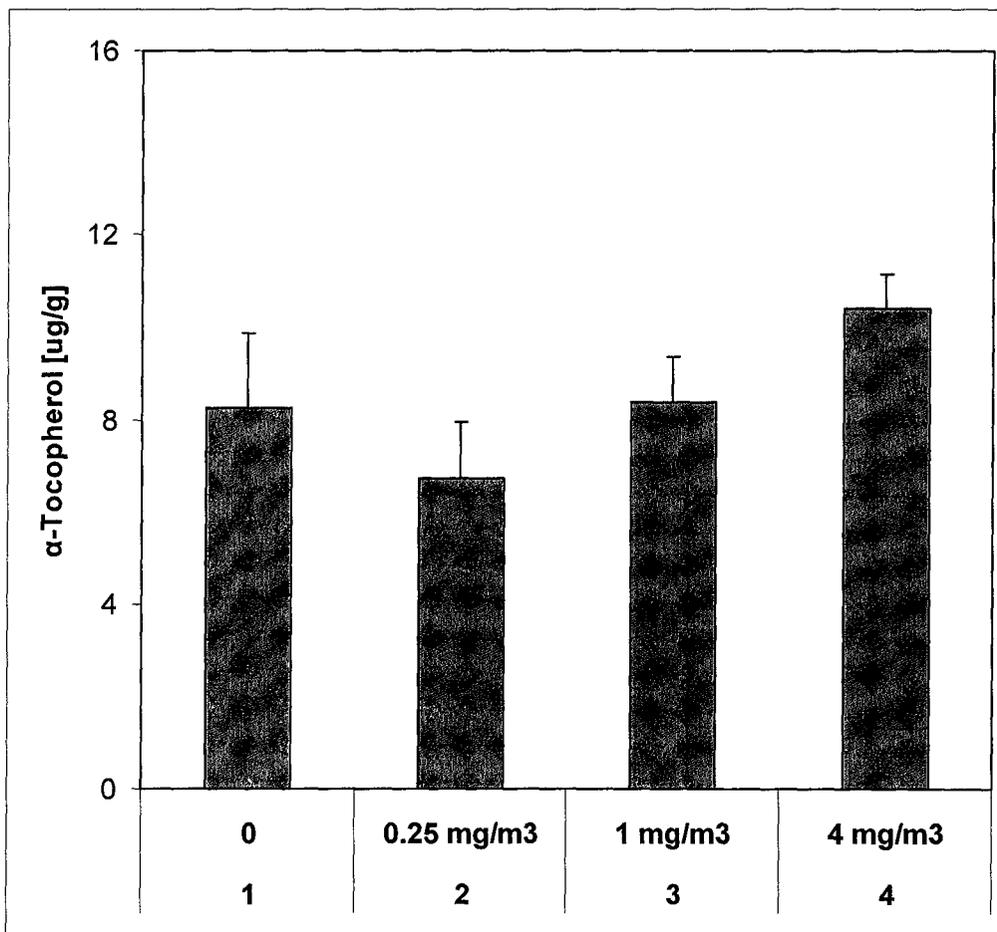


Table 5 8-Isoprostane F2 α Concentrations in the Lung

Group	Animal	8-isoprostane F2 α		
		[ng/g Lung]	Mean [ng/g Lung]	Std.Dev. [ng/g Lung]
1	27	85.66	39.8	23.9
	28	55.28		
	29	n.d.		
	30	30.89		
	31	31.97		
	32	24.60		
	33	13.15		
	34	36.81		
2	75	48.17	24.7	11.1
	76	29.80		
	77	20.98		
	78	24.82		
	79	10.12		
	80	17.55		
	81	22.30		
	82	23.76		
3	123	68.46	31.5	25.5
	124	74.94		
	125	16.49		
	126	20.91		
	127	10.43		
	128	11.13		
	129	27.48		
	130	22.24		
4	171	37.39	30.5	7.2
	172	28.67		
	173	42.90		
	174	25.55		
	175	20.98		
	176	29.28		
	177	25.18		
	178	34.13		

Figure 1 α -Tocopherol Concentrations in the Lung

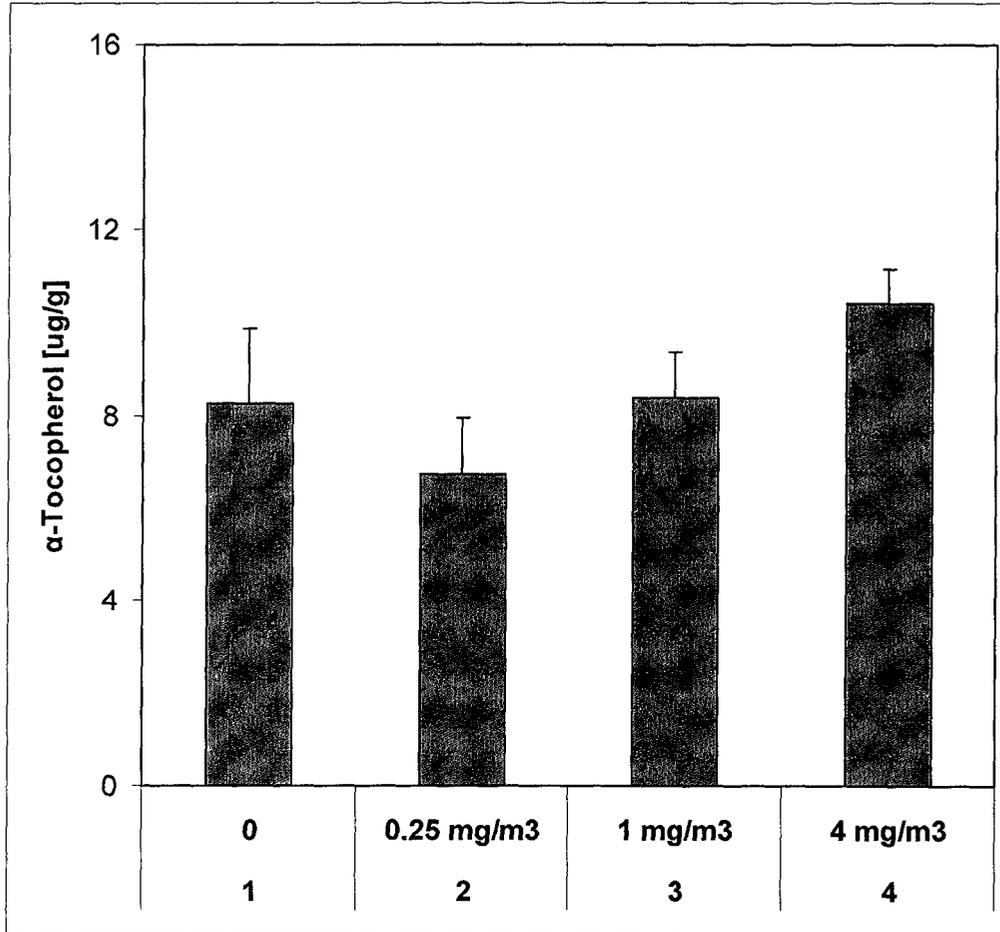


Figure 2 GSH Concentrations in the Lung

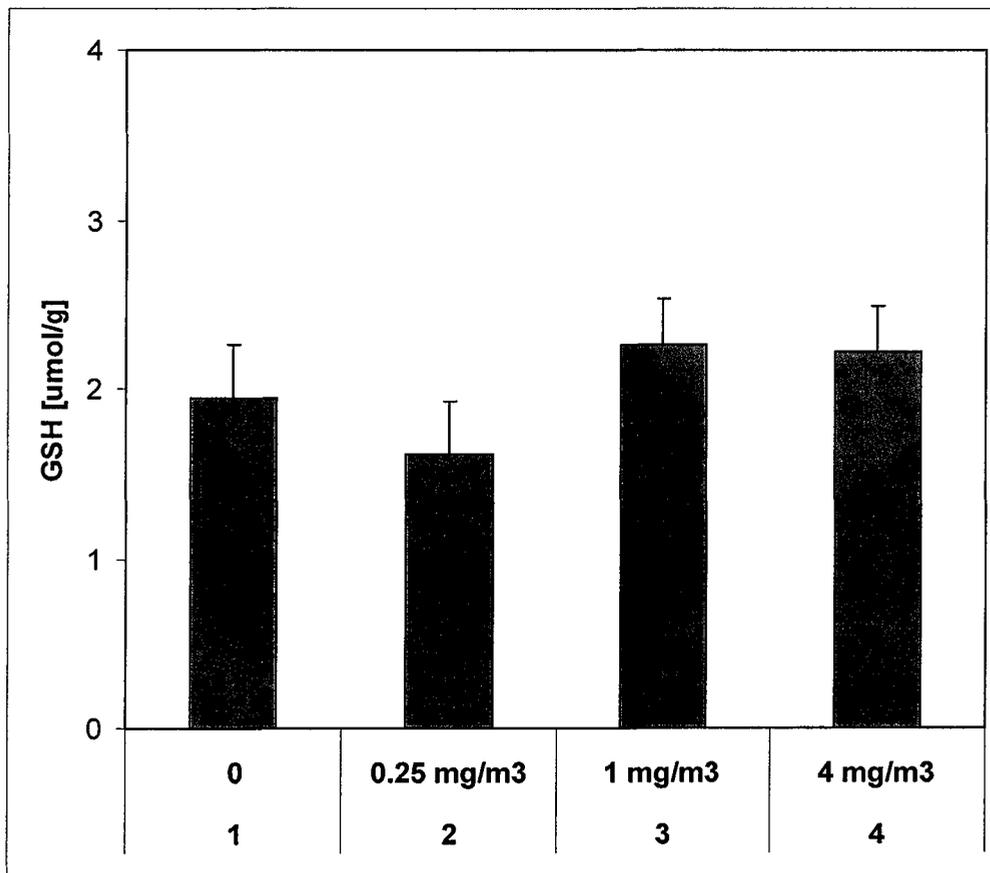


Figure 3 GSSG Concentrations in the Lung

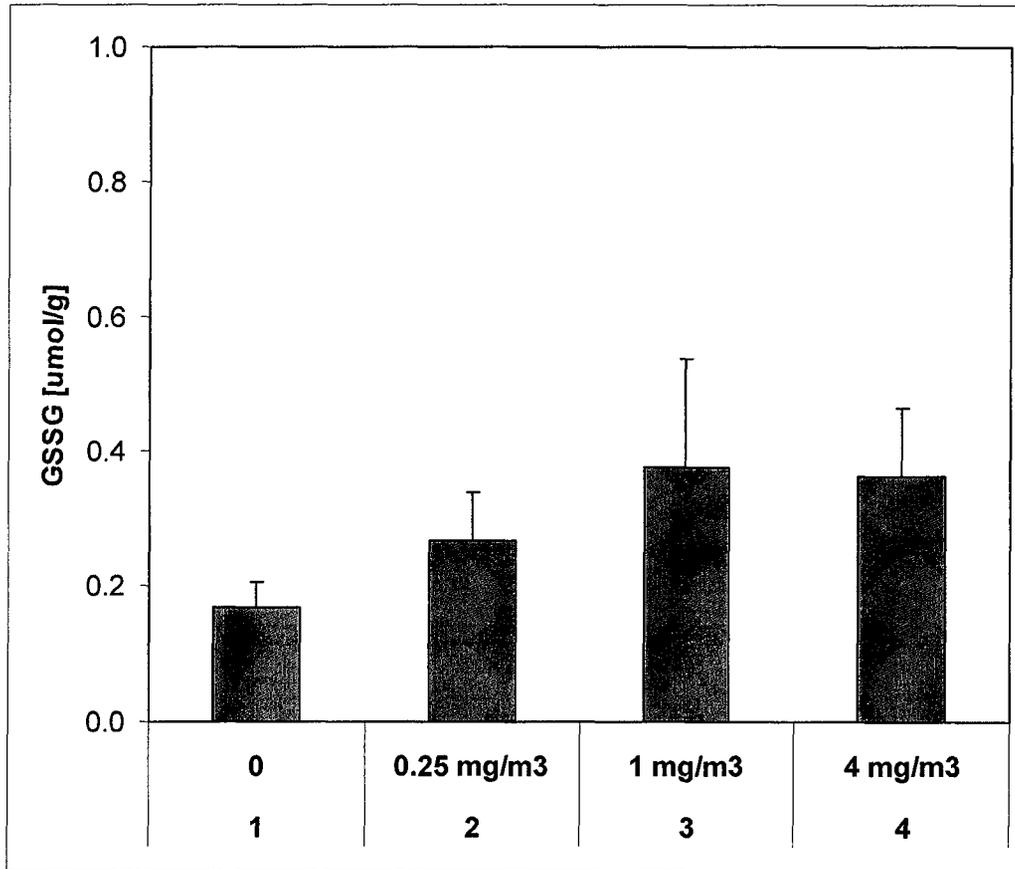


Figure 2 GSH Concentrations in the Lung

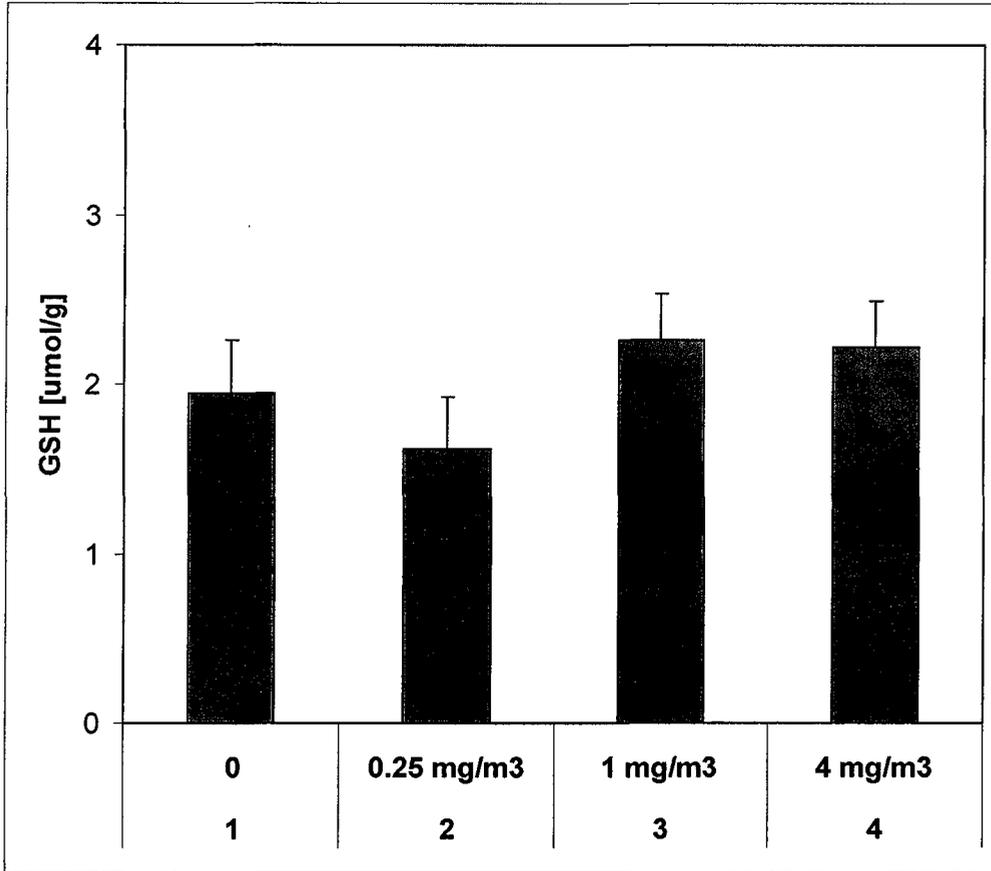


Figure 3 GSSG Concentrations in the Lung

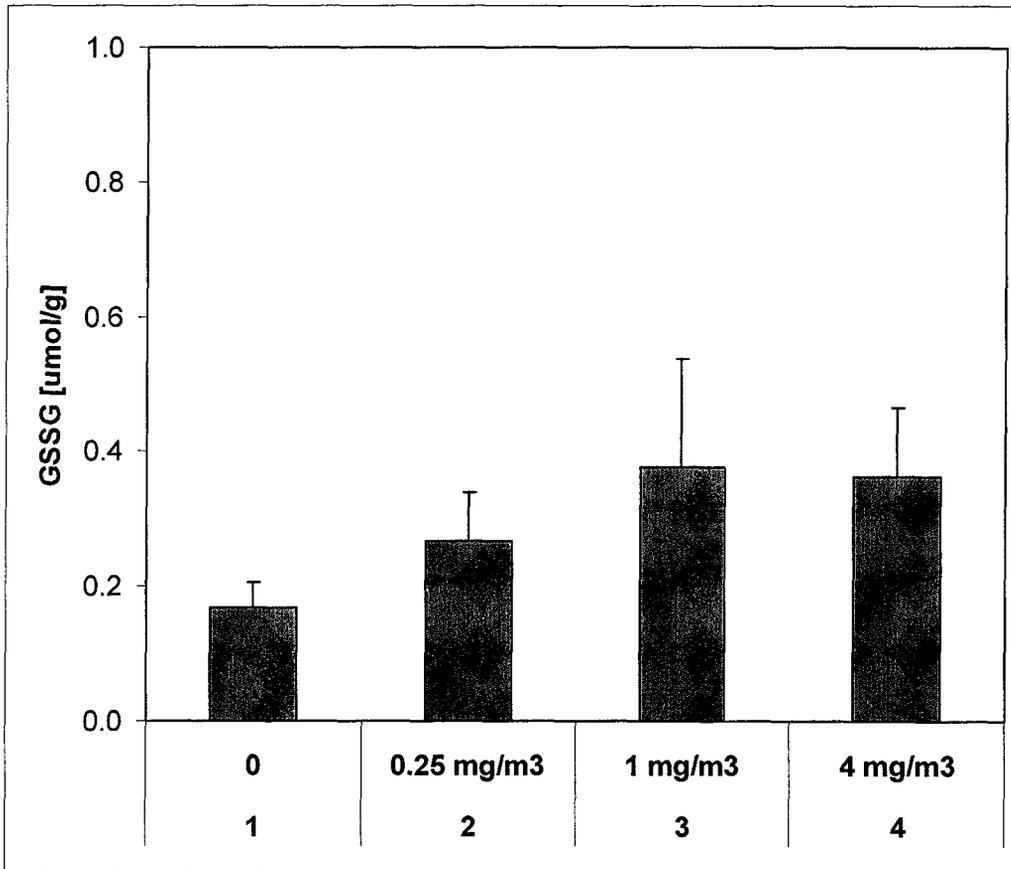


Figure 4 GSSG/GSH Ratios in the Lung

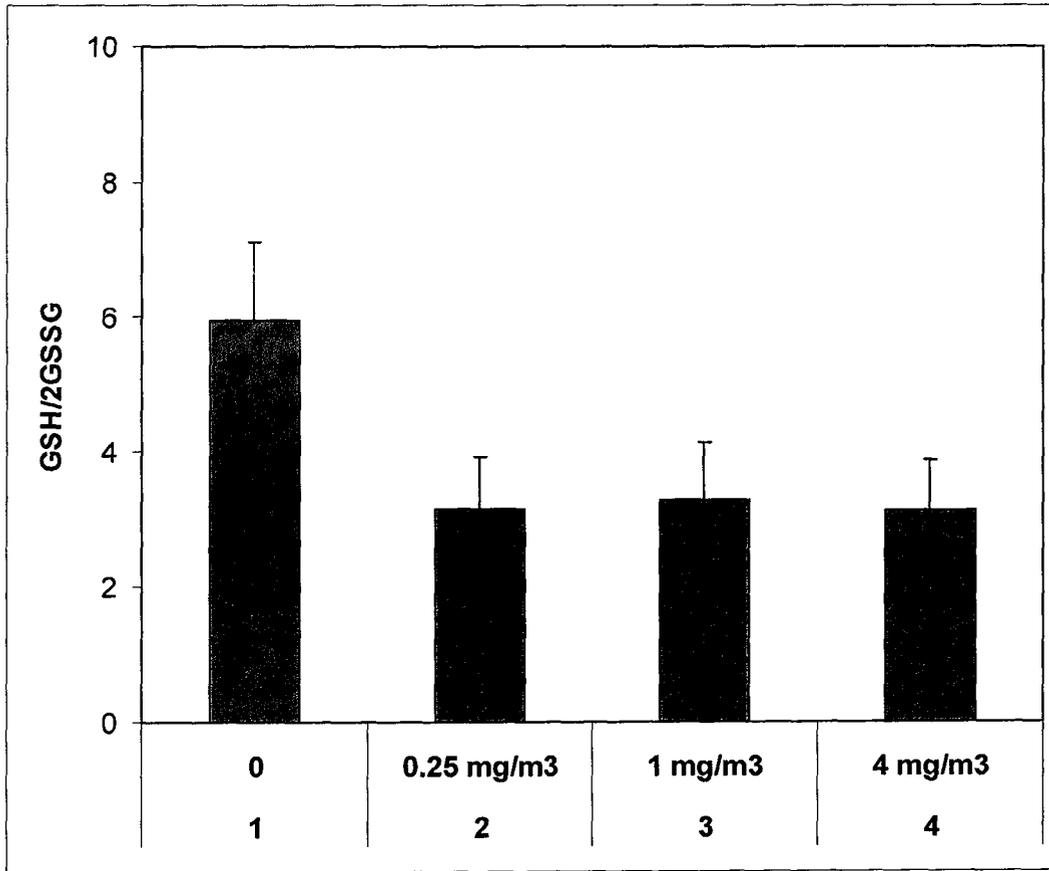


Figure 5 8-Isoprostane F2 α Concentrations in the Lung

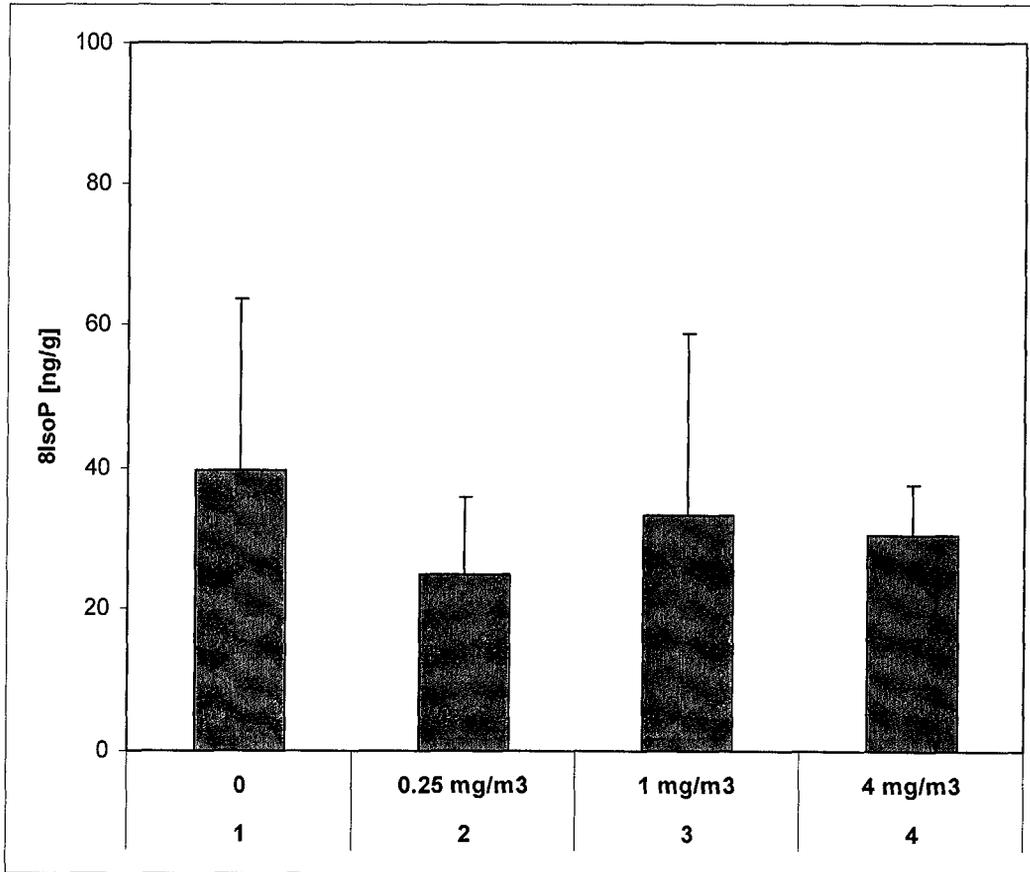


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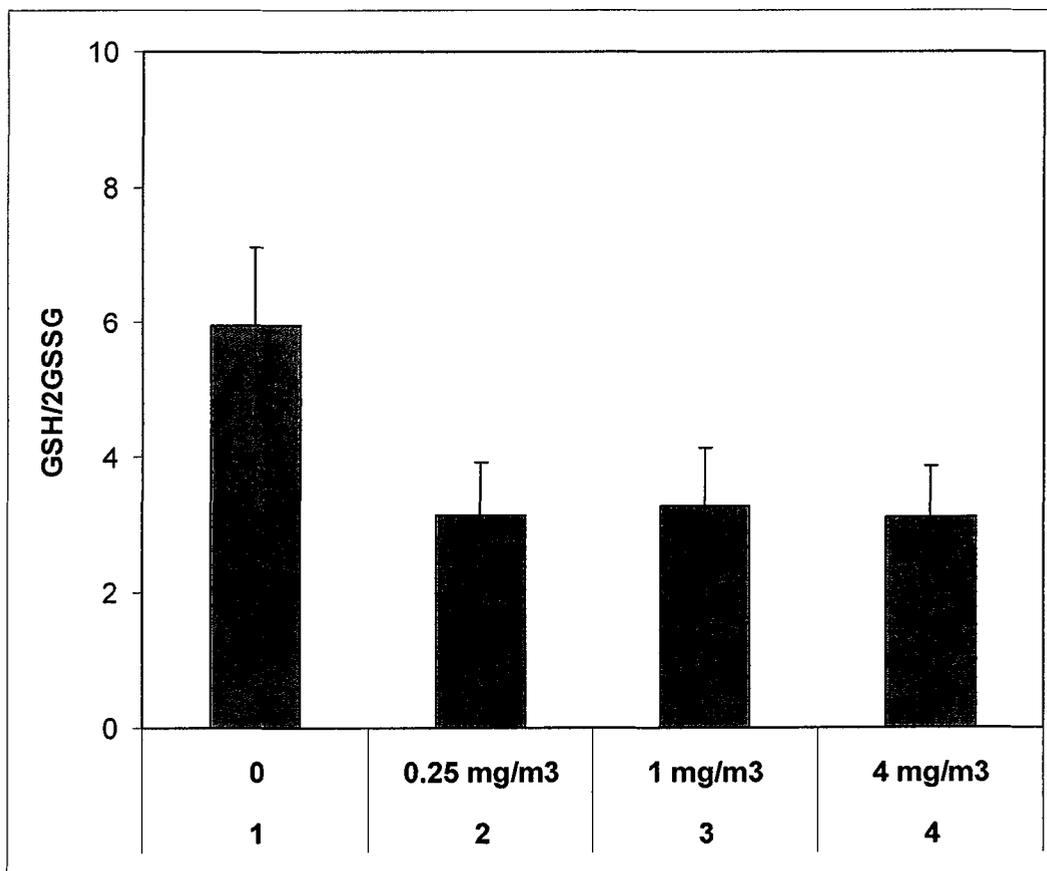
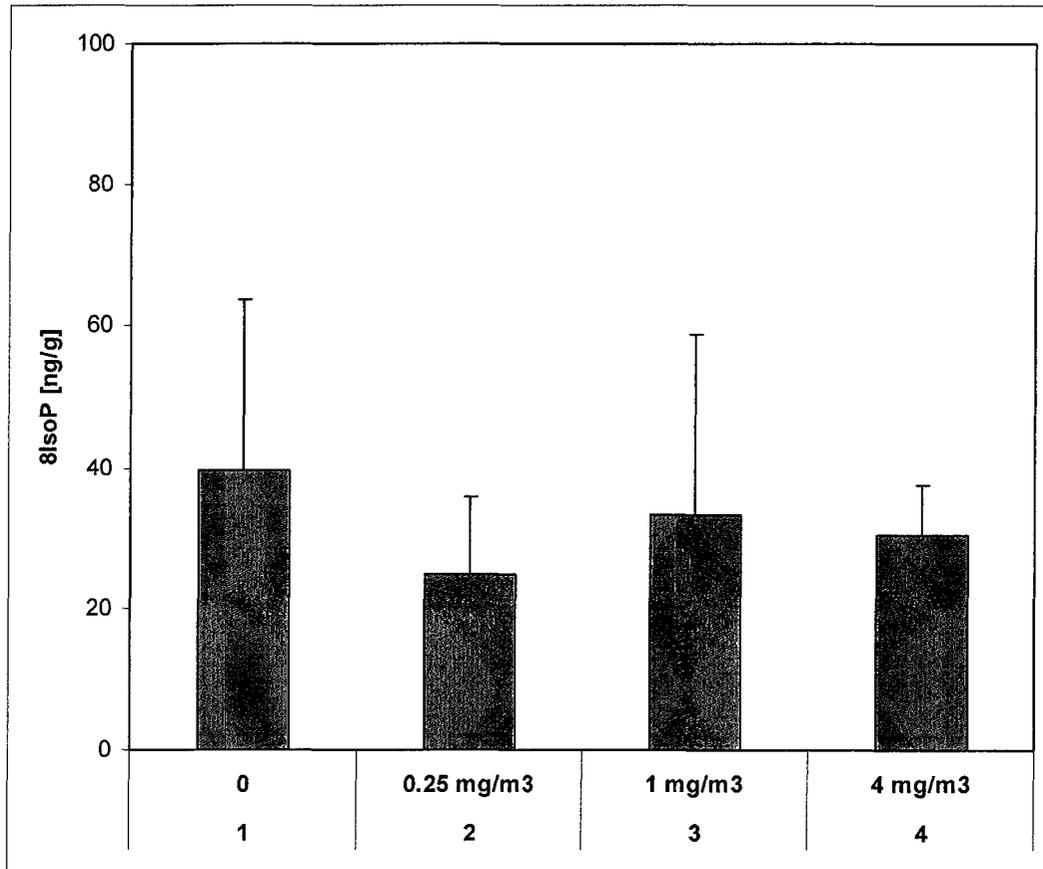


