

AR226 - 1056

252pp

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September 19, 2001

2001 SEP 26 AM 11:55

51903

CERTIFIED MAIL

3M

Document Processing Center (7407)
Attn: Section 8(e) Coordinator
Office of Toxic Substances
US Environmental Protection Agency
410 M Street, SW
Washington, DC 20460

8EHQ-0901-14523

Re: TSCA 8(e) SUBSTANTIAL RISK NOTICE (SUPPLEMENTAL) ON:
Perfluorobutanesulfonyl Fluoride: CAS# 375-72-4

Dear Sir:

3M has conducted a 28-day repeat dose inhalation study with perfluorobutanesulfonyl fluoride (PBSF) in rats at concentrations of 47, 162 and 479 ppm (6hrs/day, 5 days/week). Animals exposed to PBSF exhibited clinical signs consistent with neurotoxicity at all levels. Clinical signs were observed early in the exposure and had resolved by the next day. The functional observational battery and histopathological results were within normal limits. The mechanism of action for this neurotoxicity is not known. PBSF associated neurotoxicity was reported in a mouse inhalation toxicity screen (TSCA 8e Substantial Risk Notice, dated 30 July 1999) and in a rat acute inhalation study (TSCA 8e Substantial Risk Notice, dated September 28, 2000).

3M has established a workplace exposure limit (8-hr TWA) of 0.1 mg/m³ (0.008 ppm) for PBSF. 3M uses PBSF safely as a site limited intermediate with the following industrial hygiene practices: supplied air respiratory protection, chemical protective clothing, and local exhaust ventilation during charging.

Please contact me at 651.733-9218, for further information.

Sincerely



Larry R. Johnson, D.V.M., Ph.D., DABT
Director - Corporate Toxicology

Contain NO CBI

Enclosure: Complete Report: "T-7499 TOXICITY STUDY BY REPEAT DOSE
INHALATION ADMINISTRATION TO CD RATS FOR 4 WEEKS"



8EHQ-99-14523



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CONFIDENTIAL

MIN 252/004312

T-7499

**TOXICITY STUDY BY REPEAT DOSE INHALATION ADMINISTRATION TO CD
RATS FOR 4 WEEKS**

Sponsor

3M Medical Department,
Toxicology Services,
3M Center Building 220-2E-02,
P.O. Box 33220,
St. Paul,
Minnesota 55133-3220,
USA.

Research Laboratory

Huntingdon Life Sciences Ltd.,
Woolley Road,
Alconbury,
Huntingdon,
Cambridgeshire,
PE28 4HS,
ENGLAND.
Report issued: 22 August 2001

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid:

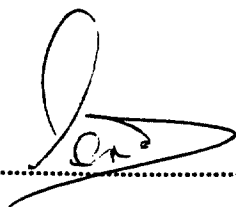
The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive, 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

United States Environmental Protection Agency, (TSCA) Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Information regarding test substance characterisation, namely the batch number and expiry date, was not available to Huntingdon Life Sciences for compliance with the Good Laboratory Practice regulations given above.



.....

Derek W. Coombs, B.Sc., M.Sc.,
Study Director,
Huntingdon Life Sciences Ltd.

22 August 2001
.....

Date



.....

Sponsor,
3M Toxicology Services.

28 August 2001
.....

Date

.....
Submitter.

.....
Date

QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study:

Study Phases Inspected	Date of Inspection	Date of Reporting
Protocol audit	31 October 2000	31 October 2000
Study based inspections		
Study preparation)	13 November 2000	13 November 2000
Exposure)		
Test substance control)		
Sampling)		
Clinical signs)		
Records audit)		
Blood sampling	8 December 2000	8 December 2000
<i>Post mortems</i>)	11 December 2000	11 December 2000
Records audit)		
Report audit	6 April 2001	9 April 2001

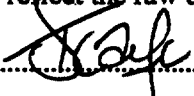
Protocol: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Study based inspections: Inspections and audits of phases of this study were conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections and audits of other routine and repetitive procedures employed on this type of study were carried out. These were promptly reported to appropriate Company Management.

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.

.....


Tracy Scarfe,
 Group Manager,
 Department of Quality Assurance,
 Huntingdon Life Sciences Ltd.

22 August 2001

Date

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SUMMARY

Three groups of rats (each of 5 males and 5 females) of the Crl:CD® BR strain were exposed to T-7499, 6 hours a day for 5 consecutive days a week for 4 weeks using a whole-body exposure system. A fourth group, acting a control, was exposed to air only.

The study mean analysed concentrations of T-7499 were 47, 162 and 459 ppm for the Low, Intermediate and High dose groups respectively.

The following comments are made in summary:

Clinical signs observed during exposure included circling movement and lethargy.

Clinical signs observed immediately post exposure included vocalising and agitation when handled, walking on toes (abnormal gait) and hyperactivity. These signs were consistent with a neurotoxic effect and generally resolved prior to exposure the following day.

The overall mean bodyweight gains for all test rats (Weeks 0 to 4) were lower than controls, attaining a degree of statistical significance for all test males.

A reduction in food consumption was evident for Groups 4 male rats.

There was no effect of treatment on the functional observational battery or haematological and blood chemistry parameters.

Necropsy revealed no treatment-related macroscopic findings and no treatment-related differences in organ weights.

Histopathological examination of the respiratory tract revealed no treatment-related findings.

Conclusion

A no observed effect level (NOEL) was not established during this study. However, clinical signs consistent with a transient effect on the nervous system had generally resolved prior to exposure the following day and there was no evidence of sustained neurotoxicity in the functional observation battery.

INTRODUCTION

The purpose of this study performed at Huntingdon Life Sciences Limited, Huntingdon, England was to assess the systemic toxic potential in rats to repeat administration by inhalation, in whole-body exposure chambers, of the test substance T-7499, for 5 consecutive days a week for 4 weeks.

The study was performed in compliance with the guidelines of the Organisation for Economic Co-operation and Development: Testing of Chemicals (412).

The test substance was administered by inhalation, a possible route for accidental exposure in man. The rat was the species of choice due to regulatory requirements and the strain was selected on account of the availability of comprehensive background data, relating to clinical and pathological parameters, at our laboratories.

Exposure levels were selected on the basis of consultation with the Sponsor and on the results obtained from a preliminary inhalation toxicity study (HLS study number MIN 251/003341).

The in-life experimental phase of the study was undertaken between 13 November and 30 January 2001.

RELEVANT STUDY DATES

Approved by:

Study Director	26 October 2000
HRC Management	26 October 2000
Study Sponsor	30 October 2000

Animals arrived at HRC: 1 November 2000

Allocation to groups: 1 November 2000

Exposures commenced: 13 November 2000

Functional observation battery (FOB) and Motor activity testing (MAT):

Week -1 (Full FOB/MAT)	9 and 10 November 2000
Week 1 (Short FOB)	18 November 2000
Week 2 (Short FOB)	25 November 2000
Week 3 (Short FOB)	2 December 2000
Week 4 (Full FOB/MAT)	9 and 10 December 2000

Haematology/blood chemistry:

Week 4	8 December 2000
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Terminal kill:

Week 5	11 December 2000
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Histopathology completed: 30 January 2001

TEST SUBSTANCE

Tradename: T-7499

Chemical name: Perfluorobutyl sulfonyl fluoride

Other name: PBSF

Intended use: None stated

Appearance: Clear colourless liquid (presented in a steel pressure vessel)

Storage conditions: Ambient temperature or in a refrigerator, unless otherwise stated by the Sponsor

Amount received: Ca. 35 kg

Batch number: None stated

Assay: Perfluorobutyl sulfonyl fluoride 96-98%
Perfluorosulfolane 2-4%

Expiry date: None stated

Date received: 26 April 2000

Supplier: Sponsor

EXPERIMENTAL PROCEDURE

ANIMALS

Fifty (25 male and 25 female) rats, aged approximately 6 weeks, of the Crl:CD[®] BR, a caesarean derived strain of Sprague-Dawley origin, were obtained from Charles River (UK) Limited, Manston Road Margate, Kent, on 1 November 2000.

On arrival, all animals were examined for abnormalities and signs of overt ill health and randomly allocated to 1 of 4 groups, each of 5 males and 5 females. The animals were then uniquely identified by numbers tattooed on the tail. The animals were acclimatised for at least 7 days before commencement of treatment.

The identification of individual rats in the 4 groups were as follows:

Group	Rat numbers	
	Male	Female
1 (Control)	16 – 20	31 – 35
2 (Low dose)	6 – 10	36 – 40
3 (Inter. dose)	11 – 15	26 – 30
4 (High dose)	1 – 5	21 – 25
Reserve	A – E	F – J

In view of the need for the Functional Observational Battery to be performed without knowledge of the treatment groups, the animal numbering system was such that it was not easy to identify a treatment group from the animal numbers.

The remaining animals, 5 males and 5 females, were assigned to the reserve group. These were identified by a letter written on the tail and were retained as potential replacements during the acclimatisation period. Following the commencement of exposures the reserves were killed.

ACCOMMODATION

The rats were housed 5 of the same sex to a cage in suspended stainless steel cages fitted with mesh front, back and floor with stainless steel sheet sides. The cages were suspended on racks. Plastic trays lined with absorbent paper were placed below each cage to collect animal excreta and the paper was changed daily. Each cage had a coloured label identifying the group and the numbers of the animals contained within it. The rats were kept in a single room and, additionally, after the start of the exposure period, each group was positioned on an individual cage battery. Each battery was in a separate ventilated cabinet within the holding room in order to avoid the possibility of inhalation of test material from the rats in other groups. Exposure took place in the same room.

The temperature and relative humidity of the holding room were recorded using a Kent Clearspan recorder. The study holding room temperature and relative humidity were set to be maintained within limits of $21 \pm 2^\circ\text{C}$ and $55 \pm 10\%$ respectively. Recorded ranges were 19.5 to 21.0°C and 39 to 58% relative humidity. Deviations from these ranges were of relatively short duration and considered not to have affected the scientific integrity of the study.

Lighting was controlled to give 12 hours light (0600 - 1800 hours) and 12 hours dark per 24 hours.

DIET

While in their cages, all rats had access to a weighed quantity of standard quality-controlled laboratory rat food (SDS Rat and Mouse No. 1 SQC modified maintenance diet, Special Diets Services, Witham, Essex).

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet. The analytical data have been lodged in Huntingdon Life Sciences Archives.

Tap water was available from moulded polypropylene water bottles at all times while the rats were in the cages. The water bottles were emptied and refilled daily and thoroughly cleaned at intervals during the study.

There was no information available to indicate that any substance likely to influence the effect of the test system could reasonably be expected to be present in the drinking water.

Results of the routine physical and chemical analyses of water at source (sampling point, Grafham Final Water) as conducted by the supplier, Anglian Water Services Ltd, have been made available to Huntingdon Life Sciences. Anglian Water takes its guidelines on water quality from the EEC directive relating to water for human consumption, viz. Council Directive 80/778/EEC.

The analytical data have been lodged in Huntingdon Life Sciences Archives.

ADMINISTRATION

The vaporous test substance was administered for 6 hours a day, for 5 consecutive days a week, for 4 weeks.

The rats were exposed to the control/test atmosphere in whole-body exposure chambers constructed from stainless steel and glass, with an internal volume of 0.75 m^3 . The test atmosphere was produced by metering the test liquid into stainless steel coil vapour generators and was diluted with clean air prior to the resultant vapour atmosphere passing into the exposure chamber.

The target concentrations for exposure were 50 ppm (Low dose), 150 ppm (Intermediate dose) and 450 ppm (High dose). Control rats received air only.

Details of administration and analysis of the test atmospheres together with the results obtained are presented in **ADMINISTRATION OF T-7499 BY INHALATION TO RATS** appended to this report.

CLINICAL INVESTIGATIONS

Dated and signed records of all activities relating to the day to day running and maintenance of the study, as well as to the group observations and examinations outlined in this procedure were recorded in the Study Daybook. In addition, observations relating to individual animals were made throughout the study were recorded.

Mortality

Throughout the study, all cages were checked in the morning and again at the end of the normal working day for dead or moribund animals.

Clinical signs

Dated and signed records of appearance, change and disappearance of clinical signs were maintained. Individual animal records were maintained on the basis of:

- any observation, considered to be of possible importance, made at any time during the study;
- any observation, considered to be of possible importance, made during transfer to exposure cages (prior to exposure), on return to holding cages (after exposure) and during daily checks;
- a careful examination made weekly commencing 1 week prior to the start of exposures when special attention was given to the detection of available respiratory sounds.

During exposure signs were recorded as a group response where all visible animals appeared to be responding similarly to the test substance. In the clinical signs individual data, death code 7 refers to terminal kill.

BODYWEIGHT

Each rat was weighed on arrival following random allocation. The weight of each rat was recorded weekly, commencing 1 week prior to the start of exposure. During the treatment period, bodyweight was recorded before exposure on the day. Bodyweight was also recorded prior to necropsy.

FOOD CONSUMPTION

The quantity of food consumed by each cage of rats was recorded weekly, commencing 1 week prior to the start of exposures until the end of the study.

FUNCTIONAL OBSERVATION BATTERY

Neurobehavioural screening

During the study, a functional observational battery and motor activity were performed at approximately the same time of day. Not all rats were tested in one day (observations made pre-treatment and in Week 4), but time of testing was balanced across the groups. Observations were made during the treatment period (on days when not exposed) and prior to study initiation.

A full functional observational battery was performed during the pre-treatment period, and during Week 4. A shortened battery was performed during Weeks 1, 2 and 3. The functional observational battery is detailed below:

The battery comprised 4 sets of observations. The first set was performed when initially handling the animal. The second set of observations was performed in the test arena and the final set (pre-treatment and Week 4 only) comprised handling/specific testing of the animal. All these observations were made with the observer blind to the treatment condition of the animal.

Observations in the hand:

- Ease of removing the animal from the cage
- Reactivity to handling (ease of handling)
- Occurrence of convulsions, tremors, twitches
- Salivation/lacrimation
- Palpebral closure
- Exophthalmus
- Piloerection
- Fur appearance
- Vocalisation on handling

Observation in the arena:

- Occurrence of convulsions, tremors, twitches
- Activity counts
- Level of arousal
- Rearing count
- Grooming
- Piloerection
- Assessment of gait/posture
- Record presence of faecal boluses, urine

Manipulations†:

Approach response
 Touch response
 Auditory startle response
 Righting reflex
 Tail pinch response
 Pupil reflex
 Grip strength (fore and hindlimb)
 Landing footsplay
 Body temperature (°C)
 Bodyweight (g)

At any point during the observations, additional comments were made as free text where considered appropriate.

† The manipulations were only made during the pre-treatment period and Week 4.

Although bodyweight was recorded, no discussion of any possible effects of treatment on bodyweight is presented in this section as this has been covered elsewhere in the report.

For the observations, if all animals in all groups failed to show a given sign such as lacrimation this sign has been omitted from presentation in the report although it has been recorded on the raw data sheet.

Motor activity was performed before initiation of treatment and during the fourth week of treatment and was monitored using a Coulbourn Infra-Red Activity Monitoring System¹.

This system uses an infra-red detector to monitor activity. The following categories of activity are recorded: the time spent in no movement, locomotor and non-locomotor activity. The number of occurrences (events) of each category is also recorded. For reporting this data, only the time spent in locomotor activity is presented.

For testing, designated animals were placed singly into observation cages. Once all animals had been placed into the cages, the test session programme was started. The test session for each animal was 1 hour. Data was collected every 2 minutes and written onto a floppy disk.

The functional observational battery was performed in Room 011 and the motor activity monitoring was performed in Room 007.

Analysis and presentation of the behavioural screening data

The following data were routinely subjected to statistical analysis: rearing and activity counts, grip strength, hind limb splay, bodyweight and temperature. These data were analysed using a one-way analysis of variance followed by Williams' test (Williams 1971/2) for a dose-related response. Pre-treatment data were analysed by analysis of variance followed by Student's 't' test.

1 System supplied by Coulbourn Instruments, Lehigh Valley, PA, U.S.A.

The reporting of the categorical data for the observational battery has been handled in the following manner. The observational endpoints such as ease of handling, arousal, etc., have been tabulated for frequency of occurrence for each group. Although during recording, some responses were classified in terms of the degree or type of response (i.e. startle: no reaction, an ear twitch, a flinch, etc.), for the purposes of reporting, as there were no remarkable differences between the groups, for a given endpoint the response has been reported as being either present or absent.

As there were no remarkable differences in the incidence of observations, no statistical analyses were performed on the categorical data.

The Coulbourn activity data were analysed using a one-way analysis of variance followed by Williams' test (Williams 1971/2) for a dose-related response. Pre-treatment data were analysed by analysis of variance followed by Student's 't' test.

When the categorical data suggested a possible difference between control animals and treated groups, the data was analysed using Jonckheere-Tepstra test (Hollander & Wolfe, 1973; StatXact, 1992). A two tailed test has been reported, unless the responses were directional in nature.

Key to the functional observational battery

Ease of removal from cage

- 2 easy (little resistance)
- 3 slightly awkward

Ease of handling

- 2 easy (little resistance)
- 3 slightly awkward

Salivation (only scored if present)

- Y sign observed

The numbers associated with salivation indicate the degree of effect
(1, 2, 3 increasing degree of effect)

- N sign not observed

Arousal

- 3 reduced alertness
- 4 alert (normal)
- 5 somewhat high (slight excitement, tense, over reaction to external stimuli)

Gait

- T walking on toes
- Hu hunched
- U unable to assess - see additional comments
- A abnormal
- normal gait

The numbers associated with gait indicate the degree of effect
(1, 2, 3 increasing degree of effect)

Mobility impaired: mobility of the rat impaired due to gait abnormalities

Approach

- 1 no reaction
- 2 sniffs only
- 3 approaches and sniffs
- 4 freezes
- 5 back/turns away
- 6 walks past probe
- O other reaction - see additional comments

Touch

- 1 no reaction
- 2 turns
- 3 walks away
- 4 freezes
- 5 turns to opposite side
- 6 walks backwards
- O other reaction - see additional comments

Startle

- 1 no reaction
- 2 ear twitch only
- 3 normal flinch
- 4 noticeable response
- 5 exaggerated response
- O other reaction - see additional comments

Tail pinch

- 1 no reaction
- 2 turns
 - 2 turns immediately
 - 3 violent turn
- 3 walks away
- 4 freezes
- 5 jumps forward
- 6 runs away
- O other reaction - see additional comments

A 1 with a response that is not a turn indicates that the response included a turn such as walks away with a turn

Righting reflex

- 1 immediate reaction

Vocalising

- Y sign observed
- N sign not observed

The numbers associated with vocalising indicate the degree of effect (1, 2, 3 increasing loudness of vocalising)

Pupil reflex

- B reflex observed both eyes

Urine

- N none observed
- S small amount observed
- M moderate amount observed
- L large amount observed

Rearing and activity counts

Counts were made of rearing and activity when the animals were in the arena. A count for rearing was counted every time the animal lifted both fore feet clear of a supporting surface. The floor of the arena was marked off into 6 equal areas ("squares"). A count for activity was made whenever the animal moved all four feet into one of these squares.

LABORATORY INVESTIGATIONS

Sample collection

Blood samples for haematology and blood chemistry were collected from all animals following overnight deprivation of food, prior to exposure on Day 26.

Samples of venous blood were withdrawn from the retro-orbital sinus using sterile glass pipettes while the rats were held under isoflurane anaesthesia.

The blood samples collected were put into tubes containing the following anticoagulants:

- EDTA - for haematological investigations (0.5 ml whole blood)
- Citrate - for clotting tests (0.5 ml whole blood)
- Heparin - for blood chemical investigations (0.7 ml whole blood)

The haematological and blood chemical investigations performed are listed below, together with an abbreviated title (for use in appendices and tables), methods and the units of measurement.

Haematology

The following estimations were performed using a Bayer-Technicon H*1E haematology analyser:

	Units
Haematocrit (Hct)	L/L
Haemoglobin (Hb)	g/dL
Red cell count (RBC)	$\times 10^{12}/L$
Mean corpuscular haemoglobin (MCH)	pg
Mean corpuscular haemoglobin concentration (MCHC)	g/dL
Mean corpuscular volume (MCV)	fL
Total white cell count (WBC)	$\times 10^9/L$
Differential WBC count	
Neutrophils (Neutrophil)	$\times 10^9/L$
Lymphocytes (Lymphocyte)	
Eosinophils (Eosinophil)	
Basophils (Basophil)	
Monocytes (Monocyte)	
Large unstained cells (LUC)	

Units

Cell morphology: the most common morphological changes (anisocytosis, micro/macrocytosis, hypo/hyperchromasia) were recorded as follows:

-	=	no abnormalities detected
+	=	slight
++	=	moderate
+++	=	marked

Platelet count (Plt)	$\times 10^9/L$
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The following estimations were performed using the appropriate methodology, as described below:

Reticulocyte count (Retic) – Sysmex R3000 Reticulocyte Counter	% (of red cells)
--	------------------

The following were performed using an ACL 3000 Plus analyser using the appropriate IL reagents:

Prothrombin time (PT) - method of Quick, H.A., (1942)	sec
---	-----

Activated Partial Thromboplastin Time (APTT) - method of Proctor, R.R and Rapaport, S.I., (1961)	sec
--	-----

Blood chemistry

The following parameter was analysed with an Hitachi 917 clinical chemistry analyser using, except where indicated, standard Hitachi 917 methodologies:

Triglycerides (Trig)	mmol/L
Total protein (Total Prot)	g/L
Protein electrophoretogram (Beckman Paragon system)	
Albumin (Alb)	g/L and %
Alpha 1 globulin (a1 Glob)	g/L and %
Alpha 2 globulin (a2 Glob)	g/L and %
Beta globulin (Beta Glob)	g/L and %
Gamma globulin (Gamma Glob)	g/L and %
Albumin/globulin ratio (A/G Ratio)	

	Units
Urea (Urea)	mmol/L
Alkaline phosphatase (Alk. Phos) - reaction temperature 37°C	U/L
Bilirubin - total (Bili. Total)	umol/L
Creatinine (Creat)	umol/L
Sodium (Na)	mmol/L
Potassium (K)	mmol/L
Calcium (Ca Total)	mmol/L
Phosphorus (Phos)	mmol/L
Chloride (Cl)	mmol/L
Cholesterol - total (Chol Total)	mmol/L
Glucose (Gluc) - hexokinase mediated	mmol/L
Alanine aminotransferase (ALT) - reaction temperature 37°C	U/L
Aspartate aminotransferase (AST) - reaction temperature 37°C	U/L

SACRIFICE

All groups were killed following 5 consecutive days of exposure a week, for 4 weeks. The terminal kill was performed on Day 29 of the study.

Animals were killed by an intraperitoneal injection of pentobarbitone sodium followed by exsanguination from the brachial arteries.

MACROSCOPIC EXAMINATION AND ORGAN WEIGHTS

All rats were subjected to a detailed macroscopic examination.

The following organs from all animals killed at the scheduled sacrifice were dissected free of fat and weighed:

adrenals	liver
epididymides	lungs
heart	testes
kidneys	

Bilateral organs were weighed together.

FIXATION OF TISSUES

Samples, or the whole, of the following organ/tissues, together with any macroscopically abnormal entities were preserved in buffered 10% formalin, except the eyes, which were preserved in Davidson's fixative, and testes and epididymides which were fixed in Bouin's solution and then transferred to 70% alcohol.

abnormalities*	harderian glands	pituitary
adrenals (cortex and medulla)*	head ^a	prostate
alimentary tract	heart (including auricular and	salivary glands
oesophagus	ventricular regions)*	sciatic nerves
stomach	kidneys (including cortex,	seminal vesicles
duodenum	medulla and papilla regions)*	skeletal muscle (thigh)
jejunum	lachrymal glands	skin
ileum	larynx (2 levels)*	spinal cord
caecum	liver (all lobes)*	spleen*
colon	lungs (all lobes)*	sternum
rectum	lymph nodes (mandibular,	testes*
animal identification mark	mesenteric and	thymus
aorta (thoracic)	tracheobronchial)	thyroids with parathyroids
brain	mammary area (caudal)	tongue
bronchi*	nasal turbinates (3 levels)*	trachea (including bifurcation)*
epididymides*	optic nerves	urinary bladder
eyes	ovaries	uterus with cervix
femur	pancreas	vagina

^a Including nasal cavity, paranasal sinuses and nasopharynx

HISTOPATHOLOGY

Histopathological examinations were performed on all scheduled tissues (marked with *) for Groups 1 and 4. These tissues were embedded in paraffin wax and sections approximately 4-5 μm thick were cut, processed and stained with haematoxylin and eosin for examination by light microscope. Sections cut from the testes were stained with PAS-haematoxylin.

For testes tissues only (animal numbers 1-5 and 16-20), 0.5% (v/v) hydrochloric acid was used in the staining methodology to remove non-specific haematoxylin staining.

Histopathological examination was only performed on any abnormal tissues arising from Groups 2 and 3.

STATISTICAL ANALYSIS

All statistical analyses were performed separately for males and females.

For all parameters the analyses were performed using the individual animal as the experimental unit. Bodyweight data were analysed using weight gains. The following sequence of statistical tests was used for bodyweight, organ weight and clinical pathology data.

If the data consist predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise:

Bartlett's test was applied to test for heterogeneity of variance between treatments; where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), and more than two groups were being compared, group means were compared using Williams' test for a dose-related response (Williams, 1971 and 1972), or if there was evidence for a non-monotonic response, Dunnett's test (Dunnett, 1955 and 1964). For separate two-group comparisons, a Student's *t* test was used.

If significant heterogeneity of variance was present (and could not be removed by a logarithmic transformation), groups were compared using Shirley's non-parametric test for a dose-related response (Shirley, 1977), or if there was evidence for a non-monotonic response, Dunn's test (Dunn, 1964). For separate two-group comparisons, a Wilcoxon rank sum test (Wilcoxon, 1945) was used.

Where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. For organ weight data, the final bodyweight was used as covariate in an attempt to allow for differences in bodyweight which might influence the organ weights.

For microscopic findings Fisher's exact test was employed to detect treatment-related differences.

LOCATION OF STUDY RECORDS

All raw data, samples and specimens arising from the performance of this study will remain the property of the Sponsor.

Types of sample and specimen that are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences, Standard Operating Procedures.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences in its archive for a period of five years from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Chamber analysed concentration of T-7499

The data are presented in ADMINISTRATION OF T-7499 BY INHALATION TO RATS

The data are summarised below:

Group	Chamber concentration (ppm)		
	Target	Analysed	
		Mean	sd
2 (Low dose)	50	47	9
3 (Inter. dose)	150	162	22
4 (High dose)	450	459	21

The achieved chamber concentrations for Groups 2 to 4 were in good agreement with the target concentrations.

CLINICAL OBSERVATIONS

Mortality

There were no unscheduled deaths.

Clinical signs

The data are presented as follows:

Table 1	- during exposure – group distribution of observations
Table 2	- post exposure – incidence summary
Appendix 1	- pre and post exposure – individual daily observations
Appendix 2	- pre-exposure – individual weekly observations

Clinical signs observed as a group response during exposure included circling movement for Group 4 on Day 1 of the exposures. Lethargy was evident for Groups 3 and 4 during the exposures.

Clinical signs observed immediately post exposure included vocalising and agitation when handled for Groups 2, 3 and 4. Walking on toes (abnormal gait) was evident following the exposures for Groups 3 and 4. Hyperactivity was observed following the exposures for Groups 3 and 4 during Weeks 2 and 3. This sign was also evident following the exposures for Group 2 rats during Week 3.

On three occasions signs were present prior to exposure. Agitation when handled was present for a Group 3 female rat prior to exposure on Day 17. Vocalising and agitation when handled were present for two Group 3 female rats prior to exposure on Day 19. Walking on toes (abnormal gait) was present for Group 4 female rats prior to exposure on Day 26. At all other times, all of the above signs had resolved prior to exposure the following day.

At other times, dry skin abrasions to tails, irritable behaviour and pale coloured teeth were observed. These signs are considered incidental and not related to treatment.

Bodyweight

The data are presented as follows:

- Figure 1 - group mean values (g)
- Table 3 - group mean values (g)
- Appendix 3 - individual values (g)

The overall mean bodyweight gains for all test rats (Weeks 0 to 4) were lower than controls, attaining a degree of statistical significance for all test males.

Food consumption

The data are presented as follows:

- Table 4 - group mean values (g/animal)

A reduction in food consumption was evident for Group 4 male rats.

The food consumption of other test rats was considered not to be affected by treatment.

Functional observation battery

The data are presented as follows:

- Table 5 - group summary of observations
- Table 6 - activity counts – group mean values
- Table 7 - rearing counts – group mean values
- Table 8 - grip strength – group mean values
- Table 9 - landing footsplay – group mean values
- Table 10 - temperature – group mean values
- Table 11 - bodyweights – group mean values
- Table 12 - coulbour activity monitoring
- Appendix 4 - individual observations
- Appendix 5 - total time spent in locomotor activity – individual values (seconds)
- Appendix 6 - additional comments – individual observations

Functional observational battery findings for animals treated with T-7499 for four weeks, at exposure levels up to 450 ppm, showed no evidence of neurotoxicity. The following comments are made in summary.

In the hand observations

In the hand observations were unaffected by treatment.

Arena observations

Arena observations showed some inter-group variation but the differences were not considered to be treatment-related.

Compared with controls, females in all treated groups showed reduced activity and rearing scores in the arena during Week 2 of treatment, although scores for males were unaffected. In the absence of similar response patterns during preceding or subsequent weeks of treatment, these differences were considered to be due to natural variation.

Manipulations

During Week 4 of treatment, landing footsplay measurements for females receiving 150 or 450 ppm were lower than those of controls. Mean values for these groups were, however, also somewhat reduced before commencement of treatment and these differences were therefore not considered to be associated with treatment.

Motor activity

The time spent in locomotor activity for males receiving 150 ppm was lower than that shown by control animals during Week 4 of treatment whereas, in contrast, females receiving 50 or 150 ppm showed increased activity times. These differences failed to achieve statistical significance and, in view of the lack of dosage-relationship and the opposing directions of change, were considered to be due to natural variation.

LABORATORY INVESTIGATIONS

Haematology

The data are presented as follows:

Table 13	- group mean values
Appendix 7	- individual values

There were no treatment-related findings.

Blood chemistry

The data are presented as follows:

Table 14	- group mean values
Appendix 8	- individual values

There were no treatment-related findings.

TERMINAL STUDIES

Macroscopic pathology

The data are presented as follows:

Table 15	- incidence summary
Appendix 10	- individual pathological findings

There were no treatment-related findings.

Organ weights

The data are presented as follows:

Table 16	- group mean values (g)
Appendix 9	- individual values (g)

There were no treatment-related findings.

Microscopic pathology

The data are presented as follows:

Table 17	- expanded incidence summary
Appendix 10	- individual pathological findings

Treatment related findings

There were no microscopic findings that were considered to be related to treatment with T-7499.

Incidental findings

All microscopic findings were considered to be incidental and of no toxicological importance.

DISCUSSION

In this study, rats were exposed by inhalation to the vapour of T-7499 for 6 hours each day, for 5 consecutive days each week, for 4 weeks. The study mean exposure levels were 47, 162 and 459 ppm.

The overall mean bodyweight gain for all test groups was lower than controls. A reduced food consumption was also evident for male animals in Group 4.

Animals exposed at concentrations of 162 ppm (Group 3) and 459 ppm (Group 4) were lethargic during exposure procedures. Clinical signs were also noted for all test groups on occasions post exposure and included vocalisation and agitation when handled and hyperactivity. These signs were consistent with a neurotoxic effect and generally resolved prior to exposure the following day.

There was no effect of treatment on the functional observational battery or haematological and blood chemistry parameters. Necropsy revealed no treatment-related macroscopic findings and no treatment-related differences in organ weights. Histopathological examination of the respiratory tract revealed no treatment-related findings.

A no observed effect level (NOEL) was not established during this study. However, clinical signs consistent with a transient effect on the nervous system had generally resolved prior to exposure the following day and there was no evidence of sustained neurotoxicity in the functional observation battery.

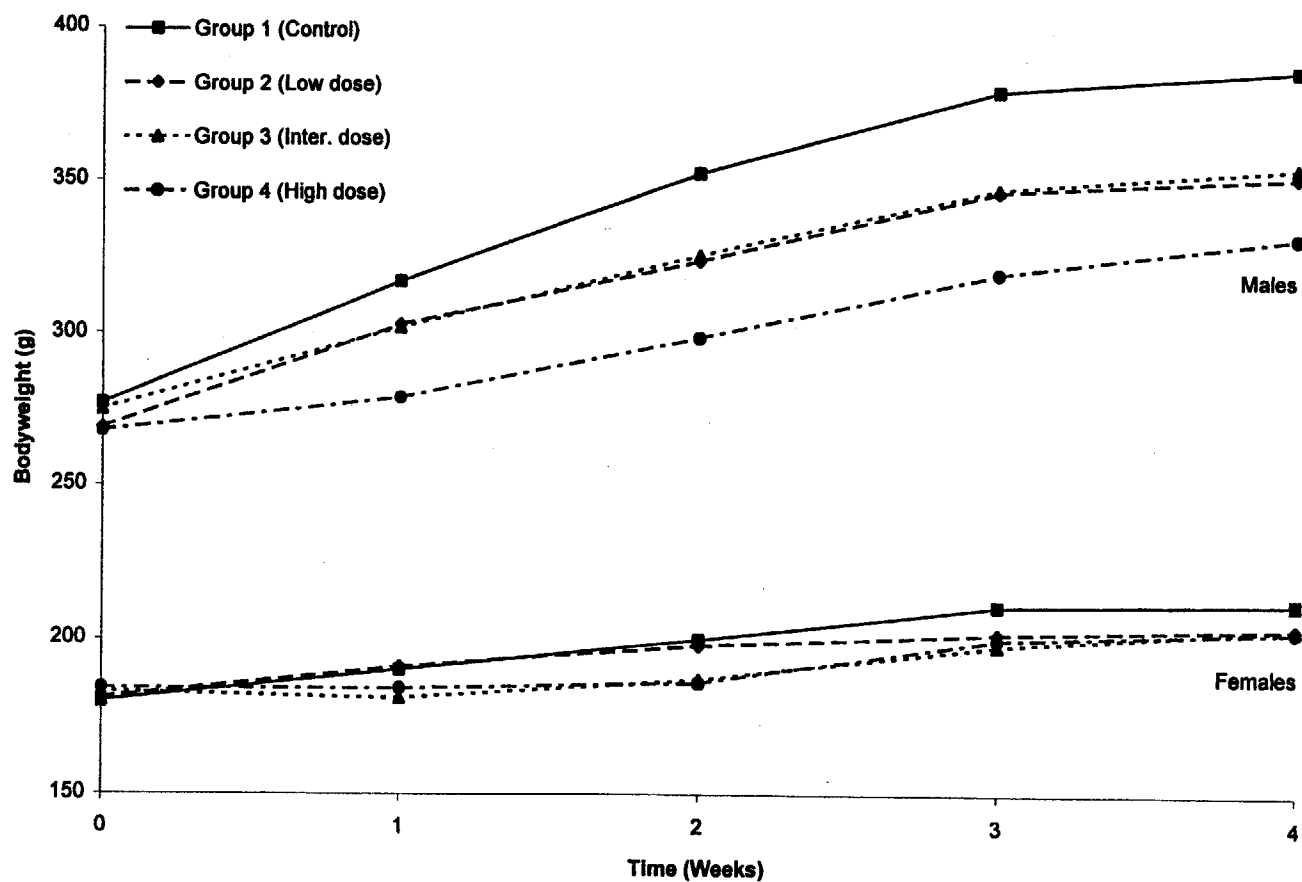
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FIGURE 1

Bodyweights – group mean values (g)



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TABLE 1

Clinical signs during exposure – group distribution of observations

Group	Sign	Exposure number																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 (Control)	No abnormalities detected	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2 (Low dose)	No abnormalities detected	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3 (Inter. dose)	No abnormalities detected										√		√								
	Lethargic	√	√	√	√	√	√	√	√	√		√		√	√	√	√	√	√	√	√
4 (High dose)	Circling movement	√																			
	Lethargic	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

TABLE 2**Clinical signs post exposure – incidence summary**

Group	Sign	Number showing sign																			
		Exposure number																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1M (Control)	No abnormalities detected	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2M (Low dose)	No abnormalities detected	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3M (Inter. dose)	No abnormalities detected	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4M (High dose)	No abnormalities detected	4	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5
	Agitated when handled	1										1									
	Vocalising when handled	1										1									

TABLE 2

(Clinical signs post exposure – continued)

Group	Sign	Number showing sign																			
		Exposure number																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1F (Control)	No abnormalities detected	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2F (Low dose)	No abnormalities detected	5	5	4	4	4	4	4	4	2	5	1	5	4	4	3	4	2	4	3	4
	Agitated when handled			1	1	1	1	1	1	3		3		1 ^a	1	1 ^b	1	3	1	2	1
	Vocalising when handled			1	1	1	1	1	1	3		2			1	2 ^b	1	3	1	2	1
	Hyperactive											3									
3F (Inter. dose)	No abnormalities detected	3	3	4	3	5	2	5	5	1		3	3	3	2	4	4	2	3	3	4
	Agitated when handled	2	1	1			1					2					1				
	Vocalising when handled	2	1	1			1					2			1		1				
	Walking on toes (abnormal gait)	1			2		2						2	2	3	1		3	2	2	1
	Hyperactive									4	5	1									
4F (High dose)	Agitated when handled	5	5	5	5	5	1					2					1				
	Vocalising when handled	5	5	5	5	5	1	2	1			2		2			1				
	Walking on toes (abnormal gait)	5	5	5	5	5	5	5	5	1	5	5	5	5	5	5	5	5	5	5	5 ^c
	Hyperactive									5		1									

^a Also present at the pre-dose observation point for animal number 36F^b Also present at the pre-dose observation point for animal numbers 36F and 40F^c Also present at the pre-dose observation point for all Group 4 female rats

TABLE 3

Bodyweight - group mean values (g)

Print No: 0008

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm): 0 50 150 450

Xyblon protocol number: MIN 252

SEX: ----- MALE -----					SEX: ----- FEMALE -----			
GROUP:	1	2	3	4	1	2	3	4
WEEK								
-1	220	217	216	216	155	158	158	160
0	277	269	275	268	180	181	183	184
1	317	303	302	279	190	191	181	184
2	353	324	326	299	200	198	187	186
3	380	347	348	320	211	202	198	200
4	387	352	355	332	212	204	204	203
Bodyweight gain (g/animal)	*	*		**				
Week 0-4	110	83	80	64	38	23	21	19
sd	10.02	22.0	18.8	21.6	7.9	12.2	7.4	12.5
% of control	-	75	73	58	-	61	55	50

sd Standard deviation

Williams' test: * p< 0.05, **p< 0.01

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TABLE 4

Food consumption - group mean values (g/animal)

Print No: 0009

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm): 0 50 150 450

Xyblon protocol number: MIN 252

SEX: ----- MALE -----				----- FEMALE -----				
GROUP:	1	2	3	4	1	2	3	4
WEEK	-----							
-1	204	197	195	203	144	140	136	143
1	211	196	199	180	131	140	119	123
2	218	192	200	180	139	134	121	125
3	226	192	198	181	137	131	128	136
4	194	185	186	174	139	128	125	130

Cumulative (g/animal)								
Week 1-4	849	765	783	715	546	533	493	514
% of control	-	90	92	84	-	98	90	94

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TABLE 5

Functional observational battery – group summary of observations

Pre-dose

Sex Group Number	Male				Female			
	1 5	2 5	3 5	4 5	1 5	2 5	3 5	4 5
OBSERVATIONS:								
<i>IN THE HAND</i>								
removing from cage, easy	5	5	5	5	5	5	5	5
handling, easy	5	5	4	3	5	5	3	4
salivation	0	0	0	0	0	0	0	0
vocalising	0	0	0	0	0	1	1	0
<i>IN THE ARENA</i>								
grooming	0	0	0	0	0	0	0	0
arousal, alert	5	5	5	5	5	5	5	4
defecation	2	2	3	1	0	0	0	0
urine	1	4	2	1	2	0	0	1
<i>GAIT</i>								
Walking on Toes	0	1	1	3	2	1	3	4
Hunched	0	0	0	0	0	0	0	0
Unable to Assess	0	0	0	0	0	0	0	0
<i>MANIPULATIONS</i>								
approach, a reaction	5	5	5	5	5	5	5	5
touch, a reaction	5	5	5	4	5	4	5	5
startle (present)	4	5	5	5	5	5	5	5
righting, immediately	5	5	5	5	5	5	5	5
tail pinch, a reaction	5	5	5	5	5	5	5	5
pupil reflex	5	5	5	5	5	5	5	5

Numbers reflect the number of animals showing the response

TABLE 5
(Functional observational battery – continued)

Week 1

Sex Group Number	Male				Female			
	1	2	3	4	1	2	3	4
	5	5	5	5	5	5	5	5
OBSERVATIONS:								
<i>IN THE HAND</i>								
removing from cage, easy	5	4	5	5	4	5	5	5
handling, easy	5	5	4	3	5	4	4	3
salivation	0	0	0	0	0	0	2	1
vocalising	0	0	0	0	1	2	1	0
<i>IN THE ARENA</i>								
grooming	0	0	0	0	0	0	0	0
arousal, alert	5	5	5	5	4	5	5	5
defecation	2	3	3	0	0	0	0	0
urine	2	3	1	1	0	1	0	0
<i>GAIT</i>								
Walking on Toes	0	2	2	2	3	3	2	3
Hunched	0	0	0	0	0	0	0	0
Unable to Assess	0	0	0	0	0	0	0	0

Numbers reflect the number of animals showing the response

TABLE 5
(Functional observational battery – continued)

Week 2

Sex Group Number	Male				Female				
	1	2	3	4	1	2	3	4	
	5	5	5	5	5	5	5	5	
OBSERVATIONS:									
IN THE HAND									
removing from cage, easy	5	5	5	5	5	5	5	5	
handling, easy	5	5	5	2	5	3	5	3	
salivation	0	0	0	0	0	0	0	0	
vocalising	0	0	0	0	2	2	0	0	
IN THE ARENA									
grooming	0	0	0	0	0	0	0	0	
arousal, alert	5	5	5	5	5	5	5	5	
defecation	2	3	1	0	0	0	0	0	
urine	2	2	1	3	0	0	0	0	
GAIT									
Walking on Toes	0	1	2	4	5	3	5	5	
Hunched	0	0	0	2	0	0	1	1	
Unable to Assess	0	0	0	0	0	1	0	0	

Numbers reflect the number of animals showing the response

TABLE 5
(Functional observational battery – continued)

Week 3

Week 3

Sex Group Number	Male				Female			
	1	2	3	4	1	2	3	4
	5	5	5	5	5	5	5	5
OBSERVATIONS:								
IN THE HAND								
removing from cage, easy	4	5	5	5	5	5	5	5
handling, easy	5	4	5	4	5	4	3	3
salivation	1	0	1	0	0	2	1	1
vocalising	0	0	0	0	3	5	0	0
IN THE ARENA								
grooming	1	0	0	0	0	0	0	0
arousal, alert	5	5	5	5	5	5	4	5
defecation	0	0	0	0	0	0	0	0
urine	2	3	2	1	0	0	0	0
GAIT								
Walking on Toes	1	2	3	3	4	5	5	5
Unable to Asses	0	0	0	0	0	0	0	0
Hunched	0	0	1	1	0	1	2	1

Numbers reflect the number of animals showing the response

TABLE 5
(Functional observational battery – continued)

Week 4

Sex Group Number	Male				Female			
	1	2	3	4	1	2	3	4
	5	5	5	5	5	5	5	5
OBSERVATIONS:								
<i>IN THE HAND</i>								
removing from cage, easy	5	5	5	5	5	5	5	5
handling, easy	5	4	5	5	5	4	5	5
salivation	0	0	0	1	0	3	0	0
vocalising	0	0	0	0	1	2	0	0
<i>IN THE ARENA</i>								
grooming	0	0	0	0	0	0	0	0
arousal, alert	5	5	5	5	5	5	5	5
defecation	0	1	0	0	0	0	0	0
urine	1	2	2	3	0	0	0	0
<i>GAIT</i>								
Walking on Toes	2	2	0	2	5	5	5	5
Hunched	0	0	0	2	1	1	2	2
Unable to Assess	0	0	1	0	0	0	0	0
<i>MANIPULATIONS</i>								
approach, a reaction	5	5	5	5	5	5	5	5
touch, a reaction	3	5	3	5	4	5	5	5
startle (present)	5	5	5	5	5	5	5	5
righting, immediately	5	5	5	5	5	5	5	5
tail pinch, a reaction	5	5	5	5	5	5	5	5
pupil reflex	5	5	5	5	5	5	5	4

Numbers reflect the number of animals showing the response

TABLE 6**Functional observation battery: Activity counts – group mean values****Pre-dose**

Group	Mean activity counts	
	Males	Females
1	10	12
2	14	13
3	11	13
4	14	12

Week 1

Group	Mean activity counts	
	Males	Females
1	11	15
2	12	14
3	9	14
4	10	10

Week 2

Group	Mean activity counts	
	Males	Females
1	12	17
2	11	9*
3	11	13*
4	11	9**

Week 3

Group	Mean activity counts	
	Males	Females
1	10	16
2	13	14
3	11	14
4	11	12

Week 4

Group	Mean activity counts	
	Males	Females
1	11	13
2	13	11
3	9	11
4	13	10

* $p < 0.05$ Williams Test** $p < 0.01$ Williams Test

TABLE 7

Functional observation battery: Rearing counts – group mean values**Pre-dose**

Group	Mean rearing counts	
	Males	Females
1	6	5
2	7	5
3	5	4
4	6	6

Week 1

Group	Mean rearing counts	
	Males	Females
1	5	7
2	7	5
3	4	7
4	4	5

Week 2

Group	Mean rearing counts	
	Males	Females
1	6	8
2	6	4
3	7	5
4	6	5**

Week 3

Group	Mean rearing counts	
	Males	Females
1	6	7
2	6	5
3	5	6
4	5	6

Week 4

Group	Mean rearing counts	
	Males	Females
1	6	6
2	6	5
3	4	5
4	5	5

** $p < 0.01$ Williams Test

TABLE 8**Functional observation battery: Grip strength – group mean values****Pre-dose**

Group	Grip strength (kg)	
	Males	Females
Forelimbs		
1	0.78	0.77
2	0.73	0.71
3	0.67	0.75
4	0.68	0.66
Hindlimbs		
1	0.69	0.77
2	0.65	0.62
3	0.65	0.69
4	0.69	0.65

Week 4

Group	Grip strength (kg)	
	Males	Females
Forelimbs		
1	1.31	1.10
2	1.17	1.12
3	1.23	1.10
4	1.28	1.08
Hindlimbs		
1	1.06	0.97
2	1.09	1.02
3	1.03	0.96
4	1.14	1.00

No differences of statistical significance ($p > 0.05$)

TABLE 9

Functional observation battery: Landing footsplay – group mean values**Pre-dose**

Group	Mean splay values (cm)	
	Males	Females
1	9.6	10.2
2	10.3	10.5
3	11.9	8.7
4	9.1	8.8

Week 4

Group	Mean splay values (cm)	
	Males	Females
1	11.2	10.0
2	11.0	9.0
3	11.8	7.1*
4	9.0	6.8**

* $p < 0.05$ Williams test** $p < 0.01$ Williams test

TABLE 10**Functional observation battery: Temperature – group mean values****Pre-dose**

Group	Mean temperature (°C)	
	Males	Females
1	38.0	37.8
2	38.0	38.4+
3	38.0	37.9
4	37.9	38.4+

Week 4

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.4
2	37.8	38.5
3	37.3	38.1
4	37.7	38.1

+ $p < 0.05$ Student's 't' -test

TABLE 11

Functional observation battery: Bodyweights – group mean values

Pre-dose

Group	Mean bodyweights (g)	
	Males	Females
1	245	166
2	242	165
3	243	166
4	238	166

Week 4

Group	Mean bodyweights (g)	
	Males	Females
1	384	208
2	349	205
3	351	199
4	323**	198

** $p < 0.01$ Williams Test

TABLE 12**Functional observation battery: Coulbourn activity monitoring – group mean values****Pre-dose**

Group	Large movements (in seconds) during 1 hour observation period	
	Males	Females
1	605	514
2	446	686
3	589	737
4	771	465

Week 4

Group	Large movements (in seconds) during 1 hour observation period	
	Males	Females
1	772	484
2	601	722
3	338	679
4	610	557

No differences of statistical significance ($p > 0.05$)

TABLE 13
Haematology – group mean values

Group		Hct	Hb	RBC	Retic	MCH	MCHC	MCV	WBC	Neutr ophil
		L/L	g/dL	x10-12/L	%	pg	g/dL	fL	x10-9/L	x10-9/L
1M	Mean	0.471	16.4	8.26	3.02	19.9	34.9	57.1	16.84	1.81
	SD	0.0117	0.53	0.235	0.395	0.73	0.52	1.60	3.968	0.633
	n	5	5	5	5	5	5	5	5	5
2M	Mean	0.461	16.3	8.22	2.30	19.8	35.4	56.1	14.87	2.13
	SD	0.0206	0.68	0.287	0.211	0.33	0.33	0.75	2.805	1.259
	n	5	5	5	5	5	5	5	5	5
3M	Mean	0.460	16.2	8.04	2.34	20.1	35.2	57.2	14.88	1.32
	SD	0.0246	0.84	0.402	0.487	0.94	0.51	2.42	3.347	0.362
	n	5	5	5	5	5	5	5	5	5
4M	Mean	0.450	16.1	8.15	2.55	19.7	35.7	55.3	16.14	2.22
	SD	0.0120	0.53	0.423	0.651	0.54	0.54	1.44	1.989	0.547
	n	4	4	4	4	4	4	4	4	4

Williams' test: * $p < 0.05$

TABLE 13
(Haematology – continued)