Figure 5 8-Isoprostane F2 α Concentrations in the Lung



APPENDIX IV - DETERMINATION OF DNA LESIONS

APPENDIX VII - COMET ASSAY

APPENDIX IV - DETERMINATION OF DNA LESIONS

APPENDIX VII - COMET ASSAY

COMET ASSAY PHASE REPORT –DRAFT

Study Title:

Vanadium Pentoxide:

16-Day Inhalation Toxicity Study in Female Mice

Subtitle:

**IN VIVO ALKALINE SINGLE CELL GEL
ELECTROPHORESIS ANALYSIS IN MOUSE LUNG
TISSUE**

Test Site: Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
D-64380 Rossdorf
Germany

Sponsor Contact: Dr D. McGregor

Study Identification: Harlan Laboratories Ltd.
Study Number : A94206
Phase Number: 1048304

1 COPY OF GLP-CERTIFICATE



Gute Laborpraxis/Good Laboratory Practice

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

HESSEN



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)

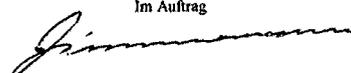
Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

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


Th. Zimmermann, 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)

COMET ASSAY PHASE REPORT –DRAFT

Study Title:

Vanadium Pentoxide:

16-Day Inhalation Toxicity Study in Female Mice

Subtitle:

**IN VIVO ALKALINE SINGLE CELL GEL
ELECTROPHORESIS ANALYSIS IN MOUSE LUNG
TISSUE**

Test Site: Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
D-64380 Rossdorf
Germany

Sponsor Contact: Dr D. McGregor

Study Identification: Harlan Laboratories Ltd.
Study Number : A94206
Phase Number: 1048304

1 COPY OF GLP-CERTIFICATE



HESSEN



Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

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In den Leppsteinswiesen 19
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Prüfungen nach Kategorien/Areas of Expertise

(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

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8 Analytische Prüfungen an biologischen Materialien

2 Toxicity studies
3 Mutagenicity studies
6 Residues
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15.08. und 27. – 29.10.2008
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

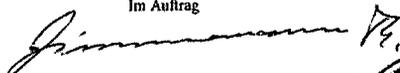
Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

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

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Im Auftrag


Th. Zimmermann, 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

Study Title: Vanadium Pentoxide:
16-Day Inhalation Toxicity Study in Female Mice

Subtitle: *In vivo* alkaline single cell gel electrophoresis
analysis in mouse lung tissue

Sponsor: Advanced Technology Institute
5300 International Blvd
N. Charleston, SC 29418 / USA

Sponsor Contact: Dr. D. McGregor
38 Shore Road
Aberdour, KY3 0TU
Scotland / United Kingdom

Study Director: Dr. D. Schuler

Test Facility: Harlan Laboratories Ltd.
Wölferstrasse 4
4414 Füllinsdorf / Switzerland

Test Site: Harlan Cytotest Cell Research GmbH (Harlan CCR)
In den Leppsteinswiesen 19
64380 Rossdorf
Germany

3.2 Responsibilities

Principal Investigator: Dr. Mandy Reichenbach

Management: Dr. Wolfgang Völkner

Head of
Quality Assurance Unit: Frauke Hermann

3.3 Schedule

Experimental Starting Date: June 02, 2009

Experimental Completion Date: June 12, 2009

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Management: Dr. Wolfgang Völkner

Head of
Quality Assurance Unit: Frauke Hermann

3.3 Schedule

Experimental Starting Date: June 02, 2009

Experimental Completion Date: June 12, 2009

3.4 Project Staff Signatures

Principal Investigator: Dr. Mandy Reichenbach

.....
Date: #3

Management: Dr. Wolfgang Völkner

.....
Date: #3

3.5 Good Laboratory Practice

The study phase was performed in compliance with:

„Chemikaliengesetz“ (Chemicals Act) of the Federal Republic of Germany, „Anhang 1“ (Annexe 1), dated July 25, 1994 („BGBl. I“ 1994, pp. 1703), last revision: June 27, 2002.

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

3.6 Guidelines

For the Comet assay no internationally accepted guideline is available. This study was conducted according to the procedures indicated by the following recommendations:

Tice, R.R. et al. (2000): Single Cell Gel/Comet Assay: Guidelines for In Vitro and In Vivo Genetic Toxicology Testing. *Environmental and Molecular Mutagenesis* 35: 206-221

Hartmann, A. et al. (2003): Recommendations for conducting the *in vivo* alkaline Comet assay. *Mutagenesis* 18 (1): 45-51

3.7 Archiving

Harlan CCR will archive the following data for 15 years:

Raw data, and Comet Assay phase report.

Microscopic slides will be archived for 12 years.

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

3.8 Deviations from the Study Plan

There were the following deviations from the study plan:

- The minced lung tissue was filtered through a 40 µm instead of a 100 µm cell strainer.
- 0.7 % Agarose was used instead of 70 % Agarose (typing error in the study plan)
- Three slides were prepared for the minced tissue but only one for the BAL cells because the amount of cells for the latter was too less for a preparation of more than one slide.
- Therefore, for BAL cells 100 cells were evaluated from that one slide. For the minced tissue one slide was kept as reserve and 50 cells per remaining slide were evaluated resulting in a total of 100 cells per animal for both preparation methods.
- For animal number 142 only 95 BAL cells instead of 100 cells were used for the Comet evaluation and 115 for the dead cell index.
- The number of nuclei from apoptotic or necrotic cells per 500 total nuclei could not be determined for the positive control samples.

These deviations, however, do not effect the validity of the study.

3.4 Project Staff Signatures

Principal Investigator: Dr. Mandy Reichenbach

.....
Date: #3

Management: Dr. Wolfgang Völkner

.....
Date: #3

3.5 Good Laboratory Practice

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Phase Number 1048304
Vanadium Pentoxide

Comet Assay Phase Report
DRAFT

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4 STATEMENT OF COMPLIANCE

Phase Number: 1048304
Test Item: Vanadium Pentoxide
Principal Investigator: Dr. Mandy Reichenbach
Study Title: Vanadium Pentoxide:
16-Day Inhalation Toxicity Study in Female Mice
Subtitle: *In vivo* alkaline single cell gel electrophoresis analysis
in mouse lung tissue

This study phase performed in the test site of Harlan Cytotest Cell Research GmbH was conducted in compliance with Good Laboratory Practice Regulations.

„Chemikaliengesetz“ (Chemicals Act) of the Federal Republic of Germany, „Anhang 1“ (Annexe 1), dated July 25, 1994 („BGBl. I“ 1994, pp. 1703), last revision: June 27, 2002.

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 phase.

Principal Investigator Harlan CCR
Dr. Mandy Reichenabch

.....
Date: #3

Phase Number 1048304
Vanadium Pentoxide

Comet Assay Phase Report
DRAFT

Page 8 / 19

5 STATEMENT OF QUALITY ASSURANCE UNIT

Phase Number: 1204500
 Test Item: Vanadium Pentoxide
 Principal Investigator: Dr. Mandy Reichenbach
 Study Title: Vanadium Pentoxide:
 16-Day Inhalation Toxicity Study in Female Mice
 Subtitle: *In vivo* alkaline single cell gel electrophoresis
 analysis in mouse lung tissue

The general facilities and activities of Harlan Cytotest Cell Research GmbH are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically and this Comet Assay phase report was audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections / Audits	Dates of Reports to	
	Principal Investigator and Test Site Management	Study Director, Lead QA and Test Facility Management
Study Plan:		
<u>Study Inspection</u>		
xxx:		
Immunotoxicology Phase Report:		

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

Head of Quality Assurance Unit

Frauke Hermann

.....

Date: #3

4 STATEMENT OF COMPLIANCE

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Dr. Mandy Reichenabch

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Head of Quality Assurance Unit

Frauke Hermann

.....

Date: #3

6 OBJECTIVE

6.1 Aims of the Study

This experiment was performed to assess the potential of the test item Vanadium Pentoxide to induce *in vivo* primary DNA breaks in individual cells of mouse lung tissue.

6.2 Introduction

The *in vivo* alkaline single cell gel electrophoresis (comet) assay can be used to investigate the genotoxic potential of substances. (Tice 1995, Fairbairn et al. 1995, Anderson et al. 1998, Rojas et al. 1999, Speit and Hartmann 1999, Singh 2000).

The Comet assay is based on the migration of DNA in an agarose gel during electrophoresis. After electrophoresis the DNA of a damaged cell has the appearance of a comet with the nuclear region of the cell representing the head and the DNA-fragments induced by the damage, which have a different migration pattern forming the tail. The alkaline version of the assay (pH of unwinding and electrophoresis buffer >13) is recommended as most suitable for regulatory purposes since it enables the detection of a broad spectrum of DNA damage. It can detect double and single strand breaks, alkali-labile sites, and single strand breaks due to incomplete excision repair.

The Comet assays primary use is that of a supplemental assay for investigations of suspected tissue specific genotoxic activity. The *in vivo* Comet assay has some advantages over other *in vivo* indicator tests with regulatory acceptance, such as the unscheduled DNA synthesis (UDS) test. The *in vivo* UDS test is generally performed in liver tissue only, while the Comet assay can be applied to virtually any organ of interest irrespective of the cell cycle or differentiation stage of the cell - provided that appropriate cell preparation has been established for each organ and cell type. In addition, the comet assay detects a broader spectrum of primary DNA lesions, including single strand breaks and oxidative base damage, which may not sensitively be detected by the UDS test.

To validate the test a reference mutagen (positive control) was tested in parallel to the test item.

In this phase of the study 2 methods were used to prepare single cell suspensions of the target tissue for evaluation of genotoxicity in the lung. The lung was perfused in order to get rid of erythrocytes and via bronchioalveolar lavage (BAL) resident immune cells were flushed out of the tissue. The gained cell suspension (BAL cells) was analysed for DNA damage. Additionally the lung tissue (pneumocytes etc.) was minced and filtered through a cell strainer to obtain a single cell suspension which was although analysed for the occurrence of DNA damage.

7 MATERIALS AND REAGENTS

7.1 Positive Control Substance

Name:	Methylmethansulfonate (MMS)
Supplier:	Sigma-Aldrich Chemie GmbH D-82018 Taufkirchen
Catalogue no.:	12,992-5
Purity:	99 %
Dissolved in:	0.9 % NaCl solution
Dosing:	200 mg/kg b.w.
Route and frequency of administration:	orally, once (4 hours before sacrifice)
Volume administered:	10 mL/kg b.w.

A solution of the positive control was prepared within one hour before administration.

The stability of the positive control substance in vehicle is unknown, but DNA damage in the expected range (tailmoment of the positive control more than 2 times higher than of the vehicle control) demonstrates appropriate biological activity.

6 OBJECTIVE

6.1 Aims of the Study

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The Comet assays primary use is that of a supplemental assay for investigations of suspected tissue specific genotoxic activity. The *in vivo* Comet assay has some advantages over other *in vivo* indicator tests with regulatory acceptance, such as the unscheduled DNA synthesis (UDS) test. The *in vivo* UDS test is generally performed in liver tissue only, while the Comet assay can be applied to virtually any organ of interest irrespective of the cell cycle or differentiation stage of the cell - provided that appropriate cell preparation has been established for each organ and cell type. In addition, the comet assay detects a broader spectrum of primary DNA lesions, including single strand breaks and oxidative base damage, which may not sensitively be detected by the UDS test.

To validate the test a reference mutagen (positive control) was tested in parallel to the test item.

In this phase of the study 2 methods were used to prepare single cell suspensions of the target tissue for evaluation of genotoxicity in the lung. The lung was perfused in order to get rid of erythrocytes and via bronchioalveolar lavage (BAL) resident immune cells were flushed out of the tissue. The gained cell suspension (BAL cells) was analysed for DNA damage. Additionally the lung tissue (pneumocytes etc.) was minced and filtered through a cell strainer to obtain a single cell suspension which was although analysed for the occurrence of DNA damage.

7 MATERIALS AND REAGENTS

7.1 Positive Control Substance

Name:	Methylmethansulfonate (MMS)
Supplier:	Sigma-Aldrich Chemie GmbH D-82018 Taufkirchen
Catalogue no.:	12,992-5
Purity:	99 %
Dissolved in:	0.9 % NaCl solution
Dosing:	200 mg/kg b.w.
Route and frequency of administration:	orally, once (4 hours before sacrifice)
Volume administered:	10 mL/kg b.w.

A solution of the positive control was prepared within one hour before administration.

The stability of the positive control substance in vehicle is unknown, but DNA damage in the expected range (tailmoment of the positive control more than 2 times higher than of the vehicle control) demonstrates appropriate biological activity.

8 METHODS

This study phase was performed in conjunction with a 16-Day toxicity study (Harlan Laboratories Ltd. Study No.: A94206). The lung samples analysed in this project were prepared at Harlan Laboratories Ltd. and brought to Harlan CCR for evaluation.

8.1 Cell Isolation

As soon as feasible after the last exposure on 03-Jun-2009 for groups 1 and 2, and on 04-Jun-2009 for groups 3 and 4, and 4 hours after the treatment of the positive control animals (group 5) on 03-Jun-2009, the following procedures were performed on all mice of allocation G.

8.1.1 Perfusion of lung tissue

After anaesthetizing the mice with 46% ketamin (Ketavet 100, Pharmacia GmbH, 76139 Karlsruhe, Germany), 23% Xylazin (Rompun 2%, Bayer HealthCare, 51368 Leverkusen, Germany) and 31% Midazolam (Dormicum, Hoffmann LaRoche, 79639 Grenzach-Wyhlen, Germany) (approx. 2 mL/kg body weight) the lung was perfused through the right ventricle with saline. Afterwards the lungs were intubated via the trachea and lavaged with approx. 20 mL mincing buffer (20 mM EDTA, 10% DMSO in HBSS pH 7.4 – 7.6) for collection of BAL cells. The lungs were then further perfused under resuscitation. The excised lung lobes were then minced to obtain a single cell suspension containing minced lung tissue cells.

8.1.2 Preparation of BAL cells

The cells isolated from lung lavage (BroncioAlveolar Lavage (BAL) cells) were centrifuged at 300xg for 10 min and resuspended in 0.7% Agarose and afterwards brought onto slides.

8.1.3 Preparation of minced lung tissue cells

The minced lung tissue cells were filtered through a 40 µm cell strainer. The cell suspension was centrifuged at about 5000xg for 1 min and the resulting pellet resuspended in 0.7% Agarose and brought onto slides.

8.2 Preparation of Microscopic Slides

Three slides per minced lung tissue cells and one slide per BAL cells per animal were prepared with 10 % cell suspension and 90 % of a 0.7 % (w/v) agarose (low melting point agarose) solution. 100 µl were applied per slide. The slides were cooled before being submerged in lysis buffer.

The following steps of protocol were performed with the slides:

Lysis	1h up to 7 days in Lysis buffer pH 10 (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1 % Triton X-100, 10 % DMSO) at 2 - 8°C in the dark
Alkaline treatment	20 min in electrophoresis buffer (0.3 M NaOH, 1 mM EDTA), pH ≥ 13 at 2 - 8°C in the dark
Electrophoresis	30 min in electrophoresis buffer 25 V (0.72 V/cm), 300 mA, at 2 - 8°C in the dark
Neutralisation	about 10 min in neutralisation buffer (0.4 M Tris pH 7.5)
Dehydration	approx. 2 min in 99 % ethanol

After dehydration the slides were air-dried and stored protected from dust and light until evaluation. Of the minced lung tissue cells one slide per animal was kept as a reserve for possible re-evaluation.

8.3 Evaluation of Results

The DNA of the cells were stained with the fluorescence dye ethidium bromide (20 µg/ml; 40 µl per slide), immediately before evaluation.

Where possible all mice per test group, 100 cells per preparation and per animal (BAL: 100 cells from one slide, minced lung tissue cells: 50 cells per slide), were evaluated on coded slides with a fluorescence microscope using a 40 x objective (except for animal 142 where only 95 BAL cells were used for evaluation). The damage of each nucleus were measured and recorded by an image analysis programme (Comet Assay IV, Perceptive Instruments).

An increasing extent of DNA migration detected with the Comet assay results in an increase of the mean of tail % intensity of one test group compared to the vehicle control. Tail % intensity is expressed as a percentage of the Comet's total intensity. Additionally, the number of nuclei from apoptotic or necrotic cells per 500 total nuclei was determined.

The following criteria are used for analysis of slides:

- Only clearly defined non-overlapping cells are scored
- Nuclei from dead/ apoptotic cells are not scored (% tail intensity above 80%)
- Cells with unusual staining artefacts are not scored
- All other normal cells, 100/animal, are scored where possible

After evaluation, the cover-slips were removed and the slides were washed once for approx. 2 min in 99 % ethanol. The slides were air-dried and stored protected from dust and light.

8 METHODS

This study phase was performed in conjunction with a 16-Day toxicity study (Harlan Laboratories Ltd. Study No.: A94206). The lung samples analysed in this project were prepared at Harlan Laboratories Ltd. and brought to Harlan CCR for evaluation.

8.1 Cell Isolation

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The minced lung tissue cells were filtered through a 40 µm cell strainer. The cell suspension was centrifuged at about 5000xg for 1 min and the resulting pellet resuspended in 0.7% Agarose and brought onto slides.

8.2 Preparation of Microscopic Slides

Three slides per minced lung tissue cells and one slide per BAL cells per animal were prepared with 10 % cell suspension and 90 % of a 0.7 % (w/v) agarose (low melting point agarose) solution. 100 μ l were applied per slide. The slides were cooled before being submerged in lysis buffer.

The following steps of protocol were performed with the slides:

Lysis	1h up to 7 days in Lysis buffer pH 10 (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1 % Triton X-100, 10 % DMSO) at 2 - 8°C in the dark
Alkaline treatment	20 min in electrophoresis buffer (0.3 M NaOH, 1 mM EDTA), pH \geq 13 at 2 - 8°C in the dark
Electrophoresis	30 min in electrophoresis buffer 25 V (0.72 V/cm), 300 mA, at 2 - 8°C in the dark
Neutralisation	about 10 min in neutralisation buffer (0.4 M Tris pH 7.5)
Dehydration	approx. 2 min in 99 % ethanol

After dehydration the slides were air-dried and stored protected from dust and light until evaluation. Of the minced lung tissue cells one slide per animal was kept as a reserve for possible re-evaluation.

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Where possible all mice per test group, 100 cells per preparation and per animal (BAL: 100 cells from one slide, minced lung tissue cells: 50 cells per slide), were evaluated on coded slides with a fluorescence microscope using a 40 x objective (except for animal 142 where only 95 BAL cells were used for evaluation). The damage of each nucleus were measured and recorded by an image analysis programme (Comet Assay IV, Perceptive Instruments).

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- All other normal cells, 100/animal, are scored where possible

After evaluation, the cover-slips were removed and the slides were washed once for approx. 2 min in 99 % ethanol. The slides were air-dried and stored protected from dust and light.

8.4 Acceptance Criteria

The study is considered valid if the following criteria are met:

- The vehicle control treatments demonstrate low levels of cytotoxicity (i.e. group mean of less than 30% clouds or 30% diffused cells indicating correct preparation of the cell suspensions)
- The vehicle control data are consistent with the laboratory's historical control data and are not overtly above the upper range

The positive control treatments demonstrate a clear increase in Comet parameters over the vehicle control.

8.5 Data management

The experimental unit of exposure for *in vivo* studies is the animal, and all analysis will be based on individual animal response.

Values obtained from each parameter will be treated as follows:

- The median is calculated for each slide
- For each animal the mean of the medians are calculated, if possible.
- The mean of the medians and standard error of the mean is calculated for each group

A test item is classified as mutagenic if it induces either a dose-related increase or a biologically relevant increase in the tail % intensity in a single dose group as compared to the historical control range. Statistical methods will be used as an aid in evaluating the results. Normally distributed data will be analysed using a one-tailed student's t-test. An F-test is first used to test for homogeneity of variances for pairwise analysis. However, the primary point of consideration will be the biological relevance of the results.

8.5.1 Historical Control Data

Data for the historical control were generated using non-treated animals.

		Minced Lung Tissue Cells	BAL Cells
Tail % intensity	Range	0.04 –3.29	0.04 – 6.34
	Mean	0.42 ± 0.60	0.66 ± 1.26
Number of animals		32	27

9 BIOMETRY

For all test groups above the control values statistical methods were used as an aid in evaluating the results as recommended by Hartmann et al 2003. Normally distributed data were analysed using a one-tailed student's t-test. An F-test was first used to test for homogeneity of variances for pairwise analysis.

10 RESULTS

10.1 % Tail Intensity of BAL Cells

Test Group	Animal Number	Amount dead cells on slides (%)	% Tail Intensity	
			Median	Group Mean ± SD*
Group 1 Air Control	43	6.0	2.59	0.97 ± 0.94
	44	2.6	0.96	
	45	0.4	0.47	
	46	0.2	0.51	
	47	animal died		
	48	2.2	0.32	
Group 2 0.25 mg/m ³ air	91	2.4	0.92	0.31 ± 0.32
	92	1.2	0.08	
	93	0.8	0.34	
	94	0.0	0.07	
	95	0.8	0.11	
	96	0.2	0.32	
Group 3 1 mg/m ³ air	139	20.6	6.23	2.34 ± 2.23
	140	6.8	2.98	
	141	1.8	0.22	
	142**	3.2	1.07	
	143	2.8	2.97	
	144	2.0	0.63	
Group 4 4 mg/m ³ air	187	5.8	0.29	0.28 ± 0.12
	188	0.6	0.20	
	189	0.4	0.17	
	190	1.2	0.29	
	191	0.2	0.27	
	192	1.2	0.52	
Group 5 200 mg/kg b.w. MMS Positive Control	193	#	57.88	65.10 ± 9.95
	194	#	62.28	
	195	#	53.92	
	196	#	75.08	
	197	#	62.16	
	198	#	79.30	

*: SD = Standard Deviation

**: only 95 cells could be evaluated for the Comet Assay and 115 cells for the dead cell index

*: the state of the cells isolated from positive control animals showed a clear DNA damage that was mostly difficult to differentiate from the structure of dead cells, no dead cell index was calculated for that group

8.4 Acceptance Criteria

The study is considered valid if the following criteria are met:

- The vehicle control treatments demonstrate low levels of cytotoxicity (i.e. group mean of less than 30% clouds or 30% diffused cells indicating correct preparation of the cell suspensions)
- The vehicle control data are consistent with the laboratory's historical control data and are not overtly above the upper range

The positive control treatments demonstrate a clear increase in Comet parameters over the vehicle control.

8.5 Data management

The experimental unit of exposure for *in vivo* studies is the animal, and all analysis will be based on individual animal response.

Values obtained from each parameter will be treated as follows:

- The median is calculated for each slide
- For each animal the mean of the medians are calculated, if possible.
- The mean of the medians and standard error of the mean is calculated for each group

A test item is classified as mutagenic if it induces either a dose-related increase or a biologically relevant increase in the tail % intensity in a single dose group as compared to the historical control range. Statistical methods will be used as an aid in evaluating the results. Normally distributed data will be analysed using a one-tailed student's t-test. An F-test is first used to test for homogeneity of variances for pairwise analysis. However, the primary point of consideration will be the biological relevance of the results.

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Data for the historical control were generated using non-treated animals.

		Minced Lung Tissue Cells	BAL Cells
Tail % intensity	Range	0.04 – 3.29	0.04 – 6.34
	Mean	0.42 ± 0.60	0.66 ± 1.26
Number of animals		32	27

9 BIOMETRY

For all test groups above the control values statistical methods were used as an aid in evaluating the results as recommended by Hartmann et al 2003. Normally distributed data were analysed using a one-tailed student's t-test. An F-test was first used to test for homogeneity of variances for pairwise analysis.

10 RESULTS

10.1% Tail Intensity of BAL Cells

Test Group	Animal Number	Amount dead cells on slides (%)	% Tail Intensity	
			Median	Group Mean ± SD*
Group 1 Air Control	43	6.0	2.59	0.97 ± 0.94
	44	2.6	0.96	
	45	0.4	0.47	
	46	0.2	0.51	
	47	animal died		
	48	2.2	0.32	
Group 2 0.25 mg/m ³ air	91	2.4	0.92	0.31 ± 0.32
	92	1.2	0.08	
	93	0.8	0.34	
	94	0.0	0.07	
	95	0.8	0.11	
	96	0.2	0.32	
Group 3 1 mg/m ³ air	139	20.6	6.23	2.34 ± 2.23
	140	6.8	2.98	
	141	1.8	0.22	
	142**	3.2	1.07	
	143	2.8	2.97	
	144	2.0	0.63	
Group 4 4 mg/m ³ air	187	5.8	0.29	0.28 ± 0.12
	188	0.6	0.20	
	189	0.4	0.17	
	190	1.2	0.29	
	191	0.2	0.27	
	192	1.2	0.52	
Group 5 200 mg/kg b.w. MMS Positive Control	193	#	57.88	65.10 ± 9.95
	194	#	62.28	
	195	#	53.92	
	196	#	75.08	
	197	#	62.16	
	198	#	79.30	

*: SD = Standard Deviation

** : only 95 cells could be evaluated for the Comet Assay and 115 cells for the dead cell index

: the state of the cells isolated from positive control animals showed a clear DNA damage that was mostly difficult to differentiate from the structure of dead cells, no dead cell index was calculated for that group

10.2% Tail Intensity of Minced Lung Tissue Cells

Test Group	Animal Number	Amount dead cells on slides (%)**	% Tail Intensity	
			Mean***	Group Mean ± SD*
Group 1 Air Control	43	3.4	0.37	0.53 ± 0.43
	44	1.3	0.98	
	45	1.3	0.08	
	46	1.4	0.38	
	47	animal died		
	48	2.0	0.85	
Group 2 0.25 mg/m ³ air	91	1.5	0.22	0.62 ± 1.00
	92	2.9	0.83	
	93	0.4	0.21	
	94	2.5	0.60	
	95	1.0	0.07	
	96	0.7	1.77	
Group 3 1 mg/m ³ air	139	0.8	0.14	0.21 ± 0.14
	140	1.9	0.13	
	141	1.3	0.28	
	142	1.2	0.31	
	143	0.6	0.28	
	144	0.9	0.11	
Group 4 4 mg/m ³ air	187	4.7	0.61	0.43 ± 0.40
	188	2.3	0.14	
	189	0.7	0.05	
	190	1.2	0.77	
	191	1.3	0.47	
	192	3.0	0.53	
Group 5 200 mg/kg b.w. MMS Positive Control	193	#	40.63	48.34 ± 11.88
	194	#	41.52	
	195	#	40.94	
	196	#	62.04	
	197	#	41.57	
	198	#	63.36	

*. SD = Standard Deviation

**.: animal mean of the amount of dead cells of two scored slides

***. mean of the median % tail intensity value of two scored slides

#: the state of the cells isolated from positive control animals showed a clear DNA damage that was mostly difficult to differentiate from the structure of dead cells, no dead cell index was calculated for that group

10.3 Biometry

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Student's t-test (test item groups).