Group		Lymph ocyte x10-9/L	Eosin ophil x10-9/L	Baso phil x10-9/L	Mono cyte x10-9/L	LUC x10-9/L	Plt x10-9/L	PT sec	APTT sec
1M	Mean	14.46	0.16	0.07	0.18	0.16	959	13.0	18.7
	SD	3.702	0.059	0.038	0.073	0.076	295.6	1.03	1.87
	n	5	5	5	5	5	5	5	5
2M	Mean	12.20	0.13	0.05	0.17	0.18	1116	12.9	18.1
	SD	1.741	0.039	0.017	0.045	0.041	83.1	0.26	2.86
	n	5	5	5	5	5	5	5	5
3M			*						
	Mean	13.18	0.09	0.06	0.12	0.11	1124	13.5	19.5
	SD	3.111	0.013	0.026	0.046	0.062	122.3	0.61	1.05
	n	5	5	5	5	5	5	5	5
4M			*						
	Mean	13.26	0.10	0.07	0.26	0.24	1023	13.3	16.8
	SD	2.032	0.028	0.017	0.050	0.042	245.8	0.80	2.82
	n	4	4	4	4	4	4	5	5

Williams' test: * $p < 0.05$

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TABLE 13

(Haematology – continued)

Group		Hct	Hb	RBC	Retic	MCH	MCHC	MCV	WBC	Neutr ophil
		L/L	g/dL	x10-12/L	%	pg	g/dL	fL	x10-9/L	x10-9/L
1F	Mean	0.433	15.3	7.71	2.52	19.9	35.4	56.1	10.12	0.95
	SD	0.0160	0.40	0.300	0.321	0.49	0.66	0.89	2.122	0.358
	n	5	5	5	5	5	5	5	5	5
2F		*	*							
	Mean	0.409	14.5	7.39	2.03	19.7	35.6	55.4	7.75	0.84
	SD	0.0077	0.40	0.161	0.182	0.66	0.55	1.18	3.126	0.235
	n	5	5	5	5	5	5	5	5	5
3F		*	*						*	
	Mean	0.401	14.4	7.24	2.17	19.9	35.9	55.4	5.70	0.88
	SD	0.0133	0.49	0.204	0.507	0.54	0.13	1.26	1.223	0.296
	n	5	5	5	5	5	5	5	5	5
4F		*	*						*	
	Mean	0.411	14.9	7.47	2.22	19.9	36.2	55.0	7.76	1.25
	SD	0.0209	0.63	0.332	0.712	0.61	0.83	2.25	1.170	0.536
	n	5	5	5	5	5	5	5	5	5

Williams' test: * $p < 0.05$

TABLE 13

(Haematology – continued)

Group		Lymph ocyte x10-9/L	Eosin ophil x10-9/L	Baso phil x10-9/L	Mono cyte x10-9/L	LUC x10-9/L	Plt x10-9/L	PT sec	APTT sec
1F	Mean	8.68	0.18	0.03	0.14	0.14	1071	13.7	17.1
	SD	1.983	0.077	0.012	0.052	0.050	171.9	0.48	1.40
	n	5	5	5	5	5	5	5	5
2F			*	*					
	Mean	6.62	0.10	0.01	0.10	0.08	1049	13.8	16.8
	SD	2.810	0.021	0.009	0.053	0.036	94.5	0.49	2.38
	n	5	5	5	5	5	5	5	5
3F		*	*	*	*	##			
	Mean	4.62	0.09	0.01	0.07	0.04	965	13.5	14.3
	SD	1.105	0.027	0.007	0.025	0.005	155.8	1.47	2.41
	n	5	5	5	5	5	5	5	5
4F		*	**	*	*	#		†	
	Mean	6.27	0.09	0.02	0.07	0.07	1075	14.4	16.3
	SD	1.089	0.036	0.008	0.021	0.016	83.7	0.11	1.46
	n	5	5	5	5	5	5	5	5

Williams' test: * $p < 0.05$, ** $p < 0.01$ Dunnett's test: # $p < 0.05$, ## $p < 0.01$ Shirley's test: † $p < 0.05$

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TABLE 14

Blood chemistry – group mean values

Group		Alk. Phos U/L	ALT U/L	AST U/L	Bili. Total umol/L	Urea mmol/L	Creat umol/L	Gluc mmol/L	Chol Total mmol/L	Trig mmol/L
1M	Mean	436	44	86	2	5.75	43	7.40	1.50	0.71
	SD	109.0	4.3	16.1	0.5	0.728	1.8	0.622	0.181	0.243
	n	5	5	5	5	5	5	5	5	5
2M	Mean	447	43	75	2	5.57	41	7.22	1.69	0.81
	SD	56.8	3.8	5.2	0.5	0.589	2.2	0.622	0.250	0.185
	n	5	5	5	5	5	5	5	5	5
3M	Mean	446	38	72	1	5.22	41	6.81	1.38	0.50
	SD	62.4	5.4	5.5	0.5	0.557	1.6	0.873	0.191	0.140
	n	5	5	5	5	5	5	5	5	5
4M	Mean	381	42	94	2	5.77	44	7.07	1.16*	0.61
	SD	33.5	5.6	6.1	0.7	0.615	4.3	0.924	0.166	0.245
	n	5	5	5	5	5	5	5	5	5

Williams' test: * $p < 0.05$

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TABLE 14
(Blood chemistry – continued)

Group		Na	K	Cl	Ca Total	Phos	Total Prot	Alb	a1 Glob	a2 Glob
		mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	g/L	g/L	g/L	g/L
1M	Mean	140	4.2	100	2.74	2.37	64	31	14	4
	SD	1.5	0.65	1.0	0.089	0.080	0.8	1.3	1.0	0.0
	n	5	5	5	5	5	5	5	5	5
2M	Mean	140	3.8	101	*		*		**	
	SD	1.0	0.20	1.1	2.63	2.16	62	32	11	4
	n	5	5	5	0.086	0.114	2.1	0.5	0.9	0.8
3M	Mean	139	3.9	100	*		**		**	
	SD	0.8	0.17	1.1	2.63	2.43	61	30	12	4
	n	5	5	5	0.071	0.329	1.1	1.5	0.8	0.0
4M	Mean	140	3.7	101	**		**		**	††
	SD	0.9	0.27	0.5	2.58	2.24	61	32	12	3
	n	5	5	5	0.078	0.069	2.2	1.7	0.8	0.0

Williams' test: * $p < 0.05$, ** $p < 0.01$

Shirley's test: †† $p < 0.01$

TABLE 14
(Blood chemistry – continued)

Group		Beta Glob g/L	Gamma Glob g/L	A/G Ratio
1M	Mean	12	3	0.96
	SD	0.4	0.5	0.083
	n	5	5	5
2M	Mean	12	3	1.06
	SD	1.3	0.9	0.098
	n	5	5	5
3M	Mean	11	3	1.01
	SD	1.1	0.5	0.076
	n	5	5	5
4M	Mean	11	3	1.13
	SD	0.8	0.4	0.074
	n	5	5	5

Williams' test: ** $p < 0.01$

TABLE 14
(Blood chemistry – continued)

Group		Alb t	a1 Glob t	a2 Glob t	Beta Glob t	Gamma Glob t
1M	Mean	49.1	21.6	6.3	18.9	4.1
	SD	2.50	1.25	0.32	0.65	1.03
	n	5	5	5	5	5
2M			*			
	Mean	51.1	18.6	6.2	19.9	4.3
	SD	2.40	1.32	0.78	1.46	0.98
	n	5	5	5	5	5
3M			*			
	Mean	50.1	20.0	7.0	18.5	4.4
	SD	2.07	1.63	0.17	1.60	0.95
	n	5	5	5	5	5
4M		*	*	##		
	Mean	52.9	19.2	5.0	18.2	4.6
	SD	1.58	1.47	0.28	1.52	0.97
	n	5	5	5	5	5

Williams' test: * $p < 0.05$

Dunnett's test: ## $p < 0.01$

TABLE 14
(Blood chemistry – continued)

Group		Alk. Phos U/L	ALT U/L	AST U/L	Bili. Total umol/L	Urea mmol/L	Creat umol/L	Gluc mmol/L	Chol Total mmol/L	Trig mmol/L
1F	Mean	237	38	81	3	7.15	44	6.79	2.09	0.53
	SD	36.7	3.8	3.6	0.9	0.981	4.1	0.738	0.433	0.194
	n	5	5	5	5	5	5	5	5	5
2F	Mean	215	41	81	*	6.52	47	7.45	1.91	0.50
	SD	51.0	7.8	13.0	0.5	0.812	3.1	0.521	0.154	0.253
	n	5	5	5	5	5	5	5	5	5
3F	Mean	266	33	83	*	5.74	44	6.59	1.52	0.46
	SD	36.4	5.3	10.9	0.4	0.458	2.4	1.043	0.085	0.129
	n	5	5	5	5	5	5	5	5	5
4F	Mean	240	33	76	*	6.19	43	7.03	1.23	0.51
	SD	39.6	5.7	8.0	0.4	1.069	1.1	0.659	0.345	0.347
	n	5	5	5	5	5	5	5	5	5

Williams' test: * $p < 0.05$, ** $p < 0.01$

TABLE 14
(Blood chemistry – continued)

Group		Na	K	Cl	Ca Total	Phos	Total Prot	Alb	a1 Glob	a2 Glob
		mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	g/L	g/L	g/L	g/L
1F	Mean	140	3.7	101	2.66	1.91	64	34	11	4
	SD	0.8	0.11	1.1	0.082	0.174	1.8	0.9	0.5	0.4
	n	5	5	5	5	5	5	5	5	5
2F	Mean	140	3.5	101	2.59	1.73	64	35	11	4
	SD	0.9	0.21	1.5	0.135	0.227	2.6	2.9	0.4	0.4
	n	5	5	5	5	5	5	5	5	5
3F					*					
	Mean	139	3.5	101	2.50	1.72	59	33	10	4
	SD	0.8	0.30	1.4	0.084	0.200	5.3	3.0	1.0	0.5
	n	5	5	5	5	5	5	5	5	5
4F		**			*					*
	Mean	137	3.4	100	2.51	1.80	60	33	10	3
	SD	1.1	0.25	1.8	0.083	0.133	3.3	2.2	1.5	0.5
	n	5	5	5	5	5	5	5	5	5

Williams' test: * $p < 0.05$, ** $p < 0.01$

TABLE 14
(Blood chemistry – continued)

Group		Beta Glob g/L	Gamma Glob g/L	A/G Ratio
1F	Mean	12	3	1.15
	SD	0.9	0.5	0.069
	n	5	5	5
2F	Mean	12	3	1.19
	SD	1.1	0.4	0.135
	n	5	5	5
3F	Mean	10	3	1.22
	SD	1.1	0.8	0.025
	n	5	5	5
4F	Mean	11	3	1.20
	SD	1.3	0.8	0.078
	n	5	5	5

No differences of statistical significance ($p > 0.05$)

TABLE 14
(Blood chemistry – continued)

Group		Alb	a1 Glob	a2 Glob	Beta Glob	Gamma Glob
		†	†	†	†	†
1F	Mean	53.5	16.7	6.3	18.1	5.3
	SD	1.47	0.74	0.45	1.14	0.97
	n	5	5	5	5	5
2F	Mean	54.3	17.3	6.2	17.9	4.3
	SD	2.94	0.73	0.49	1.83	0.69
	n	5	5	5	5	5
3F	Mean	54.5	16.9	6.2	17.7	4.8
	SD	0.70	0.54	0.13	1.07	0.59
	n	5	5	5	5	5
4F				*		
	Mean	54.4	16.7	5.7	17.8	5.3
	SD	1.10	1.76	0.41	1.83	1.45
	n	5	5	5	5	5

Williams' test: * $p < 0.05$

TABLE 15

Macroscopic pathology - group distribution of findings

Print No: 0013

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm): 0 50 150 450

Xybion protocol number: MIN 252

--- NUMBER - OF - ANIMALS - AFFECTED ---								
SEX: -----MALE----- FEMALE-----								
GROUP: -1- -2- -3- -4- -1- -2- -3- -4-								
ORGAN AND KEYWORD(S) OR PHRASE	NUMBER:	5	5	5	5	5	5	5

** TOP OF LIST **								
ADRENALS	NUMBER EXAMINED:	5	5	5	5	5	5	5
EPIDIDYMIDES	NUMBER EXAMINED:	5	5	5	5	0	0	0
HEART	NUMBER EXAMINED:	5	5	5	5	5	5	5
KIDNEYS	NUMBER EXAMINED:	5	5	5	5	5	5	5
PELVIC DILATION		0	0	1	0	1	0	0
LARYNX	NUMBER EXAMINED:	5	5	5	5	5	5	5
LIVER	NUMBER EXAMINED:	5	5	5	5	5	5	5
MEDIAN CLEFT PALE AREA(S)		0	0	0	0	1	0	0
LOBE(S) NECROTIC		0	0	0	0	1	0	0
LUNGS & BRONCHI	NUMBER EXAMINED:	5	5	5	5	5	5	5
DARK AREA(S)		0	0	0	1	0	0	1
NASAL TURBINATES	NUMBER EXAMINED:	5	5	5	5	5	5	5
SPLEEN	NUMBER EXAMINED:	5	5	5	5	5	5	5
TESTES	NUMBER EXAMINED:	5	5	5	5	0	0	0
TRACHEA	NUMBER EXAMINED:	5	5	5	5	5	5	5
TRACHEAL BIFURC.	NUMBER EXAMINED:	5	5	5	5	5	5	5
LN MANDIBULAR	NUMBER EXAMINED:	5	5	5	5	5	5	5
CYSTIC ENLARGEMENT		1	0	0	0	0	0	0
ENLARGED		0	0	0	1	0	0	1

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TABLE 15
(Macroscopic pathology -- continued)

Print No: 0013

Printed: 26-FEB-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Xybion protocol number: MIN 252

--- NUMBER - OF - ANIMALS - AFFECTED ---								
ORGAN AND KEYWORD(S) OR PHRASE	SEX: -----MALE-----				-----FEMALE-----			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:	5	5	5	5	5	5	5	5

** FROM PREVIOUS PAGE **								
LN MANDIBULAR	NUMBER EXAMINED:	5	5	5	5	5	5	5
CONGESTED		0	0	0	1	0	0	0
LN PANCREATIC	NUMBER EXAMINED:	5	5	5	5	5	5	5
ENLARGED		0	0	0	1	0	0	0
SKIN	NUMBER EXAMINED:	5	5	5	5	5	5	5
SCAB(S)		0	0	0	1	0	0	0
STOMACH	NUMBER EXAMINED:	5	5	5	5	5	5	5
ANTRUM WHITE NODULE(S)		0	0	0	1	0	1	1
UTERUS	NUMBER EXAMINED:	0	0	0	0	5	5	5
FLUID DISTENTION		0	0	0	0	0	1	2
VAGINA	NUMBER EXAMINED:	0	0	0	0	5	5	5
PALE AREA(S)		0	0	0	0	0	1	0
** END OF LIST **								

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TABLE 16

Organ weights - group mean values (g)

Print No: 0014

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control T-7499
 Exposure level (ppm): 0 50 150 450

Xyblon protocol number: MIN 252

ABSOLUTE ORGAN WEIGHTS					BODYWEIGHTS ADJUSTED VALUES			
SEX:	MALE							
GROUP:	---1---	---2---	---3---	---4---	---1---	---2---	---3---	---4---
NUMBER:	5	5	5	5				
TERMINAL BODY WEIGHT (g)								
N	:	5	5	5	**			
MEAN	:	386.0	357.7	358.2	5			
sd	:	11.2	37.0	23.1	331.7			
	:				34.0			
ADRENALS								
N	:	5	5	5	5			
MEAN	:	0.054	0.059	0.058	0.054			
sd	:	0.007	0.005	0.004	0.011			
EPIDIDYIMIDES								
N	:	5	5	5	5			
MEAN	:	0.978	0.972	0.920	0.904			
sd	:	0.032	0.092	0.083	0.061			
HEART					HEART			
N	:	5	5	5	N	:	5	5
MEAN	:	1.305	1.549	1.277	MEAN	:	1.168	1.552
sd	:	0.174	0.234	0.205			1.278	1.239
	:							
KIDNEYS					KIDNEYS			
N	:	5	5	5	N	:	5	5
MEAN	:	2.44	2.39	2.35	MEAN	:	2.27	2.39
sd	:	0.05	0.30	0.19			2.35	2.38
	:							
Williams' test:		** p< 0.01						
Dunnett's test:		## p< 0.01						

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TABLE 16

(Organ weights - continued)

Print No: 0014

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm) : 0 50 150 450

Xybion protocol number: MIN 252

ABSOLUTE ORGAN WEIGHTS					BODYWEIGHTS ADJUSTED VALUES				
SEX:	MALE								
GROUP:	1	2	3	4	1	2	3	4	
NUMBER:	5	5	5	5					
LIVER					LIVER				
N	5	5	5	5	N	5	5	5	5
MEAN	14.77	13.07	12.43	12.69	MEAN	13.83	13.09	12.44	13.61
sd	1.10	2.06	0.87	1.79					
LUNGS					LUNGS				
N	5	5	5	5	N	5	5	5	5
MEAN	1.259	1.306	1.248	1.180	MEAN	1.203	1.308	1.249	1.234
sd	0.072	0.158	0.095	0.054					
TESTES									
N	5	5	5	5					
MEAN	3.31	3.48	3.29	3.31					
sd	0.22	0.58	0.10	0.26					

Williams' test: * p< 0.05

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TABLE 16

(Organ weights - continued)

Print No: 0015

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm) : 0 50 150 450

Xybion protocol number: MIN 252

ABSOLUTE ORGAN WEIGHTS					BODYWEIGHTS ADJUSTED VALUES				
SEX:	FEMALE								
GROUP:	---1---	---2---	---3---	---4---	---1---	---2---	---3---	---4---	
NUMBER:	5	5	5	5					
TERMINAL BODY WEIGHT (g)									
N	5	5	5	5					
MEAN	214.8	210.8	206.7	205.0					
sd	12.9	13.9	11.1	18.8					
ADRENALS					ADRENALS				
N	5	5	5	5	N	5	5	5	5
MEAN	0.057	0.069	0.053	0.069	MEAN	0.055	0.069	0.053	0.070
sd	0.010	0.008	0.004	0.010					
HEART					HEART				
N	5	5	5	5	N	5	5	5	5
MEAN	0.924	0.898	0.891	0.840	MEAN	0.903	0.893	0.901	0.856
sd	0.163	0.100	0.124	0.121					
KIDNEYS									
N	5	5	5	5					
MEAN	1.37	1.48	1.59	1.66					
sd	0.14	0.10	0.37	0.36					
LIVER					LIVER				
N	5	5	5	5	N	5	5	5	5
MEAN	7.81	7.68	7.34	7.56	MEAN	7.63	7.63	7.41	7.69
sd	1.00	0.52	0.50	0.54					

Dunnett's test: # $p < 0.05$

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TABLE 16

(Organ weights - continued)

Print No: 0015

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xyblon protocol number: MIN 252

	ABSOLUTE ORGAN WEIGHTS			
SEX:	-----FEMALE-----			
GROUP:	---1---	---2---	---3---	---4---
NUMBER:	5	5	5	5

	LUNGS				
N	:	5	5	5	5
MEAN	:	0.998	0.938	0.946	0.949
sd	:	0.090	0.060	0.067	0.064

No differences of statistical significance

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TABLE 17

Microscopic pathology - expanded incidence summary

Print No: 0016

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm) : 0 50 150 450

Xyblon protocol number: MIN 252

--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---								
ORGAN AND FINDING DESCRIPTION	SEX: -----MALE-----				-----FEMALE-----			
	GROUP: -1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:	5	5	5	5	5	5	5	5
** TOP OF LIST **								
HEART NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--VALVULAR ENDOCARDITIS	0	0	0	0	0	0	0	1
KIDNEYS NUMBER EXAMINED:	5	0	1	5	5	0	0	5
--CORTICAL TUBULAR BASOPHILIA	3	0	1	1	0	0	0	0
--MEDULLARY TUBULAR BASOPHILIA	1	0	0	0	0	0	0	0
--DILATED MEDULLARY TUBULES CONTAINING EOSINOPHILIC COLLOID	0	0	1	0	0	0	0	0
--TUBULAR HYPERPLASIA, SIMPLE	1	0	0	0	0	0	0	0
--INTERSTITIAL INFLAMMATION	0	0	0	0	0	0	0	1
--UNILATERAL PELVIC DILATATION	1	0	1	0	1	0	0	0
LARYNX NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--EPITHELIAL HYPERPLASIA - ARYTENOIDS	1	0	0	1	1	0	0	0
--EPITHELIAL HYPERPLASIA - VENTRAL POUCH	1	0	0	0	0	0	0	0
--INFLAMMATORY CELLS IN LAMINA PROPRIA (VENTRAL AND VENTROLATERAL)	1	0	0	0	0	0	0	0
--INFLAMMATORY CELLS IN LAMINA PROPRIA (VENTRAL)	0	0	0	0	0	0	0	1
LIVER NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--SIDEROPHAGES, BILE DUCT HYPERPLASIA, FIBROSIS AND MINERALISATION - RIGHT ANTERIOR LOBE	0	0	0	0	1	0	0	0
LUNGS & BRONCHI NUMBER EXAMINED:	5	0	0	5	5	0	1	5
--ALVEOLITIS	2	0	0	0	2	0	1	0
--FOAMY ALVEOLAR MACROPHAGES	0	0	0	0	0	0	1	0
--ALVEOLAR OSSEOUS METAPLASIA	2	0	0	0	0	0	0	0
--EXTRAVASATION OF EOSINOPHILS	0	0	0	0	1	0	0	0
--SUBPLEURAL INFLAMMATION	0	0	0	1	0	0	0	0
NASAL TURBINATES NUMBER EXAMINED:	5	0	0	5	5	0	0	5
--TRANSITIONAL EPITHELIAL HYPERPLASIA	2	0	0	0	0	0	0	0

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TABLE 17

(Microscopic pathology - continued)

Print No: 0016

Printed: 26-FEB-01

Group : 1 2 3 4
 Compound : Control ----- T-7499 -----
 Exposure level (ppm): 0 50 150 450

Xybion protocol number: MIN 252

--- NUMBER - OF - ANIMALS - AFFECTED ---								
SEX: -----MALE----- FEMALE-----								
GROUP: -1- -2- -3- -4- -1- -2- -3- -4-								
ORGAN AND FINDING DESCRIPTION	NUMBER:	5	5	5	5	5	5	5

** FROM PREVIOUS PAGE **								
NASAL TURBINATES	NUMBER EXAMINED:	5	0	0	5	5	0	0
--RESPIRATORY EPITHELIAL HYPERPLASIA		1	0	0	0	0	0	0
--OEDEMA IN STENO'S GLAND		0	0	0	0	0	0	1
TRACHEA	NUMBER EXAMINED:	5	0	0	5	5	0	0
--SUBEPITHELIAL INFLAMMATION		3	0	0	1	2	0	0
LN MANDIBULAR	NUMBER EXAMINED:	1	0	0	2	0	0	1
--CYSTIC SINUSES		1	0	0	0	0	0	0
--INCREASED CELLULARITY - GENERALISED		0	0	0	1	0	0	1
--SINUS ERYTHROCYTOSIS/ERYTHROPHAGOCYTOSIS		0	0	0	1	0	0	0
LN PANCREATIC	NUMBER EXAMINED:	0	0	0	1	0	0	0
--SINUS ERYTHROCYTOSIS/ERYTHROPHAGOCYTOSIS		0	0	0	1	0	0	0
SKIN	NUMBER EXAMINED:	0	0	0	1	0	0	0
--SCAB		0	0	0	1	0	0	0
--EPIDERMAL HYPERPLASIA		0	0	0	1	0	0	0
--EPIDERMAL ULCERATION		0	0	0	1	0	0	0
--DERMAL INFLAMMATION		0	0	0	1	0	0	0
STOMACH	NUMBER EXAMINED:	0	0	0	1	0	1	1
--ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR MUCOSA, FOCAL		0	0	0	1	0	1	1
UTERUS	NUMBER EXAMINED:	0	0	0	0	0	1	2
--LUMINAL DILATATION		0	0	0	0	0	1	2
VAGINA	NUMBER EXAMINED:	0	0	0	0	0	0	1
--PROMINENT PROSTATIC TISSUE ON SEROSAL ASPECT		0	0	0	0	0	0	1
** END OF LIST **								

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APPENDIX 1

Clinical signs pre and post exposure – individual daily observations

Group	Animal number	Observations
1M (Control)	16 - 20	No abnormalities detected
2M (Low dose)	6 - 10	No abnormalities detected
3M (Inter. dose)	11 - 15	No abnormalities detected
4M (High dose)	1 2 3 4 5	No abnormalities detected Agitated and vocalising when handled, Exposure 1 Agitated and vocalising when handled, Exposure 11 No abnormalities detected No abnormalities detected
1F (Control)	31 - 35	No abnormalities detected
2F (Low dose)	36 37 38 39 40	Agitated when handled, Exposures 3 to 9, 11, 14 and 16 to 20 and <i>pre-exposures 13 and 15</i> . Vocalising when handled, Exposures 3 to 9, 11 and 14 to 20 and <i>pre-exposure 15</i> Agitated and vocalising when handled, Exposures 9, 11 and 17. Hyperactive, Exposure 11 Agitated and vocalising when handled, Exposure 19 Hyperactive, Exposure 11 Agitated when handled, Exposures 9, 11, 15 and 17 and <i>pre-exposure 15</i> . Vocalising when handled, Exposures 9, 15 and 17 and <i>pre-exposure 15</i> . Hyperactive, Exposure 11
3F (Inter. dose)	26 27 28 29 30	Agitated when handled, Exposure 11. Vocalising when handled, Exposures 11 and 14. Hyperactive, Exposures 9 to 11. Walking on toes (abnormal gait), Exposure 14 Walking on toes (abnormal gait), Exposures 4, 6, 12 to 14, 17, 18, and 20. Hyperactive, Exposures 9 and 10 Agitated and vocalising when handled, Exposure 1. Hyperactive, Exposures 9 and 10 Agitated and vocalising when handled, Exposure 16. Walking on toes (abnormal gait), Exposures 2, 4, 6, 12 to 15, 17 and 19. Hyperactive, Exposures 9 and 10 Agitated and vocalising when handled, Exposures 1 to 3, 6 and 11. Walking on toes (abnormal gait), Exposure 17 to 19. Hyperactive, Exposure 10
4F (High dose)	21 22 23 24 25	Agitated and vocalising when handled, Exposures 1 to 5. Walking on toes (abnormal gait), Exposures 2 to 20 and <i>pre-exposure 20</i> . Hyperactive, Exposure 9 Agitated and vocalising when handled, Exposures 1 to 5. Walking on toes (abnormal gait), Exposures 2 to 8 and 10 to 20 and <i>pre-exposure 20</i> . Hyperactive, Exposure 9 Agitated when handled, Exposures 1 to 6. Vocalising when handled, Exposures 1 to 7 and 13. Walking on toes (abnormal gait), Exposures 2 to 8 and 10 to 20 and <i>pre-exposure 20</i> . Hyperactive, Exposure 9 Agitated when handled, Exposures 1 to 5 and 11. Vocalising when handled, Exposures 1 to 5, 11 and 13. Walking on toes (abnormal gait), Exposures 2 to 8 and 10 to 20 and <i>pre-exposure 20</i> . Hyperactive, Exposure 9 Agitated when handled, Exposures 1 to 5, 11 and 16. Vocalising when handled, Exposures 1 to 5, 7, 8, 11 and 16. Walking on toes (abnormal gait), Exposures 2 to 8 and 10 to 20 and <i>pre-exposure 20</i> . Hyperactive, Exposures 9 and 11

APPENDIX 2

Clinical signs (pre-exposure) – individual weekly observations

Print No: 0019

Printed: 26-FEB-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Xybion protocol number: MIN 252

CATEGORY GROUP: 1M
ANIMAL DEATH WK OF KEYWORD WEEKS 1-5
NUMBER CODE DEATH QUALIFIER

16 7 5 ANIMAL HAS NO SIGNIFICANT FINDINGS
17 7 5 ANIMAL HAS NO SIGNIFICANT FINDINGS
18 7 5 ANIMAL HAS NO SIGNIFICANT FINDINGS
19 7 5 ANIMAL HAS NO SIGNIFICANT FINDINGS
20 7 5 ANIMAL HAS NO SIGNIFICANT FINDINGS

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybian protocol number: MIN 252

			CATEGORY	GROUP: 2M	
ANIMAL DEATH	WK OF		KEYWORD		WEEKS 1-5
NUMBER CODE	DEATH		QUALIFIER		
6	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
7	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
8	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
9	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
10	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

			CATEGORY	GROUP: 3M	
ANIMAL DEATH WK OF			KEYWORD		WEEKS 1-5
NUMBER CODE DEATH			QUALIFIER		

11	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
12	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
13	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
14	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
15	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybion protocol number: MIN 252

			CATEGORY	GROUP: 4M	
ANIMAL DEATH	WK OF		KEYWORD		WEEKS 1-5
NUMBER CODE	DEATH		QUALIFIER		
1	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
2	7	5	STAINING		
			BROWN		
			DORSAL BODY SURFACE		5
3	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
4	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
5	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

			CATEGORY	GROUP: 1F	
ANIMAL DEATH WK OF	KEYWORD				WEEKS 1-5
NUMBER CODE DEATH	QUALIFIER				

31	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
32	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
33	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
34	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS
35	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Xybion protocol number: MIN 252

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 2F	WEEKS 1-5
36	7	5	SKIN ABRASION DRY TAIL		4
37	7	5	BEHAVIOUR IRRITABLE		2
38	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
39	7	5	SKIN ABRASION DRY TAIL		3-5
40	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybion protocol number: MIN 252

			CATEGORY	GROUP: 3F	WEEKS 1-5
ANIMAL	DEATH	WK OF	KEYWORD		
NUMBER	CODE	DEATH	QUALIFIER		
26	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
27	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
28	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
29	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
30	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		

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APPENDIX 2

(Clinical signs (pre-exposure) – continued)

Print No: 0019

Printed: 26-FEB-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybio protocol number: MIN 252

ANIMAL DEATH WK OF			CATEGORY	GROUP: 4F	WEEKS 1-5
NUMBER	CODE	DEATH	KEYWORD QUALIFIER		

21	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
22	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
23	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
24	7	5	ANIMAL HAS NO SIGNIFICANT FINDINGS		
25	7	5	TEETH ABNORMAL COLOUR PALE		4-5

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APPENDIX 3

Bodyweights - individual values (g)

Print No: 0020

Printed: 26-FEB-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Xybion protocol number: MIN 252

GROUP	ANIMAL	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4
1M	16	292	336	374	393	401
	17	275	312	345	366	373
	18	279	322	359	389	397
	19	277	318	349	376	379
	20	263	296	336	375	386
2M	6	274	306	332	361	371
	7	293	333	357	378	389
	8	241	263	272	289	289
	9	275	307	329	348	348
	10	265	303	331	361	364
3M	11	267	281	313	332	338
	12	278	306	327	355	356
	13	281	315	346	381	391
	14	277	308	329	350	355
	15	273	298	315	325	333
4M	1	264	277	290	311	323
	2	250	255	273	285	298
	3	293	306	334	363	388
	4	270	286	308	334	343
	5	265	271	292	308	306

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APPENDIX 3

(Bodyweights – continued)

Print No: 0021

Printed: 26-FEB-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Xyblon protocol number: MIN 252

GROUP	ANIMAL	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4
1F	31	165	185	197	208	208
	32	193	208	214	229	231
	33	171	181	192	200	194
	34	183	188	194	199	212
	35	188	188	205	217	216
2F	36	181	186	186	181	188
	37	189	204	214	224	220
	38	178	195	199	202	200
	39	189	190	201	200	210
	40	165	179	192	204	203
3F	26	174	161	165	191	196
	27	191	194	206	206	214
	28	166	177	175	180	190
	29	189	193	206	217	218
	30	194	182	185	198	203
4F	21	186	178	177	187	186
	22	163	163	170	181	184
	23	190	195	199	225	222
	24	202	197	203	213	219
	25	179	185	180	194	207

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APPENDIX 4

Functional observational battery – individual observations

Pre-dose

Group 1 Males

OBSERVATIONS	Animal number				
	16	17	18	19	20
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	11	6	9	10	16
Arousal	4	4	4	4	4
Rearing count	5	1	9	5	8
Bolus count	2	0	0	0	2
Urine present	N	N	N	N	L
Gait	-	-	-	-	-
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	2	2	2	2
Startle	3	3	2	4	1
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns	-	-	-	-	-
vocalises	-	2	2	-	-
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.6	37.8	38.0	38.1	38.3
Bodyweight (g)	248	249	238	253	238
GRIP STRENGTH (KG) ‡					
forelimb	0.69	0.80	0.88	0.80	0.71
hindlimb	0.80	0.85	0.52	0.69	0.61
LANDING FOOTSPRAY (cm) ‡	7.6	11.2	12.3	9.0	8.1

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 2 Males

OBSERVATIONS	Animal number				
	6	7	8	9	10
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	12	12	15	16	15
Arousal	4	4	4	4	4
Rearing count	8	4	9	6	8
Bolus count	3	1	0	0	0
Urine present	M	M	S	N	M
Gait	-	-	-	T1	-
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	2	2	0	2	3
Startle	3	2	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	5	6	3	3	3
turns	-	-	-	-	-
vocalises	2	2	1	-	-
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.2	37.3	38.4	38.1	37.9
Bodyweight (g)	244	252	226	247	241
GRIP STRENGTH (KG) ‡					
forelimb	0.58	0.75	0.58	0.88	0.87
hindlimb	0.47	0.70	0.61	0.77	0.69
LANDING FOOTSPRAY (cm) ‡	10.1	9.7	8.4	14.2	9.0

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 3 Males

OBSERVATIONS	Animal number				
	11	12	13	14	15
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Vocalising degree	N	N	N	N	N
	-	-	-	-	-
IN THE ARENA					
Activity count	5	15	15	11	10
Arousal	4	4	4	4	4
Rearing count	0	10	7	5	3
Bolus count	2	0	0	2	8
Urine present	S	N	N	N	S
Gait	-	T1	-	-	-
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	2	0	2	2	2
Startle	3	3	3	2	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns	-	-	-	-	-
vocalises	2	2	-	-	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.1	37.8	37.9	37.8	38.4
Bodyweight (g)	237	242	242	253	243
GRIP STRENGTH (KG) ‡					
forelimb	0.82	0.51	0.47	0.77	0.80
hindlimb	0.56	0.55	0.56	0.81	0.76
LANDING FOOTSPRAY (cm) ‡	13.1	11.2	9.6	13.2	12.7

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 4 Males

OBSERVATIONS	Animal number				
	1	2	3	4	5
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	3	2	2	3
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	16	14	21	8	11
Arousal	4	4	4	4	4
Rearing count	5	6	8	6	4
Bolus count	0	0	0	1	0
Urine present	N	N	N	N	S
Gait	T1	T1	T1	-	-
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	2	2	6	1	2
Startle	3	3	3	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	2	3	3
turns	-	-	2	-	-
vocalises	1	-	2	-	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.2	37.8	37.7	38.0	37.9
Bodyweight (g)	228	221	259	244	240
GRIP STRENGTH (KG) ‡					
forelimb	0.62	0.54	0.68	0.71	0.84
hindlimb	0.79	0.65	0.58	0.66	0.79
LANDING FOOTSPRAY (cm) ‡	9.2	11.7	9.2	8.5	7.1

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 1 Females

OBSERVATIONS	Animal number				
	31	32	33	34	35
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	7	6	13	16	16
Arousal	4	4	4	4	4
Rearing count	2	2	6	7	7
Bolus count	0	0	0	0	0
Urine present	N	N	S	N	S
Gait	-	-	-	T1	T2
MANIPULATIONS					
Approach	2	3	3	3	3
Touch	2	2	2	6	2
Startle	3	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	6	5	3	3
turns	-	-	-	-	-
vocalises	2	3	-	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.1	37.8	37.7	38.6	37.8
Bodyweight (g)	155	176	161	159	179
GRIP STRENGTH (KG) ‡					
forelimb	0.83	0.83	0.63	0.80	0.77
hindlimb	0.63	0.75	0.65	0.79	0.80
LANDING FOOTSPRAY (cm) ‡	10.6	10.1	6.7	12.9	10.7

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 2 Females

OBSERVATIONS	Animal number				
	36	37	38	39	40
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	2	-	-	-	-
IN THE ARENA					
Activity count	13	12	13	11	16
Arousal	4	4	4	4	4
Rearing count	2	3	6	6	6
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	-	-	-	-	T1
MANIPULATIONS					
Approach	3	3	3	2	3
Touch	2	2	2	1	2
Startle	4	2	2	3	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns	-	-	-	-	-
vocalises	2	1	-	2	-
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.9	38.4	38.4	37.7	38.6
Bodyweight (g)	164	170	168	169	153
GRIP STRENGTH (KG) ‡					
forelimb	0.82	0.75	0.73	0.64	0.63
hindlimb	0.68	0.57	0.73	0.63	0.49
LANDING FOOTSPRAY (cm) ‡	11.5	11.8	11.4	9.2	8.9

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 3 Females

OBSERVATIONS	Animal number				
	26	27	28	29	30
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	3	2	2	2
Vocalising	N	N	N	N	Y
degree	-	-	-	-	1
IN THE ARENA					
Activity count	11	12	10	22	11
Arousal	4	4	4	4	4
Rearing count	2	6	3	6	4
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	-	T1	-	T1	T1
MANIPULATIONS					
Approach	3	3	2	3	3
Touch	3	0	2	2	2
Startle	3	2	4	2	2
Righting reflex	1	1	1	1	1
Tail pinch	3	3	5	3	3
turns	-	-	-	-	-
vocalises	1	2	3	-	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.1	38.0	37.4	37.5	38.5
Bodyweight (g)	155	170	159	171	175
GRIP STRENGTH (KG) ‡					
forelimb	0.67	0.83	0.75	0.86	0.64
hindlimb	0.54	0.86	0.54	0.83	0.68
LANDING FOOTSPRAY (cm) ‡	5.1	7.2	11.5	10.8	8.8

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Pre-dose

Group 4 Females

OBSERVATIONS	Animal number				
	21	22	23	24	25
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	3	2	2	2
Vocalising degree	N	N	N	N	N
	-	-	-	-	-
IN THE ARENA					
Activity count	11	12	14	9	13
Arousal	4	4	4	5	4
Rearing count	7	9	6	3	4
Bolus count	0	0	0	0	0
Urine present	N	N	N	S	N
Gait	T2	T1	T1	-	T1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	2	6	2	2	2
Startle	2	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns	-	-	-	-	-
vocalises	1	-	1	-	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.5	38.5	38.1	38.4	38.7
Bodyweight (g)	167	154	172	179	160
GRIP STRENGTH (KG) ‡					
forelimb	0.69	0.55	0.79	0.80	0.51
hindlimb	0.51	0.65	0.80	0.64	0.66
LANDING FOOTSPRAY (cm) ‡	10.1	8.1	11.4	5.8	8.6

‡ Values represent the mean of 2 trials

APPENDIX 4**(Functional observational battery – continued)****Week 1****Group 1 Males**

OBSERVATIONS	Animal number				
	16	17	18	19	20
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	11	12	10	7	17
Arousal	4	4	4	4	4
Rearing count	4	5	9	3	5
Bolus count	2	0	0	4	0
Urine present	N	M	N	N	M
Gait	-	-	-	-	-

APPENDIX 4

(Functional observational battery – continued)

Week 1

Group 2 Males

OBSERVATIONS	Animal number				
	6	7	8	9	10
IN THE HAND					
Removing	3	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	8	17	10	15	8
Arousal	4	4	4	4	4
Rearing count	9	7	5	6	6
Bolus count	1	3	0	0	4
Urine present	M	S	N	N	S
Gait	-	T1	-	T1	-

APPENDIX 4**(Functional observational battery – continued)****Week 1****Group 3 Males**

OBSERVATIONS	Animal number				
	11	12	13	14	15
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	4	12	13	9	7
Arousal	4	4	4	4	4
Rearing count	0	11	5	1	3
Bolus count	3	0	0	2	3
Urine present	S	N	N	N	N
Gait	T1	-	-	T1	-

APPENDIX 4

(Functional observational battery – continued)

Week 1

Group 4 Males

OBSERVATIONS	Animal number				
	1	2	3	4	5
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	3	2	3
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	12	7	9	11	9
Arousal	4	4	4	4	4
Rearing count	6	4	6	3	2
Bolus count	0	0	0	0	0
Urine present	S	N	N	N	N
Gait	T2	-	T1	-	-

APPENDIX 4

(Functional observational battery – continued)

Week 1

Group 1 Females

OBSERVATIONS	Animal number				
	31	32	33	34	35
IN THE HAND					
Removing	2	2	3	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	Y	N	N	N	N
degree	2	-	-	-	-
IN THE ARENA					
Activity count	11	9	17	21	15
Arousal	4	4	5	4	4
Rearing count	7	4	9	11	6
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	-	-	T1	T1	T1

APPENDIX 4

(Functional observational battery – continued)

Week 1

Group 2 Females

OBSERVATIONS	Animal number				
	36	37	38	39	40
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	Y	Y	N	N
degree	-	2	2	-	-
IN THE ARENA					
Activity count	16	14	12	14	12
Arousal	4	4	4	4	4
Rearing count	4	8	2	7	5
Bolus count	0	0	0	0	0
Urine present	S	N	N	N	N
Gait	-	T1	-	T1	T1

APPENDIX 4**(Functional observational battery – continued)****Week 1****Group 3 Females**

OBSERVATIONS	Animal number				
	26	27	28	29	30
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Salivation	N	N	Y	N	Y
degree	-	-	1	-	1
Vocalising	N	N	N	N	Y
degree	-	-	-	-	2
IN THE ARENA					
Activity count	17	11	14	20	9
Arousal	4	4	4	4	4
Rearing count	6	7	7	9	5
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	-	-	T1	T2	-

APPENDIX 4

(Functional observational battery – continued)

Week 1

Group 4 Females

OBSERVATIONS	Animal number				
	21	22	23	24	25
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	3	2
Salivation	N	Y	N	N	N
degree	-	1	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	9	8	13	12	6
Arousal	4	4	4	4	4
Rearing count	5	1	6	6	6
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T2HU1	T2HU1	T1	-	-

APPENDIX 4

(Functional observational battery – continued)

Week 2

Group 1 Males

OBSERVATIONS	Animal number				
	16	17	18	19	20
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising degree	N	N	N	N	N
	-	-	-	-	-
IN THE ARENA					
Activity count	12	11	9	12	14
Arousal	4	4	4	4	4
Rearing count	11	4	7	4	4
Bolus count	0	0	0	2	1
Urine present	N	M	N	N	M
Gait	-	-	-	-	-

APPENDIX 4**(Functional observational battery – continued)****Week 2****Group 2 Males**

OBSERVATIONS	Animal number				
	6	7	8	9	10
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	11	13	12	11	10
Arousal	4	4	4	4	4
Rearing count	9	5	5	3	9
Bolus count	2	5	0	0	3
Urine present	M	N	N	N	S
Gait	-	T2	-	-	-

APPENDIX 4

(Functional observational battery – continued)

Week 2

Group 3 Males

OBSERVATIONS	Animal number				
	11	12	13	14	15
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	8	10	14	12	10
Arousal	4	4	4	4	4
Rearing count	7	8	6	6	7
Bolus count	2	0	0	0	0
Urine present	N	N	N	S	N
Gait	T1	T1	-	-	-

APPENDIX 4**(Functional observational battery – continued)****Week 2****Group 4 Males**

OBSERVATIONS	Animal number				
	1	2	3	4	5
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	3	3	2	3
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	11	13	13	9	10
Arousal	4	4	4	4	4
Rearing count	5	10	6	4	5
Bolus count	0	0	0	0	0
Urine present	M	S	N	S	N
Gait	T1	T2	T2HU1	-	T1HU1

APPENDIX 4

(Functional observational battery – continued)

Week 2

Group 1 Females

OBSERVATIONS	Animal number				
	31	32	33	34	35
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	Y	Y	N	N	N
degree	2	2	-	-	-
IN THE ARENA					
Activity count	14	12	19	17	23
Arousal	4	4	4	4	4
Rearing count	7	6	9	9	8
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T2	T1	T1	T1	T2

APPENDIX 4

(Functional observational battery – continued)

Week 2

Group 2 Females

OBSERVATIONS	Animal number				
	36	37	38	39	40
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	3	2	3
Vocalising	Y	Y	N	N	N
degree	2	1	-	-	-
IN THE ARENA					
Activity count	8	12	14	5	7
Arousal	4	4	4	4	4
Rearing count	3	5	6	2	3
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	-	T1	T2	U	T1

APPENDIX 4

(Functional observational battery – continued)

Week 2

Group 3 Females

OBSERVATIONS	Animal number				
	26	27	28	29	30
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	20	12	8	13	13
Arousal	4	4	4	4	4
Rearing count	8	4	4	5	5
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T1	T2	T1	T2HU

APPENDIX 4

(Functional observational battery – continued)

Week 2

Group 4 Females

OBSERVATIONS	Animal number				
	21	22	23	24	25
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	3	2
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	11	10	5	9	12
Arousal	4	4	4	4	4
Rearing count	5	9	2	5	5
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T2HU1	T2	T2	T2	T2

APPENDIX 4

(Functional observational battery – continued)

Week 3

Group 1 Males

OBSERVATIONS	Animal number				
	16	17	18	19	20
IN THE HAND					
Removing	2	2	2	2	3
Handling	2	2	2	2	2
Salivation	N	Y	N	N	N
degree	-	1	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Grooming	N	N	N	N	Y
Activity count	10	9	11	8	12
Arousal	4	4	4	4	4
Rearing count	9	4	4	4	9
Bolus count	0	0	0	0	0
Urine present	N	N	S	N	S
Gait	-	T1	-	-	-

APPENDIX 4**(Functional observational battery – continued)****Week 3****Group 2 Males**

OBSERVATIONS	Animal number				
	6	7	8	9	10
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	13	16	11	9	16
Arousal	4	4	4	4	4
Rearing count	7	7	3	3	8
Bolus count	0	0	0	0	0
Urine present	S	S	N	N	S
Gait	-	-	T2	-	T1

APPENDIX 4**(Functional observational battery – continued)****Week 3****Group 3 Males**

OBSERVATIONS	Animal number				
	11	12	13	14	15
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	Y	N	N
degree	-	-	1	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	13	11	11	11	9
Arousal	4	4	4	4	4
Rearing count	4	8	7	5	3
Bolus count	0	0	0	0	0
Urine present	N	S	N	S	N
Gait	T1	T1	-	T1	T1HU1

APPENDIX 4

(Functional observational battery – continued)

Week 3

Group 4 Males

OBSERVATIONS	Animal number				
	1	2	3	4	5
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	3	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	12	6	14	13	9
Arousal	4	4	4	4	4
Rearing count	4	1	8	6	4
Bolus count	0	0	0	0	0
Urine present	S	N	N	N	N
Gait	-	T2	T2HU1	T1	-

APPENDIX 4

(Functional observational battery – continued)

Week 3

Group 1 Females

OBSERVATIONS	Animal number				
	31	32	33	34	35
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	Y	Y	N	Y	N
degree	2	1	-	1	-
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	11	14	25	12	20
Arousal	4	4	4	4	4
Rearing count	7	5	11	6	8
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	-	T1	T2	T2

APPENDIX 4

(Functional observational battery – continued)

Week 3

Group 2 Females

OBSERVATIONS	Animal number				
	36	37	38	39	40
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	3	2	2	2
Salivation	N	N	Y	N	Y
degree	-	-	1	-	1
Vocalising	Y	Y	Y	Y	Y
degree	2	2	2	2	2
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	15	14	10	16	16
Arousal	4	5	4	4	4
Rearing count	3	7	3	6	8
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T2	T1	T2HU1	T1

APPENDIX 4

(Functional observational battery – continued)

Week 3

Group 3 Females

OBSERVATIONS	Animal number				
	26	27	28	29	30
IN THE HAND					
Removing	2	2	2	2	2
Handling	3	2	2	2	3
Salivation	N	N	Y	N	N
degree	-	-	1	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	20	17	10	14	10
Arousal	4	4	4	4	4
Rearing count	10	8	2	6	5
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T2	T2	T2HU1	T2HU1

APPENDIX 4

(Functional observational battery – continued)

Week 3

Group 4 Females

OBSERVATIONS	Animal number				
	21	22	23	24	25
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	3	2	3	2
Salivation	N	N	N	Y	N
degree	-	-	-	1	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Grooming	N	N	N	N	N
Activity count	17	8	8	13	14
Arousal	4	4	4	4	4
Rearing count	9	6	2	9	4
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T2	T2	T1	T1	T2HU1

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 1 Males

OBSERVATIONS	Animal number				
	16	17	18	19	20
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	8	9	14	9	17
Arousal	4	4	4	4	4
Rearing count	3	5	7	5	8
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	M
Gait	T1	-	T1	-	-
MANIPULATIONS					
Approach	3	0	3	3	3
Touch	6	1	1	6	6
Startle	3	2	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	5	3	3	3	3
turns	-	-	-	-	-
vocalises	-	Y	Y	-	Y
degree	-	2	2	-	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.2	37.9	38.1	37.9	37.8
Bodyweight (g)	395	362	398	379	384
GRIP STRENGTH (KG) ‡					
forelimb	1.14	1.32	1.51	1.07	1.51
hindlimb	1.15	0.93	1.36	0.97	0.92
LANDING FOOTSPRAY (cm) ‡	10.9	14.8	11.3	8.9	10.1