BAL Cells

Vehicle control versus test group	%Tail intensity	
	Significance	p
Group 2 0.25 mg/m ³ air	n.t.	-
Group 3 1 mg/m ³ air	-	0.234
Group 4 4 mg/m ³ air	n.t.	-
200 mg/kg b.w. MMS	+	<0.001

- = not significant
+ = significant
n.t. = not tested because obtained mean values were below the mean value of the respective vehicle control.

Mincing Lung Tissue Cells

Vehicle control versus test group	%Tail intensity	
	Significance	p
Group 2 0.25 mg/m ³ air	-	0.804
Group 3 1 mg/m ³ air	n.t.	-
Group 4 4 mg/m ³ air	n.t.	-
200 mg/kg b.w. MMS	+	0.004

- = not significant
+ = significant
n.t. = not tested because obtained mean values were below the mean value of the respective vehicle control.

10.2% Tail Intensity of Minced Lung Tissue Cells

Test Group	Animal Number	Amount dead cells on slides (%)**	% Tail Intensity	
			Mean***	Group Mean ± SD*
Group 1 Air Control	43	3.4	0.37	0.53 ± 0.43
	44	1.3	0.98	
	45	1.3	0.08	
	46	1.4	0.38	
	47	animal died		
	48	2.0	0.85	
Group 2 0.25 mg/m ³ air	91	1.5	0.22	0.62 ± 1.00
	92	2.9	0.83	
	93	0.4	0.21	
	94	2.5	0.60	
	95	1.0	0.07	
	96	0.7	1.77	
Group 3 1 mg/m ³ air	139	0.8	0.14	0.21 ± 0.14
	140	1.9	0.13	
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	142	1.2	0.31	
	143	0.6	0.28	
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** : animal mean of the amount of dead cells of two scored slides

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11 DISCUSSION

Lung tissue samples of animals treated with Vanadium Pentoxide in a 16-Day repeated dose toxicity assay were analysed for primary DNA damage.

The analysis of cells obtained from bronchioalveolar lavage (BAL cells) or mincing of the lung tissue (minced lung tissue cells) showed that neither the treatment of the animals with the vehicle nor the treatment of the animals with the indicated doses of the test item induced DNA damage. The obtained values for the treated animals were near to those treated with air control. The mean % tail Intensities of the animals treated with 0.25, 1 and 4 mg/m³ air were 0.307, 2.343 and 0.288 for BAL cells and 0.615, 0.210 and 0.428 for minced lung tissue cells, respectively. The variations in the values between the dose groups were not significant. All obtained values were within the historical control range.

The number of nuclei from apoptotic or necrotic cells per 500 total nuclei was determined for each sample to indicate the quality of the slide preparation. Neither the slides prepared from animals treated with the negative control nor from those treated with any dose level of the test item showed an remarkable increase in number of dead cells indicating a good slide preparation. However, for both preparation types (BAL cells or minced lung tissue cells) the amount of dead cells could not be determined for the positive control groups. There, the cells showed a structure very close to dead cells complicating the macroscopic differentiation. Nevertheless, the validity of the study was not jeopardized because the comet results where dead cells (% tail intensity above 80%) could be excluded showed a clear and statistically significant increase in DNA damage in cells treated with the positive control.

For BAL cells and for minced lung tissue cells the vehicle control was in the range to ensure a valid performance of the study. An appropriate reference mutagen [MMS, 200 mg/kg b.w. oral] was used as a positive control. Treatment with the positive control substance led to a distinct and statistically significant increase of DNA damage as detected by % Tail Intensity analysis.

In conclusion, it can be stated that under the described circumstances as compared to the controls the test item **did not** induce any DNA damage in the *in vivo* Comet assay performed in BAL cells and minced lung tissue cells.

12 REFERENCES

- Anderson, D., Yu, T.W., McGregor, D.B. (1998): Comet assay responses as indicators of carcinogen exposure. *Mutagenesis* 13, 539-555
- Fairbairn, D.W., Olive, P.L., and O'Neill, K.L. (1995): The Comet assay: A comprehensive review. *Mutation Research* 339: 37-59
- FDA, Guidance for Industry and Review Staff Resommended Approaches to Integration of Genetic Toxicology Study Results, FDA (2006)
- Hartmann, A. et al. (2003): Recommendations for conducting the *in vivo* alkaline Comet assay. *Mutagenesis* 18 (1): 45-51
- Rojas, E. Lopez, M.C., and Valverde, M. (1999): Single cell gel electrophoresis assay: Methodology and applications. *J Chromatology B* 722: 225-254
- Singh, N.P. (2000): Microgels for estimation of DNA stand breaks, DNA protein crosslinks and apoptosis. *Mutation Res* 455: 111-127
- Speit, G. and Hartmann, A. (1999): The comet assay (single-cell gel test)-A sensitive genotoxicity test for the detection of DNA-damage and repair, in: D.S. Henderson (Ed.), *Methods in Molecular Biology Vol 113: DNA Reair Protocols: Eucaryotic Systems*, Humana Press Inc., Totowa, NJ., pp. 203-212
- Tice, R.R: (1995): The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells, in D.H. Phillips and S. Venitt (Eds), *Environmental Mutagenesis*, Bios Scientific Publishers, Oxford, pp. 315-339
- Tice, R.R. et al. (2000): Single Cell Gel/Comet Assay: Guidelines for In Vitro and In Vivo Genetic Toxicology Testing. *Environmental and Molecular Mutagenesis* 35: 206-221

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11 DISCUSSION

Lung tissue samples of animals treated with Vanadium Pentoxide in a 16-Day repeated dose toxicity assay were analysed for primary DNA damage.

The analysis of cells obtained from bronchioalveolar lavage (BAL cells) or mincing of the lung tissue (minced lung tissue cells) showed that neither the treatment of the animals with the vehicle nor the treatment of the animals with the indicated doses of the test item induced DNA damage. The obtained values for the treated animals were near to those treated with air control. The mean % tail Intensities of the animals treated with 0.25, 1 and 4 mg/m³ air were 0.307, 2.343 and 0.288 for BAL cells and 0.615, 0.210 and 0.428 for minced lung tissue cells, respectively. The variations in the values between the dose groups were not significant. All obtained values were within the historical control range.

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For BAL cells and for minced lung tissue cells the vehicle control was in the range to ensure a valid performance of the study. An appropriate reference mutagen [MMS, 200 mg/kg b.w. oral] was used as a positive control. Treatment with the positive control substance led to a distinct and statistically significant increase of DNA damage as detected by % Tail Intensity analysis.

In conclusion, it can be stated that under the described circumstances as compared to the controls the test item **did not** induce any DNA damage in the *in vivo* Comet assay performed in BAL cells and minced lung tissue cells.

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- FDA, Guidance for Industry and Review Staff Resommended Approaches to Integration of Genetic Toxicology Study Results, FDA (2006)
- Hartmann, A. et al. (2003): Recommendations for conducting the *in vivo* alkaline Comet assay. *Mutagenesis* 18 (1): 45-51
- Rojas, E. Lopez, M.C., and Valverde, M. (1999): Single cell gel electrophoresis assay: Methodology and applications. *J Chromatology B* 722: 225-254
- Singh, N.P. (2000): Microgels for estimation of DNA stand breaks, DNA protein crosslinks and apoptosis. *Mutation Res* 455: 111-127
- Speit, G. and Hartmann, A. (1999): The comet assay (single-cell gel test)-A sensitive genotoxicity test for the detection of DNA-damage and repair, in: D.S. Henderson (Ed.), *Methods in Molecular Biology Vol 113: DNA Reair Protocols: Eucaryotic Systems*, Humana Press Inc., Totowa, NJ., pp. 203-212
- Tice, R.R: (1995): The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells, in D.H. Phillips and S. Venitt (Eds), *Environmental Mutagenesis*, Bios Scientific Publishers, Oxford, pp. 315-339
- Tice, R.R. et al. (2000):Single Cell Gel/Comet Assay: Guidelines for In Vitro and In Vivo Genetic Toxicology Testing. *Environmental and Molecular Mutagenesis* 35: 206-221

13 DISTRIBUTION OF THE PHASE REPORT

Contracting Institute:	1x copy
Principal Investigator:	1x original

14 ANNEX 1: INDIVIDUAL RESULTS FOR MINCED LUNG TISSUE CELLS

dose group	animal number	median % Tail Intensity			mean % Tail Intensity per group	standard deviation
		slide 1	slide 2	mean		
Group 1 Air Control	43	0.69	0.06	0.37	0.53	0.43
	44	1.10	0.86	0.98		
	45	0.12	0.04	0.08		
	46	0.68	0.08	0.38		
	47	animal died				
	48	0.62	1.08	0.85		
Group 2 0.25 mg/m ³ air	91	0.24	0.19	0.22	0.62	1.00
	92	1.52	0.14	0.83		
	93	0.31	0.12	0.21		
	94	0.62	0.57	0.60		
	95	0.07	0.06	0.07		
	96	3.51	0.03	1.77		
Group 3 1 mg/m ³ air	139	0.02	0.26	0.14	0.21	0.14
	140	0.25	0.01	0.13		
	141	0.44	0.12	0.28		
	142	0.35	0.27	0.31		
	143	0.34	0.21	0.28		
	144	0.19	0.03	0.11		
Group 4 4 mg/m ³ air	187	0.72	0.51	0.61	0.43	0.40
	188	0.22	0.05	0.14		
	189	0.05	0.05	0.05		
	190	0.50	1.03	0.77		
	191	0.10	0.85	0.47		
	192	0.03	1.04	0.53		
Group 5 200 mg/kg b.w. MMS Positive Control	193	38.68	42.58	40.63	48.34	11.88
	194	37.91	45.13	41.52		
	195	38.45	43.43	40.94		
	196	58.02	66.06	62.04		
	197	52.36	30.77	41.57		
	198	63.57	63.16	63.36		

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REPORT

Vanadium Pentoxide

16-Day Inhalation Toxicity Study in Female Mice

Study Director: Dr. D. Schuler

Test Facility: **Harlan Laboratories Ltd.**
Wölferstrasse 4
4414 Füllinsdorf / Switzerland

Sponsor: **Advanced Technology Institute**
5300 International Blvd
N. Charleston, SC 29418 / USA

Study Identification: Harlan Laboratories Study **A94206**

Version: Draft 1

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GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Harlan Laboratories Study: A94206
Test Item: Vanadium Pentoxide
Study Director: Dr. D. Schuler
Study Title: 16-Day Inhalation Toxicity Study in Female Mice

This study has been performed in compliance with the:

Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted on November 26th, 1997 by decision of the OECD Council [C (97)186/Final].

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

Although not being included in a national GLP compliance-monitoring program, CEA/Grenoble has been chosen as a test location, because they are recognized experts in their fields of work.

Study Director: Dr. D. Schuler

.....
Date:

QUALITY ASSURANCE GLP STATEMENT

Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen / Switzerland

Harlan Laboratories Study: A94206
Test Item: Vanadium Pentoxide
Study Director: Dr. D. Schuler
Study Title: 16-Day Inhalation Toxicity Study in Female Mice

The general facilities and activities are inspected at least once a year and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the quality assurance. The dates are given below.

Dates and Types of QA Inspections	Dates of Reports to the Study Director and Test Facility Management

This statement also confirms that this final report reflects the raw data.

In addition this final report includes all QA-statements issued by the test site quality assurances.

Quality Assurance: T. Frei

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Date:

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SIGNATURE(S) OF ADDITIONAL SCIENTIST(S)

Analytical Chemistry: Dr. D. Flade

.....
Date:

Biomarker Analysis: Dr. P. Sagelsdorff

.....
Date:

The signatures of the principal investigators (PI(s)) are included in the respective phase reports which are attached as appendices to this report.

PREFACE

General Information

Test Item: Vanadium Pentoxide

Study Title: 16-Day Inhalation Toxicity Study in Female Mice

Sponsor: Advanced Technology Institute
5300 International Blvd
N. Charleston, SC 29418 / USA

Sponsor Contact: Dr. D. McGregor
38 Shore Road
Aberdour, KY3 0TU
Scotland / United Kingdom

Test Facility: Harlan Laboratories Ltd. (a)
Wölferstrasse 4
4414 Füllinsdorf / Switzerland

Test Sites: Harlan Cytotest Cell Research GmbH (b)
In den Leppsteinswiesen 19
64380 Rossdorf / Germany
AnaPath GmbH (c)
Buchsweg 56
4625 Oberbuchsitzen / Switzerland

Test Location: CEA/Grenoble (d)
17 rue des Martyrs
38054 Grenoble Cedex 9 / France

Lead QA: Harlan Laboratories Ltd.
Quality Assurance GLP
Zelgliweg 1
4452 Itingen / Switzerland

Test Site QAs: Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Rossdorf / Germany
Harlan Laboratories Ltd
Quality Assurance GLP
4452 Itingen / Switzerland

SIGNATURE(S) OF ADDITIONAL SCIENTIST(S)

Analytical Chemistry: Dr. D. Flade

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Harlan Laboratories Ltd
Quality Assurance GLP
4452 Itingen / Switzerland

Responsibilities

Study Director:	Dr. D. Schuler (a)
Deputy Study Director:	Dr. D. Pothmann (a)
Study Coordinator:	H. Huber (a)
Inhalation Technologist:	M. Walter (a)
Deputy Inhalation Technologist:	P. Hardy (a)
Senior Animal Technician:	B. Madörin (a)
Necropsy / Histotechnique:	Dr. K. Weber (a)
Analytical Chemistry (Vanadium Pentoxide Analysis in Aerosol, Blood and Lungs):	Dr. D. Flade (a)
Biomarker Analysis:	Dr. P. Sagelsdorff (a)

Principal Investigator(s):

Study Phase Comet Assay:	Dr. M. Reichenbach (b)
Study Phase Pathology, Cell Proliferation:	Dr. H.-J. Chevalier (c)

Responsible for (non-GLP):

Study Phase DNA Lesions:	Dr. J.-L. Ravanat (d)
--------------------------	-----------------------

Quality Assurance:

Head of Lead QA:	T. Fink
------------------	---------

Schedule

Delivery of Mice:	05-May-2009
Experimental Starting Date:	07-May-2009
Quarantine:	05- to 06-May-2009 (sub groups A, B, C) 05- to 07-May-2009 (sub groups D and E, and for groups 1, 2 and 5 also sub groups F and G) 05- to 08-May-2009 (sub groups F and G of groups 3 and 4)
Acclimatization:	07- to 17-May-2009 (sub groups A, B, C) 08- to 18-May-2009 (sub groups D and E, and for groups 1 and 2 also sub groups F and G) 09- to 19-May-2009 (sub groups F and G of groups 3 and 4) 09-May to 02-Jun-2009 (group 5)
Administration / Treatment:	18-May to 02-Jun-2009 (sub groups A and C) 18- to 24-May-2009 (sub group B) 19-May to 03-Jun-2009 (sub groups D and E, and for groups 1 and 2 also sub groups F and G) 20-May to 04-Jun-2009 (sub groups F and G of groups 3 and 4) 03-Jun-2009 (group 5)
Termination (Necropsy):	02-Jun-2009 (sub group A) 25-May-2009 (sub group B) 03-Jun-2009 (sub group C, and for groups 1, 2, and 5 also sub groups F and G) 04-Jun-2009 (sub groups D, E, and for groups 3 and 4 also sub groups F and G)
Experimental Completion Date:	November 2009

Animal Welfare

This study was performed in an AAALAC-accredited laboratory in accordance with the Swiss Animal Protection Law under license no. 45.

Responsibilities

Study Director:	Dr. D. Schuler (a)
Deputy Study Director:	Dr. D. Pothmann (a)
Study Coordinator:	H. Huber (a)
Inhalation Technologist:	M. Walter (a)
Deputy Inhalation Technologist:	P. Hardy (a)
Senior Animal Technician:	B. Madörin (a)
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Principal Investigator(s):

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Study Phase Pathology, Cell Proliferation:	Dr. H.-J. Chevalier (c)

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Experimental Completion Date:	November 2009

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Archiving

Harlan Laboratories Ltd. (4452 Itingen / Switzerland) retain the study plan, raw data, pathology phase raw data, sample of test item, specimens (as long as the quality permits evaluation), the pathology phase report and the final report of the present study for at least ten years.

No data are discarded without the Sponsor's written consent.

The raw data and the phase report of the study phase performed by the principal investigator for Comet Assay are archived at her test site for at least 15 years.

The raw data and the phase report of the study phase performed by the principal investigator for DNA Lesions are archived at his test location for at least 10 years.

1 SUMMARY

General

In this toxicity study, Vanadium Pentoxide was administered by nose-only inhalation to female mice for a period of 16 days (6 hours per day) at target concentrations of 0.25, 1.0 and 4.0 mg/m³ test item in air. In this study 198 female B6C3F1/Hsd mice were allocated to 4 groups of 48 mice each. The mice of Group 1 served as air controls. The mice of each group were further allocated in eight sub groups to evaluate the range of toxicity on specific end-points in the lungs and to obtain data on the concentration of Vanadium Pentoxide in blood and lungs. One additional group of six mice (group 5) was used as positive controls for a comet assay test.

Throughout the treatment period all mice were observed daily for viability and clinical signs. In addition, the body weight of each mouse was recorded weekly during the acclimatization and treatment period. After completion of treatment the animals of each sub group were subjected to determination of Vanadium Pentoxide in blood and lungs, to necropsy, to weighing of the lungs, to lung preparation for cell proliferation analysis after 7 and 16 days of treatment, respectively, to biomarker analysis in lung tissues, to investigations on DNA lesions in the lungs and/or to a comet assay with BAL and pulmonary cells.

The results of the study are summarized as follows:

Technical Data

Aerosol concentrations are detailed in the table below. They were considered to be very close to the respective targets. The Mass Median Aerodynamic Diameters of the generated aerosols (ranging between 1.22 and 1.43 µm) as well as temperature, relative humidity and oxygen concentration during exposure were considered to be suitable for this type of study.

Group	Chemical aerosol concentration [mg/m ³]	Target aerosol concentration [mg/m ³]	Chemical aerosol concentration relative to target
2	0.246 ± 0.026 (n=17, CV=10.5%)	0.25	98.2% ± 10.3% (n=17)
3	0.993 ± 0.122 (n=18, CV=12.3%)	1.0	99.3% ± 12.2% (n=18)
4	4.01 ± 0.55 (n=18, CV=13.8%)	4.0	100.1% ± 13.8% (n=18)

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3	0.993 ± 0.122 (n=18, CV=12.3%)	1.0	99.3% ± 12.2% (n=18)
4	4.01 ± 0.55 (n=18, CV=13.8%)	4.0	100.1% ± 13.8% (n=18)

Mortality / Viability

There were no deaths during the course of this study considered to be related to treatment.

Clinical Signs

No clinical signs were noted during the course of this study.

Body Weights and Body Weight Gain

A transient body weight loss of marginal degree was noted in groups 3 and 4.

Vanadium Pentoxide in Whole Blood and Lungs

The amount of Vanadium Pentoxide in the lungs increased approximately dose-proportional from group 2 to group 3 and less than dose proportional from group 3 to group 4. Values for whole blood were similar.

Organ Weights

Lung weights showed a dose-related increase in groups 3 and 4.

Macroscopic Findings

No gross findings were recorded at scheduled necropsy.

Microscopic Findings / Cell Proliferation

Alveolar histiocytosis, subacute alveolitis and/or granulocytic infiltration was noted in all examined mice of groups 3 and 4, generally in a dose-dependent degree.

Immunohistochemical analysis of the lungs after 7 or 16 days of treatment revealed a dose-dependently and generally time-dependently increased proliferation rate in groups 3 and 4.

Biomarker Determination

In the lungs of groups 2 and 4 slightly reduced and increased α -tocopherol concentrations were noted, respectively. The concentrations of GSSG were increased in groups 2 to 4 along with decreased GSH/GSSG ratios as well as with slightly decreased concentrations of GSH of group 2. No effects of treatment with the test item on F2-Isoprostane concentrations in the lungs were noted.

Determination of DNA Lesions

\\ Will be included in the next draft version.

Comet Assay

The analysis of BAL cells or pulmonary cells did not demonstrate any DNA damage in the in vivo Comet assay induced by Vanadium Pentoxide.

Conclusion

It was considered that these findings are unlikely to be related to oxidative stress but possibly related with the removal of Vanadium Pentoxide particles from the alveoli resulting in increased proliferation of histiocytes. (\\ To be up-dated when results of analysis for DNA lesions are available.)

Based on these findings a No-Observed-Adverse-Effect-Level (NOAEL) of 0.246, mg/m³ may be established under the conditions of this study.

Mortality / Viability

There were no deaths during the course of this study considered to be related to treatment.

Clinical Signs

No clinical signs were noted during the course of this study.

Body Weights and Body Weight Gain

A transient body weight loss of marginal degree was noted in groups 3 and 4.

Vanadium Pentoxide in Whole Blood and Lungs

The amount of Vanadium Pentoxide in the lungs increased approximately dose-proportional from group 2 to group 3 and less than dose proportional from group 3 to group 4. Values for whole blood were similar.

Organ Weights

Lung weights showed a dose-related increase in groups 3 and 4.

Macroscopic Findings

No gross findings were recorded at scheduled necropsy.

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2 PURPOSE

The purpose of this 16-day study was to evaluate the range of toxicity on specific end-points in the lungs of mice following nose-only inhalation of Vanadium Pentoxide. Additionally, data was obtained on the concentration in blood and lungs associated with the exposure.

The results were used to provide a rational basis for the assessment of the toxicological risk to man.

3 MATERIALS AND METHODS

3.1 Test System

Animals:	Mouse, B6C3F1/Hsd
Rationale:	Recognized by international guidelines as a recommended test system.
Breeder:	Harlan Laboratories Ltd. 5190 Dominion Drive Dublin, VA 24084 / USA
Number of Groups:	5 (1 air control, 3 dose groups, 1 positive control)
Number of Mice in the Study:	Group 1: 48 females Group 2: 48 females Group 3: 48 females Group 4: 48 females Group 5: 6 females
Total Number of Mice Ordered	211 females (including 13 females reserve mice for possible replacement of mice showing clinical signs during acclimatization).
Age (at Delivery):	7 to 8 weeks
Body Weight Range (at Acclimatization):	16.9 to 23.2 g ($\pm 17\%$)
Identification:	By unique cage number and by individual unique tail numbers with an indelible felt-tip pen.
Randomization:	Computer-generated random algorithm.
Acclimatization:	At least 7 days under laboratory conditions, after clinical health examination. Only mice without any visible signs of illness were used for the study. The mice of groups 1 to 4 were accustomed to the restraint tubes and the exposure conditions for 3 daily periods of approximately, 1, 3 and 5 hours, respectively.
Reserve Mice:	Thirteen female reserve mice from the same delivery batch were retained during the acclimatization period for the replacement of unsuitable mice before the start of the treatment period. No substitution was necessary; all reserve mice were removed from the study towards the end of the treatment period.

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3.2 Allocation

The group identification and animal numbers assigned to treatment are stated in the following table:

Sub Groups:	Group 1 Air Control	Group 2 0.25 mg/m³ Air	Group 3 1.0 mg/m³ Air	Group 4 4.0 mg/m³ Air	Group 5 Positive Control
A Vanadium Pentoxide	1 - 6	49 - 54	97 - 102	145 - 150	---
B Cell Proliferation (day 7)	7 - 12	55 -60	103 - 108	151 - 156	---
C Cell Proliferation (day 16), Histopathology	13 - 18	61 - 66	109 - 114	157 - 162	---
D Biomarkers in Lung Tissue (Glutathione, α -tocopherol)	19 -26	67 - 74	115 - 122	163 - 170	---
E Biomarkers in Lung Tissue (F2-isoprostanes)	27 - 34	75 - 82	123 - 130	171 - 178	---
F DNA lesions	35 - 42	83 - 90	131 - 138	179 - 186	---
G Comet Assay	43 - 48	91 - 96	139 - 144	187 - 192	193 - 198

Reserve mice were numbered 199 to 211.

3.3 Husbandry

Room Numbers, Füllinsdorf:

Rooms 3.12 and 3.14

Conditions:

Optimal Hygienic Conditions behind a barrier system in an air-conditioned facility with approximately 10 - 15 air changes per hour, continuously monitored environmental conditions (temp. range: 22 ± 3 °C; relative humidity range: 30 - 70%). Values outside of the humidity range occasionally occurred, usually following room cleaning, and are considered not to have any influence on the study. Therefore, these data are not reported but are retained at Harlan Laboratories Ltd. There was 12-hour fluorescent light/12-hour dark cycle with music during the light period.

Accommodation:

The mice were housed individually in Makrolon® type-2 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne GmbH & Co, KG, 73494 Rosenberg / Germany, imported by Provimi Kliba AG, 4303 Kaiseraugst / Switzerland).

Diet:

Pelleted standard Kliba Nafag 3433 (batch no. 76/08) mouse maintenance diet (Provimi Kliba SA, 4303 Kaiseraugst / Switzerland) was available *ad libitum*. The feed batch was analyzed for contaminants.

Results of respective analyses for contaminants are included in Appendix I on p. 166 .

Water:

Community tap-water from Itingen was available *ad libitum* in water bottles. Results of bacteriological assay, chemical and contaminant analyses of respective samples are included in Appendix II on p. 169 .

3.2 Allocation

The group identification and animal numbers assigned to treatment are stated in the following table:

Sub Groups:	Group 1 Air Control	Group 2 0.25 mg/m³ Air	Group 3 1.0 mg/m³ Air	Group 4 4.0 mg/m³ Air	Group 5 Positive Control
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C Cell Proliferation (day 16), Histopathology	13 - 18	61 - 66	109 - 114	157 - 162	---
D Biomarkers in Lung Tissue (Glutathione, α -tocopherol)	19 - 26	67 - 74	115 - 122	163 - 170	---
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G Comet Assay	43 - 48	91 - 96	139 - 144	187 - 192	193 - 198

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Water:

Community tap-water from Itingen was available *ad libitum* in water bottles. Results of bacteriological assay, chemical and contaminant analyses of respective samples are included in Appendix II on p. 169 .

3.4 Test Item, Positive Control Item and Reference Item

3.4.1 Test Item

Data as supplied by the Sponsor.

Identification:	Vanadium Pentoxide
Description:	Solid, yellow-orange
Batch Number:	H060391
Molecular Weight:	182
Purity:	99.8%
Expiry Date (Retest Date):	January 2010
Storage Conditions:	At room temperature (range of 20 ± 5 °C), under dry conditions
Safety Precautions:	Laboratory overall, gloves and face mask were sufficient to ensure personnel health and safety during the handling of the test item. The face mask was replaced by a full face, filtered, positive air-supplied respirator during the inhalation exposure.
Preparation of Test Item:	The test item was used as supplied by the Sponsor.