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 2 Males

OBSERVATIONS	Animal number				
	6	7	8	9	10
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	3
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	9	14	13	17	13
Arousal	4	4	4	4	4
Rearing count	6	5	7	7	6
Bolus count	4	0	0	0	0
Urine present	S	N	N	S	N
Gait	-	T1	-	-	T1
MANIPULATIONS					
Approach	3	3	3	3	2
Touch	5	6	3	3	3
Startle	3	2	3	2	3
Righting reflex	1	1	1	1	1
Tail pinch	6	3	3	3	3
turns	1	-	-	-	-
vocalises	-	-	-	-	-
degree	-	-	-	-	-
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.8	37.3	38.7	38.0	37.0
Bodyweight (g)	366	372	291	350	368
GRIP STRENGTH (KG) ‡					
forelimb	0.99	1.23	1.05	1.31	1.28
hindlimb	0.83	1.11	0.99	1.24	1.27
LANDING FOOTSPRAY (cm) ‡	12.8	12.4	8.6	12.4	8.9

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 3 Males

OBSERVATIONS	Animal number				
	11	12	13	14	15
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	10	13	11	9	2
Arousal	4	4	4	4	4
Rearing count	8	4	5	2	2
Bolus count	0	0	0	0	0
Urine present	N	S	N	S	N
Gait	-	-	-	-	U
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	1	3	1	3
Startle	2	3	2	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	3
turns	-	-	-	-	-
vocalises	-	-	-	Y	-
degree	-	-	-	2	-
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.6	37.2	37.0	37.0	37.8
Bodyweight (g)	333	355	381	352	332
GRIP STRENGTH (KG) ‡					
forelimb	1.32	1.06	1.16	1.40	1.23
hindlimb	0.91	0.88	0.91	1.30	1.17
LANDING FOOTSPRAY (cm) ‡	14.3	11.4	9.4	11.5	12.6

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 4 Males

OBSERVATIONS	Animal number				
	1	2	3	4	5
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	Y	N	N	N
degree	-	1	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	12	11	18	17	9
Arousal	4	4	4	4	4
Rearing count	3	5	4	9	2
Bolus count	0	0	0	0	0
Urine present	S	N	S	S	N
Gait	HU1	T1HU1	-	-	T1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	0	2	6	3
Startle	2	3	3	2	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	6	3	3
turns	-	-	-	-	-
vocalises	-	Y	Y	-	-
degree	-	1	1	-	-
Pupil reflex	B	B	B	B	B
Temperature (°C)	37.6	37.4	37.8	38.8	37.3
Bodyweight (g)	311	288	378	337	302
GRIP STRENGTH (KG) ‡					
forelimb	1.35	1.02	1.49	1.12	1.37
hindlimb	1.18	1.14	1.12	1.25	1.01
LANDING FOOTSPRAY (cm) ‡					
	10.8	11.6	8.6	7.4	6.3

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 1 Females

OBSERVATIONS	Animal number				
	31	32	33	34	35
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	Y	N	N	N	N
degree	1	-	-	-	-
IN THE ARENA					
Activity count	7	13	18	9	16
Arousal	4	4	4	4	4
Rearing count	2	8	7	3	9
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T1	T2	T2HU1	T2
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	2	1	6	3	3
Startle	3	2	4	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	6	3	6	3
turns	-	-	-	-	-
vocalises	Y	Y	-	Y	Y
degree	2	3	-	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.4	38.5	38.2	38.6	38.3
Bodyweight (g)	208	221	196	208	207
GRIP STRENGTH (KG) ‡					
forelimb	1.13	1.05	0.95	1.16	1.22
hindlimb	0.95	1.10	0.54	1.22	1.06
LANDING FOOTSPRAY (cm) ‡	10.9	8.5	7.4	11.7	11.9

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 2 Females

OBSERVATIONS	Animal number				
	36	37	38	39	40
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	3	2	2
Salivation	N	Y	Y	N	Y
degree	-	1	1	-	1
Vocalising	Y	Y	N	N	N
degree	2	1	-	-	-
IN THE ARENA					
Activity count	6	9	6	22	11
Arousal	4	4	4	4	4
Rearing count	2	6	2	12	5
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T1	T1HU1	T1	T2	T2
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	6	0	3	0	3
Startle	4	2	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	6	5	5
turns	-	-	-	-	-
vocalises	Y	Y	Y	Y	Y
degree	2	2	2	2	2
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.8	38.2	38.2	38.6	38.6
Bodyweight (g)	188	220	204	209	202
GRIP STRENGTH (KG) ‡					
forelimb	1.15	1.06	1.08	1.12	1.23
hindlimb	0.93	1.08	1.08	1.24	0.79
LANDING FOOTSPRAY (cm) ‡	9.2	7.0	9.4	9.8	9.7

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 3 Females

OBSERVATIONS	Animal number				
	26	27	28	29	30
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	15	8	8	15	9
Arousal	4	4	4	4	4
Rearing count	8	4	1	5	5
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T2	T1	T2HU1	T2	T2HU1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	3	3	0	6
Startle	3	3	3	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	2	3	6
turns	-	-	2	-	-
vocalises	Y	-	Y	Y	Y
degree	1	-	2	1	1
Pupil reflex	B	B	B	B	B
Temperature (°C)	38.9	37.8	38.0	37.7	38.3
Bodyweight (g)	189	213	182	214	199
GRIP STRENGTH (KG) ‡					
forelimb	0.95	1.21	1.10	1.15	1.08
hindlimb	0.60	1.17	1.19	1.13	0.72
LANDING FOOTSPRAY (cm) ‡	5.0	6.6	8.8	9.2	6.2

‡ Values represent the mean of 2 trials

APPENDIX 4

(Functional observational battery – continued)

Week 4

Group 4 Females

OBSERVATIONS	Animal number				
	21	22	23	24	25
IN THE HAND					
Removing	2	2	2	2	2
Handling	2	2	2	2	2
Salivation	N	N	N	N	N
degree	-	-	-	-	-
Vocalising	N	N	N	N	N
degree	-	-	-	-	-
IN THE ARENA					
Activity count	9	6	9	13	12
Arousal	4	4	4	4	4
Rearing count	4	4	2	5	8
Bolus count	0	0	0	0	0
Urine present	N	N	N	N	N
Gait	T3HU1	T2HU1	T1	T1	T1
MANIPULATIONS					
Approach	3	3	3	3	3
Touch	3	3	2	6	0
Startle	2	2	2	3	3
Righting reflex	1	1	1	1	1
Tail pinch	3	3	3	3	5
turns	-	-	-	-	-
vocalises	Y	Y	Y	-	Y
degree	2	2	1	-	2
Pupil reflex	B	B	R	B	B
Temperature (°C)	38.2	38.2	37.7	38.7	37.9
Bodyweight (g)	180	178	223	209	202
GRIP STRENGTH (KG) ‡					
forelimb	1.03	0.99	1.19	1.23	0.97
hindlimb	0.83	0.81	1.20	1.09	1.06
LANDING FOOTSPRAY (cm) ‡	7.4	6.5	7.4	4.6	8.1

‡ Values represent the mean of 2 trials

APPENDIX 5**Total time spent in locomotor activity – individual values (seconds)**

Animal	Predose	Week 4
Group 1M (Control)		
16	741	486
17	234	426
18	709	1073
19	635	888
20	705	985
Group 2M (Low dose)		
6	462	495
7	492	728
8	545	545
9	550	1072
10	180	163
Group 3M (Inter. dose)		
11	587	321
12	878	655
13	269	148
14	538	250
15	674	315
Group 4M (High dose)		
1	522	639
2	426	864
3	1198	649
4	987	517
5	721	381

APPENDIX 5**(Total time spent in locomotor activity – continued)**

Animal	Predose	Week 4
Group 1F (Control)		
31	401	681
32	786	552
33	674	550
34	273	229
35	437	410
Group 2F (Low dose)		
36	872	617
37	418	915
38	603	1172
39	727	631
40	810	277
Group 3F (Inter. dose)		
26	876	937
27	483	446
28	1000	797
29	975	917
30	351	297
Group 4F (High dose)		
21	568	789
22	280	516
23	448	672
24	330	2
25	699	806

APPENDIX 6

Additional comments from functional observational battery – individual observations

Pre-dose

Group 1 Sex M

No additional comments

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 2 Sex M

7 (7)

Slight brown nasal staining
In the arena: Scratching

8 (8)

Touch response: Rears

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 3 Sex M

11 (11)

Slight brown nasal staining

12 (12)

Touch response: Rears

14 (14)

Slight brown nasal staining

15 (15)

During manipulations: Soft pale faeces

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 4 Sex M

4 (4)

Slight brown nasal staining

5 (5)

Temperature: Slight body tremors

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 1 Sex F

31 (31)

Slight brown nasal staining

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 2 Sex F

36 (36)

During manipulations: Vocalising moderately
Landing footsplay: Awkward to handle

37 (37)

Slight brown nasal staining

40 (40)

In the arena: Scratching

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 3 Sex F

27 (27)

Touch response: Rears

30 (30)

Slight brown nasal staining

APPENDIX 6

(Additional comments from functional observational battery – continued)

Pre-dose

Group 4 Sex F

21 (21)

Slight brown nasal staining

22 (22)

In the arena: Scratching

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 1 Sex M

16 (16)

In the arena: Outside digit right hindlimb curled under

19 (19)

Slight brown nasal staining

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 2 Sex M

6 (6)

Slight brown nasal staining

8 (8)

Slight brown nasal staining

10 (10)

Slight brown nasal staining
Scab left forepaw

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 3 Sex M

12 (12)

Slight brown nasal staining

15 (15)

Slight brown nasal staining

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 4 Sex M

4 (4)

Slight brown nasal staining
Slight hair loss forelimbs

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 1 Sex F

No additional comments

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 2 Sex F

37 (37)

Slight brown nasal staining

40 (40)

Slight brown staining head and ears

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 3 Sex F

27 (27)

Slight hair loss face

29 (29)

Slight hair loss neck

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 1

Group 4 Sex F

21 (21)

Slight brown nasal staining

22 (22)

In the arena: Scratching

23 (23)

Slight brown nasal staining

In the arena: Climbed on front edge of arena

25 (25)

Lower teeth pale

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 1 Sex M

18 (18)

Slight brown nasal staining

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 2 Sex M

8 (8)

Slight brown nasal staining

9 (9)

Slight brown nasal staining

10 (10)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 3 Sex M

11 (11)

Slight brown nasal staining

12 (12)

In the arena: Climbed on front edge of arena

14 (14)

Slight brown nasal staining

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 4 Sex M

2 (2)

Slight brown nasal staining

3 (3)

Slight brown staining head and neck

4 (4)

Slight brown nasal staining and slight brown staining head and neck
In the arena: Climbed on front edge of arena

5 (5)

Slight brown nasal staining and slight brown staining head and neck

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 1 Sex F

31 (31)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 2 Sex F

36 (36)

In the arena: Climbed on front edge of arena

37 (37)

In the arena: Climbed on front edge of arena

38 (38)

In the arena: Climbed on front edge of arena

39 (39)

Tip of tail missing

In the arena: Climbed and walked on front edge of arena

40 (40)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 3 Sex F

27 (27)

Slight hair loss head

In the arena: Climbed on front edge of arena

28 (28)

In the arena: Climbed on front edge of arena

29 (29)

Moderate hair loss head and neck

In the arena: Scratching and climbed on front edge of arena

30 (30)

Scabs right ear and slight hair loss neck

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 2

Group 4 Sex F

21 (21)

Slight brown nasal staining

In the arena: Scratching, eyes slight to $\frac{1}{2}$ closed on occasions and climbed on front edge of arena

22 (22)

In the arena: Climbed on front edge of arena

23 (23)

In the arena: Climbed and walked on front edge of arena

24 (24)

Slight lack of grooming rump

In the arena: Climbed on front edge of arena

25 (25)

Eyes slight to $\frac{1}{2}$ closed on occasions and lower teeth pale

In the arena: Climbed on front edge of arena and jumped out of arena

APPENDIX 6

(Additional comments from functional observational battery -- continued)

Week 3

Group 1 Sex M

17 (17)

Slight lack of grooming rump

20 (20)

Slight lack of grooming rump

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 2 Sex M

6 (6)

In the arena: Climbed on front edge of arena

7 (7)

In the arena: Climbed on front edge of arena

8 (8)

Moderate brown nasal staining
Slight brown staining head, neck and muzzle

9 (9)

Slight brown nasal staining
Slight brown staining head and neck

10 (10)

Slight brown nasal staining
Slight lack of grooming rump
In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 3 Sex M

12 (12)

In the arena: Climbed on front edge of arena

13 (13)

In the arena: Climbed on front edge of arena

14 (14)

Slight brown nasal staining

In the arena: Climbed on front edge of arena

15 (15)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 4 Sex M

1 (1)

Slight brown staining neck
In the arena: Hunched on occasions

2 (2)

Slight brown staining neck
In the arena: Eyes slightly closed on occasions

3 (3)

Slight brown staining head and neck

4 (4)

Moderate brown nasal staining
Slight brown staining neck
Slight lack of grooming rump

5 (5)

Slight brown nasal staining
Slight brown staining head and neck

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 1 Sex F

31 (31)

In the arena: Climbed on front edge of arena

33 (33)

Slight brown nasal staining

In the arena: Climbed on front edge of arena

34 (34)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 2 Sex F

36 (36)

Moderate brown staining head, neck and ears

Slight lack of grooming rump

In the arena: Climbed and walked on front edge of arena

37 (37)

Slight brown staining head and neck

Slight hair loss neck

In the arena: Climbed and walked on front edge of arena

38 (38)

Slight brown nasal staining

In the arena: Climbed and walked on front edge of arena

39 (39)

Slight brown staining head and neck

Tip of tail missing

In the arena: Climbed on front edge of arena

40 (40)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 3 Sex F

26 (26)

In the arena: Climbed on front edge of arena

27 (27)

In the arena: Climbed on front edge of arena

28 (28)

Slight hair loss neck

Slight brown staining ears

In the arena: Climbed and walked on front edge of arena

29 (29)

In the arena: Climbed on front edge of arena

30 (30)

Slight hair loss and brown staining neck

Slight brown nasal staining

Small scab right ear

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 3

Group 4 Sex F

21 (21)

Slight lack of grooming rump

22 (22)

Slight brown nasal staining

Slight lack of grooming rump

In the arena: Climbed on front edge of arena

23 (23)

Slight hair loss dorsal

Slight brown staining head

In the arena: Climbed and walked on front edge of arena

24 (24)

Slight lack of grooming rump

Small nick right ear

In the arena: Climbed on front edge of arena

25 (25)

Lower teeth pale

Slight lack of grooming rump

Slight brown staining head

Eyes slightly closed on occasions

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 1 Sex M

16 (16)

Slight brown nasal staining

In the arena: Climbed on front edge of arena

17 (17)

Approach response: Bit probe

18 (18)

Slight brown staining head

19 (19)

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 2 Sex M

6 (6)

Slight brown nasal staining
In the arena: Climbed on front edge of arena

7 (7)

Slight brown nasal staining
In the arena: Climbed on front edge of arena

8 (8)

Slight brown nasal staining
Slight brown staining head and neck
In the arena: Climbed on front edge of arena

9 (9)

Slight brown staining head and neck

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 3 Sex M

11 (11)

Slight brown nasal staining

In the arena: Climbed on front edge of arena

12 (12)

Slight brown staining head and neck

In the arena: Climbed on front edge of arena

13 (13)

In the arena: Climbed on front edge of arena

14 (14)

In the arena: Climbed on front edge of arena

15 (15)

Slight brown nasal staining

Moderate brown staining head and neck

In the arena: Climbed and sat on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 4 Sex M

1 (1)

Slight brown staining neck
Slight brown nasal staining
In the arena: Climbed on front edge of arena

2 (2)

Moderate brown staining head and neck
In the arena: Scratching
Touch response: Rears

3 (3)

Approach response: Bit probe

4 (4)

In the arena: Climbed on front edge of arena and scratching

5 (5)

Moderate brown staining head and neck
Slight lack of grooming rump

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 1 Sex F

31 (31)

Slight brown staining ears

In the arena: Climbed on front edge of arena

During manipulations: Moderately vocalising throughout

32 (32)

Slight brown staining ears and head

33 (33)

In the arena: Climbed on front edge of arena

34 (34)

Slight brown staining ears and head

Slight lack of grooming rump

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 2 Sex F

36 (36)

Moderate brown staining head and neck

In the arena: Climbed and walked on front edge of arena

During manipulations: Loud/aggressively vocalising throughout and awkward to handle

37 (37)

Moderate brown staining head and neck

In the arena: Climbed and walked on front edge of arena

Approach response: Attempted to bite probe

Touch response: Rears

38 (38)

Slight brown staining head and neck

In the arena: Climbed and walked on front edge of arena

Temperature: Moderately vocalising

39 (39)

Slight lack of grooming rump

Tip of tail missing

Slight brown staining head and neck

In the arena: Climbed and walked on front edge of arena

Touch response: Rears

40 (40)

Slight lack of grooming rump

In the arena: Climbed and walked on front edge of arena

During manipulations: Moderately vocalising throughout

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 3 Sex F

26 (26)

In the arena: Climbed on front edge of arena

27 (27)

Slight hair loss right hindlimb

Slight brown staining head and neck

In the arena: Climbed and walked on front edge of arena

28 (28)

Slight brown nasal staining

Slight hair loss head

In the arena: Climbed and walked on front edge of arena

Pupil reflex: Right eye pupil dilated under light at 1st attempt

29 (29)

In the arena: Climbed on front edge of arena

Touch response: Rears

30 (30)

Slight lack of grooming rump

Slight brown staining head

Small nick out of Right ear

In the arena: Climbed on front edge of arena

APPENDIX 6

(Additional comments from functional observational battery – continued)

Week 4

Group 4 Sex F

21 (21)

In the arena: Eyes slight to $\frac{1}{2}$ closed on occasions and climbed on front edge of arena

22 (22)

Slight brown nasal staining

Slight brown staining head

In the arena: Climbed and walked on front edge of arena

23 (23)

Slight brown staining head and neck

Slight lack of grooming rump

In the arena: Climbed and walked on front edge of arena

Pupil reflex: Left pupil dilated under light on first attempt, on 2nd attempt constricted as normal

24 (24)

Moderate lack of grooming rump

Small nick out of right ear

25 (25)

Moderate lack of grooming rump

Lower teeth pale

In the arena: Climbed on front edge of arena

Approach response: Bit Probe

Touch response: Rears

APPENDIX 7

Haematology – individual values

Group	Animal	Hct	Hb	RBC	Retic	MCH	MCHC	MCV	WBC	Neutr ophil
		L/L	g/dL	x10-12/L	%	pg	g/dL	fL	x10-9/L	x10-9/L
1M	16	0.488	17.2	8.23	3.06	21.0	35.3	59.3	21.75	1.62
	17	0.477	16.8	8.59	2.37	19.6	35.3	55.5	16.51	1.08
	18	0.459	16.1	7.93	3.16	20.3	35.1	57.9	11.16	1.49
	19	0.462	16.0	8.31	3.44	19.2	34.6	55.6	15.68	2.13
	20	0.471	16.1	8.25	3.09	19.5	34.1	57.0	19.11	2.72
2M	6	0.452	16.1	8.00	2.47	20.2	35.7	56.5	16.21	1.67
	7	0.471	16.4	8.37	2.49	19.6	34.9	56.3	14.04	2.14
	8	0.434	15.4	7.92	2.00	19.4	35.3	54.8	11.45	0.97
	9	0.459	16.4	8.19	2.38	20.0	35.7	56.1	13.77	1.63
	10	0.489	17.3	8.63	2.17	20.0	35.3	56.7	18.88	4.26
3M	11	0.483	16.8	8.64	2.68	19.4	34.7	55.9	12.01	1.73
	12	0.453	16.3	7.85	2.05	20.8	36.0	57.7	19.98	1.61
	13	0.483	16.9	7.90	2.52	21.4	35.1	61.2	16.54	1.35
	14	0.455	16.0	8.23	2.80	19.5	35.2	55.3	13.28	0.91
	15	0.424	14.8	7.60	1.63	19.4	34.8	55.8	12.59	1.00
4M	1	0.463	16.3	8.65	2.38	18.9	35.2	53.5	16.93	2.12
	2	0.453	16.5	8.27	1.83	19.9	36.4	54.8	14.59	2.92
	3	0.434	15.3	7.64	3.40	20.0	35.3	56.8	18.58	2.24
	4	CTD	CTD	CTD	CTD	CTD	CTD	CTD	CTD	CTD
	5	0.450	16.1	8.04	2.58	20.0	35.7	56.0	14.44	1.59

CTD Clotted sample

APPENDIX 7

(Haematology -- continued)

Group	Animal	Lymph ocyte x10-9/L	Eosin ophil x10-9/L	Baso phil x10-9/L	Mono cyte x10-9/L	LUC x10-9/L	Plt x10-9/L	PT sec	APTT sec
1M	16	19.41	0.26	0.13	0.21	0.13	654	12.5	15.7
	17	14.81	0.15	0.06	0.20	0.21	1181	13.8	20.5
	18	9.26	0.17	0.03	0.12	0.10	1091	14.4	19.8
	19	13.16	0.14	0.05	0.10	0.08	1241	12.0	18.2
	20	15.67	0.10	0.08	0.28	0.26	627	12.4	19.2
2M	6	13.99	0.08	0.07	0.19	0.20	1026	12.9	13.3
	7	11.25	0.14	0.05	0.22	0.23	1040	12.6	18.4
	8	10.06	0.09	0.03	0.14	0.15	1221	13.0	19.1
	9	11.71	0.15	0.05	0.11	0.13	1126	13.3	20.9
	10	13.99	0.17	0.07	0.20	0.20	1167	12.8	18.9
3M	11	10.04	0.08	0.04	0.06	0.06	1028	13.4	18.8
	12	17.80	0.09	0.10	0.15	0.22	1305	13.5	18.2
	13	14.75	0.08	0.08	0.17	0.11	1013	14.5	19.3
	14	12.05	0.08	0.06	0.08	0.10	1187	12.8	20.6
	15	11.24	0.11	0.04	0.12	0.08	1085	13.5	20.5
4M	1	14.27	0.07	0.06	0.22	0.20	954	13.7	19.6
	2	11.08	0.11	0.06	0.21	0.21	773	13.5	19.7
	3	15.57	0.08	0.09	0.31	0.29	1360	13.9	16.1
	4	CTD	CTD	CTD	CTD	CTD	CTD	11.9	13.3
	5	12.13	0.13	0.05	0.29	0.26	1004	13.5	15.1

CTD Clotted sample

APPENDIX 7

(Haematology – continued)

Group	Animal	Aniso cyto	Micro cyto	Macro cyto	Hypo chrom	Hyper chrom
1M	16	-	-	-	-	-
	17	-	-	-	-	-
	18	-	-	-	-	-
	19	-	-	-	-	-
	20	-	-	-	-	-
2M	6	-	-	-	-	-
	7	-	-	-	-	-
	8	-	-	-	-	-
	9	-	-	-	-	-
	10	-	-	-	-	-
3M	11	-	-	-	-	-
	12	-	-	-	-	-
	13	-	-	-	-	-
	14	-	-	-	-	-
	15	-	-	-	-	-
4M	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-
	4	CTD	CTD	CTD	CTD	CTD
	5	-	-	-	-	-
CTD	Clotted sample					

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APPENDIX 7
(Haematology - continued)

Group	Animal	Hct L/L	Hb g/dL	RBC x10-12/L	Retic %	MCH pg	MCHC g/dL	MCV fL	WBC x10-9/L	Neutr ophil x10-9/L
1F	31	0.455	15.6	8.00	2.51	19.6	34.4	56.9	9.91	1.13
	32	0.417	14.8	7.53	2.80	19.6	35.5	55.4	9.90	1.47
	33	0.432	15.2	7.77	2.87	19.5	35.1	55.6	13.13	0.88
	34	0.441	15.8	7.96	2.14	19.9	35.8	55.5	7.17	0.56
	35	0.418	15.1	7.29	2.26	20.7	36.1	57.3	10.51	0.72
2F	36	0.396	14.0	7.29	1.95	19.3	35.4	54.4	6.12	0.75
	37	0.414	15.0	7.25	1.94	20.7	36.3	57.1	13.33	1.22
	38	0.415	14.6	7.66	2.08	19.1	35.2	54.2	6.66	0.90
	39	0.408	14.3	7.35	2.33	19.4	35.0	55.6	6.47	0.66
	40	0.412	14.8	7.38	1.87	20.1	36.0	55.8	6.18	0.66
3F	26	0.387	13.9	6.94	2.52	20.0	35.8	55.8	6.21	1.39
	27	0.408	14.7	7.25	2.05	20.3	36.0	56.3	4.21	0.70
	28	0.386	13.8	7.22	2.83	19.1	35.8	53.5	4.79	0.76
	29	0.412	14.8	7.29	1.88	20.4	36.0	56.6	7.31	0.89
	30	0.412	14.7	7.51	1.56	19.6	35.7	54.8	5.99	0.67
4F	21	0.390	14.6	7.28	1.06	20.1	37.5	53.6	6.48	1.18
	22	0.392	14.2	7.51	2.59	18.9	36.1	52.2	7.03	0.81
	23	0.417	14.7	7.20	2.80	20.4	35.2	57.9	8.73	2.18
	24	0.441	15.9	8.03	2.02	19.7	36.0	54.9	9.24	1.05
	25	0.414	14.9	7.34	2.63	20.3	36.0	56.4	7.34	1.04

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APPENDIX 7

(Haematology – continued)