3.4.2 Positive Control Item

Data as supplied by the Principal Investigator for Comet Assay

Name:	Methylmethansulfonate (MMS)
Supplier:	Sigma-Aldrich Chemie GmbH 82018 Taufkirchen / Germany
Catalogue no.:	12,992-5
Purity:	99%
Storage Conditions:	at 2 – 8 °C
Dissolved in:	0.9 % NaCl solution

3.4.3 Reference Item

The reference item was used as analytical standard.

Data as provided by the responsible for analytical chemistry

Identification:	Vanadium 1000 µg/mL AAS/ICP
Supplier, Art. No.:	Baker 6945
Lot Number:	15.0090409
Content:	1000 µg/mL
Expiry Date (Retest Date):	September 2012
Storage Conditions:	No particular precaution required - storage at Harlan Laboratories Ltd. at room temperature (range of 20 ± 5 °C)

3.5 Treatment

Method:	Groups 1 to 4 Inhalation by nose-only, flow past exposure. Group 5 Oral, by gavage
Rationale for Method:	Inhalation is a possible route of human exposure (Groups 1 to 4) The oral administration of MMS resulted in significantly increased percentage tail DNA intensities of the analysed lung cells in the lungs as demonstrated in Harlan CCR validation study 1048302 (Group 5)
Frequency of Administration:	Groups 1 to 4 16 consecutive days (except allocation B mice: 7 consecutive days). Group 5 Single administration
Duration of Daily Exposure:	6 hours (Groups 1 to 4)
Target Aerosol Concentration:	Group 1: 0 mg/m ³ air (Air control) Group 2: 0.25 mg/m ³ air Group 3: 1.0 mg/m ³ air Group 4: 4.0 mg/m ³ air
Oral Dose:	Group 5: 200 mg/kg b.w. (Dose Volume: 10 mL/kg b.w.)

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Oral Dose:	Group 5: 200 mg/kg b.w. (Dose Volume: 10 mL/kg b.w.)

Rationale for Aerosol Concentration Selection:

The target aerosol concentrations were proposed by the Sponsor and are based on the results obtained within the previous National Toxicology Program (*Toxicology and Carcinogenesis Studies of Vanadium Pentoxide (CAS No. 1314-62-1) in F344/N Rats and B6C3F₁ Mice (Inhalation Studies)*). NTP Technical Report Series No. 507. NIH Publication No. 03-4441. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, Research Triangle Park, NC.

Rationale for Oral Dose:

This dose level (200 mg MMS/kg b.w.) resulted in significantly increased percentage tail DNA intensities of the analyzed lung cells as demonstrated in Harlan CCR validation study 1048302.

3.6 Inhalation Exposure System

Inhalation exposure was performed using a system similar to that originally described by Sachs et al. (see References (1) and (2)). The mice are confined separately in restraint tubes which are positioned radially around the flow-past, nose-only exposure chamber described by Cannon et al. (see References (3)). The design of this chamber is based upon the fluid dynamic modeling of the test aerosol flow.

The exposure system ensured a uniform distribution and provided a constant flow of test material to each exposure tube. The flow of air at each tube was 0.5 L/min, which was sufficient to minimize re-breathing of the test aerosol as it was more than twice the respiratory minute volume of a mouse.

Before commencement of the exposure of the group(s), technical trials were conducted (without mice) using the inhalation system foreseen for the study. The technical trials were conducted using established procedures based on GLP, but were not inspected or audited by the Harlan Laboratories Quality Assurance. Technical trial data were documented in the raw data but not reported.

3.7 Test Aerosol Generation

A dust aerosol was generated from the test item using a rotating brush aerosol generator connected to a micronizing jet mill. The aerosol generated was then discharged into the exposure chamber through a ⁶³Ni charge neutraliser. The ⁶³Ni charge neutralizer was used to electrostatically discharge the generated aerosol prior to arrival at the exposure chamber. An air-vacuum dilution system was used to achieve the target aerosol concentration for groups 2 and 3.

Air control mice (Group 1) were exposed to air under the same conditions as mice treated with the test item.

The air for aerosol generation was delivered by a model 'Atlas Copco ZT30-8' oil-free, rotating gear-wheel compressor system'. Additionally, there was an air-cleaning filter system mounted at the entry in the exposure room.

3.8 Exposure System Monitoring

The aerosol concentrations of the test item determined gravimetrically and chemically, particle size distribution determined gravimetrically and chemically, relative humidity, temperature and oxygen concentration, were measured on test aerosol samples collected directly from the delivery tube in the breathing zone of the mice, at an empty port of the exposure chamber, as specified below. Airflow rates were measured for the collection of samples for the determination of test item concentration and particle size using a dry-test meter ('Schlumberger Industries SA', City of Geneva) and/or a pressure gauge (Timeus & Co., Zürich), calibrated with a reference dry-test meter.

3.8.1 Nominal Determination of Aerosol Concentrations

The test item usage was measured by weighing the generator cylinder containing the test item before and after exposure to determine the quantity of test item used. The weight used was then divided by the total air-flow volume to give the nominal concentration. These data were used for the purpose of monitoring the performance of the generation system.

3.8.2 Gravimetric Determination of Aerosol Concentrations

Gravimetric determinations of the aerosol concentration were performed once daily for Groups 2 to 4 using Millipore® durapore filters, Type HVLP, (Polyvinylidenedifluoride membrane, pore size 0.45 µm) l loaded in a 47 mm in-line stainless steel filter sampling device (Gelman Science Inc., Ann Arbor, Michigan / U.S.A.).

Rationale for Aerosol Concentration Selection:

The target aerosol concentrations were proposed by the Sponsor and are based on the results obtained within the previous National Toxicology Program (*Toxicology and Carcinogenesis Studies of Vanadium Pentoxide (CAS No. 1314-62-1) in F344/N Rats and B6C3F1 Mice (Inhalation Studies)*). NTP Technical Report Series No. 507. NIH Publication No. 03-4441. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP, Research Triangle Park, NC.

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3.8.3 Chemical Determination of Aerosol Concentrations

Chemical analyses to determine the concentration of Vanadium Pentoxide in the generated aerosol were performed once daily for Groups 2 to 4 using the filters collected for gravimetric determinations. After weighing, filter samples and a blank filter were transferred to appropriately labeled vials, and sent at ambient temperature to the study scientist for Vanadium Pentoxide analysis. The samples were analyzed for Vanadium using an AAS method and reported as Vanadium Pentoxide after recalculation. Analytical chemistry results are included in Appendix III on p. 173 .

3.8.4 Particle Size Distributions and Mass Median Aerodynamic Diameters

The distribution of particle size in the generated aerosol was by gravimetry twice during the 16-day treatment period in each of groups 3 and 4 using a Cascade Impactor. In group 3, sampling was performed over a period of 4 consecutive days. The impactor samples were also analyzed chemically. They were transferred to appropriately labeled vials and sent at ambient temperature to the study scientist for Vanadium Pentoxide analysis. The samples were analyzed for Vanadium using an AAS method and reported as Vanadium Pentoxide after recalculation. Analytical chemistry results are included in Appendix III on p. 173 .

The Mass Median Aerodynamic Diameter (MMAD) and the Geometric Standard Deviation (GSD) were calculated on the basis of the results from the impactors, using Microsoft Excel Software. In addition, the group-specific mean MMAD and GSD were calculated on the basis of the mean stage specific cumulative weight percentages of all impactors of the respective group in the study.

3.8.5 Oxygen Concentration

The oxygen concentration in the chamber was measured continuously during each exposure using a calibrated device. The oxygen concentration was maintained above 19% during the exposure period. The results were reported three times during each daily exposure.

3.8.6 Relative Humidity / Temperature

The relative humidity and temperature in the chamber were measured continuously during each exposure using a calibrated device. The results were reported three times during each daily exposure.

3.8.7 Airflow Rate

The exposure airflow rate was adjusted as appropriate before the start of the exposure using calibrated flow-meters and / or pressure gauges. The actual airflow rates were recorded three times during each daily exposure.

3.9 Observations

Viability / Mortality:

Twice daily, prior to and following exposure and at least once daily during the quarantine and the acclimatization periods.

These data were used only for the purpose of routine health monitoring and no tables were included in the report.

Clinical Signs:

Clinical signs were recorded on the first day of the acclimatization period. During the 16-day treatment period, clinical signs were observed at least once daily following exposure.

These data were used only for the purpose of routine health monitoring and no tables were included in the report.

Body Weights:

Each mouse was weighed approximately weekly during the acclimatization and treatment periods (before exposure/dosing).

3.10 Vanadium Pentoxide in Whole Blood and Lungs

Within 5 minutes after the end of the last exposure on 02-Jun-2009, blood samples were collected from the vena cava of allocation A mice whilst they were maintained under isoflurane (Forene[®]) anaesthesia. Approximately 0.8 mL (0.6 - 1.0 mL) of blood were sampled. Blood samples were collected in appropriately labeled tubes containing lithium heparin anticoagulant and stored between -15 and -25°C in the dark, before dispatch for analysis in dry ice to the attention of the study scientist for Vanadium Pentoxide analysis.

Thereafter, the lungs were removed and the time recorded. The main stem bronchi and the mediastinal tissue containing the mediastinal lymph nodes were resected and discarded. The lungs were weighed and inserted into appropriately labeled plastic bags and stored between -15 and -25°C in the dark, before dispatch for analysis in dry ice to the attention of the study scientist for Vanadium Pentoxide analysis.

The mice mentioned above were killed by exsanguination and discarded without further examination.

All samples were analyzed for Vanadium using an AAS method validated under Harlan Laboratories Study B34154 and reported as Vanadium Pentoxide after recalculation. Results of this investigation are included in Appendix III on p. 173 .

3.8.3 Chemical Determination of Aerosol Concentrations

Chemical analyses to determine the concentration of Vanadium Pentoxide in the generated aerosol were performed once daily for Groups 2 to 4 using the filters collected for gravimetric determinations. After weighing, filter samples and a blank filter were transferred to appropriately labeled vials, and sent at ambient temperature to the study scientist for Vanadium Pentoxide analysis. The samples were analyzed for Vanadium using an AAS method and reported as Vanadium Pentoxide after recalculation. Analytical chemistry results are included in Appendix III on p. 173 .

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The oxygen concentration in the chamber was measured continuously during each exposure using a calibrated device. The oxygen concentration was maintained above 19% during the exposure period. The results were reported three times during each daily exposure.

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The relative humidity and temperature in the chamber were measured continuously during each exposure using a calibrated device. The results were reported three times during each daily exposure.

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The exposure airflow rate was adjusted as appropriate before the start of the exposure using calibrated flow-meters and / or pressure gauges. The actual airflow rates were recorded three times during each daily exposure.

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Twice daily, prior to and following exposure and at least once daily during the quarantine and the acclimatization periods.

These data were used only for the purpose of routine health monitoring and no tables were included in the report.

Clinical Signs:

Clinical signs were recorded on the first day of the acclimatization period. During the 16-day treatment period, clinical signs were observed at least once daily following exposure.

These data were used only for the purpose of routine health monitoring and no tables were included in the report.

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Each mouse was weighed approximately weekly during the acclimatization and treatment periods (before exposure/dosing).

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Thereafter, the lungs were removed and the time recorded. The main stem bronchi and the mediastinal tissue containing the mediastinal lymph nodes were resected and discarded. The lungs were weighed and inserted into appropriately labeled plastic bags and stored between -15 and -25°C in the dark, before dispatch for analysis in dry ice to the attention of the study scientist for Vanadium Pentoxide analysis.

The mice mentioned above were killed by exsanguination and discarded without further examination.

All samples were analyzed for Vanadium using an AAS method validated under Harlan Laboratories Study B34154 and reported as Vanadium Pentoxide after recalculation. Results of this investigation are included in Appendix III on p. 173 .

3.11 Pathology

3.11.1 Necropsy

All surviving mice of allocation B and C were necropsied after 7 days and 16 days of exposure, respectively.

After 7 Days of Exposure: 25-May-2009

After 16 Days of Exposure: 03-Jun-2009

The mice were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. All mice surviving to the end of the observation period were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. The lung weight was recorded at the scheduled dates of necropsy. Samples of the lungs were collected and fixed in neutral phosphate buffered 4% formaldehyde solution. The lungs were instilled with the fixative.

3.11.2 Histotechnique

The lungs from all allocation B and C mice were processed, embedded in paraffin wax, cut at a nominal thickness of 4 micrometers. The lung slides were stained for the two endogenous proliferation markers PCNA and Ki67.

In addition, the lung slides of allocation C mice were stained with hematoxylin and eosin.

3.11.3 Investigations on Cell Proliferation

The slides stained for two endogenous proliferation markers (PCNA and Ki67) of all lungs from allocation B (7 days of exposure) and allocation C (16 days of exposure) mice were examined.

A description of all abnormalities is included in the pathology phase report (see Appendix IV on p. 189).

3.11.4 Histopathology

The slides stained with hematoxylin and eosin of all lungs of allocation C mice (16 days of exposure) were examined.

A description of all abnormalities is included in the pathology phase report (see Appendix IV on p. 189). Attempts were made to correlate gross observations with microscopic findings.

3.11.5 Investigations on Biomarkers in Lung Tissue

One day after the last exposure, the lungs were removed from allocation D and E mice whilst they were maintained under sodium pentobarbitone anaesthesia. The main stem bronchi and the mediastinal tissue containing the mediastinal lymph nodes were resected and discarded.

After 16 Days of Exposure: 04-Jun-2009

The lungs / lung lobes were allocated to the following analyses:

Allocation D Mice: Right lung lobes Analyzed for glutathione
Left lung lobes Analyzed for α -tocopherol

Allocation E Mice: Lung, whole Analyzed for F2-Isoprostanes

Following sampling, the weight of the lung lobes (allocation D) or of the whole lung (allocation E) was recorded and the samples transferred in appropriately labelled plastic bags. Then the organs were shock-frozen in liquid nitrogen, before transfer as soon as possible on dry ice to responsible for Biomarkers. The lungs and lung lobes were stored at -80 ± 10 °C until analysis.

The responsible for biomarkers in lung tissues provided results for inclusion in the report of this study as an appendix.

The results of this investigation are presented in Appendix V on p. 247 .

3.11 Pathology

3.11.1 Necropsy

All surviving mice of allocation B and C were necropsied after 7 days and 16 days of exposure, respectively.

After 7 Days of Exposure: 25-May-2009

After 16 Days of Exposure: 03-Jun-2009

The mice were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. All mice surviving to the end of the observation period were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. The lung weight was recorded at the scheduled dates of necropsy. Samples of the lungs were collected and fixed in neutral phosphate buffered 4% formaldehyde solution. The lungs were instilled with the fixative.

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One day after the last exposure, the lungs were removed from allocation D and E mice whilst they were maintained under sodium pentobarbitone anaesthesia. The main stem bronchi and the mediastinal tissue containing the mediastinal lymph nodes were resected and discarded.

After 16 Days of Exposure: 04-Jun-2009

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Allocation D Mice: Right lung lobes Analyzed for glutathione
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Following sampling, the weight of the lung lobes (allocation D) or of the whole lung (allocation E) was recorded and the samples transferred in appropriately labelled plastic bags. Then the organs were shock-frozen in liquid nitrogen, before transfer as soon as possible on dry ice to responsible for Biomarkers. The lungs and lung lobes were stored at -80 ± 10 °C until analysis.

The responsible for biomarkers in lung tissues provided results for inclusion in the report of this study as an appendix.

The results of this investigation are presented in Appendix V on p. 247 .

3.11.6 Investigation on DNA Lesions

As soon as feasible after the last exposure on 03-Jun-2009 for groups 1 and 2, and on 04-Jun-2009 for groups 3 and 4, the following procedures were performed on all mice of allocation F.

The procedure detailed below was performed at Harlan Laboratories Ltd, Füllindorf, under the responsibility and by staff of Harlan Cytotest Cell Research GmbH.

After anaesthetizing the mice with 46% Ketamin (Ketavet 100, Pharmacia GmbH, 76139 Karlsruhe, Germany), 23% Xylazin (Rompun 2%, Bayer HealthCare, 51368 Leverkusen, Germany) and 31% Midazolam (Dormicum, Hoffmann LaRoche, 79639 Grenzach-Wyhlen, Germany) (approx. 2 mL/kg body weight) the lung were perfused through the right ventricle with saline. Afterwards the lungs were intubated via the trachea and lavaged with approx. 20 mL saline. The lungs were then further perfused under resuscitation using saline. The lung lobes were then excised and shock frozen in liquid nitrogen. After completion of the sampling, lung samples were shipped on dry ice to the person responsible for the determination of DNA lesions:

Lung samples were analysed for DNA lesions. The phase report for determination of DNA lesions provided by the PI for DNA lesions is included in Appendix VI on p. \\. (report not yet available)

3.11.7 Comet Assay

The procedure detailed below was performed at Harlan Laboratories Ltd, Füllindorf, under the responsibility and by staff of Harlan Cytotest Cell Research GmbH.

As soon as feasible after the last exposure on 03-Jun-2009 for groups 1 and 2, and on 04-Jun-2009 for groups 3 and 4, and 4 hours after the treatment of the positive controls (Group 5) on 03-Jun-2009, the following procedures were performed on all mice of allocation G. The lungs were perfused as described above except that for the lung lavage mincing buffer (20 mM EDTA, 10 % DMSO in HBSS pH 7.4-7.6) was used instead of saline. The excised lung lobes were then minced in 1 ml ice-cold mincing buffer using fine scissors. The resulting cell suspension (minced lung tissue cells) was filtered through a 100 µm cell strainer. The cell suspension was then centrifuged at about 5000 g for 1 min and the resulting pellet resuspended in 70% agarose. The cells isolated from lung lavage (BronchioAlveolar Lavage (BAL) cells) were centrifuged at 300 xg for 10 min and resuspended in 70% agarose.

Lung samples were analysed for DNA strand breaks using the comet assay. Details are described in Appendix III. The principal investigator for the comet assay provided a phase report which is included in Appendix VII on p. 267 .

3.12 Data Compilation

The RCC-TOX CONTROL LIMS computer was used to sort and present suitable data for inclusion in the report. All electronically recorded data are conserved on a magnetic medium.

Individual values were rounded before printing. All derived values that appear in the tables represent the rounded results of calculations that used the exact raw data value.

3.13 Statistical Analysis

The following statistical methods were used to analyze body weighs and organ weights:

- The Dunnett-test [see References (4)] (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex.

Group means and standard deviations were calculated for continuous data.

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Group means and standard deviations were calculated for continuous data.

4 RESULTS

4.1 Inhalation Technical Data

Numeric results are presented in the following format, where appropriate:
Mean value \pm standard deviation (number of determinations [n], coefficient of variation [CV])

4.1.1 Exposure Conditions

(See Individual Tables on p. 45)

Temperature, relative humidity and oxygen concentration during exposure were considered to be satisfactory for this type of study.

Group	Temperature [°C]	Relative humidity [%]	Oxygen concentration [%]
1	22.7 \pm 0.3 (n=17)	1.0 \pm 0.3 (n=17)	20.7 \pm 0.0 (n=17)
2	22.1 \pm 0.1 (n=17)	3.8 \pm 0.1 (n=17)	20.6 \pm 0.0 (n=17)
3	21.8 \pm 0.1 (n=18)	1.9 \pm 0.8 (n=18)	20.6 \pm 0.0 (n=18)
4	21.2 \pm 0.1 (n=18)	0.7 \pm 0.6 (n=18)	20.4 \pm 0.0 (n=18)

4.1.2 Aerosol Concentrations

(See Individual Tables on p. 49)

The chemical aerosol concentrations were very close to targets for all groups. In addition, the aerosol concentrations were stable during the whole treatment period as demonstrated by the low CVs (below 14% for the chemical aerosol concentration of all groups).

Group	Gravimetric aerosol concentration [mg/m ³]	Chemical aerosol concentration [mg/m ³]	Target aerosol concentration [mg/m ³]	Chemical aerosol concentration relative to target
2	0.231 \pm 0.070 (n=17, CV=30.3%)	0.246 \pm 0.026 (n=17, CV=10.5%)	0.25	98.2% \pm 10.3% (n=17)
3	1.03 \pm 0.12 (n=18, CV=12.0%)	0.993 \pm 0.122 (n=18, CV=12.3%)	1.0	99.3% \pm 12.2% (n=18)
4	4.02 \pm 0.45 (n=18, CV=11.3%)	4.01 \pm 0.55 (n=18, CV=13.8%)	4.0	100.1% \pm 13.8% (n=18)

Animals of Group 5 were treated orally with 200 mg/kg MMS at a dose volume of 10 mL/kg body weight.

4.1.3 Particle Size Determination

(See Individual Tables on p. 52)

The values for gravimetrically and chemically determined Mass Median Aerodynamic Diameter (MMAD) were between 1.22 μ and 1.43 μ m on all occasions as stated in the following table. Determination of particle size distribution in group 2 was not feasible due to the low aerosol concentration. However, the MMAD for group 2 was considered to be similar to the other groups because one single aerosol generation system was used for all three treatment groups. The MMADs were at the lower limit of the target range of 1 to 3 μ m, therefore deposition of the particles can be assumed to have occurred mainly in the lower but also in the upper respiratory tract.

Gravimetric determination of particle size distribution:

Group	Range of MMAD [μ m]	Range of GSD	Number of determinations	Mean percentage of particles < 3 μ m [%]
3	1.22 - 1.26	1.89 - 1.92	2	91.5%
4	1.24 - 1.43	1.89 - 1.92	2	89.2%

Chemical determination of particle size distribution:

Group	Range of MMAD [μ m]	Range of GSD	Number of determinations	Mean percentage of particles < 3 μ m [%]
3	1.27 - 1.34	1.77 - 1.86	2	91.9%
4	1.33 - 1.41	1.75 - 1.82	2	91.2%

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4	1.33 - 1.41	1.75 - 1.82	2	91.2%

4.2 Observations

4.2.1 Viability / Mortality

One mouse of group 3 (allocation group C) was found dead prior to the end of exposure during the second week of treatment. This death was considered as incidental and not related to treatment in the absence of clinical signs and of a relation to dose and normal body weight gain was recorded in week 1.

There were no further unscheduled deaths.

4.2.2 Clinical Signs

No clinical signs were noted during the course of this study. Therefore, no tables are presented.

4.2.3 Body Weights

(See Figures on p. 37 , Summary Tables on p. 55 , Individual Tables on p. 83)

Marginal body weight loss was noted in groups 3 and 4 in week 1. The differences to control attained statistical significance in group 4. At the end of the treatment period these animals recovered and body weights were similar across all groups.

There were no effects on the body weight development in group 2.

4.3 Vanadium Pentoxide in Whole Blood and Lungs

The concentration of Vanadium Pentoxide in the lungs increased dose-proportional from group 2 to group 3. Vanadium Pentoxide values for blood and lung samples in group 4 were higher when compared with group 3 but less than dose proportionally.

Group	Vanadium pentoxide in whole blood [µg/L]	Vanadium pentoxide in lung samples [µg/g]
2	- *	14.31 ± 1.50
3	91.27 ± 10.78	55.36 ± 3.97
4	285.8 ± 29.4	114.9 ± 13.3

* below lowest calibration point (25 µg/L)

4.4 Pathology

4.4.1 Organ Weights

(See Summary Tables on p. 61 , Individual Tables on p. 105)

After 16 days of vanadium pentoxide exposure, lung weights showed a dose-related increase in groups 3 and 4 (allocations A, C, D, E). Differences to controls were statistically significant. At the interim kill after 7 days (allocation B), there were no effects on the lung weights in group 3 and the statistically significant increased lung weight in group 4 was less pronounced.

There were no effects on the lung weight in group 2 that were considered to be related to treatment. The statistically significant increase in the weight of the right lung lobe of allocation D animals was considered to be incidental in the absence of corresponding histopathological findings and as there were no effects in the other sub-groups.

4.4.2 Macroscopic Findings

(See Summary Tables on p. 80 , Individual Tables on p. 135)

At necropsy of allocation B and C animals, no gross findings of the lung were recorded that distinguished test item exposed mice from controls.

A reddish discolored lung was noted in the animal of group B which prematurely died. This kind of lesion is a common alteration in decedents and, therefore, was considered to be related to a certain degree of tissue autolysis due to delayed necropsy of this animal and not to represent of a primary treatment-related effect.

4.4.3 Microscopic Findings and Cell Proliferation

(See Appendix IV on p. 189)

Multifocal/diffuse alveolar histiocytosis and multifocal subacute alveolitis was noted with a dose-related increase in severity in almost all mice of allocation C in groups 3 and 4. In addition, multifocal granulocytic infiltration was noted in 4 out of 6 mice of group 3 and 5 out of 6 mice of group 4.

Focal alveolar histiocytosis of minimal degree which was noted in 1 mouse of group 2. This finding was considered to be incidental.

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Focal alveolar histiocytosis of minimal degree which was noted in 1 mouse of group 2. This finding was considered to be incidental.

Immunohistochemistry (based on the mean numbers of marker-positive cells in animals of allocation B and C):

Ki67: The mean grades of proliferation rate after 7 or 16 days were time-dependent and dose-related increased in groups 3 and 4. The grades in group 2 were similar to controls.

PCNA: The mean grades of proliferation rate were dose-related increased in groups 3 and 4 after 7 days and time-dependently increased in group 4 after 16 days. The grades in group 2 as well as after 16 days in group 3 were similar to controls.

4.5 Biomarker Determination

(See Appendix V on p. 247)

Slightly but statistically significantly reduced mean α -tocopherol concentrations were recorded in the lungs of group 2 animals compared with controls while slightly but statistically significantly increased values were noted for group 4 (allocation D). No effect was noted in group 3.

Slightly but statistically significantly decreased mean concentrations of reduced glutathione (GSH) were observed in the lungs of group 2 compared with controls and no effects were noted in groups 3 and 4 (allocation D). The mean concentrations of oxidised glutathione (GSSG) were statistically significantly increased in groups 2 to 4 and, accordingly, the GSH/GSSG ratios were statistically significantly decreased.

No effects of treatment with the test item on F2-Isoprostane concentrations in the lungs were noted in groups 2 to 4 (allocation E).

4.6 Determination of DNA Lesions

(See Appendix VI on p. \\\)

\\ Will be included in the next draft version. (allocation F)

4.7 Comet Assay

(See Appendix VII on p. 267)

The analysis of cells obtained from bronchioalveolar lavage or from minced lung tissue of animals of allocation G showed that treatment of mice with Vanadium Pentoxide did not induce any DNA damage in the in vivo Comet assay.

5 DISCUSSION AND CONCLUSION

Administration of Vanadium Pentoxide at concentrations of 0.246, 0.993 or 4.01 mg/m³ air for at least 16 days to mice by nose-only, flow-past inhalation exposure resulted mainly in pulmonary effects (increased weight, inflammatory lesions and increased proliferation rate) at the mid and high dose in a dose-dependent severity.

The slight decrease in α -tocopherol in the lungs of group 2 was considered to be of no biological significance. The slight increase in group 4 might indicate an adaptive response to a weak but sustained oxidative stress and is probably not biologically significant. Furthermore, GSSG, as an oxidation product of GSH, was slightly increased, probably as a result of scavenging reactive oxygen by GSH. GSH is much less affected due to the 10 fold constitutive excess. In addition, the de novo synthesis of GSH as well as the glutathione reductase (which recycles GSSG to GSH) are known to be very fast inducible by oxidative stress, thereby compensating for a depletion of GSH as noted in group 2. Finally, the unchanged isoprostane levels confirm that the natural defense mechanisms of the lung against oxidative stress, are sufficient to protect the tissue against oxidative damage, which might be induced by vanadium pentoxide.

Inflammatory lesions in the lungs which consisted of alveolar histiocytosis, alveolitis and granulocytic infiltration which were noted in groups 3 and 4 correspond to increased lung weights and are possibly related to the marginal body weight loss in during the first week. These findings are unlikely to be related to oxidative stress but they may be due to an overload of the lungs with Vanadium Pentoxide particles resulting in incomplete lung clearance indicated by proliferation of histiocytes, inflammatory changes and increased proliferation rate. The latter was demonstrated immunohistochemically.

(\ To be up-dated when results of analysis for DNA lesions are available.)

The dose response of these findings was in line with the Vanadium Pentoxide levels in the lungs.

Based on these findings a No-Observed-Adverse-Effect-Level (NOAEL) of 0.246 mg/m³ may be established under the conditions of this study.

Immunohistochemistry (based on the mean numbers of marker-positive cells in animals of allocation B and C):

Ki67: The mean grades of proliferation rate after 7 or 16 days were time-dependent and dose-related increased in groups 3 and 4. The grades in group 2 were similar to controls.

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(\ To be up-dated when results of analysis for DNA lesions are available.)

The dose response of these findings was in line with the Vanadium Pentoxide levels in the lungs.

Based on these findings a No-Observed-Adverse-Effect-Level (NOAEL) of 0.246 mg/m³ may be established under the conditions of this study.

6 REFERENCES

1. K. Sachsse, L. Ullmann, G. Voss and R. Hess:
Measurements of Inhalation Toxicity of Aerosols in Small Laboratory Animals. In:
Proceedings of the Europ. Soc. For the Study of Drug Toxicity, 15, 239-251, 1973.
2. K. Sachsse, L. Ullmann and K. Zbinden:
Toxikologische Prüfungen von Aerosolen im Tierexperiment. „Chemische
Rundschau“ 29, 38, 1, 1976.
3. W.C. Cannon, E.F. Blanton and K.E. McDonald:
The Flow-Past Chamber: An Improved Nose-Only Exposure System for Rodents. Am.
Ind. Hyg. Assoc. J., 44, 923-928, 1983.
4. C.W. Dunnett:
A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J.
Amer. Stat. Assoc. 50, 1096-1121 (1955).

7 FIGURES

6 REFERENCES

1. K. Sachsse, L. Ullmann, G. Voss and R. Hess:
Measurements of Inhalation Toxicity of Aerosols in Small Laboratory Animals. In:
Proceedings of the Europ. Soc. For the Study of Drug Toxicity, 15, 239-251, 1973.
2. K. Sachsse, L. Ullmann and K. Zbinden:
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Rundschau“ 29, 38, 1, 1976.
3. W.C. Cannon, E.F. Blanton and K.E. McDonald:
The Flow-Past Chamber: An Improved Nose-Only Exposure System for Rodents. Am.
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4. C.W. Dunnett:
A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J.
Amer. Stat. Assoc. 50, 1096-1121 (1955).

7 FIGURES

**BODY WEIGHTS (G) - GRAPHICS
MAIN STUDY**

Date: 25-AUG-09 16:40:34

User: HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)
Option: Y-Axis Optimized

**BODY WEIGHTS (G) - GRAPHICS
MAIN STUDY**

Data excluded from Summary Report

Not Reported

**BODY WEIGHTS (G) - GRAPHICS
MAIN STUDY**

Date: 25-AUG-09 16:40:34

User: HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)
Option: Y-Axis Optimized

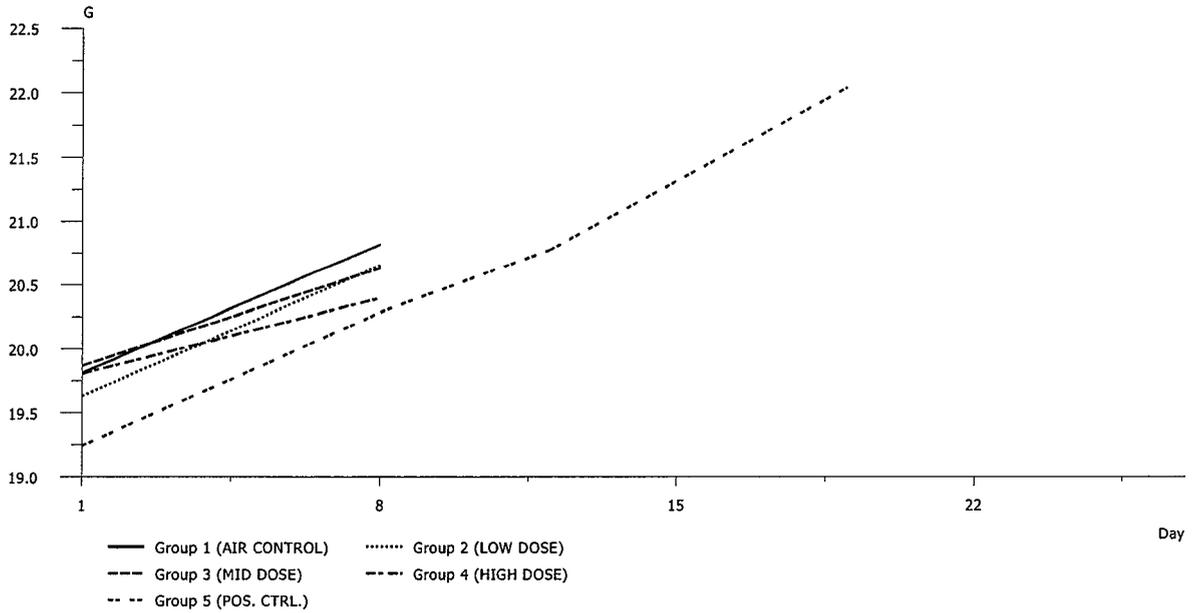
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MAIN STUDY**

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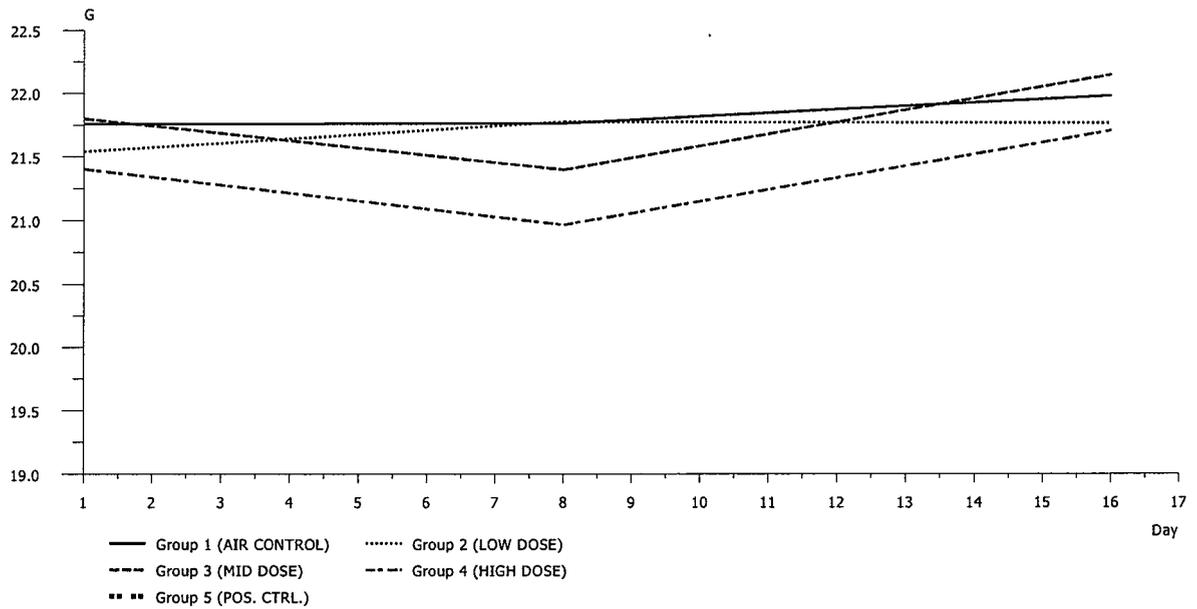
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BODY WEIGHTS (G) - GRAPHICS
MAIN STUDY
FEMALES

ACCLIMATISATION



TREATMENT



**BODY WEIGHT GAIN (%) - GRAPHICS
MAIN STUDY**

Date: 25-AUG-09 16:46:51

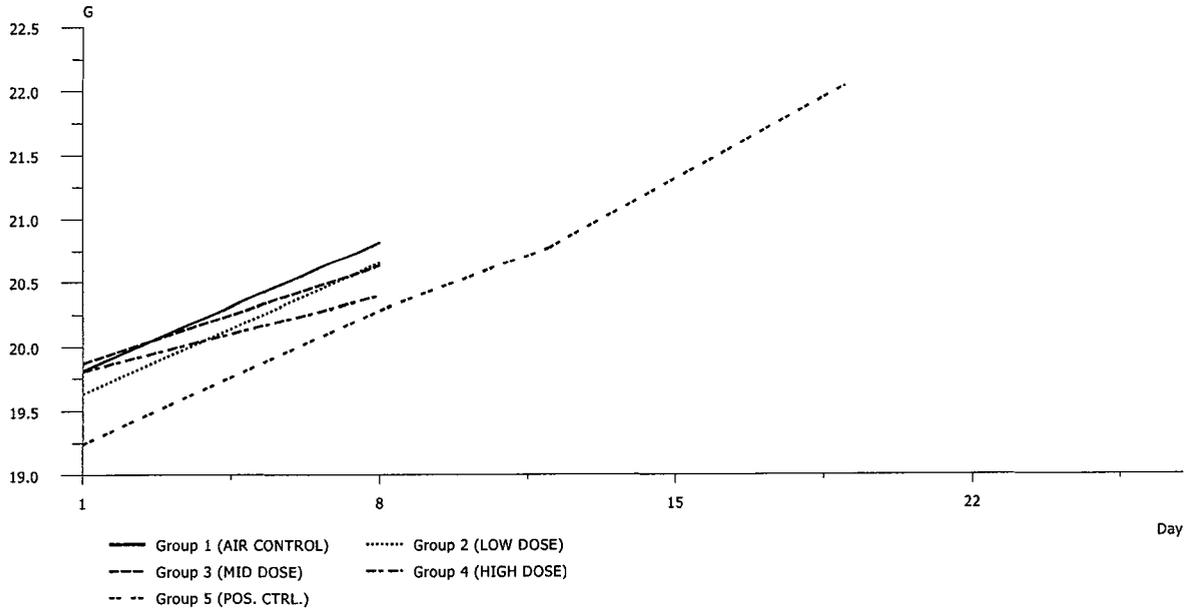
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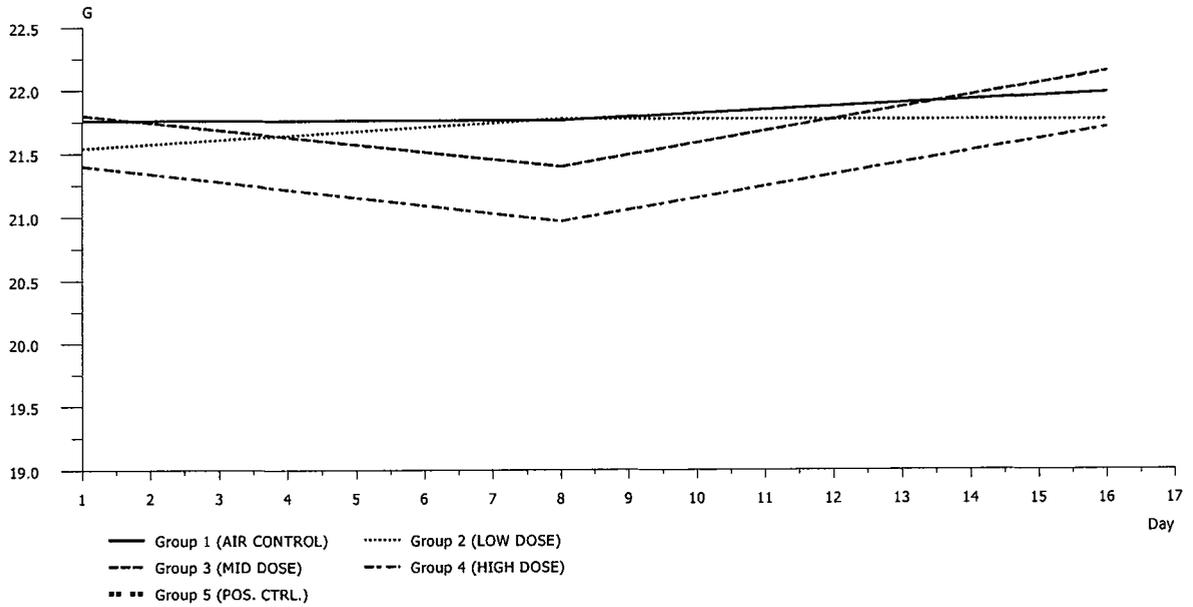
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Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)
Option: Y-Axis Optimized

BODY WEIGHTS (G) - GRAPHICS
MAIN STUDY
FEMALES

ACCLIMATISATION



TREATMENT



**BODY WEIGHT GAIN (%) - GRAPHICS
MAIN STUDY**

Date: 25-AUG-09 16:46:51

User: HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)
Option: Y-Axis Optimized

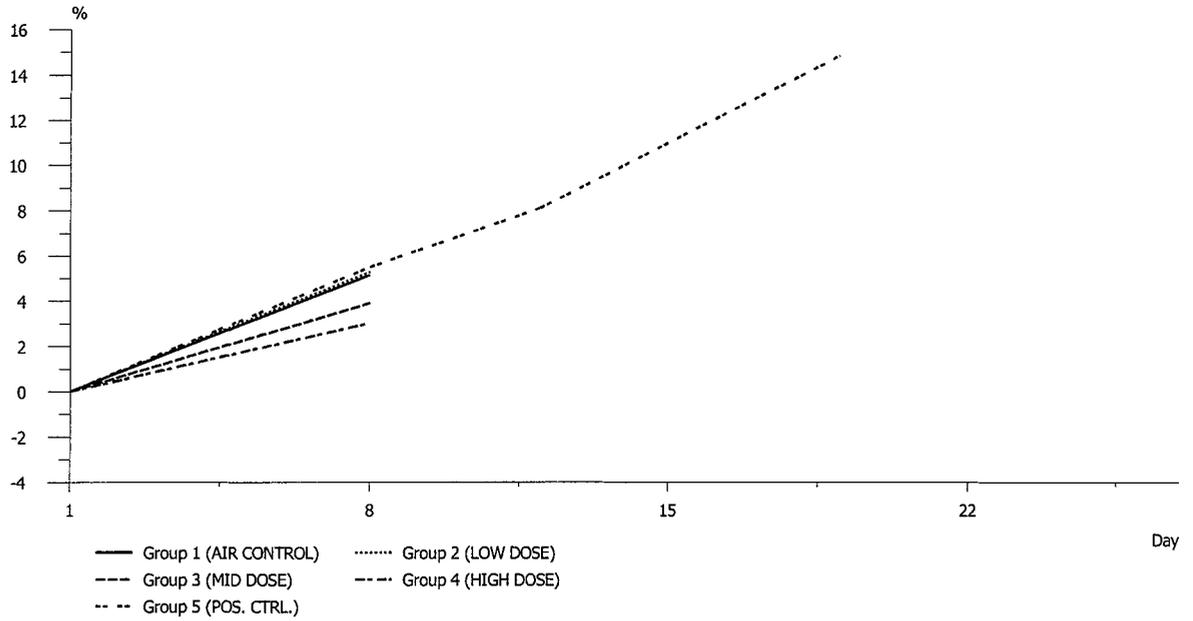
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MAIN STUDY**

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BODY WEIGHT GAIN (%) - GRAPHICS
MAIN STUDY
FEMALES

ACCLIMATISATION



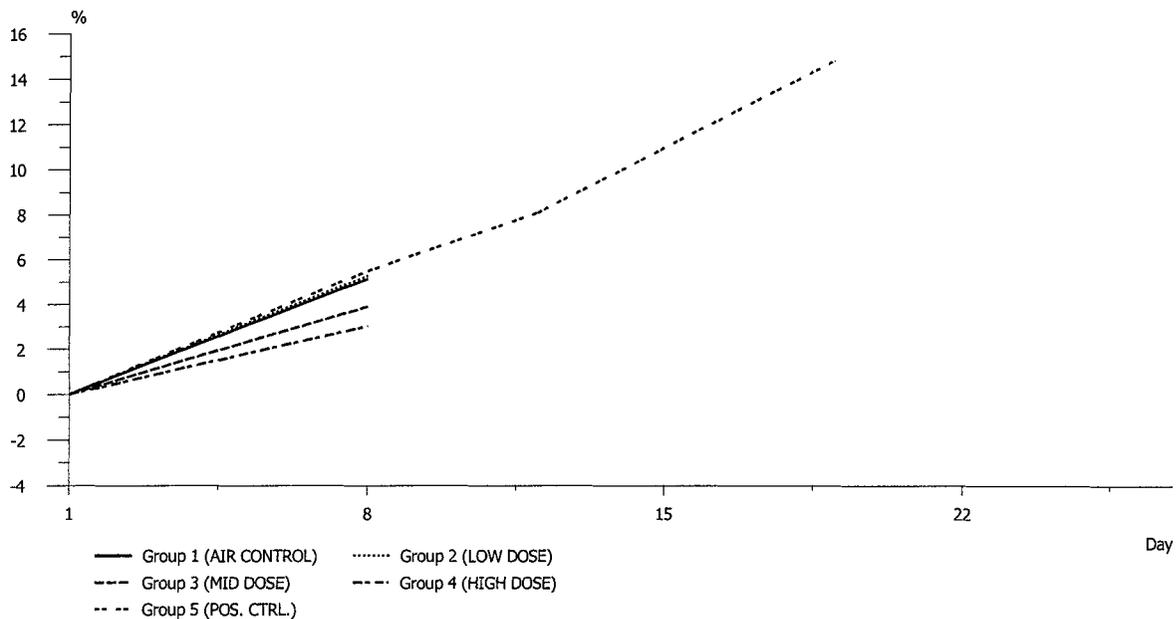
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MAIN STUDY**

Data excluded from Summary Report

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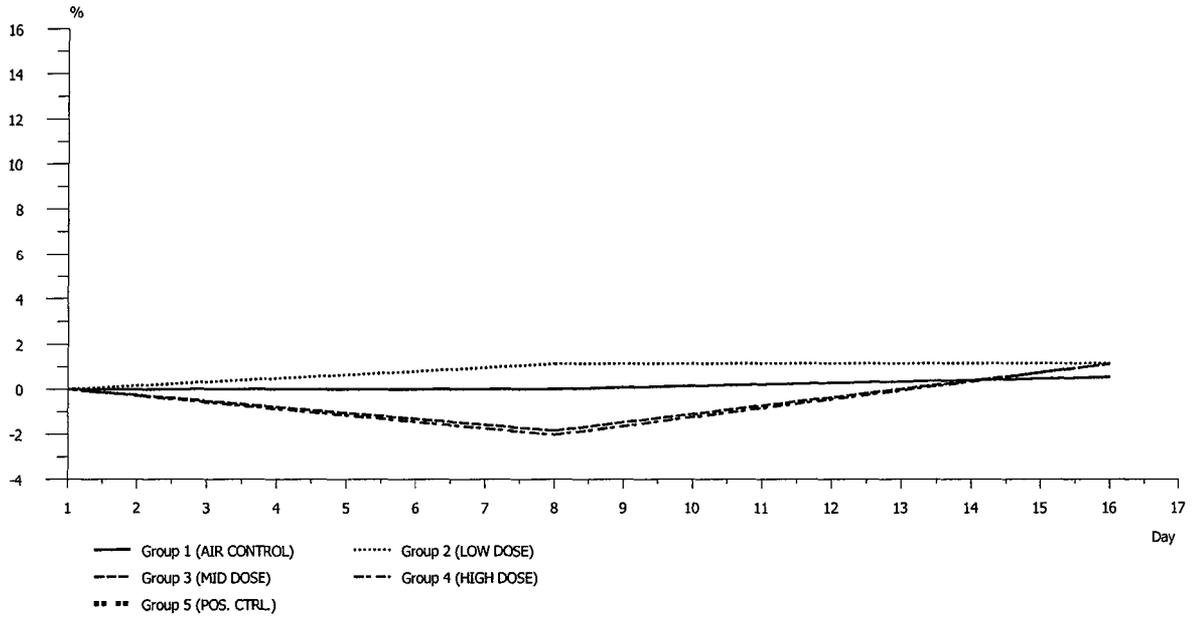
BODY WEIGHT GAIN (%) - GRAPHICS
MAIN STUDY
FEMALES

ACCLIMATISATION



BODY WEIGHT GAIN (%) - GRAPHICS
MAIN STUDY
FEMALES

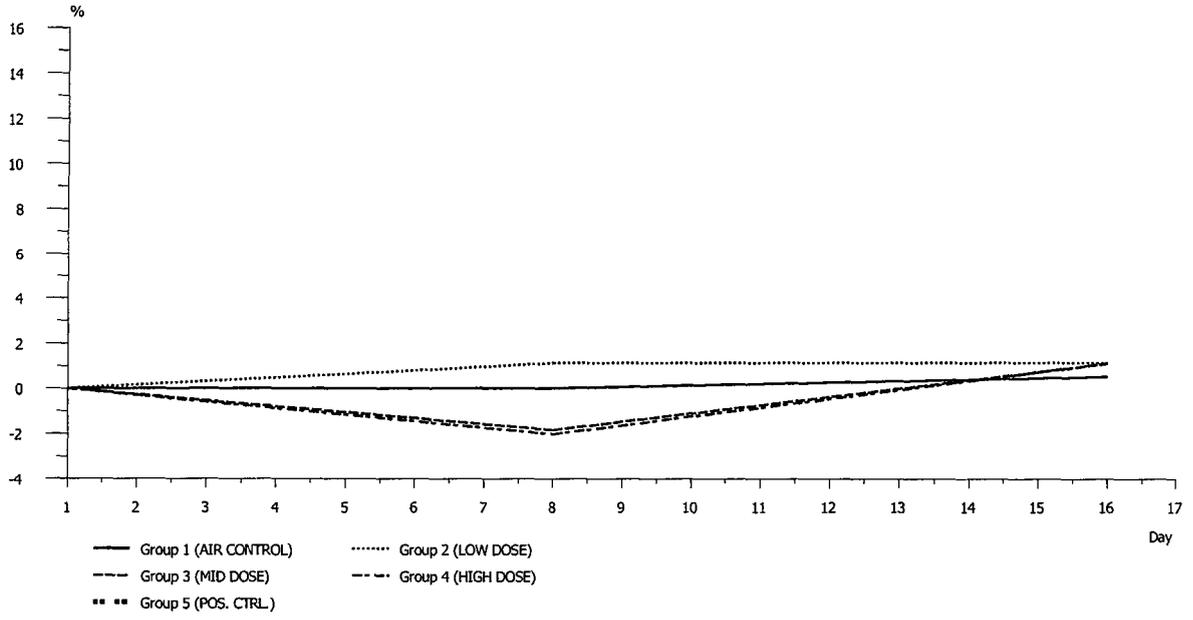
TREATMENT



8 INHALATION TABLES

BODY WEIGHT GAIN (%) - GRAPHICS
MAIN STUDY
FEMALES

TREATMENT



8 INHALATION TABLES

Exposure Conditions							
Group 1		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	22.5	3	1.1	3	20.7	3
19-May-09	2	22.6	3	0.9	3	20.7	3
20-May-09	3	22.7	3	0.7	3	20.7	3
21-May-09	4	23.0	3	1.0	3	20.7	3
22-May-09	5	23.2	3	1.2	3	20.7	3
23-May-09	6	22.7	3	0.9	3	20.7	3
24-May-09	7	22.9	3	1.2	3	20.7	3
25-May-09	8	23.1	3	1.2	3	20.7	3
26-May-09	9	22.9	3	1.3	3	20.7	3
27-May-09	10	22.7	3	0.8	3	20.7	3
28-May-09	11	22.5	3	0.6	3	20.7	3
29-May-09	12	22.2	3	1.4	3	20.7	3
30-May-09	13	22.2	3	1.1	3	20.7	3
31-May-09	14	22.4	3	1.4	3	20.7	3
01-Jun-09	15	22.6	3	0.5	3	20.7	3
02-Jun-09	16	22.6	3	0.8	3	20.7	3
03-Jun-09	17	22.5	3	0.8	3	20.7	3

Exposure Conditions							
Group 2		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	22.1	3	3.7	3	20.6	3
19-May-09	2	22.2	3	3.6	3	20.6	3
20-May-09	3	22.1	3	3.7	3	20.6	3
21-May-09	4	22.0	3	3.8	3	20.6	3
22-May-09	5	22.1	3	3.8	3	20.6	3
23-May-09	6	22.0	3	3.7	3	20.6	3
24-May-09	7	21.9	3	3.9	3	20.6	3
25-May-09	8	21.8	3	3.9	3	20.6	3
26-May-09	9	22.0	3	4.0	3	20.6	3
27-May-09	10	22.1	3	3.7	3	20.6	3
28-May-09	11	22.1	3	3.7	3	20.6	3
29-May-09	12	22.0	3	3.7	3	20.6	3
30-May-09	13	22.2	3	3.8	3	20.6	3
31-May-09	14	22.2	3	3.9	3	20.6	3
01-Jun-09	15	22.2	3	4.0	3	20.6	3
02-Jun-09	16	22.1	3	3.7	3	20.6	3
03-Jun-09	17	22.1	3	3.8	3	20.6	3

Exposure Conditions							
Group 1		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	22.5	3	1.1	3	20.7	3
19-May-09	2	22.6	3	0.9	3	20.7	3
20-May-09	3	22.7	3	0.7	3	20.7	3
21-May-09	4	23.0	3	1.0	3	20.7	3
22-May-09	5	23.2	3	1.2	3	20.7	3
23-May-09	6	22.7	3	0.9	3	20.7	3
24-May-09	7	22.9	3	1.2	3	20.7	3
25-May-09	8	23.1	3	1.2	3	20.7	3
26-May-09	9	22.9	3	1.3	3	20.7	3
27-May-09	10	22.7	3	0.8	3	20.7	3
28-May-09	11	22.5	3	0.6	3	20.7	3
29-May-09	12	22.2	3	1.4	3	20.7	3
30-May-09	13	22.2	3	1.1	3	20.7	3
31-May-09	14	22.4	3	1.4	3	20.7	3
01-Jun-09	15	22.6	3	0.5	3	20.7	3
02-Jun-09	16	22.6	3	0.8	3	20.7	3
03-Jun-09	17	22.5	3	0.8	3	20.7	3

Exposure Conditions							
Group 2		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	22.1	3	3.7	3	20.6	3
19-May-09	2	22.2	3	3.6	3	20.6	3
20-May-09	3	22.1	3	3.7	3	20.6	3
21-May-09	4	22.0	3	3.8	3	20.6	3
22-May-09	5	22.1	3	3.8	3	20.6	3
23-May-09	6	22.0	3	3.7	3	20.6	3
24-May-09	7	21.9	3	3.9	3	20.6	3
25-May-09	8	21.8	3	3.9	3	20.6	3
26-May-09	9	22.0	3	4.0	3	20.6	3
27-May-09	10	22.1	3	3.7	3	20.6	3
28-May-09	11	22.1	3	3.7	3	20.6	3
29-May-09	12	22.0	3	3.7	3	20.6	3
30-May-09	13	22.2	3	3.8	3	20.6	3
31-May-09	14	22.2	3	3.9	3	20.6	3
01-Jun-09	15	22.2	3	4.0	3	20.6	3
02-Jun-09	16	22.1	3	3.7	3	20.6	3
03-Jun-09	17	22.1	3	3.8	3	20.6	3