Group	Animal	Lymph ocyte x10-9/L	Eosin ophil x10-9/L	Baso phil x10-9/L	Mono cyte x10-9/L	LUC x10-9/L	Plt x10-9/L	PT sec	APTT sec
1F	31	8.20	0.27	0.03	0.09	0.19	1046	13.0	16.1
	32	7.94	0.21	0.02	0.15	0.11	995	13.5	18.7
	33	11.72	0.12	0.05	0.17	0.18	1262	14.1	17.7
	34	6.35	0.08	0.02	0.08	0.07	838	13.7	15.3
	35	9.21	0.21	0.03	0.20	0.13	1216	14.2	17.9
2F	36	5.08	0.10	0.01	0.10	0.08	1095	14.4	18.1
	37	11.63	0.12	0.03	0.18	0.14	1183	13.3	17.1
	38	5.49	0.13	0.01	0.05	0.08	933	14.1	19.0
	39	5.63	0.09	0.01	0.05	0.04	1008	13.8	16.9
	40	5.26	0.08	0.01	0.10	0.07	1027	13.3	12.8
3F	26	4.66	0.08	0.01	0.05	0.03	916	13.3	11.1
	27	3.35	0.06	0.00	0.07	0.03	1162	13.9	14.6
	28	3.79	0.13	0.01	0.06	0.04	886	11.1	13.0
	29	6.14	0.11	0.02	0.11	0.04	1083	14.5	17.5
	30	5.14	0.09	0.01	0.05	0.04	776	14.8	15.3
4F	21	4.96	0.15	0.02	0.10	0.08	972	14.2	14.4
	22	6.02	0.08	0.02	0.05	0.05	1059	14.4	17.2
	23	6.31	0.06	0.01	0.08	0.09	1036	14.3	18.1
	24	7.98	0.07	0.03	0.05	0.06	1194	14.5	15.4
	25	6.08	0.08	0.01	0.07	0.06	1113	14.4	16.5

APPENDIX 7

(Haematology – continued)

Group	Animal	Aniso cyto	Micro cyto	Macro cyto	Hypo chrom	Hyper chrom
1F	31	-	-	-	-	-
	32	-	-	-	-	-
	33	-	-	-	-	-
	34	-	-	-	-	-
	35	-	-	-	-	-
2F	36	-	-	-	-	-
	37	-	-	-	-	-
	38	-	-	-	-	-
	39	-	-	-	-	-
	40	-	-	-	-	-
3F	26	-	-	-	-	-
	27	-	-	-	-	-
	28	-	-	-	-	-
	29	-	-	-	-	-
	30	-	-	-	-	-
4F	21	-	-	-	-	-
	22	-	-	-	-	-
	23	-	-	-	-	-
	24	-	-	-	-	-
	25	-	-	-	-	-

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APPENDIX 8

Blood chemistry – individual values

Group	Animal	Alk. Phos U/L	ALT U/L	AST U/L	Bili. Total umol/L	Urea mmol/L	Creat umol/L	Gluc mmol/L	Chol Total mmol/L	Trig mmol/L
1M	16	370	41	75	3	4.95	43	7.42	1.64	0.92
	17	335	45	82	2	6.00	43	6.36	1.35	0.90
	18	462	48	79	2	6.58	43	7.90	1.73	0.54
	19	612	39	78	3	5.02	46	7.48	1.32	0.82
	20	400	49	114	2	6.19	41	7.86	1.44	0.37
2M	6	498	41	79	2	6.05	42	7.88	2.01	0.88
	7	406	47	79	2	4.60	38	7.28	1.86	0.92
	8	396	43	70	2	5.87	41	6.94	1.67	0.50
	9	518	37	77	1	5.44	40	6.31	1.38	0.77
	10	416	45	68	1	5.90	44	7.67	1.54	0.96
3M	11	534	41	71	1	5.39	39	6.85	1.52	0.46
	12	466	36	71	1	4.91	43	5.83	1.36	0.59
	13	457	46	81	1	6.13	40	6.12	1.49	0.70
	14	386	32	66	2	4.91	42	7.99	1.49	0.42
	15	385	36	73	2	4.78	42	7.28	1.06	0.35
4M	1	389	39	88	2	6.69	45	6.86	0.99	0.26
	2	379	42	87	2	5.39	42	6.70	1.36	0.46
	3	337	52	100	2	5.06	42	5.84	1.31	0.84
	4	430	39	95	3	5.89	51	8.18	1.08	0.66
	5	372	39	99	1	5.84	40	7.77	1.05	0.81

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Na mmol/L	K mmol/L	Cl mmol/L	Ca Total mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	a1 Glob g/L	a2 Glob g/L
1M	16	142	3.4	101	2.78	2.43	63	32	13	4
	17	139	4.2	99	2.75	2.45	64	32	13	4
	18	140	4.2	100	2.81	2.36	65	30	15	4
	19	139	4.0	99	2.79	2.34	65	33	14	4
	20	138	5.2	101	2.59	2.25	64	30	15	4
2M	6	139	3.9	100	2.56	2.23	60	32	11	3
	7	139	4.0	101	2.63	2.31	63	31	11	4
	8	141	3.7	102	2.52	2.02	59	32	11	3
	9	141	3.9	101	2.70	2.15	62	31	13	4
	10	140	3.5	99	2.72	2.09	64	32	11	5
3M	11	139	4.1	100	2.66	2.48	62	32	13	4
	12	140	3.9	100	2.68	2.45	60	30	12	4
	13	140	4.1	100	2.69	2.94	61	32	11	4
	14	138	3.9	98	2.56	2.11	59	29	13	4
	15	139	3.7	101	2.54	2.17	61	29	12	4
4M	1	141	3.7	102	2.45	2.20	60	32	11	3
	2	140	3.6	101	2.61	2.18	63	34	11	3
	3	139	3.7	101	2.63	2.35	60	30	12	3
	4	139	4.2	101	2.56	2.26	59	32	13	3
	5	139	3.5	102	2.64	2.20	64	34	12	3

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Beta Glob g/L	Gamma Glob g/L	A/G Ratio
1M	16	12	2	1.03
	17	12	3	1.00
	18	13	3	0.86
	19	12	2	1.03
	20	12	3	0.88
2M	6	11	2	1.14
	7	14	4	0.97
	8	11	2	1.19
	9	12	2	1.00
	10	13	3	1.00
3M	11	10	2	1.07
	12	12	3	1.00
	13	11	3	1.10
	14	11	2	0.97
	15	13	3	0.91
4M	1	11	3	1.14
	2	11	3	1.17
	3	12	3	1.00
	4	10	2	1.19
	5	12	3	1.13

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Alb g	a1 Glob g	a2 Glob g	Beta Glob g	Gamma Glob g
1M	16	51.5	20.8	6.1	18.5	3.0
	17	50.1	20.3	6.0	18.4	5.1
	18	46.1	23.0	6.8	20.0	4.1
	19	51.1	21.1	6.1	18.6	3.1
	20	46.8	22.9	6.3	18.9	5.1
2M	6	53.5	17.9	5.8	19.0	3.7
	7	48.8	17.8	5.8	22.0	5.6
	8	53.9	19.1	5.4	18.1	3.6
	9	49.4	20.7	6.4	20.1	3.4
	10	50.0	17.5	7.4	20.1	5.0
3M	11	51.8	20.6	7.2	16.8	3.7
	12	49.8	19.8	6.8	19.2	4.4
	13	52.3	17.6	7.0	17.6	5.6
	14	49.5	22.1	7.0	18.0	3.3
	15	47.1	20.0	6.8	20.9	5.1
4M	1	53.6	18.5	4.7	17.9	5.2
	2	53.4	17.9	5.4	17.8	5.5
	3	50.1	20.1	4.8	20.8	4.2
	4	53.8	21.3	5.1	16.7	3.1
	5	53.7	18.1	5.1	18.0	5.0

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Alk. Phos U/L	ALT U/L	AST U/L	Bili. Total umol/L	Urea mmol/L	Creat umol/L	Gluc mmol/L	Chol Total mmol/L	Trig mmol/L
1F	31	201	42	78	2	6.23	37	6.69	2.55	0.77
	32	258	42	87	3	7.93	47	7.71	1.96	0.46
	33	287	35	81	4	7.73	47	6.33	2.26	0.43
	34	203	34	79	2	5.94	44	7.32	1.41	0.30
	35	238	37	79	2	7.92	45	5.88	2.26	0.69
2F	36	287	32	76	2	5.41	48	7.72	2.04	0.42
	37	167	39	63	2	6.50	45	7.03	1.71	0.94
	38	167	38	89	2	7.56	50	6.76	2.04	0.31
	39	240	53	97	1	6.16	42	7.78	1.78	0.44
	40	215	43	79	1	6.96	48	7.95	1.97	0.37
3F	26	246	31	83	2	5.74	43	6.76	1.61	0.44
	27	230	27	71	2	5.13	43	6.03	1.39	0.45
	28	320	36	100	2	5.48	42	5.76	1.53	0.68
	29	249	41	86	2	6.26	47	6.08	1.58	0.37
	30	286	32	77	1	6.10	47	8.34	1.51	0.36
4F	21	241	31	65	2	4.64	41	6.71	1.70	1.05
	22	201	28	83	1	7.57	43	6.48	1.28	0.35
	23	233	37	74	2	6.49	43	6.49	1.36	0.67
	24	305	27	75	2	5.83	42	7.53	0.82	0.21
	25	218	40	85	2	6.41	44	7.92	0.97	0.29

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Na mmol/L	K mmol/L	Cl mmol/L	Ca Total mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	a1 Glob g/L	a2 Glob g/L
1F	31	139	3.7	100	2.69	2.05	65	35	10	5
	32	140	3.7	99	2.65	1.96	66	34	11	4
	33	140	3.9	101	2.61	2.05	66	35	11	4
	34	141	3.7	102	2.58	1.63	62	33	10	4
	35	139	3.6	101	2.79	1.88	63	35	11	4
2F	36	140	3.5	101	2.54	1.51	64	32	11	5
	37	140	3.7	99	2.79	1.95	68	39	12	4
	38	140	3.8	102	2.58	1.92	61	32	11	4
	39	140	3.3	102	2.42	1.47	63	35	11	4
	40	138	3.4	99	2.62	1.81	65	36	11	4
3F	26	140	3.5	103	2.45	1.49	55	30	9	3
	27	140	3.3	101	2.61	1.71	66	36	11	4
	28	139	3.9	101	2.44	1.98	53	29	9	3
	29	138	3.8	99	2.58	1.85	62	34	11	4
	30	139	3.2	101	2.44	1.57	61	34	10	4
4F	21	136	3.7	98	2.59	1.74	64	36	13	4
	22	138	3.4	102	2.60	1.84	57	32	10	4
	23	137	3.7	98	2.49	1.95	62	33	10	3
	24	139	3.2	101	2.40	1.86	56	30	9	3
	25	137	3.2	99	2.48	1.60	60	32	10	3

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Beta Glob g/L	Gamma Glob g/L	A/G Ratio
1F	31	12	3	1.17
	32	13	4	1.06
	33	11	4	1.13
	34	11	3	1.14
	35	11	3	1.25
2F	36	13	3	1.00
	37	10	2	1.34
	38	12	3	1.10
	39	11	3	1.25
	40	12	3	1.24
3F	26	11	2	1.20
	27	12	4	1.20
	28	9	2	1.21
	29	10	3	1.21
	30	10	3	1.26
4F	21	9	3	1.29
	22	10	2	1.28
	23	12	4	1.14
	24	10	3	1.15
	25	12	4	1.14

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APPENDIX 8

(Blood chemistry – continued)

Group	Animal	Alb g	a1 Glob g	a2 Glob g	Beta Glob g	Gamma Glob g
1F	31	54.2	15.4	7.0	19.2	4.2
	32	51.4	17.2	6.5	19.2	5.7
	33	53.3	17.1	6.2	16.7	6.6
	34	53.2	16.8	6.1	18.3	5.6
	35	55.4	17.0	5.8	17.2	4.5
2F	36	50.0	17.8	7.1	19.9	5.3
	37	57.8	17.9	5.9	15.1	3.4
	38	53.0	17.6	6.1	19.1	4.1
	39	55.4	16.8	6.0	17.7	4.2
	40	55.3	16.2	6.1	17.8	4.5
3F	26	53.7	16.5	6.1	19.4	4.3
	27	53.9	16.7	6.2	17.8	5.4
	28	54.7	17.2	6.4	17.5	4.2
	29	54.8	17.7	6.1	16.9	4.5
	30	55.4	16.4	6.1	16.7	5.4
4F	21	55.6	19.7	5.9	14.7	4.0
	22	55.4	16.9	6.2	17.9	3.6
	23	53.2	15.6	5.6	18.8	6.9
	24	54.4	15.4	5.7	18.5	6.0
	25	53.4	16.0	5.1	19.3	6.2

: 175 :

MIN 252/004312

APPENDIX 9

Absolute organ weights - individual values (g)

Print No: 0024

Printed: 05-MAR-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Kyblon protocol number: MIN 252

		TERMINAL							
GROUP	ANIMAL	BODY WT (g)	ADRENALS	EPIDIDYMIID	HEART	KIDNEYS	LIVER	LUNGS	TESTES
1M	16	397.8	0.054	1.033	1.208	2.47	14.00	1.254	3.57
	17	371.8	0.065	0.948	1.072	2.47	14.36	1.217	3.18
	18	396.8	0.048	0.966	1.299	2.48	14.62	1.167	3.34
	19	379.7	0.054	0.967	1.447	2.37	14.20	1.310	3.00
	20	383.8	0.049	0.976	1.499	2.39	16.69	1.347	3.45
2M	6	375.3	0.062	1.048	1.825	2.41	14.77	1.482	3.96
	7	394.5	0.054	1.069	1.603	2.67	14.01	1.279	4.13
	8	296.4	0.053	0.887	1.272	1.94	9.85	1.056	3.04
	9	356.7	0.064	0.991	1.347	2.27	12.18	1.345	3.49
	10	365.7	0.062	0.867	1.698	2.64	14.53	1.370	2.79
3M	11	341.2	0.063	0.813	1.088	2.24	12.15	1.115	3.19
	12	360.0	0.060	0.920	1.403	2.21	12.00	1.188	3.42
	13	395.4	0.060	0.977	1.574	2.64	13.55	1.334	3.36
	14	357.8	0.055	0.868	1.170	2.22	11.39	1.333	3.25
	15	336.6	0.054	1.020	1.148	2.45	13.07	1.271	3.24
4M	1	325.2	0.060	0.912	1.115	2.09	12.55	1.225	3.63
	2	299.6	0.052	0.886	0.913	1.89	10.49	1.101	3.18
	3	385.6	0.068	0.860	1.371	2.61	14.78	1.228	3.20
	4	340.5	0.038	0.856	1.044	2.03	11.49	1.149	3.01
	5	307.6	0.054	1.006	1.089	2.48	14.16	1.198	3.52

Epididymid = Epididymides

MIN 252/004312

APPENDIX 9

(Absolute organ weights – continued)

Print No: 0025

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Printed: 05-MAR-01

Xybion protocol number: MIN 252

		TERMINAL					
GROUP	ANIMAL	BODY WT (g)	ADRENALS	HEART	KIDNEYS	LIVER	LUNGS
1F	31	211.1	0.046	0.927	1.45	7.89	0.930
	32	234.3	0.071	1.142	1.51	8.78	1.004
	33	198.8	0.054	0.704	1.14	6.52	0.931
	34	212.0	0.052	0.995	1.34	7.10	1.149
	35	217.7	0.061	0.850	1.43	8.75	0.978
2F	36	188.0	0.063	0.825	1.53	7.09	0.999
	37	224.4	0.071	0.868	1.61	8.51	0.957
	38	211.0	0.082	0.966	1.37	7.72	0.937
	39	211.7	0.067	1.036	1.40	7.53	0.961
	40	219.1	0.062	0.797	1.50	7.52	0.838
3F	26	200.8	0.052	0.763	1.32	6.94	0.866
	27	213.4	0.059	0.797	1.59	7.59	0.958
	28	191.5	0.048	0.956	1.40	6.67	0.900
	29	220.0	0.054	1.070	1.41	7.77	1.038
	30	207.9	0.050	0.871	2.23	7.72	0.970
4F	21	194.2	0.075	0.904	1.52	8.19	1.001
	22	177.8	0.059	0.722	1.45	6.91	0.843
	23	222.1	0.064	0.721	1.49	7.14	0.958
	24	220.2	0.084	1.003	1.54	7.99	0.995
	25	210.5	0.064	0.849	2.31	7.58	0.948

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MIN 252/004312

APPENDIX 10

Individual pathological findings

The initial examination was undertaken by the study pathologist, the results of which were then subjected to a routine peer review by a second pathologist. The diagnoses reported here represent the consensus opinions of both pathologists.

Study pathologist: Sheila E. Begg, M.A., Vet.M.B., M.R.C.V.S., F.R.C.Path.,
Consultant Pathologist,
Department of Pathology.

Peer review: David J. Lewis, Ph.D., F.R.C.Path.,
Consultant Pathologist,
Department of Pathology.

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xyblon protocol number: MIN 252

ANIMAL NUMBER: 0016	SEX: MALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 397.8 GRAMS

NECROPSY

PATHOLOGY OBSERVATIONS

HISTOPATHOLOGY

KIDNEYS :

-CORTICAL TUBULAR BASOPHILIA, -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0017	SEX: MALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 371.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

KIDNEYS :

-CORTICAL TUBULAR BASOPHILIA, -MINIMAL, FOCAL

LUNGS & BRONCHI :

-ALVEOLITIS, -MINIMAL, FOCAL

-ALVEOLAR OSSEOUS METAPLASIA, -MINIMAL, FOCAL

NASAL TURBINATES :

-TRANSITIONAL EPITHELIAL HYPERPLASIA, -MINIMAL, FOCAL

>NOTE:>LESION IN LATERAL WALL

TRACHEA :

-SUBEPITHELIAL INFLAMMATION, -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 180 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm): 0 50 150 450

Print No: 0026

Printed: 05-MAR-01

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0018 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 396.8 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

KIDNEYS :

-UNILATERAL PELVIC DILATATION, -SLIGHT

LUNGS & BRONCHI :

-ALVEOLITIS, -MINIMAL, FOCAL

NASAL TURBINATES :

-TRANSITIONAL EPITHELIAL HYPERPLASIA, -MINIMAL, FOCAL

>NOTE:>LESION IN LATERAL WALL AND VENTRAL PART OF NASOTURBinate

TRACHEA :

-SUBEPITHELIAL INFLAMMATION, -MINIMAL, MULTI-FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 181 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0019	SEX: MALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 379.7 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

KIDNEYS :

- MEDULLARY TUBULAR BASOPHILIA, -MINIMAL, FOCAL
- TUBULAR HYPERPLASIA, SIMPLE, -SLIGHT, FOCAL

LARYNX :

- EPITHELIAL HYPERPLASIA - ARYTENOIDS, -SLIGHT
- EPITHELIAL HYPERPLASIA - VENTRAL POUCH, -MODERATE
- INFLAMMATORY CELLS IN LAMINA PROPRIA (VENTRAL AND VENTROLATERAL), -SLIGHT

LUNGS & BRONCHI :

- ALVEOLAR OSSEOUS METAPLASIA, -MINIMAL, FOCAL

TRACHEA :

- SUBEPITHELIAL INFLAMMATION, -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0020	SEX: MALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 383.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

LN MANDIBULAR :
-CYSTIC ENLARGEMENT; RIGHT, ONE, 10MM.

KIDNEYS :
-CORTICAL TUBULAR BASOPHILIA, -MINIMAL, FOCAL

LN MANDIBULAR :
-CYSTIC SINUSES, -MODERATE

NASAL TURBINATES :
-RESPIRATORY EPITHELIAL HYPERPLASIA, -SLIGHT, FOCAL
>NOTE:>LESION IN DORSAL MEATUS

: 183 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0006	SEX: MALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 375.3 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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MIN 252/004312

APPENDIX 10

(Individual pathological findings -- continued)

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Print No: 0026

Printed: 05-MAR-01

Xyblon protocol number: MIN 252

ANIMAL NUMBER: 0007	SEX: MALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 394.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 185 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings -- continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0008	SEX: MALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 296.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0009	SEX: MALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 356.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group : 1 2 3 4
Compound : Control ----- T-7499 -----
Exposure level (ppm) : 0 50 150 450

Xyblon protocol number: MIN 252

ANIMAL NUMBER: 0010 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 365.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0011	SEX: MALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 341.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

: 190 :

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0012	SEX: MALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 360.0 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0013	SEX: MALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 395.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0014	SEX: MALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 357.8 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

KIDNEYS :
-PELVIC DILATION, MINIMAL; LEFT.

KIDNEYS :
-CORTICAL TUBULAR BASOPHILIA, -MINIMAL, FOCAL
-DILATED MEDULLARY TUBULES CONTAINING EOSINOPHILIC COLLOID, -
MINIMAL, FOCAL
-UNILATERAL PELVIC DILATATION, -MINIMAL

: 192 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0015	SEX: MALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 336.6 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xyblon protocol number: MIN 252

ANIMAL NUMBER: 0001	SEX: MALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 325.2 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

LN MANDIBULAR :
-ENLARGED; ONE.

LN MANDIBULAR :
-INCREASED CELLULARITY - GENERALISED, -SLIGHT

LN PANCREATIC :
-ENLARGED, MINIMAL; ONE.

LN PANCREATIC :
-SINUS ERYTHROCYTOSIS/ERYTHROPHAGOCYTOSIS, -MINIMAL

: 194 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Kybion protocol number: MIN 252

ANIMAL NUMBER: 0002	SEX: MALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 299.6 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

LN MANDIBULAR :
-CONGESTED, MINIMAL; RIGHT, ONE.

LUNGS & BRONCHI :
-DARK AREA(S); RIGHT ANTERIOR LOBE, ONE, SUBPLEURAL, 2MM.

KIDNEYS :
-CORTICAL TUBULAR BASOPHILIA, -MINIMAL, FOCAL

LN MANDIBULAR :
-SINUS ERYTHROCYTOSIS/ERYTHROPHAGOCYTOSIS, -SLIGHT

LUNGS & BRONCHI :
-SUBPLEURAL INFLAMMATION, -MINIMAL, FOCAL

: 195 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0003	SEX: MALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 385.6 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

SKIN :

-SCAB(S); TAIL, A FEW, 1MM. (ON TATTOO)

SKIN :

-SCAB, -PRESENT
 -EPIDERMAL HYPERPLASIA, -MODERATE, FOCAL
 -EPIDERMAL ULCERATION, -MINIMAL, FOCAL
 -DERMAL INFLAMMATION, -SLIGHT, FOCAL

STOMACH :

-ANTRUM WHITE NODULE(S); MUCOSA, ONE, NEAR TO LIMITING RIDGE, 1MM.

STOMACH :

-ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR MUCOSA, FOCAL, -PRESENT

: 196 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Kybion protocol number: MIN 252

ANIMAL NUMBER: 0004	SEX: MALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 340.5 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

LARYNX :
 -EPITHELIAL HYPERPLASIA - ARYTENOIDS, -SLIGHT
 TRACHEA :
 -SUBEPITHELIAL INFLAMMATION, -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 197 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0005	SEX: MALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 307.6 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybian protocol number: MIN 252

ANIMAL NUMBER: 0031	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 211.1 GRAMS

NECROPSY

P A T H O L O G Y O B S E R V A T I O N S

HISTOPATHOLOGY

LIVER :

-MEDIAN CLEFT PALE AREA(S); ONE, SUBCAPSULAR, 2MM.
-LOBE(S) NECROTIC; RIGHT ANTERIOR LOBE.

LIVER :

-SIDEROPHAGES, BILE DUCT HYPERPLASIA, FIBROSIS AND
MINERALISATION - RIGHT ANTERIOR LOBE, -MODERATE

LUNGS & BRONCHI :

-ALVEOLITIS, -MINIMAL, FOCAL
-EXTRAVASATION OF EOSINOPHILS, -MINIMAL, FOCAL

: 199 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0032	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 234.3 GRAMS

NECROPSY

PATHOLOGY OBSERVATIONS

HISTOPATHOLOGY

TRACHEA :
-SUBEPITHELIAL INFLAMMATION, -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 200 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Printed: 05-MAR-01

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0033	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 198.8 GRAMS

NECROPSY

P A T H O L O G Y O B S E R V A T I O N S

HISTOPATHOLOGY

LUNGS & BRONCHI :
-ALVEOLITIS, -MINIMAL, FOCAL

TRACHEA :
-SUBEPITHELIAL INFLAMMATION, -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 201 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0034	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 212.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

KIDNEYS :
-PELVIC DILATION, MINIMAL; RIGHT.

KIDNEYS :
-UNILATERAL PELVIC DILATATION, -MINIMAL

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0035	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 217.7 GRAMS

NECROPSY

PATHOLOGY OBSERVATIONS

HISTOPATHOLOGY

LARYNX :

-EPITHELIAL HYPERPLASIA - ARYTENOIDS, -SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybio protocol number: MIN 252

ANIMAL NUMBER: 0036	SEX: FEMALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 188.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

STOMACH :
-ANTRUM WHITE NODULE(S); MUCOSA, ONE, NEAR TO LIMITING
RIDGE, 2MM.