Exposure Conditions							
Group 3		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	21.7	3	1.3	3	20.6	3
19-May-09	2	21.8	3	1.0	3	20.6	3
20-May-09	3	21.8	3	1.0	3	20.6	3
21-May-09	4	21.7	3	1.3	3	20.6	3
22-May-09	5	21.8	3	1.4	3	20.6	3
23-May-09	6	21.8	3	1.3	3	20.5	3
24-May-09	7	21.6	3	1.9	3	20.6	3
25-May-09	8	21.5	3	1.8	3	20.6	3
26-May-09	9	21.7	3	2.5	3	20.6	3
27-May-09	10	21.8	3	2.6	3	20.6	3
28-May-09	11	21.8	3	2.4	3	20.6	3
29-May-09	12	21.8	3	3.0	3	20.6	3
30-May-09	13	21.8	3	4.2	3	20.6	3
31-May-09	14	21.8	2	1.4	2	20.6	2
01-Jun-09	15	21.8	2	1.5	2	20.6	2
02-Jun-09	16	21.8	3	2.4	3	20.6	3
03-Jun-09	17	21.8	3	1.2	3	20.6	3
04-Jun-09	18	21.8	3	1.8	3	20.6	3

Exposure Conditions							
Group 4		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	21.3	3	1.0	3	20.3	3
19-May-09	2	21.3	3	0.3	3	20.4	3
20-May-09	3	21.2	3	0.5	3	20.4	3
21-May-09	4	21.1	3	0.6	3	20.3	3
22-May-09	5	21.2	3	0.9	3	20.3	3
23-May-09	6	21.3	3	0.3	3	20.4	3
24-May-09	7	21.1	3	2.2	3	20.4	3
25-May-09	8	21.0	3	1.2	3	20.4	3
26-May-09	9	21.1	3	2.3	3	20.3	3
27-May-09	10	21.4	3	0.8	3	20.4	3
28-May-09	11	21.4	3	0.5	3	20.4	3
29-May-09	12	21.2	3	0.6	3	20.4	3
30-May-09	13	21.2	3	0.2	3	20.4	3
31-May-09	14	21.3	3	0.2	3	20.4	3
01-Jun-09	15	21.3	3	0.2	3	20.4	3
02-Jun-09	16	21.2	3	0.6	3	20.4	3
03-Jun-09	17	21.3	3	0.5	3	20.4	3
04-Jun-09	18	21.3	3	0.5	3	20.4	3

Exposure Conditions							
Group 3		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	21.7	3	1.3	3	20.6	3
19-May-09	2	21.8	3	1.0	3	20.6	3
20-May-09	3	21.8	3	1.0	3	20.6	3
21-May-09	4	21.7	3	1.3	3	20.6	3
22-May-09	5	21.8	3	1.4	3	20.6	3
23-May-09	6	21.8	3	1.3	3	20.5	3
24-May-09	7	21.6	3	1.9	3	20.6	3
25-May-09	8	21.5	3	1.8	3	20.6	3
26-May-09	9	21.7	3	2.5	3	20.6	3
27-May-09	10	21.8	3	2.6	3	20.6	3
28-May-09	11	21.8	3	2.4	3	20.6	3
29-May-09	12	21.8	3	3.0	3	20.6	3
30-May-09	13	21.8	3	4.2	3	20.6	3
31-May-09	14	21.8	2	1.4	2	20.6	2
01-Jun-09	15	21.8	2	1.5	2	20.6	2
02-Jun-09	16	21.8	3	2.4	3	20.6	3
03-Jun-09	17	21.8	3	1.2	3	20.6	3
04-Jun-09	18	21.8	3	1.8	3	20.6	3

Exposure Conditions							
Group 4		Temperature [°C]		Relative humidity [%]		Oxygen [%]	
Date	Day of treatment	Mean	Number of determinations	Mean	Number of determinations	Mean	Number of determinations
18-May-09	1	21.3	3	1.0	3	20.3	3
19-May-09	2	21.3	3	0.3	3	20.4	3
20-May-09	3	21.2	3	0.5	3	20.4	3
21-May-09	4	21.1	3	0.6	3	20.3	3
22-May-09	5	21.2	3	0.9	3	20.3	3
23-May-09	6	21.3	3	0.3	3	20.4	3
24-May-09	7	21.1	3	2.2	3	20.4	3
25-May-09	8	21.0	3	1.2	3	20.4	3
26-May-09	9	21.1	3	2.3	3	20.3	3
27-May-09	10	21.4	3	0.8	3	20.4	3
28-May-09	11	21.4	3	0.5	3	20.4	3
29-May-09	12	21.2	3	0.6	3	20.4	3
30-May-09	13	21.2	3	0.2	3	20.4	3
31-May-09	14	21.3	3	0.2	3	20.4	3
01-Jun-09	15	21.3	3	0.2	3	20.4	3
02-Jun-09	16	21.2	3	0.6	3	20.4	3
03-Jun-09	17	21.3	3	0.5	3	20.4	3
04-Jun-09	18	21.3	3	0.5	3	20.4	3

Aerosol Concentrations							
Group 2		Gravimetric Determination of Test Item			Chemical Determination of Vanadium Pentoxide		
Date	Day of treatment	Air volume [L]	Amount on filter [mg]	Aerosol concentration [mg/m3]	Air volume [L]	Amount on filter [µg]	Aerosol concentration [mg/m3]
18-May-09	1	369.8	0.117	0.316	369.8	85.58	0.231
19-May-09	2	375.6	0.111	0.295	375.6	86.38	0.230
20-May-09	3	377.4	0.128	0.339	377.4	95.45	0.253
21-May-09	4	373.9	0.065	0.174	373.9	69.71	0.186
22-May-09	5	364.9	0.098	0.269	364.9	92.71	0.254
23-May-09	6	358.0	0.113	0.316	358.0	102.6	0.287
24-May-09	7	359.7	0.057	0.158	359.7	87.95	0.244
25-May-09	8	362.1	0.053	0.146	362.1	84.99	0.235
26-May-09	9	362.1	0.071	0.196	362.1	80.59	0.223
27-May-09	10	362.1	0.086	0.238	362.1	104.3	0.288
28-May-09	11	362.1	0.105	0.290	362.1	101.5	0.280
29-May-09	12	367.2	0.040	0.109	367.2	97.29	0.265
30-May-09	13	362.8	0.073	0.201	362.8	79.68	0.220
31-May-09	14	362.1	0.083	0.229	362.1	90.11	0.249
01-Jun-09	15	362.1	0.095	0.262	362.1	88.69	0.245
02-Jun-09	16	366.5	0.095	0.259	366.5	84.93	0.232
03-Jun-09	17	365.2	0.050	0.137	365.2	92.29	0.253

Aerosol Concentrations							
Group 3		Gravimetric Determination of Test Item			Chemical Determination of Vanadium Pentoxide		
Date	Day of treatment	Air volume [L]	Amount on filter [mg]	Aerosol concentration [mg/m ³]	Air volume [L]	Amount on filter [µg]	Aerosol concentration [mg/m ³]
18-May-09	1	357.1	0.388	1.09	357.1	356.4	0.998
19-May-09	2	363.3	0.395	1.09	363.3	361.9	0.996
20-May-09	3	346.6	0.419	1.21	346.6	410.8	1.19
21-May-09	4	346.6	0.251	0.724	346.6	267.6	0.772
22-May-09	5	349.9	0.352	1.01	349.9	381.5	1.09
23-May-09	6	345.0	0.414	1.20	345.0	440.8	1.28
24-May-09	7	347.0	0.319	0.919	347.0	372.8	1.07
25-May-09	8	348.3	0.330	0.948	348.3	321.1	0.922
26-May-09	9	348.6	0.335	0.961	348.6	317.0	0.909
27-May-09	10	349.0	0.416	1.19	349.0	367.8	1.05
28-May-09	11	348.6	0.408	1.17	348.6	356.5	1.02
29-May-09	12	349.9	0.346	0.989	349.9	336.9	0.963
30-May-09	13	349.0	0.305	0.874	349.0	276.5	0.792
31-May-09	14	349.0	0.376	1.08	349.0	337.7	0.968
01-Jun-09	15	349.0	0.365	1.05	349.0	327.6	0.939
02-Jun-09	16	353.5	0.357	1.01	353.5	318.5	0.901
03-Jun-09	17	348.0	0.357	1.03	348.0	356.4	1.02
04-Jun-09	18	349.0	0.359	1.03	349.0	344.0	0.986

Aerosol Concentrations							
Group 2		Gravimetric Determination of Test Item			Chemical Determination of Vanadium Pentoxide		
Date	Day of treatment	Air volume [L]	Amount on filter [mg]	Aerosol concentration [mg/m ³]	Air volume [L]	Amount on filter [µg]	Aerosol concentration [mg/m ³]
18-May-09	1	369.8	0.117	0.316	369.8	85.58	0.231
19-May-09	2	375.6	0.111	0.295	375.6	86.38	0.230
20-May-09	3	377.4	0.128	0.339	377.4	95.45	0.253
21-May-09	4	373.9	0.065	0.174	373.9	69.71	0.186
22-May-09	5	364.9	0.098	0.269	364.9	92.71	0.254
23-May-09	6	358.0	0.113	0.316	358.0	102.6	0.287
24-May-09	7	359.7	0.057	0.158	359.7	87.95	0.244
25-May-09	8	362.1	0.053	0.146	362.1	84.99	0.235
26-May-09	9	362.1	0.071	0.196	362.1	80.59	0.223
27-May-09	10	362.1	0.086	0.238	362.1	104.3	0.288
28-May-09	11	362.1	0.105	0.290	362.1	101.5	0.280
29-May-09	12	367.2	0.040	0.109	367.2	97.29	0.265
30-May-09	13	362.8	0.073	0.201	362.8	79.68	0.220
31-May-09	14	362.1	0.083	0.229	362.1	90.11	0.249
01-Jun-09	15	362.1	0.095	0.262	362.1	88.69	0.245
02-Jun-09	16	366.5	0.095	0.259	366.5	84.93	0.232
03-Jun-09	17	365.2	0.050	0.137	365.2	92.29	0.253

Aerosol Concentrations							
Group 3		Gravimetric Determination of Test Item			Chemical Determination of Vanadium Pentoxide		
Date	Day of treatment	Air volume [L]	Amount on filter [mg]	Aerosol concentration [mg/m ³]	Air volume [L]	Amount on filter [µg]	Aerosol concentration [mg/m ³]
18-May-09	1	357.1	0.388	1.09	357.1	356.4	0.998
19-May-09	2	363.3	0.395	1.09	363.3	361.9	0.996
20-May-09	3	346.6	0.419	1.21	346.6	410.8	1.19
21-May-09	4	346.6	0.251	0.724	346.6	267.6	0.772
22-May-09	5	349.9	0.352	1.01	349.9	381.5	1.09
23-May-09	6	345.0	0.414	1.20	345.0	440.8	1.28
24-May-09	7	347.0	0.319	0.919	347.0	372.8	1.07
25-May-09	8	348.3	0.330	0.948	348.3	321.1	0.922
26-May-09	9	348.6	0.335	0.961	348.6	317.0	0.909
27-May-09	10	349.0	0.416	1.19	349.0	367.8	1.05
28-May-09	11	348.6	0.408	1.17	348.6	356.5	1.02
29-May-09	12	349.9	0.346	0.989	349.9	336.9	0.963
30-May-09	13	349.0	0.305	0.874	349.0	276.5	0.792
31-May-09	14	349.0	0.376	1.08	349.0	337.7	0.968
01-Jun-09	15	349.0	0.365	1.05	349.0	327.6	0.939
02-Jun-09	16	353.5	0.357	1.01	353.5	318.5	0.901
03-Jun-09	17	348.0	0.357	1.03	348.0	356.4	1.02
04-Jun-09	18	349.0	0.359	1.03	349.0	344.0	0.986

Aerosol Concentrations							
Group 4		Gravimetric Determination of Test Item			Chemical Determination of Vanadium Pentoxide		
Date	Day of treatment	Air volume [L]	Amount on filter [mg]	Aerosol concentration [mg/m3]	Air volume [L]	Amount on filter [µg]	Aerosol concentration [mg/m3]
18-May-09	1	357.7	1.380	3.86	357.7	1351	3.78
19-May-09	2	362.9	1.386	3.82	362.9	1278	3.52
20-May-09	3	341.2	1.503	4.41	341.2	1646	4.82
21-May-09	4	341.5	0.950	2.78	341.5	1102	3.23
22-May-09	5	345.8	1.377	3.98	345.8	1571	4.54
23-May-09	6	340.9	1.589	4.66	340.9	1840	5.40
24-May-09	7	341.9	1.262	3.69	341.9	1504	4.40
25-May-09	8	345.8	1.401	4.05	345.8	1346	3.89
26-May-09	9	345.8	1.369	3.96	345.8	1277	3.69
27-May-09	10	346.7	1.618	4.67	346.7	1519	4.38
28-May-09	11	345.4	1.562	4.52	345.4	1443	4.18
29-May-09	12	347.7	1.438	4.14	347.7	1372	3.95
30-May-09	13	345.8	1.153	3.33	345.8	1079	3.12
31-May-09	14	345.8	1.466	4.24	345.8	1353	3.91
01-Jun-09	15	345.8	1.419	4.10	345.8	1349	3.90
02-Jun-09	16	350.6	1.449	4.13	350.6	1349	3.85
03-Jun-09	17	345.8	1.395	4.03	345.8	1313	3.80
04-Jun-09	18	347.0	1.380	3.98	347.0	1296	3.73

GRAVIMETRIC PARTICLE SIZE DETERMINATION

Data of Individual Impactors (Gravimetrically Determined) - Group 3													
Date of Sampling	Total Amount Collected [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
22-May-09	1026	3.9	2.2	7.8	26.8	31.5	17.5	3.2	7.0	1.26	1.92	0.96	90.9
29-May-09	1018	2.7	1.9	11.4	22.5	34.3	15.7	4.3	7.3	1.22	1.89	0.97	92.2

Data of Individual Impactors (Gravimetrically Determined) - Group 4													
Date of Sampling	Total Amount Collected [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
18-May-09	1073	6.2	2.4	8.3	32.9	25.9	17.9	2.6	3.8	1.43	1.89	0.97	87.6
25-May-09	1259	2.6	3.0	10.3	32.6	25.1	13.9	3.0	9.4	1.24	1.92	0.95	91.3

Aerosol Concentrations							
Group 4		Gravimetric Determination of Test Item			Chemical Determination of Vanadium Pentoxide		
Date	Day of treatment	Air volume [L]	Amount on filter [mg]	Aerosol concentration [mg/m ³]	Air volume [L]	Amount on filter [µg]	Aerosol concentration [mg/m ³]
18-May-09	1	357.7	1.380	3.86	357.7	1351	3.78
19-May-09	2	362.9	1.386	3.82	362.9	1278	3.52
20-May-09	3	341.2	1.503	4.41	341.2	1646	4.82
21-May-09	4	341.5	0.950	2.78	341.5	1102	3.23
22-May-09	5	345.8	1.377	3.98	345.8	1571	4.54
23-May-09	6	340.9	1.589	4.66	340.9	1840	5.40
24-May-09	7	341.9	1.262	3.69	341.9	1504	4.40
25-May-09	8	345.8	1.401	4.05	345.8	1346	3.89
26-May-09	9	345.8	1.369	3.96	345.8	1277	3.69
27-May-09	10	346.7	1.618	4.67	346.7	1519	4.38
28-May-09	11	345.4	1.562	4.52	345.4	1443	4.18
29-May-09	12	347.7	1.438	4.14	347.7	1372	3.95
30-May-09	13	345.8	1.153	3.33	345.8	1079	3.12
31-May-09	14	345.8	1.466	4.24	345.8	1353	3.91
01-Jun-09	15	345.8	1.419	4.10	345.8	1349	3.90
02-Jun-09	16	350.6	1.449	4.13	350.6	1349	3.85
03-Jun-09	17	345.8	1.395	4.03	345.8	1313	3.80
04-Jun-09	18	347.0	1.380	3.98	347.0	1296	3.73

GRAVIMETRIC PARTICLE SIZE DETERMINATION

Data of Individual Impactors (Gravimetrically Determined) - Group 3													
Date of Sampling	Total Amount Collected [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
22-May-09	1026	3.9	2.2	7.8	26.8	31.5	17.5	3.2	7.0	1.26	1.92	0.96	90.9
29-May-09	1018	2.7	1.9	11.4	22.5	34.3	15.7	4.3	7.3	1.22	1.89	0.97	92.2

Data of Individual Impactors (Gravimetrically Determined) - Group 4													
Date of Sampling	Total Amount Collected [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
18-May-09	1073	6.2	2.4	8.3	32.9	25.9	17.9	2.6	3.8	1.43	1.89	0.97	87.6
25-May-09	1259	2.6	3.0	10.3	32.6	25.1	13.9	3.0	9.4	1.24	1.92	0.95	91.3

CHEMICAL PARTICLE SIZE DETERMINATION

Data of Individual Impactors (Chemically Determined) - Group 3													
Date of Sampling	Total Test Item on Filter [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
22-May-09	1289	2.6	2.5	8.4	28.3	33.5	18.4	3.4	2.9	1.34	1.77	0.97	92.2
29-May-09	1307	2.6	2.8	11.5	21.3	35.3	16.4	5.5	4.6	1.27	1.86	0.98	91.6

Data of Individual Impactors (Chemically Determined) - Group 3													
Date of Sampling	Total Test Item on Filter [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
18-May-09	1027	2.3	2.2	8.3	34.8	27.5	19.0	3.0	3.1	1.33	1.75	0.97	92.7
25-May-09	1600	3.0	3.8	11.2	33.9	26.3	14.6	4.4	2.8	1.41	1.82	0.97	89.7

9 SUMMARY TABLES

CHEMICAL PARTICLE SIZE DETERMINATION

Data of Individual Impactors (Chemically Determined) - Group 3													
Date of Sampling	Total Test Item on Filter [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
22-May-09	1289	2.6	2.5	8.4	28.3	33.5	18.4	3.4	2.9	1.34	1.77	0.97	92.2
29-May-09	1307	2.6	2.8	11.5	21.3	35.3	16.4	5.5	4.6	1.27	1.86	0.98	91.6

Data of Individual Impactors (Chemically Determined) - Group 3													
Date of Sampling	Total Test Item on Filter [µg]	Percentages: Stage No. Effective Cut-Off Diameter [µm]								MMAD [µm]	GSD	Corr. Coeff. (R)	% < 3 µm
		1	2	3	4	5	6	7	8				
		--	4.6	3.0	2.13	1.6	1.06	0.715	0.325				
18-May-09	1027	2.3	2.2	8.3	34.8	27.5	19.0	3.0	3.1	1.33	1.75	0.97	92.7
25-May-09	1600	3.0	3.8	11.2	33.9	26.3	14.6	4.4	2.8	1.41	1.82	0.97	89.7

9 SUMMARY TABLES

**BODY WEIGHTS (G) - SUMMARY
MAIN STUDY**

Date: 25-AUG-09 16:40:33

User: HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

**BODY WEIGHTS (G) - SUMMARY
MAIN STUDY**

Data excluded from Summary Report

Not Reported

**BODY WEIGHTS (G) - SUMMARY
MAIN STUDY**

Date: 25-AUG-09 16:40:33

User: HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

**BODY WEIGHTS (G) - SUMMARY
MAIN STUDY**

Data excluded from Summary Report

Not Reported

BODY WEIGHTS (G) - SUMMARY
MAIN STUDY
FEMALES

		Group 1	Group 2	Group 3	Group 4	Group 5
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	POS. CTRL.
ACCLIMATISATION						
Day 1	MEAN	19.8	19.6 -	19.9 -	19.8 -	19.2 -
	ST.DEV.	1.0	1.2	1.0	1.1	1.3
	N	48	48	48	48	6
Day 8	MEAN	20.8	20.7 -	20.6 -	20.4 -	20.3 -
	ST.DEV.	1.0	1.3	1.0	1.1	1.5
	N	48	48	48	48	6
Day 12	MEAN	---	---	---	---	20.8
	ST.DEV.	---	---	---	---	1.3
	N	0	0	0	0	6
Day 19	MEAN	---	---	---	---	22.0
	ST.DEV.	---	---	---	---	1.1
	N	0	0	0	0	6
TREATMENT						
Day 1	MEAN	21.8	21.5 -	21.8 -	21.4 -	21.1 -
	ST.DEV.	1.1	1.3	1.1	1.3	0.2
	N	48	48	48	48	6
Day 8	MEAN	21.8	21.8 -	21.4 -	21.0 *	---
	ST.DEV.	1.4	1.4	1.2	1.4	---
	N	48	48	48	48	0
Day 16	MEAN	22.0	21.8 -	22.1 -	21.7 -	---
	ST.DEV.	1.3	1.6	1.5	1.9	---
	N	42	42	41	42	0

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

**BODY WEIGHT GAIN (%) - SUMMARY
MAIN STUDY**

Date: 25-AUG-09 16:46:50

User: HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

**BODY WEIGHTS (G) - SUMMARY
MAIN STUDY
FEMALES**

		Group 1	Group 2	Group 3	Group 4	Group 5
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	POS. CTRL.
ACCLIMATISATION						
Day 1	MEAN	19.8	19.6 -	19.9 -	19.8 -	19.2 -
	ST.DEV.	1.0	1.2	1.0	1.1	1.3
	N	48	48	48	48	6
Day 8	MEAN	20.8	20.7 -	20.6 -	20.4 -	20.3 -
	ST.DEV.	1.0	1.3	1.0	1.1	1.5
	N	48	48	48	48	6
Day 12	MEAN	---	---	---	---	20.8
	ST.DEV.	---	---	---	---	1.3
	N	0	0	0	0	6
Day 19	MEAN	---	---	---	---	22.0
	ST.DEV.	---	---	---	---	1.1
	N	0	0	0	0	6
TREATMENT						
Day 1	MEAN	21.8	21.5 -	21.8 -	21.4 -	21.1 -
	ST.DEV.	1.1	1.3	1.1	1.3	0.2
	N	48	48	48	48	6
Day 8	MEAN	21.8	21.8 -	21.4 -	21.0 *	---
	ST.DEV.	1.4	1.4	1.2	1.4	---
	N	48	48	48	48	0
Day 16	MEAN	22.0	21.8 -	22.1 -	21.7 -	---
	ST.DEV.	1.3	1.6	1.5	1.9	---
	N	42	42	41	42	0

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

**BODY WEIGHT GAIN (%) - SUMMARY
MAIN STUDY**

Date: 25-AUG-09 16:46:50

User: HUH

Report Data

Activity: BW - BODY WEIGHT

Timing: 10 - BODY WEIGHT

Satellite: A-G - ALLOCATION A - G

Study Phase(s) : Acclimatisation (1 - 27)
Treatment (1 - 17)

**BODY WEIGHT GAIN (%) - SUMMARY
MAIN STUDY**

Data excluded from Summary Report

Not Reported

BODY WEIGHT GAIN (%) - SUMMARY
MAIN STUDY
FEMALES

		Group 1	Group 2	Group 3	Group 4	Group 5
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	POS. CTRL.
ACCLIMATISATION						
Day 1	MEAN	0.0	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0	0.0
	N	48	48	48	48	6
Day 8	MEAN	5.2	5.3 -	3.9 -	3.0 -	5.5 -
	ST.DEV.	4.3	4.9	4.1	3.6	5.1
	N	48	48	48	48	6
Day 12	MEAN	---	---	---	---	8.1
	ST.DEV.	---	---	---	---	4.7
	N	0	0	0	0	6
Day 19	MEAN	---	---	---	---	14.8
	ST.DEV.	---	---	---	---	6.5
	N	0	0	0	0	6
TREATMENT						
Day 1	MEAN	0.0	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0	0.0
	N	48	48	48	48	6
Day 8	MEAN	0.0	1.1 -	-1.8 -	-2.0 *	---
	ST.DEV.	3.8	4.5	4.0	4.0	---
	N	48	48	48	48	0
Day 16	MEAN	0.6	1.2 -	1.1 -	1.2 -	---
	ST.DEV.	5.1	4.5	4.3	5.6	---
	N	42	42	41	42	0

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**BODY WEIGHT GAIN (%) - SUMMARY
MAIN STUDY**

Data excluded from Summary Report

Not Reported

BODY WEIGHT GAIN (%) - SUMMARY
MAIN STUDY
FEMALES

		Group 1	Group 2	Group 3	Group 4	Group 5
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	POS. CTRL.
ACCLIMATISATION						
Day 1	MEAN	0.0	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0	0.0
	N	48	48	48	48	6
Day 8	MEAN	5.2	5.3 -	3.9 -	3.0 -	5.5 -
	ST.DEV.	4.3	4.9	4.1	3.6	5.1
	N	48	48	48	48	6
Day 12	MEAN	---	---	---	---	8.1
	ST.DEV.	---	---	---	---	4.7
	N	0	0	0	0	6
Day 19	MEAN	---	---	---	---	14.8
	ST.DEV.	---	---	---	---	6.5
	N	0	0	0	0	6
TREATMENT						
Day 1	MEAN	0.0	0.0	0.0	0.0	0.0
	ST.DEV.	0.0	0.0	0.0	0.0	0.0
	N	48	48	48	48	6
Day 8	MEAN	0.0	1.1 -	-1.8 -	-2.0 *	---
	ST.DEV.	3.8	4.5	4.0	4.0	---
	N	48	48	48	48	0
Day 16	MEAN	0.6	1.2 -	1.1 -	1.2 -	---
	ST.DEV.	5.1	4.5	4.3	5.6	---
	N	42	42	41	42	0

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE**

Date : 26-AUG-09 10:22:08

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 3 - VANADIUM

Satellite: A - ALLOCATION A

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE

Exclusions from Summary

Not Reported

Selection of Organs

All Lu.Lob

Body W.

Further organs not reported

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE**

Date : 26-AUG-09 10:22:08

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 3 - VANADIUM

Satellite: A - ALLOCATION A

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE**

Exclusions from Summary

Not Reported

Selection of Organs

All Lu.Lob
Body W.

Further organs not reported

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES**

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	19.7	19.3 -	20.2 -	18.6 -
	ST.DEV.	0.6	0.6	1.3	1.7
	MINIMUM	18.7	18.3	19.0	15.6
	MAXIMUM	20.3	20.0	22.3	20.8
	N	6	6	6	6
ALL LU.LOB	MEAN	0.120	0.124 -	0.147 **	0.186 **
	ST.DEV.	0.004	0.006	0.006	0.018
	MINIMUM	0.115	0.117	0.136	0.154
	MAXIMUM	0.126	0.133	0.152	0.207
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	19.7	19.3 -	20.2 -	18.6 -
	ST.DEV.	0.6	0.6	1.3	1.7
	MINIMUM	18.7	18.3	19.0	15.6
	MAXIMUM	20.3	20.0	22.3	20.8
	N	6	6	6	6
ALL LU.LOB	MEAN	0.611	0.645 -	0.728 **	0.999 **
	ST.DEV.	0.027	0.022	0.036	0.027
	MINIMUM	0.580	0.613	0.673	0.952
	MAXIMUM	0.649	0.679	0.784	1.026
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**ORGAN WEIGHTS (GRAM) - SUMMARY
 ALLOC. A / VANADIUM PENTOXIDE
 AFTER 16 DAYS OF TREATMENT
 FEMALES**

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	19.7	19.3 -	20.2 -	18.6 -
	ST.DEV.	0.6	0.6	1.3	1.7
	MINIMUM	18.7	18.3	19.0	15.6
	MAXIMUM	20.3	20.0	22.3	20.8
	N	6	6	6	6
ALL LU.LOB	MEAN	0.120	0.124 -	0.147 **	0.186 **
	ST.DEV.	0.004	0.006	0.006	0.018
	MINIMUM	0.115	0.117	0.136	0.154
	MAXIMUM	0.126	0.133	0.152	0.207
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. A / VANADIUM PENTOXIDE
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	19.7	19.3 -	20.2 -	18.6 -
	ST.DEV.	0.6	0.6	1.3	1.7
	MINIMUM	18.7	18.3	19.0	15.6
	MAXIMUM	20.3	20.0	22.3	20.8
	N	6	6	6	6
ALL LU.LOB	MEAN	0.611	0.645 -	0.728 **	0.999 **
	ST.DEV.	0.027	0.022	0.036	0.027
	MINIMUM	0.580	0.613	0.673	0.952
	MAXIMUM	0.649	0.679	0.784	1.026
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. B / CELL PROFILARATION**

Date : 26-AUG-09 10:24:21

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 1 - 7 TAGE

Satellite: B - ALLOCATION B

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. B / CELL PROFILARATION

Exclusions from Summary

Not Reported

Selection of Organs

All organs reported

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	19.1	19.4 -	19.1 -	19.2 -
	ST.DEV.	1.0	1.4	1.1	1.0
	MINIMUM	17.9	17.1	17.2	17.8
	MAXIMUM	20.6	20.7	20.3	20.6
	N	6	6	6	6
LUNGS	MEAN	0.158	0.155 -	0.153 -	0.177 *
	ST.DEV.	0.011	0.009	0.011	0.010
	MINIMUM	0.151	0.144	0.136	0.166
	MAXIMUM	0.179	0.166	0.169	0.195
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	19.1	19.4 -	19.1 -	19.2 -
	ST.DEV.	1.0	1.4	1.1	1.0
	MINIMUM	17.9	17.1	17.2	17.8
	MAXIMUM	20.6	20.7	20.3	20.6
	N	6	6	6	6
LUNGS	MEAN	0.829	0.801 -	0.802 -	0.924 **
	ST.DEV.	0.045	0.030	0.025	0.045
	MINIMUM	0.784	0.761	0.772	0.881
	MAXIMUM	0.894	0.842	0.833	1.000
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. B / CELL PROLIFERATION
AFTER 7 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	19.1	19.4 -	19.1 -	19.2 -
	ST.DEV.	1.0	1.4	1.1	1.0
	MINIMUM	17.9	17.1	17.2	17.8
	MAXIMUM	20.6	20.7	20.3	20.6
	N	6	6	6	6
LUNGS	MEAN	0.158	0.155 -	0.153 -	0.177 *
	ST.DEV.	0.011	0.009	0.011	0.010
	MINIMUM	0.151	0.144	0.136	0.166
	MAXIMUM	0.179	0.166	0.169	0.195
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. B / CELL PROFILARATION
AFTER 7 DAYS OF TREATMENT
FEMALES

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	19.1	19.4 -	19.1 -	19.2 -
	ST.DEV.	1.0	1.4	1.1	1.0
	MINIMUM	17.9	17.1	17.2	17.8
	MAXIMUM	20.6	20.7	20.3	20.6
	N	6	6	6	6
LUNGS	MEAN	0.829	0.801 -	0.802 -	0.924 **
	ST.DEV.	0.045	0.030	0.025	0.045
	MINIMUM	0.784	0.761	0.772	0.881
	MAXIMUM	0.894	0.842	0.833	1.000
	N	6	6	6	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. C / CELL PROFILARATION**

Date : 26-AUG-09 10:26:17

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 2 - 16 Tage

Satellite: C - ALLOCATION C

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. C / CELL PROFILARATION**

Exclusions from Summary

Not Reported

Selection of Organs

All organs reported

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. C / CELL PROFILARATION**

Date : 26-AUG-09 10:26:17

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 2 - 16 Tage

Satellite: C - ALLOCATION C

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. C / CELL PROFILARATION**

Exclusions from Summary

Not Reported

Selection of Organs

All organs reported

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	18.9	19.4 -	19.5 -	18.5 -
	ST.DEV.	1.2	1.7	0.9	1.2
	MINIMUM	17.9	17.5	18.2	17.3
	MAXIMUM	21.1	21.5	20.5	20.2
	N	6	6	5	6
LUNGS	MEAN	0.138	0.151 -	0.168 **	0.199 **
	ST.DEV.	0.007	0.017	0.009	0.009
	MINIMUM	0.129	0.127	0.155	0.184
	MAXIMUM	0.149	0.172	0.179	0.208
	N	6	5	5	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	18.9	19.4 -	19.5 -	18.5 -
	ST.DEV.	1.2	1.7	0.9	1.2
	MINIMUM	17.9	17.5	18.2	17.3
	MAXIMUM	21.1	21.5	20.5	20.2
	N	6	6	5	6
LUNGS	MEAN	0.729	0.763 -	0.865 **	1.073 **
	ST.DEV.	0.024	0.062	0.035	0.032
	MINIMUM	0.698	0.712	0.815	1.030
	MAXIMUM	0.757	0.835	0.906	1.109
	N	6	5	5	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. C / CELL PROLIFERATION
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	18.9	19.4 -	19.5 -	18.5 -
	ST.DEV.	1.2	1.7	0.9	1.2
	MINIMUM	17.9	17.5	18.2	17.3
	MAXIMUM	21.1	21.5	20.5	20.2
	N	6	6	5	6
LUNGS	MEAN	0.138	0.151 -	0.168 **	0.199 **
	ST.DEV.	0.007	0.017	0.009	0.009
	MINIMUM	0.129	0.127	0.155	0.184
	MAXIMUM	0.149	0.172	0.179	0.208
	N	6	5	5	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. C / CELL PROFILARATION
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	18.9	19.4 -	19.5 -	18.5 -
	ST.DEV.	1.2	1.7	0.9	1.2
	MINIMUM	17.9	17.5	18.2	17.3
	MAXIMUM	21.1	21.5	20.5	20.2
	N	6	6	5	6
LUNGS	MEAN	0.729	0.763 -	0.865 **	1.073 **
	ST.DEV.	0.024	0.062	0.035	0.032
	MINIMUM	0.698	0.712	0.815	1.030
	MAXIMUM	0.757	0.835	0.906	1.109
	N	6	5	5	6

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG**

Date : 26-AUG-09 10:31:09

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 4 - 16 TAGE, ALL. D

Satellite: D - ALLOCATION D

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG

Exclusions from Summary

Not Reported

Selection of Organs

Body W.

Left Lobe

Right Lungs

Further organs not reported

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG

Date : 26-AUG-09 10:31:09

User : HUH

Report Data

Types: Weights
 Body Ratios

Statistic Tests Weights : DUNNETT
 Body Ratios : DUNNETT

Necropsies: 4 - 16 TAGE, ALL. D

Satellite: D - ALLOCATION D

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG**

Exclusions from Summary

Not Reported

Selection of Organs

Body W.

Left Lobe

Right Lungs

Further organs not reported

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES**

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	20.4	19.2 -	19.4 -	19.2 -
	ST.DEV.	1.0	0.8	1.7	0.9
	MINIMUM	18.7	18.2	17.4	17.7
	MAXIMUM	21.7	20.6	23.0	20.6
	N	8	8	8	8
RIGHT LUNG	MEAN	0.086	0.100 **	0.107 **	0.127 **
	ST.DEV.	0.006	0.004	0.009	0.008
	MINIMUM	0.076	0.095	0.093	0.113
	MAXIMUM	0.095	0.105	0.120	0.139
	N	8	8	8	8
LEFT LOBE	MEAN	0.043	0.047 -	0.052 **	0.062 **
	ST.DEV.	0.003	0.002	0.004	0.005
	MINIMUM	0.038	0.044	0.045	0.053
	MAXIMUM	0.048	0.050	0.056	0.070
	N	8	8	8	8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1 AIR CONTROL	Group 2 LOW DOSE	Group 3 MID DOSE	Group 4 HIGH DOSE
BODY W.	MEAN	20.4	19.2 -	19.4 -	19.2 -
	ST. DEV.	1.0	0.8	1.7	0.9
	MINIMUM	18.7	18.2	17.4	17.7
	MAXIMUM	21.7	20.6	23.0	20.6
	N	8	8	8	8
RIGHT LUNG	MEAN	0.422	0.519 **	0.556 **	0.659 **
	ST. DEV.	0.023	0.021	0.056	0.036
	MINIMUM	0.396	0.485	0.452	0.607
	MAXIMUM	0.461	0.547	0.638	0.716
	N	8	8	8	8
LEFT LOBE	MEAN	0.211	0.244 **	0.269 **	0.320 **
	ST. DEV.	0.012	0.014	0.019	0.021
	MINIMUM	0.197	0.228	0.230	0.299
	MAXIMUM	0.233	0.275	0.283	0.361
	N	8	8	8	8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	20.4	19.2 -	19.4 -	19.2 -
	ST.DEV.	1.0	0.8	1.7	0.9
	MINIMUM	18.7	18.2	17.4	17.7
	MAXIMUM	21.7	20.6	23.0	20.6
	N	8	8	8	8
RIGHT LUNG	MEAN	0.086	0.100 **	0.107 **	0.127 **
	ST.DEV.	0.006	0.004	0.009	0.008
	MINIMUM	0.076	0.095	0.093	0.113
	MAXIMUM	0.095	0.105	0.120	0.139
	N	8	8	8	8
LEFT LOBE	MEAN	0.043	0.047 -	0.052 **	0.062 **
	ST.DEV.	0.003	0.002	0.004	0.005
	MINIMUM	0.038	0.044	0.045	0.053
	MAXIMUM	0.048	0.050	0.056	0.070
	N	8	8	8	8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

ORGAN/BODY WEIGHT RATIOS (%) - SUMMARY
ALLOC. D / BIOMARKERS IN LUNG
AFTER 16 DAYS OF TREATMENT
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	20.4	19.2 -	19.4 -	19.2 -
	ST.DEV.	1.0	0.8	1.7	0.9
	MINIMUM	18.7	18.2	17.4	17.7
	MAXIMUM	21.7	20.6	23.0	20.6
	N	8	8	8	8
RIGHT LUNG	MEAN	0.422	0.519 **	0.556 **	0.659 **
	ST.DEV.	0.023	0.021	0.056	0.036
	MINIMUM	0.396	0.485	0.452	0.607
	MAXIMUM	0.461	0.547	0.638	0.716
	N	8	8	8	8
LEFT LOBE	MEAN	0.211	0.244 **	0.269 **	0.320 **
	ST.DEV.	0.012	0.014	0.019	0.021
	MINIMUM	0.197	0.228	0.230	0.299
	MAXIMUM	0.233	0.275	0.283	0.361
	N	8	8	8	8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**) or not sig. (-)

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOCATION E**

Date : 03-AUG-09 14:51:24

User : HUH

Report Data

Types: Weights

Statistic Tests Weights : DUNNETT

Necropsies: 5 - 16 TAGE, ALL. E

Satellite: E - ALLOCATION E

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOCATION E**

Exclusions from Summary

Not Reported

Selection of Organs

Body W.

Lungs

Further organs not reported

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOCATION E**

Date : 03-AUG-09 14:51:24

User : HUH

Report Data

Types: Weights

Statistic Tests Weights : DUNNETT

Necropsies: 5 - 16 TAGE, ALL. E

Satellite: E - ALLOCATION E

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOCATION E**

Exclusions from Summary

Not Reported

Selection of Organs

Body W.
Lungs

Further organs not reported

ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOCATION E
AFTER 16 DAYS, ALL. E
FEMALES

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	19.0	18.6 -	19.1 -	18.7 -
	ST.DEV.	1.0	1.6	1.0	1.0
	MINIMUM	17.9	17.0	17.6	17.3
	MAXIMUM	20.7	21.7	20.7	19.8
	N	8	8	8	8
LUNGS	MEAN	0.140	0.136 -	0.156 *	0.197 **
	ST.DEV.	0.004	0.013	0.011	0.018
	MINIMUM	0.136	0.118	0.141	0.178
	MAXIMUM	0.146	0.154	0.168	0.236
	N	8	8	8	8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

MACROSCOPICAL FINDINGS - SUMMARY
ALLOCATION A-E
ALL NECROPSIES

Not Reported

**ORGAN WEIGHTS (GRAM) - SUMMARY
ALLOCATION E
AFTER 16 DAYS, ALL. E
FEMALES**

		Group 1	Group 2	Group 3	Group 4
		AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY W.	MEAN	19.0	18.6 -	19.1 -	18.7 -
	ST.DEV.	1.0	1.6	1.0	1.0
	MINIMUM	17.9	17.0	17.6	17.3
	MAXIMUM	20.7	21.7	20.7	19.8
	N	8	8	8	8
LUNGS	MEAN	0.140	0.136 -	0.156 *	0.197 **
	ST.DEV.	0.004	0.013	0.011	0.018
	MINIMUM	0.136	0.118	0.141	0.178
	MAXIMUM	0.146	0.154	0.168	0.236
	N	8	8	8	8

*/**/- : DUNNETT-Test based on pooled variance sig. at 5% (*), 1% (**), or not sig. (-)

MACROSCOPICAL FINDINGS - SUMMARY
ALLOCATION A-E
ALL NECROPSIES

Not Reported

MACROSCOPICAL FINDINGS - SUMMARY
ALLOCATION A-E
ALL NECROPSIES
FEMALES

	Group 1	Group 2	Group 3	Group 4
	AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS EXAMINED	34	34	34	34
ANIMALS COMPLETED	34	34	34	34
ANIMALS WITHOUT FINDINGS	34	34	33	34
ANIMALS AFFECTED				
LUNGS				
- DISCOLORATION	0 (0%)	0 (0%) -	1 (3%) -	0 (0%) -

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

10 INDIVIDUAL TABLES

MACROSCOPICAL FINDINGS - SUMMARY
ALLOCATION A-E
ALL NECROPSIES
FEMALES

	Group 1	Group 2	Group 3	Group 4
	AIR CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS EXAMINED	34	34	34	34
ANIMALS COMPLETED	34	34	34	34
ANIMALS WITHOUT FINDINGS	34	34	33	34
ANIMALS AFFECTED				
LUNGS				
- DISCOLORATION	0 (0%)	0 (0%) -	1 (3%) -	0 (0%) -

*/**/- : Fisher's Exact Test significant at 5% (*), 1% (**) or not significant (-)

10 INDIVIDUAL TABLES

BODY WEIGHTS (G)
MAIN STUDY

Date : 25-AUG-09 16:40:11

User : HUH

Report Data

Activity: BW - BODY WEIGHT

Timing: 10 - BODY WEIGHT

Satellite: A-G - ALLOCATION A - G

Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

BODY WEIGHTS (G)
MAIN STUDY

Comments

Data excluded from Summary Report

Not Reported

BODY WEIGHTS (G)
MAIN STUDY

Date : 25-AUG-09 16:40:11

User : HUH

Report Data

Activity: BW - BODY WEIGHT
Timing: 10 - BODY WEIGHT
Satellite: A-G - ALLOCATION A - G
Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

BODY WEIGHTS (G)
MAIN STUDY

Comments

Data excluded from Summary Report

Not Reported

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 1 (AIR CONTROL)

Animal	1	2	3	4	5	6	7	
ACCLIMATISATION								
Day	1	19.4	20.2	19.1	20.9	20.4	19.3	20.4
	8	21.1	20.3	19.2	21.2	20.8	19.2	21.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	1	2	3	4	5	6	7	
TREATMENT								
Day	1	22.3	21.9	20.1	22.0	20.7	22.0	22.1
	8	23.4	22.4	20.0	21.9	21.3	21.7	20.8
	16	20.3	19.4	19.5	20.3	20.0	18.7	---
Animal	8	9	10	11	12	13	14	
ACCLIMATISATION								
Day	1	18.4	20.3	18.4	18.9	19.0	20.7	18.0
	8	19.4	21.3	19.3	19.3	21.4	21.1	20.0
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	8	9	10	11	12	13	14	
TREATMENT								
Day	1	20.4	21.5	20.3	20.3	21.3	20.9	21.4
	8	19.1	21.6	19.8	18.6	19.1	22.1	20.6
	16	---	---	---	---	---	21.4	20.7
Animal	15	16	17	18	19	20	21	
ACCLIMATISATION								
Day	1	19.0	21.4	20.1	18.7	20.7	20.0	22.3
	8	19.5	22.2	20.2	20.0	21.8	19.9	23.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	15	16	17	18	19	20	21	
TREATMENT								
Day	1	19.9	23.3	21.3	21.2	23.2	20.8	23.5
	8	20.2	23.8	21.5	21.4	23.7	21.2	25.3
	16	20.7	23.9	22.0	21.5	23.7	22.0	24.3
Animal	22	23	24	25	26	27	28	
ACCLIMATISATION								
Day	1	20.0	19.5	19.5	19.0	21.3	21.1	20.6
	8	20.7	21.7	20.8	21.3	21.3	21.4	22.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)

MAIN STUDY

FEMALES

Group 1 (AIR CONTROL)

Animal		22	23	24	25	26	27	28
TREATMENT								
Day	1	20.5	23.7	21.4	23.3	23.0	23.5	22.8
	8	21.1	21.9	21.3	22.4	22.2	24.4	23.1
	16	22.6	22.3	22.3	22.1	23.0	23.3	23.2
Animal		29	30	31	32	33	34	35
ACCLIMATISATION								
Day	1	20.3	20.5	21.0	20.6	19.3	20.3	19.4
	8	23.0	20.5	21.1	20.5	19.9	20.4	21.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		29	30	31	32	33	34	35
TREATMENT								
Day	1	23.5	21.6	22.8	21.1	21.5	22.2	22.3
	8	23.5	21.6	23.1	21.4	22.0	22.1	21.4
	16	23.8	22.0	23.1	22.6	22.4	22.5	22.1
Animal		36	37	38	39	40	41	42
ACCLIMATISATION								
Day	1	20.4	18.2	20.0	19.1	19.8	18.9	18.4
	8	20.8	19.4	19.6	20.7	20.2	20.5	20.2
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		36	37	38	39	40	41	42
TREATMENT								
Day	1	21.6	19.5	21.2	21.7	22.2	20.9	20.5
	8	21.6	20.1	21.1	21.3	21.4	21.5	20.8
	16	21.9	21.2	22.3	22.5	21.9	22.2	20.6
Animal		43	44	45	46	47	48	
ACCLIMATISATION								
Day	1	20.6	20.5	19.1	18.9	20.3	19.4	
	8	23.0	22.7	21.0	21.0	21.7	20.6	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal		43	44	45	46	47	48	
TREATMENT								
Day	1	23.7	23.2	22.5	21.5	21.5	21.0	
	8	22.8	23.7	22.4	22.1	22.9	22.0	
	16	24.3	23.8	21.7	22.3	21.2	21.3	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 1 (AIR CONTROL)

Animal	1	2	3	4	5	6	7	
ACCLIMATISATION								
Day	1	19.4	20.2	19.1	20.9	20.4	19.3	20.4
	8	21.1	20.3	19.2	21.2	20.8	19.2	21.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	1	2	3	4	5	6	7	
TREATMENT								
Day	1	22.3	21.9	20.1	22.0	20.7	22.0	22.1
	8	23.4	22.4	20.0	21.9	21.3	21.7	20.8
	16	20.3	19.4	19.5	20.3	20.0	18.7	---
Animal	8	9	10	11	12	13	14	
ACCLIMATISATION								
Day	1	18.4	20.3	18.4	18.9	19.0	20.7	18.0
	8	19.4	21.3	19.3	19.3	21.4	21.1	20.0
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	8	9	10	11	12	13	14	
TREATMENT								
Day	1	20.4	21.5	20.3	20.3	21.3	20.9	21.4
	8	19.1	21.6	19.8	18.6	19.1	22.1	20.6
	16	---	---	---	---	---	21.4	20.7
Animal	15	16	17	18	19	20	21	
ACCLIMATISATION								
Day	1	19.0	21.4	20.1	18.7	20.7	20.0	22.3
	8	19.5	22.2	20.2	20.0	21.8	19.9	23.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	15	16	17	18	19	20	21	
TREATMENT								
Day	1	19.9	23.3	21.3	21.2	23.2	20.8	23.5
	8	20.2	23.8	21.5	21.4	23.7	21.2	25.3
	16	20.7	23.9	22.0	21.5	23.7	22.0	24.3
Animal	22	23	24	25	26	27	28	
ACCLIMATISATION								
Day	1	20.0	19.5	19.5	19.0	21.3	21.1	20.6
	8	20.7	21.7	20.8	21.3	21.3	21.4	22.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)

MAIN STUDY

FEMALES

Group 1 (AIR CONTROL)

Animal		22	23	24	25	26	27	28
TREATMENT								
Day	1	20.5	23.7	21.4	23.3	23.0	23.5	22.8
	8	21.1	21.9	21.3	22.4	22.2	24.4	23.1
	16	22.6	22.3	22.3	22.1	23.0	23.3	23.2
Animal		29	30	31	32	33	34	35
ACCLIMATISATION								
Day	1	20.3	20.5	21.0	20.6	19.3	20.3	19.4
	8	23.0	20.5	21.1	20.5	19.9	20.4	21.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		29	30	31	32	33	34	35
TREATMENT								
Day	1	23.5	21.6	22.8	21.1	21.5	22.2	22.3
	8	23.5	21.6	23.1	21.4	22.0	22.1	21.4
	16	23.8	22.0	23.1	22.6	22.4	22.5	22.1
Animal		36	37	38	39	40	41	42
ACCLIMATISATION								
Day	1	20.4	18.2	20.0	19.1	19.8	18.9	18.4
	8	20.8	19.4	19.6	20.7	20.2	20.5	20.2
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		36	37	38	39	40	41	42
TREATMENT								
Day	1	21.6	19.5	21.2	21.7	22.2	20.9	20.5
	8	21.6	20.1	21.1	21.3	21.4	21.5	20.8
	16	21.9	21.2	22.3	22.5	21.9	22.2	20.6
Animal		43	44	45	46	47	48	
ACCLIMATISATION								
Day	1	20.6	20.5	19.1	18.9	20.3	19.4	
	8	23.0	22.7	21.0	21.0	21.7	20.6	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal		43	44	45	46	47	48	
TREATMENT								
Day	1	23.7	23.2	22.5	21.5	21.5	21.0	
	8	22.8	23.7	22.4	22.1	22.9	22.0	
	16	24.3	23.8	21.7	22.3	21.2	21.3	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 2 (LOW DOSE)

Animal	49	50	51	52	53	54	55	
ACCLIMATISATION								
Day	1	18.9	18.5	17.8	18.8	19.3	19.6	19.2
	8	19.5	19.3	19.7	20.7	20.3	20.7	20.7
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	49	50	51	52	53	54	55	
TREATMENT								
Day	1	20.2	19.8	20.6	21.3	20.3	21.8	22.2
	8	21.4	21.3	21.7	21.9	21.9	21.6	21.6
	16	19.2	19.6	18.3	20.0	19.1	19.6	---
Animal	56	57	58	59	60	61	62	
ACCLIMATISATION								
Day	1	19.1	19.5	21.1	19.8	18.8	20.9	21.4
	8	20.2	20.5	22.0	22.0	20.0	20.9	22.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	56	57	58	59	60	61	62	
TREATMENT								
Day	1	20.6	21.0	23.0	23.6	20.3	22.6	24.0
	8	20.2	19.8	21.8	20.9	18.3	23.7	24.0
	16	---	---	---	---	---	23.8	24.4
Animal	63	64	65	66	67	68	69	
ACCLIMATISATION								
Day	1	18.9	17.8	20.7	19.3	18.6	18.7	20.0
	8	19.0	18.6	21.8	19.9	19.5	19.8	20.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	63	64	65	66	67	68	69	
TREATMENT								
Day	1	19.8	20.2	22.0	20.6	20.6	21.0	22.2
	8	20.4	19.5	21.6	21.7	20.7	20.9	21.6
	16	20.7	20.6	22.8	21.5	21.3	20.9	21.4
Animal	70	71	72	73	74	75	76	
ACCLIMATISATION								
Day	1	20.0	19.4	19.4	20.0	20.1	23.2	18.0
	8	20.7	25.1	19.3	21.5	21.0	23.1	20.0
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 2 (LOW DOSE)

Animal	70	71	72	73	74	75	76	
TREATMENT								
Day	1	21.4	22.1	21.7	23.4	21.8	23.5	20.9
	8	21.5	22.7	21.5	22.8	22.8	24.3	21.6
	16	21.3	22.7	21.9	23.3	22.3	23.9	20.8
Animal	77	78	79	80	81	82	83	
ACCLIMATISATION								
Day	1	19.4	19.1	20.2	20.7	19.1	20.5	17.9
	8	19.6	20.8	21.0	21.6	19.7	21.5	19.5
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	77	78	79	80	81	82	83	
TREATMENT								
Day	1	20.4	22.0	21.1	22.9	19.9	22.8	21.0
	8	21.0	21.8	21.8	22.8	21.3	22.2	20.3
	16	20.7	22.3	21.9	23.5	21.1	22.4	20.9
Animal	84	85	86	87	88	89	90	
ACCLIMATISATION								
Day	1	22.0	20.1	20.8	19.3	16.9	19.9	17.5
	8	22.8	20.3	20.0	21.0	17.7	22.2	18.2
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	84	85	86	87	88	89	90	
TREATMENT								
Day	1	23.6	20.9	20.7	21.7	18.5	22.5	20.0
	8	23.8	21.1	22.7	22.1	18.9	22.9	19.4
	16	24.0	21.4	22.7	22.7	19.3	23.0	19.4
Animal	91	92	93	94	95	96		
ACCLIMATISATION								
Day	1	21.4	19.2	21.6	19.7	20.7	19.7	
	8	22.0	20.0	21.3	21.2	21.5	20.1	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal	91	92	93	94	95	96		
TREATMENT								
Day	1	24.0	22.0	22.1	22.3	22.2	21.4	
	8	24.1	22.7	24.3	22.9	23.6	22.0	
	16	23.7	22.5	24.3	23.0	22.9	23.1	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 2 (LOW DOSE)

Animal	49	50	51	52	53	54	55	
ACCLIMATISATION								
Day	1	18.9	18.5	17.8	18.8	19.3	19.6	19.2
	8	19.5	19.3	19.7	20.7	20.3	20.7	20.7
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	49	50	51	52	53	54	55	
TREATMENT								
Day	1	20.2	19.8	20.6	21.3	20.3	21.8	22.2
	8	21.4	21.3	21.7	21.9	21.9	21.6	21.6
	16	19.2	19.6	18.3	20.0	19.1	19.6	---
Animal	56	57	58	59	60	61	62	
ACCLIMATISATION								
Day	1	19.1	19.5	21.1	19.8	18.8	20.9	21.4
	8	20.2	20.5	22.0	22.0	20.0	20.9	22.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	56	57	58	59	60	61	62	
TREATMENT								
Day	1	20.6	21.0	23.0	23.6	20.3	22.6	24.0
	8	20.2	19.8	21.8	20.9	18.3	23.7	24.0
	16	---	---	---	---	---	23.8	24.4
Animal	63	64	65	66	67	68	69	
ACCLIMATISATION								
Day	1	18.9	17.8	20.7	19.3	18.6	18.7	20.0
	8	19.0	18.6	21.8	19.9	19.5	19.8	20.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	63	64	65	66	67	68	69	
TREATMENT								
Day	1	19.8	20.2	22.0	20.6	20.6	21.0	22.2
	8	20.4	19.5	21.6	21.7	20.7	20.9	21.6
	16	20.7	20.6	22.8	21.5	21.3	20.9	21.4
Animal	70	71	72	73	74	75	76	
ACCLIMATISATION								
Day	1	20.0	19.4	19.4	20.0	20.1	23.2	18.0
	8	20.7	25.1	19.3	21.5	21.0	23.1	20.0
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)

MAIN STUDY

FEMALES

Group 2 (LOW DOSE)

Animal		70	71	72	73	74	75	76
TREATMENT								
Day	1	21.4	22.1	21.7	23.4	21.8	23.5	20.9
	8	21.5	22.7	21.5	22.8	22.8	24.3	21.6
	16	21.3	22.7	21.9	23.3	22.3	23.9	20.8
Animal		77	78	79	80	81	82	83
ACCLIMATISATION								
Day	1	19.4	19.1	20.2	20.7	19.1	20.5	17.9
	8	19.6	20.8	21.0	21.6	19.7	21.5	19.5
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		77	78	79	80	81	82	83
TREATMENT								
Day	1	20.4	22.0	21.1	22.9	19.9	22.8	21.0
	8	21.0	21.8	21.8	22.8	21.3	22.2	20.3
	16	20.7	22.3	21.9	23.5	21.1	22.4	20.9
Animal		84	85	86	87	88	89	90
ACCLIMATISATION								
Day	1	22.0	20.1	20.8	19.3	16.9	19.9	17.5
	8	22.8	20.3	20.0	21.0	17.7	22.2	18.2
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		84	85	86	87	88	89	90
TREATMENT								
Day	1	23.6	20.9	20.7	21.7	18.5	22.5	20.0
	8	23.8	21.1	22.7	22.1	18.9	22.9	19.4
	16	24.0	21.4	22.7	22.7	19.3	23.0	19.4
Animal		91	92	93	94	95	96	
ACCLIMATISATION								
Day	1	21.4	19.2	21.6	19.7	20.7	19.7	
	8	22.0	20.0	21.3	21.2	21.5	20.1	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal		91	92	93	94	95	96	
TREATMENT								
Day	1	24.0	22.0	22.1	22.3	22.2	21.4	
	8	24.1	22.7	24.3	22.9	23.6	22.0	
	16	23.7	22.5	24.3	23.0	22.9	23.1	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 3 (MID DOSE)