UTERUS :
-FLUID DISTENTION

STOMACH :
-ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR MUCOSA, FOCAL,-
PRESENT

UTERUS :
-LUMINAL DILATATION,-SLIGHT

: 205 :

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0037	SEX: FEMALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 224.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

: 206 :

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0038	SEX: FEMALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 211.0 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0039	SEX: FEMALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 211.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 207 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0040	SEX: FEMALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 219.1 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0026	SEX: FEMALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 200.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

LN MANDIBULAR :
-ENLARGED, MINIMAL

LN MANDIBULAR :
-INCREASED CELLULARITY - GENERALISED, -SLIGHT

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0027	SEX: FEMALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 213.4 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

UTERUS :
-FLUID DISTENTION

UTERUS :
-LUMINAL DILATATION, -SLIGHT

: 210 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0028	SEX: FEMALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 191.5 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

UTERUS :
-FLUID DISTENTION

UTERUS :
-LUMINAL DILATATION, -SLIGHT

: 211 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0029	SEX: FEMALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 220.0 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

LUNGS & BRONCHI :
-DARK AREA(S); LEFT, ONE, SUBPLEURAL, 2MM.

LUNGS & BRONCHI :
-ALVEOLITIS,-MINIMAL, FOCAL
-FOAMY ALVEOLAR MACROPHAGES,-MINIMAL, FOCAL

VAGINA :
-PALE AREA(S); SEROSAL ASPECT, ONE, RAISED, 2X2MM.

VAGINA :
-PROMINENT PROSTATIC TISSUE ON SEROSAL ASPECT,-PRESENT

: 212 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xyblon protocol number: MIN 252

ANIMAL NUMBER: 0030	SEX: FEMALE	DOSE GROUP: 3	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 207.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

STOMACH :
-ANTRUM WHITE NODULE(S); MUCOSA, THREE, NEAR TO LIMITING
RIDGE, 1MM.

STOMACH :
-ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR MUCOSA, FOCAL,-
PRESENT

: 213 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0021	SEX: FEMALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 194.2 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

NASAL TURBINATES :
-OEDEMA IN STENO'S GLAND, -SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 214 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Kybion protocol number: MIN 252

ANIMAL NUMBER: 0022	SEX: FEMALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 177.8 GRAMS

NECROPSY

PATHOLOGY OBSERVATIONS

HISTOPATHOLOGY

UTERUS :
-FLUID DISTENTION

UTERUS :
-LUMINAL DILATATION, -SLIGHT

: 215 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings – continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm)	:	0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0023	SEX: FEMALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 222.1 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

KIDNEYS :

-INTERSTITIAL INFLAMMATION, -MINIMAL, FOCAL

UTERUS :

-FLUID DISTENTION, MINIMAL

UTERUS :

-LUMINAL DILATATION, -SLIGHT

: 216 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):	:	0	50	150	450

Xyblon protocol number: MIN 252

ANIMAL NUMBER: 0024	SEX: FEMALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 220.2 GRAMS

NECROPSY

PATHOLOGY OBSERVATIONS

HISTOPATHOLOGY

HEART :

-VALVULAR ENDOCARDITIS, -MINIMAL, FOCAL

LARYNX :

-INFLAMMATORY CELLS IN LAMINA PROPRIA (VENTRAL), -MINIMAL, FOCAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

: 217 :

MIN 252/004312

APPENDIX 10

(Individual pathological findings - continued)

Print No: 0026

Printed: 05-MAR-01

Group	:	1	2	3	4
Compound	:	Control	-----	T-7499	-----
Exposure level (ppm):		0	50	150	450

Xybion protocol number: MIN 252

ANIMAL NUMBER: 0025	SEX: FEMALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11-DEC-00	STUDY DAY OF DEATH: 29	STUDY WEEK OF DEATH: 5	TERMINAL BODY WEIGHT: 210.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

MIN 252/004312

**ADMINISTRATION OF T-7499
BY INHALATION TO RATS**

Author

Stuart Cracknell

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TEST SUBSTANCE AND ADMINISTRATION

TEST SUBSTANCE

The test substance, which is referred to throughout this report as T-7499, is 96 – 98% perfluorobutane sulfonyl fluoride (PBSF) and 2 – 4% perfluorosulfolane. It was supplied as a liquid in a small cylinder (net contents 35 kg/cylinder) and was received at these laboratories on 26 April 2000. Information from the Sponsor indicated that T-7499 was sufficiently stable for use in this study. The T-7499 was administered to the rats as a vapour diluted with air.

ADMINISTRATION

The T-7499 vapour was administered to the rats by whole-body exposure in chambers described below. The chamber atmospheres were produced by metering the liquid test substance into stainless steel coil vapour generators through which a stream of dried air was passed. The atmosphere produced by the generation system was further diluted with air to give the final chamber concentrations of test aerosol.

The in-line airflow to the vapour generation apparatus and the diluent air supply were verified using a dry type gas meter during the preliminary phase of the study. During the study the airflow to the atmosphere generation system was monitored throughout each of the exposures using calibrated in-line tapered tube gas flowmeters.

The settings of the test substance metering system required to obtain the target chamber concentrations were determined during the preliminary generation trials without animals, based on the gas chromatographic (GC) analysis of atmosphere samples. Minor adjustments were made to the test material delivery rates in order to maintain chamber concentrations close to target.

Animals assigned to the control group received an exposure to compressed air only, from the same source as used for the generation of the test atmospheres.

The duration of administration was a single 6-hour exposure on 5 consecutive days of each week for 4 weeks.

The combined usage of T-7499 was determined, for each day of treatment, for the three test groups.

EXPOSURE SYSTEM

Each exposure system comprised a whole-body inhalation exposure chamber, a vapour generator, a tapered needle fluid flow control valve, diluent air control valves, a compressed air supply and control valves to each generator and in-line airflow monitoring flowmeters. Components of the generation system that were common to all groups were the test material cylinder, a load cell and its associated display for determining the test material usage (weight loss from the cylinder) and the stainless steel test material supply pipework.

Schematic diagrams of the vapour generation system and an exposure chamber system are presented in Figures A and B. The component parts of the systems are described in further detail below:

Vapour generation

The vapour generation system for Groups 2, 3 and 4 (Low, Intermediate and High dose) comprised a common reservoir of liquid T-7499, which was maintained at a pressure of 40 psi under Nitrogen. The pressurised vessel supplied the test substance to separate metering valves (Nupro S Series Needle Valves^a) to each of the test chambers. The liquid delivery line to the metering valves was fitted with toggle valves to allow isolation of the test substance supply and was also fitted with a particulate filter (stainless steel, 15 µm pore size^a). Fluid passing through the metering valves was delivered directly into the air inlet to stainless steel vaporisation coils that were immersed in a water bath maintained at a temperature of approximately 60°C. The test substance vapour and air mixture was subsequently carried to secondary dilution vessels constructed of glass and stainless steel, adjacent to the inhalation exposure chambers, through PolyTetraFluoroEthylene (PTFE) tubing with an external diameter of 6 mm. The T-7499 vapour in air mixture from the generators was further diluted with air in the secondary vessels to produce the exposure atmospheres.

The air supplied to the vapour generators and secondary dilution vessels was filtered to remove any residual particulate and was dried (dew point ~-2°C).

The test material reservoir (the original cylinder supplied by the Sponsor) was mounted on an electronic load cell^b and the weight of the reservoir and contents were displayed continuously. The contents of the cylinder were maintained at a constant pressure (40 psi) under dried nitrogen.

Liquid was transported from the base of the reservoir to the metering valves. Particulate filters (stainless steel, 7 µm pore size)^a were incorporated into each liquid delivery line between the distribution manifold and the valves to protect them from blockage by any particulate that might have been present. The liquid metered by the valves was delivered to the vaporisation coils through PTFE tubing of approximately 2 mm internal diameter.

A line drawing of the generation system is given in Figure A.

^a Nupro Co, Willoughby, Ohio 44094, USA

^b Huntleigh Industrial Controls Ltd, Load Cell Division, Portman Moor Industrial Estate, East Moors, Cardiff, South Glamorgan, CF22 2HB

For all groups exposed to T-7499, the vapour/air mixture produced in the vapour generators was passed into the base of the secondary dilution vessel and was mixed with a further supply of clean and dry air sufficient to ensure a total chamber airflow of approximately 150 l/min. The test atmosphere was then passed through flexible ducting to a tangential inlet mounted at the apex of the appropriate exposure chamber.

The control group received clean air only at a rate of approximately 150 l/min.

Inhalation chamber

The exposure chambers were of stainless steel and glass construction and consisted of a cuboidal body fitted with a pyramidal base and top. The internal volume of each chamber was approximately 0.75 m^3 . At the apex of the upper pyramidal figure was the tangentially mounted air duct. Immediately below this was a perforated canister, which ensured equal distribution of the test atmosphere within the chamber.

Access to the chamber was through the front of the box section *via* a hinged door with a glass panel and stainless steel frame. The door was sealed using moulded rubber sealing strip.

Exposure cages constructed of stainless steel mesh were suspended on a framework arranged on 4 levels. Each level is able to hold 4 cages, with each cage capable of housing 4 rats individually. This gave a potential animal exposure capacity of 64 rats. In this investigation, 10 animal compartments were used on level 2 and air samples were withdrawn for analysis from this level. No cages were present on levels 1 or 4.

A wet and dry alcohol bulb thermohygrometer was suspended in the chamber. This was visible through the glass-panelled door and was used to monitor chamber temperature and relative humidity.

The pyramidal base of each chamber was fitted with a 2-inch drain. The drain connected with a common drainage system *via* a ball valve.

A square tubular exhaust plenum, 3 inches in diameter and perforated along the ventral surface, was situated in the pyramidal base. This connected to the main extract system.

The total chamber airflow was 150 litres/minute. Air entered the chamber through the inlet duct. Diluent air flow was measured using a tapered tube flow meter situated at the front of a purpose-built stainless steel trolley on which the secondary dilution vessel was mounted. Generation air was measured on a similar flowmeter mounted on the vapour generation trolley.

A Magnehelic pressure gauge (0-25mm water gauge) was connected with each chamber by a nylon tube. This was mounted on the secondary dilution vessel trolley and was used to monitor the atmosphere pressure inside the chamber, relative to the exposure room.

Extraction of the chambers was accomplished by means of a single fan mounted on the outside wall of the building withdrawing air through a manifold to which all chambers were connected. The chamber air extract was vented to atmosphere *via* an exhaust stack.

Extract flow was adjusted using gate valves mounted in the extract ducting between the chamber and filters. The internal pressure within each chamber was maintained in the range -2 to -4 mm water below ambient pressure when operational.

The control animals were exposed using a similar system to that used for the test groups, but received air only.

PROCEDURE

A separate exposure chamber was used for each group. The control animals were exposed using an identical exposure chamber to that used for the test groups. The inhalation system was set up as described above.

The rats were transferred from the holding cages and placed in a predetermined sequence in the individual compartments of the exposure cages. The animals were located on level 2 of the chamber. In order to avoid any variations in the dose received due to the spatial arrangements of the animals within the chamber, the position of the animals within the chamber was changed at 7-day intervals according to a previously assigned sequence.

The diluent and generator airflows were switched on and the chamber doors were closed and secured. The chamber Magnehelic gauge was checked to ensure that operation of the chamber took place with internal pressure below that of the room.

The test substance supply toggle valves between the pressure vessel and the metering valves were opened and the exposure start time documented.

The delivery of test material to the vapour generators could be monitored during the exposures by the movement of entrained vapour bubbles in the PTFE delivery tubing.

During the first nine exposures, samples were collected at approximately hourly intervals from each test chamber. The samples were collected into 20 ml, gas tight, polypropylene syringes. Sample collection was performed by inserting the syringe needle through a septum seal located in the inhalation chamber wall and flushing the syringe with a minimum of three volumes of chamber air before drawing up the sample for analysis. After a delay of approximately 5 seconds, to allow the pressure in the syringe to equilibrate, 5 ml of the chamber air was dispensed back into the exposure chamber and the syringe then sealed. The samples were injected directly into sample loop of the gas chromatograph.

In the period from exposure 10 and throughout the remainder of the study, immediately after documenting the start time for the exposure, a sequencing device and sampling pump that controlled the automatic collection of atmosphere samples was switched on and the timer reset. This device delivered atmosphere samples from each chamber in turn (Group 4 – Group 1) to the GC system, at 15-minute intervals during the 6-hour exposure period.

The Chamber airflow, temperature and relative humidity were monitored and recorded at approximately 30-minute intervals throughout the exposure period.

At the end of the exposure the test substance supply toggle valves were switched off and the final weight of test material pressure vessel was recorded. The vapour in the test chambers was allowed to clear before the animals were removed.

At the end of this time, the rats were unloaded from the chambers and returned to their respective holding cages. The chambers were washed with hot water.

A summary of the operating conditions used is presented in Table A.

TARGET CONCENTRATIONS

The target concentrations of T-7499 were as follows:

Group	Designation	Concentration (ppm)
2	(Low dose)	50
3	(Inter. dose)	150
4	(High dose)	450

The target concentrations were selected in consultation with the Sponsor, following the review of available data.

EXPOSURE CHAMBER CONDITIONS

Analysis of chamber concentrations of T-7499

For the first nine exposures, samples of chamber air were collected in gas tight syringes and injected directly into the sample loop of the gas chromatograph. Thereafter monitoring was performed using an automated system for on-line sampling and injection. The air samples collected by both of the sample collection methods were taken in sequence from Groups 4 to 2. Methods of sample collection and analysis are described in Appendix A.

The method of analysis was adapted from a method supplied by the Sponsor to accommodate the Inhalation Toxicology Department equipment and procedures. Details of the analytical procedures used are given in Appendix A.

Airflow and pressure

The airflow into each chamber was monitored using tapered tube rotameters and checked at approximately 30-minute intervals throughout each exposure. The chamber negative pressure was displayed continuously and a documented check was performed at approximately 30 minute intervals to ensure the displayed value was in the target range.

Temperature and relative humidity

The temperature of the generation water bath and also the air in each exposure chamber was recorded at approximately 30-minute intervals during each exposure.

The wet and dry bulb temperatures of a thermohygrometer placed in each chamber were recorded at approximately 30-minute intervals throughout each exposure. Relative humidity was found using a look-up table supplied with the instrument.

CALCULATIONS

In order to minimise the cumulative errors that result from repeated rounding of numbers, much of the data in this report has been calculated continuously using unrounded numbers and only rounded for printing. Consequently, these rounded numbers will include rounding errors in the last significant figure and recalculation may lead to small apparent discrepancies with other data in the report.

Chamber concentration

Mean chamber concentrations were calculated where equal sampling intervals were employed. When adjustments to the generation system were necessary and sample intervals were varied, a time weighted average concentration was calculated.

RESULTS

VAPOUR CONCENTRATION

Analysed concentration of T-7499

The exposure mean and individual sample concentration values are presented in Table B and Appendix B, respectively.

The exposure mean concentrations for each group exposed to T-7499 are presented below and were in good agreement with target concentrations.

Group	Chamber concentration (ppm)			
	Target	Analysed		
		Mean	(sd)	(CV%)
2 (Low dose)	50	47	9.0	19.3
3 (Inter. dose)	150	162	22.1	13.7
4 (High dose)	450	459	20.6	4.5

The higher than expected coefficient of variation seen for both the Low and Intermediate exposure concentrations reflected variations in test fluid delivery to the vapour generators on each occasion that the system was switched on. This phenomenon was found to be attributable to the formation of vapour bubbles within the test fluid delivery pipes and was evident throughout the study for all test groups but was less marked at the High dose exposure concentration where the delivery rate of the test substance was highest. Minor adjustments and reconfigurations were made to the generation systems during Week 1 and 2 of exposure to minimise nucleation of vapour bubbles and associated interruptions to the liquid test substance flow.

Nominal concentration of T-7499

The data are presented in Table C and are summarised below:

Group	Study mean nominal concentration (ppm)	A/N ratio (%)
2, 3 and 4 (Low, Inter. and High dose combined)	211	106

$$A / N = \left(\frac{\text{Analysed concentration}}{\text{Nominal concentration}} \right) \times 100$$

The nominal concentration for each exposure period was calculated, for the three treated groups combined, from the mass of T-7499 delivered into the generators. The calculation assumed a constant atmospheric pressure of 760 mm Hg and used the mean chamber temperature for the three treated groups. It should be noted that use of these values may have artificially depressed the nominal concentration and increased the analysed to nominal ratio.

The calculated value of the analysed to nominal ratio was sufficiently close to 100% to confirm that the system was performing as designed.

CHAMBER TEMPERATURE AND RELATIVE HUMIDITY

The daily mean chamber temperatures and relative humidities are presented in Table D.

The chamber temperatures and humidities were similar for all groups on each day of the study. The measured relative humidities recorded during the study were lower than the target range (40% to 60% RH) reflecting the generation and dilution of the chamber atmospheres with air that was supplied from a compressor system incorporating a refrigerant drier. This deviation from the design conditions had no discernible effect upon the animals and is not considered to have affected the outcome of the study.

DISCUSSION

Control of the T-7499 vapour delivery to the exposure chambers was generally satisfactory although the greater variation between occasions of generation was evident for the Low and Intermediate exposure groups than was seen at the High dose. This was reflected in the coefficients of variation for the daily mean concentrations (19.3%, 13.7% and 4.5% respectively for Groups 2, 3 and 4).

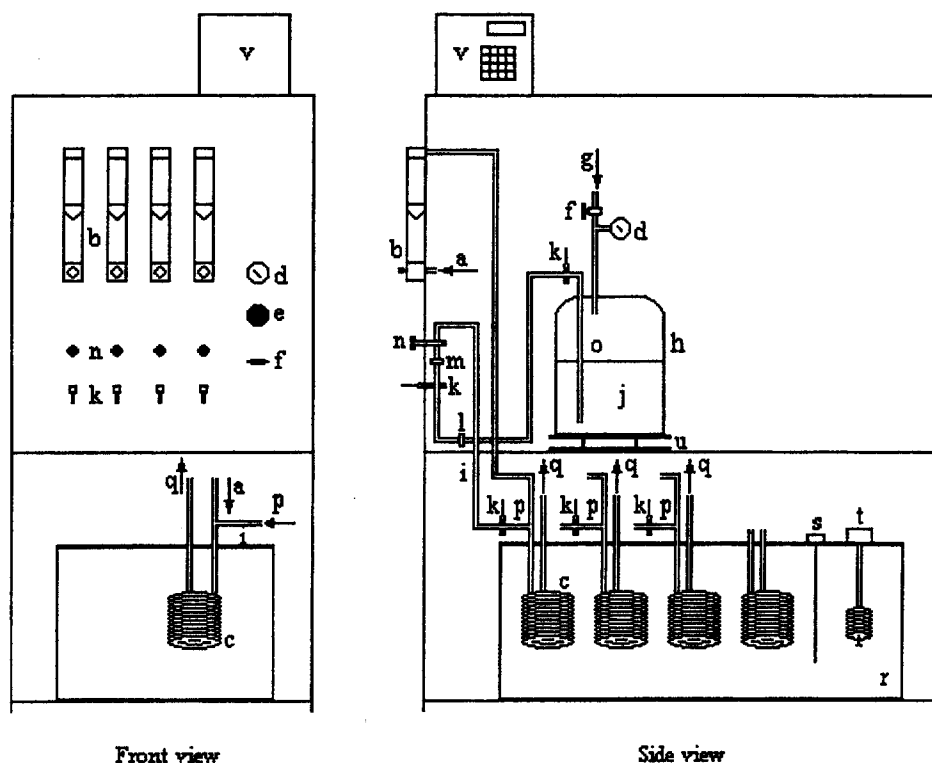
The study mean concentrations of T-7499 in each chamber were within 8% of the target concentrations for all groups.

Good agreement was calculated between the average analysed and the combined nominal chamber concentrations. The determination of a nominal concentration that was slightly below the analysed chamber concentration was attributed to a combination of factors as follows:

- the use of a single barometric pressure (760 mm Hg) in the calculations;
- the calculation of an average nominal concentration for all groups;
- use of the average chamber temperature for all three test groups in the calculations;
- the tendency of the test material to vaporise in the delivery pipe-work to the vaporisers resulting in the delivery of a mixture of vapour and liquid.

FIGURE A

Schematic diagram of vapour generation system

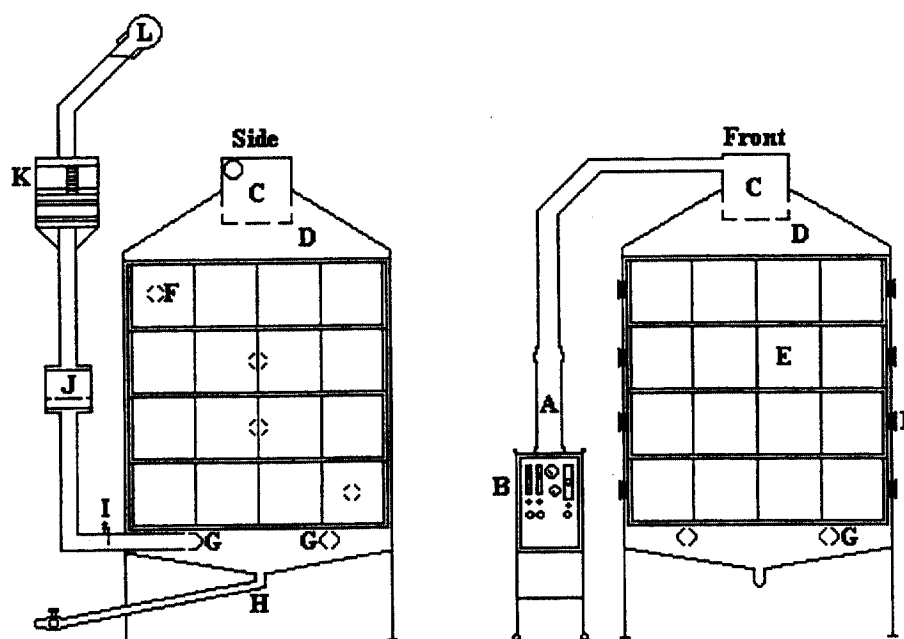


Key:

a	Air supply	l	15 µm filter
b	Rotameter and control valve	m	7 µm filter
c	Air heating coil/vapour generator	n	Micro-metering valve
d	Pressure gauge	o	Test substance from pressurised reservoir
e	Control valve (generation trolley air supply)	p	Test material feed into air supply line
f	Shut off valve	q	Vapour/air mixture (to chamber)
g	Nitrogen (pressure) inlet	r	Water bath
h	Test material tank	s	Temperature probe and digital display
i	PTFE test material delivery pipe	t	Thermo-stirrers unit
j	Test substance in pressurised reservoir	u	Load cell
k	Toggle valve	v	Load cell digital display

FIGURE B

Schematic of the exposure system used to expose rats



Key:

- | | | | |
|---|---|---|------------------------|
| A | Glass elutriator | G | Exhaust plenum |
| B | Air flow control and chamber monitoring | H | Drain |
| C | Dispersion device | I | Gate valve |
| D | Exposure chamber (0.75 m ³) | J | Pre-filter |
| E | Animal exposure cages | K | Powered extract filter |
| F | Sampling port | L | Main exhaust |

FIGURE C

Schematic of the automated sampling system

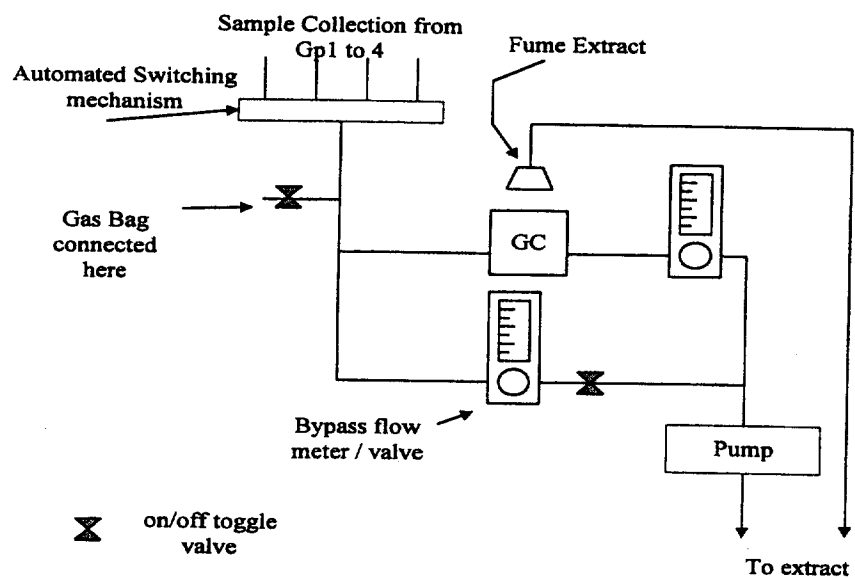


TABLE A**Operating conditions of the exposure system**

	Group			
	1 (Control)	2 (Low dose)	3 (Inter. dose)	4 (High dose)
Target concentration (ppm)	0	50	150	450
Atmosphere generation				
Test material feed	N/A	At 40 psi direct from the Sponsor supplied gas cylinder		
Test material flow control	N/A	Tapered needle flow control valve	Tapered needle flow control valve	Tapered needle flow control valve
Test material usage measurement	N/A	Weight loss from cylinder measured using a load cell		
Chamber airflows (l/min)				
Generator	N/A	50	50	50
Diluent	150	100	100	100
Chamber extract	150	150	150	150
Chamber pressure (mm water)	-2 to -4	-2 to -4	-2 to -4	-2 to -4

TABLE B**Chamber concentrations of T-7499 -- daily mean values**

Exposure No.	Chamber Concentration (ppm)		
	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
1	44	177	466
2	62	223	466
3	54	161	416
4	45	154	458
5	68	149	451
6	56	179	438
7	45	127	447
8	57	161	444
9	46	148	439
10 ^a	35	148	473
11	45	163	450
12	45	144	455
13	43	148	500
14	41	149	492
15	55	173	497
16	43	171	459
17	40	154	458
18	34	150	473
19	39	206	454
20	39	148	447
Mean	47	162	459
sd	9.0	22.1	20.6
CV (%)	19.3	13.7	4.5

sd standard deviation

CV Coefficient of Variation = $(sd \div \text{mean}) \times 100$ ^a For Exposures 10 to 20, concentration samples were collected by an automated sampling system

TABLE C

Combined nominal concentrations of T-7499 and analysed to nominal concentration ratio

Exposure No.	Wt of T-7499 used (kg)	Mean chamber Temperature (°C)	Volume of T-7499 vapour (l) ^b	Concentration (ppm)		A/N
				Nominal ^c	Analysed	
1	0.41	22	32.9	203	229	113
2	0.41	21	32.8	202	250	124
3	0.41	21	32.8	202	210	104
4	0.38	22	30.4	188	219	117
5	0.44	22	35.2	217	223	102
6	0.41	22	32.8	203	224	111
7	0.41	21	32.8	202	206	102
8	0.44	22	35.2	217	221	102
9	0.45	22	36.0	222	211	95
10 ^d	0.44	22	35.2	217	219	101
11 ^d	0.41	21	32.8	202	219	108
12	0.41	22	32.8	203	215	106
13	0.48	21	38.4	237	230	97
14	0.44	22	35.2	217	227	105
15	0.41	21	32.8	202	242	119
16	0.44	21	35.2	217	224	103
17	0.41	21	32.8	202	217	107
18	0.48	21	38.4	237	219	92
19	0.41	21	32.8	202	233	115
20	0.45	21	35.9	222	211	95
Mean	0.43	21	34.2	211	223	106
sd	0.026	0.3	2.05	12.7	10.7	8.5

sd Standard deviation

A/N Analysed/nominal concentration ratio expressed as a percentage

^b Calculated from the following equation:

$$V = \frac{W \times T \times R}{Mw} \times 1000$$

Where W = Weight of test material used in the exposure (kg)
T = Average chamber temperature (K) (= mean temp(°C) + 273)
R = Gas constant (0.08205 L Atm mol⁻¹ K⁻¹)
Mw = Molecular weight of PBSF (302.09 g/mol)

^c Calculated from the following equation:

$$\text{Nominal concentration} = \frac{V 10^6 \text{ (ppm)}}{V_a + V}$$

Where V_a = Chamber airflow (l) for the exposure (162000 litres).^d Nominal concentration included even though a discrepancy in weight loss was noted. No change in the mean or standard deviation occurred.