Animal	97	98	99	100	101	102	103
ACCLIMATISATION							
Day	1	19.2	20.1	18.9	19.9	20.3	21.5
	8	20.8	21.4	19.5	20.6	20.4	22.3
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	97	98	99	100	101	102	103
TREATMENT							
Day	1	21.4	22.5	20.5	20.8	21.6	22.0
	8	22.3	23.2	20.9	21.8	23.0	20.7
	16	19.4	22.3	19.3	19.0	20.7	---
Animal	104	105	106	107	108	109	110
ACCLIMATISATION							
Day	1	19.4	19.4	20.4	20.4	19.4	18.0
	8	19.7	20.4	20.5	21.0	20.8	19.3
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	104	105	106	107	108	109	110
TREATMENT							
Day	1	20.8	21.4	21.5	22.1	21.0	19.7
	8	20.1	19.0	20.6	21.6	21.3	19.5
	16	---	---	---	---	---	20.4
Animal	111	112	113	114	115	116	117
ACCLIMATISATION							
Day	1	20.0	20.4	19.6	19.3	20.3	19.7
	8	21.1	21.5	20.3	19.4	20.1	21.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	111	112	113	114	115	116	117
TREATMENT							
Day	1	22.6	21.9	21.8	20.8	21.8	22.2
	8	21.7	22.2	22.2	21.3	21.9	22.4
	16	22.5	22.5	22.3	21.9	22.7	23.3
Animal	118	119	120	121	122	123	124
ACCLIMATISATION							
Day	1	20.1	18.8	19.3	19.5	21.3	19.5
	8	20.6	20.7	20.4	19.3	22.1	20.5
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---

BODY WEIGHTS (G)

MAIN STUDY

FEMALES

Group 3 (MID DOSE)

Animal		118	119	120	121	122	123	124
TREATMENT								
Day	1	22.0	21.3	22.2	21.0	24.0	22.1	22.2
	8	21.2	20.4	20.5	19.6	23.4	21.7	20.9
	16	22.0	21.0	22.1	21.4	26.2	22.4	22.7
Animal		125	126	127	128	129	130	131
ACCLIMATISATION								
Day	1	21.3	19.4	20.1	20.3	19.9	18.7	19.8
	8	20.8	18.7	20.8	21.0	18.2	19.6	20.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		125	126	127	128	129	130	131
TREATMENT								
Day	1	23.0	20.7	21.9	20.7	21.1	20.3	21.6
	8	22.2	18.3	20.5	20.6	20.5	19.0	22.3
	16	23.3	20.5	23.2	22.8	22.1	20.6	22.3
Animal		132	133	134	135	136	137	138
ACCLIMATISATION								
Day	1	19.3	18.6	20.1	20.4	19.5	19.4	21.3
	8	20.1	20.9	21.0	20.6	20.6	21.6	21.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		132	133	134	135	136	137	138
TREATMENT								
Day	1	20.5	21.1	22.7	21.8	22.3	22.7	23.2
	8	20.2	21.3	21.3	21.2	21.6	22.4	22.7
	16	20.1	22.2	22.2	22.7	21.7	24.0	22.5
Animal		139	140	141	142	143	144	
ACCLIMATISATION								
Day	1	20.5	22.8	20.3	20.0	21.3	20.9	
	8	20.5	24.4	19.9	21.3	21.9	21.5	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal		139	140	141	142	143	144	
TREATMENT								
Day	1	24.0	25.8	21.5	22.3	23.2	22.6	
	8	23.1	24.0	21.0	22.0	23.1	22.8	
	16	23.9	25.7	22.1	22.5	23.8	23.9	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 3 (MID DOSE)

Animal	97	98	99	100	101	102	103	
ACCLIMATISATION								
Day	1	19.2	20.1	18.9	19.9	20.3	19.6	21.5
	8	20.8	21.4	19.5	20.6	20.4	20.9	22.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	97	98	99	100	101	102	103	
TREATMENT								
Day	1	21.4	22.5	20.5	20.8	21.6	22.1	22.0
	8	22.3	23.2	20.9	21.8	23.0	22.7	20.7
	16	19.4	22.3	19.3	19.0	20.7	20.7	---
Animal	104	105	106	107	108	109	110	
ACCLIMATISATION								
Day	1	19.4	19.4	20.4	20.4	19.4	18.0	18.0
	8	19.7	20.4	20.5	21.0	20.8	19.2	19.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	104	105	106	107	108	109	110	
TREATMENT								
Day	1	20.8	21.4	21.5	22.1	21.0	20.0	19.7
	8	20.1	19.0	20.6	21.6	21.3	20.6	19.5
	16	---	---	---	---	---	---	20.4
Animal	111	112	113	114	115	116	117	
ACCLIMATISATION								
Day	1	20.0	20.4	19.6	19.3	20.3	17.3	19.7
	8	21.1	21.5	20.3	19.4	20.1	19.7	21.6
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	111	112	113	114	115	116	117	
TREATMENT								
Day	1	22.6	21.9	21.8	20.8	21.8	20.4	22.2
	8	21.7	22.2	22.2	21.3	21.9	20.2	22.4
	16	22.5	22.5	22.3	21.9	22.7	21.2	23.3
Animal	118	119	120	121	122	123	124	
ACCLIMATISATION								
Day	1	20.1	18.8	19.3	19.5	21.3	20.1	19.5
	8	20.6	20.7	20.4	19.3	22.1	21.0	20.5
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)

MAIN STUDY

FEMALES

Group 3 (MID DOSE)

Animal		118	119	120	121	122	123	124
TREATMENT								
Day	1	22.0	21.3	22.2	21.0	24.0	22.1	22.2
	8	21.2	20.4	20.5	19.6	23.4	21.7	20.9
	16	22.0	21.0	22.1	21.4	26.2	22.4	22.7
Animal		125	126	127	128	129	130	131
ACCLIMATISATION								
Day	1	21.3	19.4	20.1	20.3	19.9	18.7	19.8
	8	20.8	18.7	20.8	21.0	18.2	19.6	20.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		125	126	127	128	129	130	131
TREATMENT								
Day	1	23.0	20.7	21.9	20.7	21.1	20.3	21.6
	8	22.2	18.3	20.5	20.6	20.5	19.0	22.3
	16	23.3	20.5	23.2	22.8	22.1	20.6	22.3
Animal		132	133	134	135	136	137	138
ACCLIMATISATION								
Day	1	19.3	18.6	20.1	20.4	19.5	19.4	21.3
	8	20.1	20.9	21.0	20.6	20.6	21.6	21.9
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		132	133	134	135	136	137	138
TREATMENT								
Day	1	20.5	21.1	22.7	21.8	22.3	22.7	23.2
	8	20.2	21.3	21.3	21.2	21.6	22.4	22.7
	16	20.1	22.2	22.2	22.7	21.7	24.0	22.5
Animal		139	140	141	142	143	144	
ACCLIMATISATION								
Day	1	20.5	22.8	20.3	20.0	21.3	20.9	
	8	20.5	24.4	19.9	21.3	21.9	21.5	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal		139	140	141	142	143	144	
TREATMENT								
Day	1	24.0	25.8	21.5	22.3	23.2	22.6	
	8	23.1	24.0	21.0	22.0	23.1	22.8	
	16	23.9	25.7	22.1	22.5	23.8	23.9	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 4 (HIGH DOSE)

Animal		145	146	147	148	149	150	151
ACCLIMATISATION								
Day	1	19.3	18.6	17.1	20.6	18.2	19.3	18.7
	8	20.4	19.6	16.6	21.2	19.6	20.2	20.5
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		145	146	147	148	149	150	151
TREATMENT								
Day	1	20.1	20.3	17.5	21.7	20.1	19.9	21.3
	8	21.0	20.0	17.3	21.9	20.4	20.2	20.9
	16	19.2	18.9	15.6	20.8	18.2	18.8	---
Animal		152	153	154	155	156	157	158
ACCLIMATISATION								
Day	1	20.7	20.2	19.5	21.4	19.3	19.7	21.3
	8	21.7	19.8	21.0	21.1	20.3	20.7	22.0
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		152	153	154	155	156	157	158
TREATMENT								
Day	1	22.0	20.4	21.2	21.7	20.3	22.0	22.7
	8	21.3	19.4	19.3	18.3	19.6	22.2	22.5
	16	---	---	---	---	---	22.0	23.1
Animal		159	160	161	162	163	164	165
ACCLIMATISATION								
Day	1	17.7	18.9	18.3	18.5	19.6	20.4	20.3
	8	17.9	19.2	19.5	18.9	20.3	20.5	20.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal		159	160	161	162	163	164	165
TREATMENT								
Day	1	18.6	19.9	20.1	19.2	22.0	21.4	21.2
	8	19.2	20.4	20.5	19.8	20.8	19.6	19.8
	16	19.8	20.6	21.8	19.5	21.7	21.3	21.9
Animal		166	167	168	169	170	171	172
ACCLIMATISATION								
Day	1	18.8	19.7	19.2	20.6	20.0	21.9	20.5
	8	19.6	20.0	19.7	20.2	20.1	21.9	21.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 4 (HIGH DOSE)

Animal	166	167	168	169	170	171	172	
TREATMENT								
Day	1	20.5	21.0	21.0	21.4	20.7	23.2	22.2
	8	19.5	19.8	20.2	21.3	20.6	21.2	21.3
	16	21.9	22.6	22.6	23.4	20.6	21.4	21.0
Animal	173	174	175	176	177	178	179	
ACCLIMATISATION								
Day	1	19.4	20.5	20.2	20.0	20.7	20.5	20.0
	8	19.6	20.6	22.2	21.5	20.7	19.8	19.4
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	173	174	175	176	177	178	179	
TREATMENT								
Day	1	21.2	22.4	23.0	22.5	22.7	20.3	21.0
	8	20.1	20.9	21.9	22.8	20.7	19.4	21.1
	16	22.5	20.0	22.8	23.8	22.9	20.9	20.5
Animal	180	181	182	183	184	185	186	
ACCLIMATISATION								
Day	1	20.3	21.0	19.9	19.7	20.6	19.4	21.8
	8	22.5	21.2	20.9	21.2	21.3	19.7	21.8
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	180	181	182	183	184	185	186	
TREATMENT								
Day	1	23.0	22.4	21.9	22.9	22.8	21.4	23.6
	8	23.1	23.2	21.6	23.0	23.4	21.1	23.4
	16	23.0	24.4	22.7	23.1	23.7	22.2	23.8
Animal	187	188	189	190	191	192		
ACCLIMATISATION								
Day	1	19.4	18.6	21.9	19.5	18.8	20.4	
	8	19.7	19.6	21.8	20.7	20.4	20.8	
	12	---	---	---	---	---	---	
	19	---	---	---	---	---	---	
Animal	187	188	189	190	191	192		
TREATMENT								
Day	1	21.3	21.2	23.9	21.7	22.0	22.5	
	8	21.8	21.3	23.9	21.7	21.3	22.4	
	16	22.8	21.3	26.8	21.8	22.9	23.1	

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 4 (HIGH DOSE)

Animal	145	146	147	148	149	150	151	
ACCLIMATISATION								
Day	1	19.3	18.6	17.1	20.6	18.2	19.3	18.7
	8	20.4	19.6	16.6	21.2	19.6	20.2	20.5
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	145	146	147	148	149	150	151	
TREATMENT								
Day	1	20.1	20.3	17.5	21.7	20.1	19.9	21.3
	8	21.0	20.0	17.3	21.9	20.4	20.2	20.9
	16	19.2	18.9	15.6	20.8	18.2	18.8	---
Animal	152	153	154	155	156	157	158	
ACCLIMATISATION								
Day	1	20.7	20.2	19.5	21.4	19.3	19.7	21.3
	8	21.7	19.8	21.0	21.1	20.3	20.7	22.0
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	152	153	154	155	156	157	158	
TREATMENT								
Day	1	22.0	20.4	21.2	21.7	20.3	22.0	22.7
	8	21.3	19.4	19.3	18.3	19.6	22.2	22.5
	16	---	---	---	---	---	22.0	23.1
Animal	159	160	161	162	163	164	165	
ACCLIMATISATION								
Day	1	17.7	18.9	18.3	18.5	19.6	20.4	20.3
	8	17.9	19.2	19.5	18.9	20.3	20.5	20.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---
Animal	159	160	161	162	163	164	165	
TREATMENT								
Day	1	18.6	19.9	20.1	19.2	22.0	21.4	21.2
	8	19.2	20.4	20.5	19.8	20.8	19.6	19.8
	16	19.8	20.6	21.8	19.5	21.7	21.3	21.9
Animal	166	167	168	169	170	171	172	
ACCLIMATISATION								
Day	1	18.8	19.7	19.2	20.6	20.0	21.9	20.5
	8	19.6	20.0	19.7	20.2	20.1	21.9	21.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHTS (G)

MAIN STUDY

FEMALES

Group 4 (HIGH DOSE)

Animal	166	167	168	169	170	171	172
TREATMENT							
Day	1	20.5	21.0	21.0	21.4	20.7	22.2
	8	19.5	19.8	20.2	21.3	20.6	21.3
	16	21.9	22.6	22.6	23.4	20.6	21.0
Animal	173	174	175	176	177	178	179
ACCLIMATISATION							
Day	1	19.4	20.5	20.2	20.0	20.7	20.0
	8	19.6	20.6	22.2	21.5	20.7	19.4
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	173	174	175	176	177	178	179
TREATMENT							
Day	1	21.2	22.4	23.0	22.5	22.7	21.0
	8	20.1	20.9	21.9	22.8	20.7	21.1
	16	22.5	20.0	22.8	23.8	22.9	20.5
Animal	180	181	182	183	184	185	186
ACCLIMATISATION							
Day	1	20.3	21.0	19.9	19.7	20.6	21.8
	8	22.5	21.2	20.9	21.2	21.3	21.8
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	180	181	182	183	184	185	186
TREATMENT							
Day	1	23.0	22.4	21.9	22.9	22.8	23.6
	8	23.1	23.2	21.6	23.0	23.4	23.4
	16	23.0	24.4	22.7	23.1	23.7	23.8
Animal	187	188	189	190	191	192	
ACCLIMATISATION							
Day	1	19.4	18.6	21.9	19.5	18.8	20.4
	8	19.7	19.6	21.8	20.7	20.4	20.8
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	187	188	189	190	191	192	
TREATMENT							
Day	1	21.3	21.2	23.9	21.7	22.0	22.5
	8	21.8	21.3	23.9	21.7	21.3	22.4
	16	22.8	21.3	26.8	21.8	22.9	23.1

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 5 (POS. CTRL.)

Animal	193	194	195	196	197	198	
ACCLIMATISATION							
Day	1	18.6	19.1	19.3	20.2	17.3	21.0
	8	20.2	20.3	19.2	22.7	18.5	21.0
	12	20.5	21.3	20.7	22.6	18.7	20.9
	19	22.5	22.8	21.7	23.3	20.3	21.7
Animal	193	194	195	196	197	198	
TREATMENT							
Day	1	21.5	20.8	21.2	21.3	20.9	21.1
	8	---	---	---	---	---	---
	16	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY

Date : 25-AUG-09 16:46:30

User : HUH

Report Data

Activity: BW - BODY WEIGHT

Timing: 10 - BODY WEIGHT

Satellite: A-G - ALLOCATION A - G

Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

BODY WEIGHTS (G)
MAIN STUDY
FEMALES

Group 5 (POS. CTRL.)

Animal	193	194	195	196	197	198
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ACCLIMATISATION

Day	1	18.6	19.1	19.3	20.2	17.3	21.0
	8	20.2	20.3	19.2	22.7	18.5	21.0
	12	20.5	21.3	20.7	22.6	18.7	20.9
	19	22.5	22.8	21.7	23.3	20.3	21.7

Animal	193	194	195	196	197	198
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TREATMENT

Day	1	21.5	20.8	21.2	21.3	20.9	21.1
	8	---	---	---	---	---	---
	16	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY

Date : 25-AUG-09 16:46:30

User : HUH

Report Data

Activity: BW - BODY WEIGHT

Timing: 10 - BODY WEIGHT

Satellite: A-G - ALLOCATION A - G

Study Phase(s): Acclimatisation (1 - 27)
Treatment (1 - 17)

BODY WEIGHT GAIN (%)
MAIN STUDY

Comments

Data excluded from Summary Report

Not Reported

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 1 (AIR CONTROL)

Animal	1	2	3	4	5	6	7
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.4	0.3	0.6	1.8	1.8	-0.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	1	2	3	4	5	6	7
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.1	2.5	-0.4	-0.5	2.7	-1.3
	16	-8.9	-11.2	-3.0	-7.7	-3.5	-14.9

Animal	8	9	10	11	12	13	14
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.5	5.2	5.3	2.2	12.6	2.0
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	8	9	10	11	12	13	14
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-6.0	0.2	-2.4	-8.4	-10.5	5.7
	16	---	---	---	---	---	2.6
							-2.9
Animal	15	16	17	18	19	20	21
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.7	3.9	0.4	6.8	5.3	-0.1
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	15	16	17	18	19	20	21
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.6	1.9	0.9	1.0	1.9	2.0
	16	4.0	2.6	3.4	1.7	2.0	6.2
							3.4
Animal	22	23	24	25	26	27	28
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.6	11.3	6.5	12.2	-0.1	1.4
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY

Comments

Data excluded from Summary Report

Not Reported

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 1 (AIR CONTROL)

Animal	1	2	3	4	5	6	7
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.4	0.3	0.6	1.8	1.8	-0.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	1	2	3	4	5	6	7
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.1	2.5	-0.4	-0.5	2.7	-1.3
	16	-8.9	-11.2	-3.0	-7.7	-3.5	-14.9

Animal	8	9	10	11	12	13	14
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.5	5.2	5.3	2.2	12.6	2.0
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	8	9	10	11	12	13	14
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-6.0	0.2	-2.4	-8.4	-10.5	5.7
	16	---	---	---	---	---	2.6
							-2.9
Animal	15	16	17	18	19	20	21
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.7	3.9	0.4	6.8	5.3	-0.1
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	15	16	17	18	19	20	21
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.6	1.9	0.9	1.0	1.9	2.0
	16	4.0	2.6	3.4	1.7	2.0	6.2
							3.4
Animal	22	23	24	25	26	27	28
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.6	11.3	6.5	12.2	-0.1	1.4
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 1 (AIR CONTROL)

Animal	22	23	24	25	26	27	28
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.0	-7.4	-0.8	-3.8	-3.4	3.8
	16	10.1	-5.7	4.0	-5.1	0.2	-0.9
1.7							
Animal	29	30	31	32	33	34	35
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	13.4	-0.1	0.5	-0.5	3.2	0.3
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
10.2							
Animal	29	30	31	32	33	34	35
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-0.2	0.0	1.3	1.2	2.4	-0.2
	16	1.1	1.7	1.2	6.7	4.1	1.5
-4.3							
-1.2							
Animal	36	37	38	39	40	41	42
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.4	7.0	-2.1	8.5	2.1	8.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
9.9							
Animal	36	37	38	39	40	41	42
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.3	3.3	-0.2	-1.7	-3.6	3.0
	16	1.5	9.0	5.2	3.9	-1.2	6.2
0.5							
Animal	43	44	45	46	47	48	
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	11.5	10.4	10.3	10.8	7.1	6.1
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	43	44	45	46	47	48	
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.8	2.0	-0.4	2.6	6.2	5.1
	16	2.6	2.7	-3.6	3.8	-1.4	1.5

BODY WEIGHT GAIN (%)

MAIN STUDY

FEMALES

Group 2 (LOW DOSE)

Animal	49	50	51	52	53	54	55
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.2	4.1	10.3	10.1	5.3	7.7
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	49	50	51	52	53	54	55
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	6.1	7.8	5.4	2.5	7.9	-1.2
	16	-4.9	-0.6	-11.2	-6.3	-6.2	-9.9

Animal	56	57	58	59	60	61	62
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.7	4.9	4.1	10.9	6.5	0.3
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	56	57	58	59	60	61	62
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-1.7	-5.6	-5.4	-11.5	-10.1	4.8
	16	---	---	---	---	---	5.3
							1.4
Animal	63	64	65	66	67	68	69
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.3	4.9	5.0	3.4	4.7	6.1
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	63	64	65	66	67	68	69
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.4	-3.1	-1.7	5.2	0.3	-0.7
	16	4.8	1.9	4.1	4.5	3.3	-0.5
							-3.5
Animal	70	71	72	73	74	75	76
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.4	29.6	-0.3	7.8	4.6	-0.5
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
							10.8

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 1 (AIR CONTROL)

Animal	22	23	24	25	26	27	28
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.0	-7.4	-0.8	-3.8	-3.4	3.8
	16	10.1	-5.7	4.0	-5.1	0.2	-0.9
Animal	29	30	31	32	33	34	35
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	13.4	-0.1	0.5	-0.5	3.2	0.3
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	29	30	31	32	33	34	35
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-0.2	0.0	1.3	1.2	2.4	-0.2
	16	1.1	1.7	1.2	6.7	4.1	1.5
Animal	36	37	38	39	40	41	42
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.4	7.0	-2.1	8.5	2.1	8.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	36	37	38	39	40	41	42
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.3	3.3	-0.2	-1.7	-3.6	3.0
	16	1.5	9.0	5.2	3.9	-1.2	6.2
Animal	43	44	45	46	47	48	
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	11.5	10.4	10.3	10.8	7.1	6.1
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	43	44	45	46	47	48	
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.8	2.0	-0.4	2.6	6.2	5.1
	16	2.6	2.7	-3.6	3.8	-1.4	1.5

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 2 (LOW DOSE)

Animal	49	50	51	52	53	54	55
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.2	4.1	10.3	10.1	5.3	5.5	7.7
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal	49	50	51	52	53	54	55
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	6.1	7.8	5.4	2.5	7.9	-1.2	-2.7
	16	-4.9	-0.6	-11.2	-6.3	-6.2	-9.9	---

Animal	56	57	58	59	60	61	62
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.7	4.9	4.1	10.9	6.5	0.3	6.8
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal	56	57	58	59	60	61	62
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-1.7	-5.6	-5.4	-11.5	-10.1	4.8	-0.1
	16	---	---	---	---	---	5.3	1.4

Animal	63	64	65	66	67	68	69
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.3	4.9	5.0	3.4	4.7	6.1	4.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal	63	64	65	66	67	68	69
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.4	-3.1	-1.7	5.2	0.3	-0.7	-2.5
	16	4.8	1.9	4.1	4.5	3.3	-0.5	-3.5

Animal	70	71	72	73	74	75	76
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.4	29.6	-0.3	7.8	4.6	-0.5	10.8
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 2 (LOW DOSE)

Animal	70	71	72	73	74	75	76
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.2	2.9	-0.6	-2.6	4.7	3.4
	16	-0.7	2.6	1.3	-0.2	2.1	1.6
							-0.7
Animal	77	78	79	80	81	82	83
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.3	9.1	3.8	4.6	3.3	4.9
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	77	78	79	80	81	82	83
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.1	-0.8	3.2	-0.2	7.1	-2.7
	16	1.7	1.3	3.7	2.7	6.2	-2.1
							-0.7
Animal	84	85	86	87	88	89	90
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.7	0.7	-3.7	9.1	4.6	11.7
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	84	85	86	87	88	89	90
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.8	1.4	9.6	1.9	2.2	1.7
	16	1.7	2.8	9.9	4.6	4.3	2.4
							-2.8
Animal	91	92	93	94	95	96	
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.8	3.9	-1.7	7.5	3.9	1.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	91	92	93	94	95	96	
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.3	3.1	10.2	2.7	6.2	3.0
	16	-1.2	2.3	9.8	3.2	3.3	8.1

BODY WEIGHT GAIN (%)

MAIN STUDY

FEMALES

Group 3 (MID DOSE)

Animal		97	98	99	100	101	102	103
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.2	6.0	3.1	3.5	0.7	6.8	4.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal		97	98	99	100	101	102	103
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.9	3.5	2.1	5.0	6.3	3.0	-6.3
	16	-9.7	-0.8	-5.8	-8.7	-4.2	-6.1	---

Animal		104	105	106	107	108	109	110
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.7	4.8	0.3	2.6	7.4	6.4	7.3
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal		104	105	106	107	108	109	110
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.6	-11.3	-4.2	-2.5	1.5	3.4	-1.3
	16	---	---	---	---	---	---	3.2

Animal		111	112	113	114	115	116	117
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.5	5.2	3.7	0.5	-1.0	13.9	9.6
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

Animal		111	112	113	114	115	116	117
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TREATMENT

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.9	1.3	2.1	2.7	0.4	-1.0	0.5
	16	-0.2	2.9	2.3	5.3	4.3	4.1	4.9

Animal		118	119	120	121	122	123	124
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ACCLIMATISATION

Day	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.3	10.4	5.9	-1.5	3.8	4.5	5.1
	12	---	---	---	---	---	---	---
	19	---	---	---	---	---	---	---

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 2 (LOW DOSE)

Animal	70	71	72	73	74	75	76
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.2	2.9	-0.6	-2.6	4.7	3.4
	16	-0.7	2.6	1.3	-0.2	2.1	1.6
							-0.7
Animal	77	78	79	80	81	82	83
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.3	9.1	3.8	4.6	3.3	4.9
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	77	78	79	80	81	82	83
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.1	-0.8	3.2	-0.2	7.1	-2.7
	16	1.7	1.3	3.7	2.7	6.2	-2.1
							-0.7
Animal	84	85	86	87	88	89	90
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.7	0.7	-3.7	9.1	4.6	11.7
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	84	85	86	87	88	89	90
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.8	1.4	9.6	1.9	2.2	1.7
	16	1.7	2.8	9.9	4.6	4.3	2.4
							-2.8
Animal	91	92	93	94	95	96	
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.8	3.9	-1.7	7.5	3.9	1.6
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	91	92	93	94	95	96	
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	0.3	3.1	10.2	2.7	6.2	3.0
	16	-1.2	2.3	9.8	3.2	3.3	8.1

BODY WEIGHT GAIN (%)
MAIN STUDY
FEMALES

Group 3 (MID DOSE)

Animal	97	98	99	100	101	102	103
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	8.2	6.0	3.1	3.5	0.7	6.8
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	97	98	99	100	101	102	103
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	3.9	3.5	2.1	5.0	6.3	3.0
	16	-9.7	-0.8	-5.8	-8.7	-4.2	-6.1

Animal	104	105	106	107	108	109	110
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	1.7	4.8	0.3	2.6	7.4	6.4
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	104	105	106	107	108	109	110
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.6	-11.3	-4.2	-2.5	1.5	3.4
	16	---	---	---	---	---	---
							3.2
Animal	111	112	113	114	115	116	117
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	5.5	5.2	3.7	0.5	-1.0	13.9
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---
Animal	111	112	113	114	115	116	117
TREATMENT							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	-3.9	1.3	2.1	2.7	0.4	-1.0
	16	-0.2	2.9	2.3	5.3	4.3	4.1
							4.9
Animal	118	119	120	121	122	123	124
ACCLIMATISATION							
Day	1	0.0	0.0	0.0	0.0	0.0	0.0
	8	2.3	10.4	5.9	-1.5	3.8	4.5
	12	---	---	---	---	---	---
	19	---	---	---	---	---	---