TABLE D

Chamber temperature and relative humidity – exposure mean values

Exposure	Mean chamber temperatures (°C) and relative humidity (%RH)							
	Group 1 (Control)		Group 2 (Low dose)		Group 3 (Intermediate dose)		Group 4 (High dose)	
	Temp	RH	Temp	RH	Temp	RH	Temp	RH
1	22	30	22	30	22	25	22	22
2	22	28	22	30	21	28	21	22
3	22	25	22	28	21	27	21	22
4	22	29	22	30	21	26	22	18
5	22	29	22	30	21	27	22	20
6	23	29	22	30	21	26	22	19
7	22	27	22	29	21	25	21	22
8	22	26	22	29	21	25	22	21
9	22	27	22	28	21	26	22	21
10	22	28	22	29	21	26	22	21
11	23	26	22	29	21	26	21	24
12	22	32	22	33	21	30	22	26
13	22	30	22	30	21	27	21	25
14	22	29	22	31	21	27	22	26
15	22	31	22	30	21	27	21	28
16	22	30	22	30	21	28	21	28
17	22	31	22	32	21	27	21	31
18	22	30	22	30	21	25	21	26
19	22	30	22	31	21	28	21	27
20	22	32	21	31	20	28	21	24
Mean	22	29	22	30	21	27	21	24
sd	0.3	2.0	0.2	1.2	0.3	1.3	0.5	3.4
CV (%)	1.4	6.9	1.0	4.0	1.5	4.9	2.4	14.4

sd standard deviation

CV Coefficient of Variation = $(sd \div \text{mean}) \times 100$

APPENDIX A

Methods of sample collection and analysis for T-7499

SAMPLE COLLECTION

Chamber concentration

All samples were taken from a sampling port located on level 2 of the exposure chamber. The first nine exposures were manually sampled by withdrawing chamber atmosphere from a sampling port on the exposure chamber into a polypropylene syringe fitted with a sealing valve. For the tenth and subsequent exposures, an automated sampling system was used to transfer samples *via* PTFE tubing to a gas chromatograph. Sampling lines were purged with the chamber atmosphere for 12 minutes prior to sample collection.

A line drawing of the automated sampling system is given in Figure A.

The automated sampling system switches sequentially between the High, Intermediate, Low and Air control chambers on a 15 minute cycle. During each cycle, the sampling line (1/4" Teflon) and GC gas sampling loop were purged with the chamber atmosphere. At approximately 12 minutes into each cycle, a supplementary timer started the GC and so injected the contents of the sampling loop. The sampling flow valves were adjusted so that most (600 ml/min) of the chamber atmosphere passed through the bypass flow meter while only 20 ml/min passed through the GC injection valve (via 1/8" Teflon tubing).

When the automated sampling system was in use, injection of gas bags was conducted by attaching each gas bag to the appropriate port. The pump was then switched on and immediately after, the gasbag tap was opened to allow the test compound to be drawn along the sample lines. For injections of the same concentration, the pump was switched on for a period of 30 seconds before starting the gas chromatograph and for dissimilar concentration, 60 seconds was used to purge the sampling lines.

METHOD OF ANALYSIS

Chamber atmosphere samples were analysed by gas chromatography. The method of sample analysis is detailed, together with a summary of the method validation, in the Inhalation Analytical Procedure at the end of this appendix.

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

CALCULATIONS

GC analysis

The samples of chamber atmosphere were injected into a gas chromatograph, which was calibrated using vapour standards prepared in gas bags. The method for calculating the concentration of T-7499 from the mass used to prepare each vapour standard is given below in equations 1 and 2.

$$\text{Concentration} = \frac{V}{V_a + V} \times 1,000,000 \text{ ppm} \quad (1)$$

$$V = \frac{W \times R \times T}{M} \times \frac{760 \text{ mm Hg}}{\text{Atm}} \quad (2)$$

where	V	=	gaseous volume of T-7499 (ml)
	W	=	mass of T-7499 (mg)
	M	=	molecular weight of PBSF (302.09 g/mole)
	R	=	0.08205 ml.atm/mmol.K
	T	=	temperature (K)
	Atm	=	atmospheric pressure (mmHg)
	V _a	=	volume of air (ml)

In order to minimise the cumulative errors that result from repeated rounding of numbers, much of the data in this report has been calculated continuously using unrounded numbers and only rounded for printing. Consequently, these rounded numbers will include rounding errors in the last significant figure and recalculation may lead to small apparent discrepancies with other data in the report.

APPENDIX A**(Methods of sample collection and analysis for T-7499 – continued)****COMPOUND SPECIFIC INHALATION ANALYTICAL PROCEDURE FOR T-7499****The analysis of T-7499 in air sample substrate**

The method outlined in this document has been validated and is considered fit for the purpose of monitoring test atmospheres in an Inhalation Toxicology study.

This document details the basic procedures for the analysis of T-7499 sampled by syringe from test atmospheres. The resulting samples, of approximate concentration 100 to 500ppm, are analysed by GC. Study specific amendments and additions will be detailed within a supplementary document.

NOTE Throughout this document, the symbol § indicates that the relevant information is not available at present, but will be included in a Study specific supplement.

EFFECTIVE DATE:	22 May 2000
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Test substance

T-7499 is mainly perfluorobutyl sulfonyl fluoride (PBSF), which has the formula $C_4F_{10}O_2S$.

Appearance	liquid
Subsample Storage of Test Mixture	An approximate temperature of +4°C. Protected from moisture.

Equipment

Balance	Sartorius	BP4100
Syringes	Hamilton Hamilton	1000 series gas-tight (100 and 25 ml) 500 series gas-tight (500 ml)
Gas sample bags	SKC INC	Tedlar® 232-series (1 and 3 dm ³ capacity)
Syringe valve	Mininert	Push button valve
Vacuum pump	AEG	ADEB 56 (or equivalent)
Flow meter	J & W Scientific	ADM1000 (acoustic displacement)

General laboratory glassware

Consumables

Syringes	Sigma Aldrich	20 ml polypropylene
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APPENDIX A**(Methods of sample collection and analysis for T-7499 – continued)****Preparation of samples for analysis**

Samples of the test atmosphere are collected using a 20 ml syringe fitted with a "Mininert" valve. The syringe needle is inserted into the sampling port and 10 ml of test atmosphere is withdrawn into the syringe. The valve is closed and the syringe removed from the sampling port for injection onto the GC.

The gas sampling valve of the GC is set to the "load" position and the syringe is placed into the injection port of the valve. The valve of the syringe is opened and the contents of the syringe are passed into the sampling valve. The valve is then switched to the "inject" position and simultaneously the run start is activated.

Preparation of calibration standards

Standards are prepared using the following method. The actual standard concentration ranges used are as detailed in the study specific supplement.

A volume of T-7499 is dispensed from the cylinder into a scintillation vial. Weigh approximately 670µg of T-7499 (400µl) and inject into a gas bag, mix thoroughly and make up to set volume with air to provide standard S1. The T-7499 is vaporised by gentle warming using a hot air blower.

Evacuate a series of gas sample bags of appropriate capacity and introduce by syringe measured volumes of air. Using gas tight syringe(s) fitted with a sealing valve, accurately dispense measured volume(s) of the T-7499 vapour into the gas sample bags *via* the injection port to produce standards covering the concentration range described in the study specific supplement.

Storage of standards and samples

The maximum storage periods for the various sample types are detailed below

Sample type	Storage conditions	Storage period
Gas standards	Room temp., light	5 days
Syringe samples	Room temp., ambient	150 minutes

APPENDIX A**(Methods of sample collection and analysis for T-7499 – continued)****Calibration and quantification**

Calibrate by injecting duplicates of each calibration standard, as detailed in the study specific supplement, at the beginning of each analytical sequence. Measure the peak area response in each injection of the calibration standard solutions and derive the line of best fit using an unweighted least squares method.

For each injection of the sample measure the peak area response and determine the amount present in the sample using the equation below:

$$\text{Amount (ppm)} = \frac{(A - I)}{S}$$

Where A = Peak area response of Perfluorobutyl sulfonyl fluoride (PBSF) in the sample chromatogram
 S = Slope of calibration line derived from calibration data
 I = Intercept of calibration line derived from calibration data

Chromatographic conditions

Analytical column	CP SIL 5CB (100% dimethyl polysiloxane), 30 m x 0.53 mm i.d. 5µm film
Carrier gas	Helium (4.25 ml/min, head pressure 18kPa, 3psig)
Split vent	Helium (20 ml/min)
Septum purge	Helium (1 ml/min)
Split ratio	1:4.7
Make up	Helium (31 ml/min)
Oxidant	Air (450 ml/min)
Fuel	Hydrogen (45 ml/min)
Injection volume	250 µl <i>via</i> gas valve injection loop
Gas valve temperature	60°C
Injector temperature	60°C
Detector temperature	100°C
Column temperature	35°C
Retention time	PBSF approximately 2.5 minutes

APPENDIX A**(Methods of sample collection and analysis for T-7499 – continued)****Quality assurance measures**

When the method is established on a chromatographic system six injections of a standard will be used to verify performance of the system. The parameters and acceptance criteria are set out below;

Parameter	Typical value	Acceptable limits
Plate count (USP)	3624	> 80%
Tailing factor (USP)	1.0958	± 20%
Repeatability (CV, n=6)	<1.4%	<5%
QC tolerance	< ±2%	< ±5%
QC tolerance at LOQ	< ±5%	< ±10%

The highest calibration standard will be compared against a standard of similar concentration prepared independently. The ratio of response factors will be acceptable if within the range 0.95 to 1.05.

A quality check standard must follow every 6 concentration samples for the analysis to be regarded as valid. The results of the quality check standards must lie within the QC tolerance limits.

A quality check standard of low concentration will be run to verify the LOQ for the run. The LOQ for the run will be regarded as the concentration of the lowest acceptable quality check standard.

Summary of method validation

The raw data for the method validation is located in study MIN/244.

Comparison of test blanks, standards and test samples showed that the analyte was well resolved from any potential interfering peak.

Precision data showed coefficients of variation for PBSF of less than 1.4% with standards in the range of 10,000 to 500 ppm and less than 2.0% to standards to 100ppm.

Unweighted least squares regression analysis of peak area response against concentration of standard (100 to 10,000 ppm) produced a correlation coefficient of 0.999991 and relative errors less than 2.1% in the range 10,000 to 100 ppm. The Limit of Quantification (LOQ) for T-7499 will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 59.45 and 17.83 ppm respectively (calculated statistically using the standard deviation obtained for a solution of concentration 100 ppm).

Standards of T-7499 in the range 100 to 10,000 ppm stored at room temperature for 5 days and subsequently analysed against fresh standards showed concentrations within 5% of their nominal.

Samples of a standard (ca 1,000 ppm) of T-7499 stored in the injection syringe for 150 minutes under ambient conditions (room temperature under normal lighting conditions) and subsequently analysed against freshly injected standards showed concentrations within 5% of their nominal concentrations.

APPENDIX A

(Methods of sample collection and analysis for T-7499 – continued)

GAS CHROMATOGRAPHS IN INHALATION TOXICOLOGY AT 25 SEPTEMBER 1997

System No.	Components of gas chromatography system		
1	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated gas sampling valve, ECD and FID.
	Hewlett Packard	18593B	} 7673 Autosampler
	Hewlett Packard	18596CX	
	Hewlett Packard	G1512AX	
	ThermoQuest*	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
2	Pye Unicam	PU4550	Chromatograph with gas valve and FID.
	Pye Unicam	PU4700	Autosampler
	ThermoQuest	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
3	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	AOC-1400	Autosampler
	Shimadzu	AOC-14	Autoinjector
	ThermoQuest	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
4	Pye Unicam	304	Chromatograph with FID.
	Pye Unicam	PU4700	Autosampler
	ThermoQuest	SP4400	Integrator
5	Pye Unicam	304	Chromatograph with FID.
	Pye Unicam	PU4700	Autosampler
	ThermoQuest	SP4400	Integrator
6	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	MGS-4	Automated gas valve
	Shimadzu	CR4-A	Integrator
7	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	MGS-4	Automated gas valve
	Shimadzu	CR4-A	Integrator
8	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated automatic gas sampling valve and FID.
	Hewlett Packard	G1513A	} 6890 Series Autosampler
	Hewlett Packard	18596CX	
	Hewlett Packard	G1512AX	
	ThermoQuest	SP4500	A/D interface
	ThermoQuest	PC1000	Integration software
9	Perkin Elmer	Autosystem XL	Chromatograph with programmable capillary inlet, heated automatic gas sampling valve and FID.

* formerly Spectra-Physics

APPENDIX A**(Methods of sample collection and analysis for T-7499 – continued)****STUDY SPECIFIC SUPPLEMENT TO THE INHALATION ANALYTICAL PROCEDURE FOR PBSF (T-7499)**

This supplement details additions and amendments to the procedure to be used for the GC assay of PBSF obtained from air samples collected on the above study.

The assay, incorporating the additions and amendments, is suitable for the analysis of PBSF, in air, at concentrations within the range of 25 to 1000ppm.

Details given in this supplement supersede those in the compound specific IAP.

EFFECTIVE DATE :	5 November 2000
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Analytical standard

Name	T-7499, PBSF
Batch number	FCS00001822
Purity	96-98% (Perfluorosulfolane 2-4%)
Expiry date	Not Stated
Supplier	Sponsor (filled by Manchester Tank)

Chromatographs

The analysis is performed using chromatograph 8.

Carrier gas	Helium (2.75 ml/min)
Split vent	Helium (24.5 ml/min)
Split ratio	1:8.9
Detector temperature	150°C
Detector Range	0
Retention time	PBSF approximately 2.7 minutes

EFFECTIVE DATE :	9 November 2000
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Summary of method validation**Manual injection**

Precision data showed coefficients of variation for PBSF of less than 0.8% with standards in the range of 1000 to 25 ppm. This data is located with the MIN/252 study data.

APPENDIX A**(Methods of sample collection and analysis for T-7499 – continued)**

Least squares regression analysis, with an unweighted linear regression, for peak area response against concentration of standard (25 to 1000 ppm) produced a correlation coefficient of 0.999984 and relative errors less than 1.3% in the range 1000 to 50 ppm and 4.3% at 25 ppm. The Limit of Quantification (LOQ) for PBSF will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 1.69 and 0.51 ppm respectively.

EFFECTIVE DATE :	10 November 2000
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Calibration and Quantification

Calibrate using standards with nominal concentrations 500 and 50 ppm.

EFFECTIVE DATE :	16 November 2000
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Preparation of standards

Prepare standards in the nominal range 25 to 600 ppm.

EFFECTIVE DATE :	17 November 2000
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Calibration and Quantification

Calibrate at 4 concentrations across the standard range.

EFFECTIVE DATE :	2 December 2000
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Summary of method validation**Automated injections**

Precision data showed coefficients of variation for PBSF of less than 4.3% with standards in the range of 600 to 25 ppm. This data is located with the MIN/252 study data.

Least squares regression analysis, with a $1/\text{concentration}^2$ weighted linear regression, for peak area response against concentration of standard (25 to 600 ppm) produced a correlation coefficient of 0.999940 and relative errors less than 1.3% in the range 600 to 25 ppm. The Limit of Quantification (LOQ) for PBSF will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 12.28 and 3.68 ppm respectively.

APPENDIX B

Chamber concentrations of T-7499 – individual sample values

Exposure No.	Sample interval (hours)	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
1	0-1	43	178	463
	1-2	44	185	467
	2-3	43	186	470
	3-4	44	164	469
	4-5	43	167	466
	5-6	44	182	459
	TWA	44	177	466
2	0-1	16	532	538
		87	255	-
		-	255	-
	1-2	89	204	505
		88	-	445
		83	-	-
	2-3	64	186	459
	3-4	62	184	452
	4-5	63	182	457
	5-6	59	179	430
	TWA	62	223	466
3	0-1	17	122	365
		64	85	-
		-	188	-
	1-2	64	190	428
		-	168	-
	2-3	58	167	419
	3-4	56	167	421
	4-5	55	167	418
	5-6	56	169	417
	TWA	54	161	416

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{Weighted concentration (ppm)} = \frac{\text{Concentration (ppm)} \times \text{"Time weighting" (min)}}{\text{Exposure duration (min)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

APPENDIX B

(Chamber concentrations of T-7499 – individual sample values – continued)

Exposure No.	Sample interval (hours)	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
4	0-1	19	52	377
		57	42	482
	1-2	52	6	472
		-	7	-
		-	199	-
		-	247	-
	2-3	48	220	473
		-	217	-
		-	215	-
	3-4	46	180	461
5		-	183	-
	4-5	39	180	465
	5-6	57	179	452
	TWA	45	154	458
	0-1	43	14	390
		-	113	-
		-	116	-
		-	301	-
	1-2	78	259	457
		-	182	-
6	2-3	91	144	469
	3-4	89	140	462
		68	-	-
	4-5	62	150	466
	5-6	56	146	439
	TWA	68	149	451
	0-1	61	229	349
		-	184	407
	1-2	35	179	480
	2-3	34	179	467
		67	-	-
	3-4	73	175	459
	4-5	71	164	443
	5-6	57	163	397
		-	-	401
	TWA	56	179	438

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{Weighted concentration (ppm)} = \frac{\text{Concentration (ppm)} \times \text{"Time weighting" (min)}}{\text{Exposure duration (min)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

APPENDIX B

(Chamber concentrations of T-7499 – individual sample values – continued)

Exposure No. ^a	Sample interval (hours)	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
7	0-1	43	128	465
	1-2	29	129	456
	2-3	47	125	448
		46	-	-
	3-4	44	127	447
	4-5	42	125	439
	5-6	61	128	429
	TWA	45	-	-
8	Mean	-	127	447
	sd	-	1.7	12.6
	0-1	40	196	502
		63	-	-
	1-2	45	158	402
	2-3	57	157	417
	3-4	51	157	432
	4-5	44	156	428
9	5-6	80	156	490
		-	-	478
	TWA	57	161	444
	0-1	38	129	390
		71	-	-
	1-2	48	147	450
	2-3	57	150	443
	3-4	28	149	434
10		44	-	-
	4-5	33	152	439
	5-6	61	153	450
	TWA	46	148	439
	0-1	53	136	467
	1-2	43	150	482
	2-3	38	149	480
	3-4	34	150	478
	4-5	13	150	458
	5-6	27	150	474
	Mean	35	148	473
	sd	13.8	5.6	9.1

sd Standard deviation

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{Weighted concentration (ppm)} = \frac{\text{Concentration (ppm)} \times \text{"Time weighting" (min)}}{\text{Exposure duration (min)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

a

Exposures 10 to 20 were monitored by automated sampling

APPENDIX B

(Chamber concentrations of T-7499 – individual sample values – continued)

Exposure No. ^a	Sample interval (hours)	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
11	0-1 ^c	57	205	478
	1-2	39	147	363
	2-3	46	150	445
	3-4	38	157	461
	4-5	35	157	466
	5-6	50	156	465
	TWA	45	163	450
12	0-1	33	129	495
	1-2	51	139	482
	2-3	55	137	398
	3-4	44	135	453
	4-5	44	170	437
	5-6	45	151	463
	Mean	45	144	455
13	sd	7.5	14.9	34.6
	0-1	47	150	482
	1-2	32	145	579
	2-3	45	148	542
	3-4	43	149	481
	4-5	40	147	486
	5-6	49	146	471
14	TWA	43	-	500
	Mean	-	148	-
	sd	-	1.9	-
	0-1	35	142	493
	1-2	48	151	546
	2-3	38	151	492
	3-4	35	151	486
	4-5	45	150	479
	5-6	47	149	479
	TWA	-	-	492
	Mean	41	149	-
	sd	6.0	3.5	-

sd Standard deviation

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{Weighted concentration (ppm)} = \frac{\text{Concentration (ppm)} \times \text{"Time weighting" (min)}}{\text{Exposure duration (min)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

^a Exposures 10 to 20 were monitored by automated sampling

^c First sample of each group was manual

APPENDIX B

(Chamber concentrations of T-7499 – individual sample values – continued)

Exposure No. ^a	Sample interval (hours)	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
15	0-1	52	145	623
	1-2	59	182	540
	2-3	41	142	521
	3-4	63	190	418
	4-5	57	194	476
	5-6	48	171	500
	TWA	55	173	497
16	0-1	45	181	462
	1-2	9	202	381
	2-3	52	205	477
	3-4	45	153	463
	4-5	39	153	458
	5-6	54	153	467
	TWA	43	171	459
17	0-1	48	152	456
	1-2	40	155	465
	2-3	19	156	464
	3-4	47	154	455
	4-5	36	150	452
	5-6	48	155	453
	TWA	40	-	-
18	Mean	-	154	458
	sd	-	2.3	5.6
	0-1	10	96	468
	1-2	31	157	484
	2-3	20	156	483
	3-4	45	155	476
	4-5	48	155	486
	5-6	52	153	438
	TWA	34	150	-
	Mean	-	-	473
	sd	-	-	18.2

sd Standard deviation

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{Weighted concentration (ppm)} = \frac{\text{Concentration (ppm)} \times \text{"Time weighting" (min)}}{\text{Exposure duration (min)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

^a Exposures 10 to 20 were monitored by automated sampling

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APPENDIX B

(Chamber concentrations of T-7499 – individual sample values – continued)

Exposure No. ^a	Sample interval (hours)	Chamber concentration (ppm)		
		Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
19	0-1	48	495	474
	1-2	39	134	468
	2-3	20	159	456
	3-4	39	156	458
	4-5	30	154	440
	5-6	51	154	428
	TWA	39	206	
20	Mean			454
	sd			17.3
	0-1	40	26	491
	1-2	23	141	413
	2-3	48	179	446
	3-4	41	163	441
	4-5	37	163	436
	5-6	32	162	453
	TWA	39	148	
	Mean			447
	sd			25.6

sd Standard deviation

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{Weighted concentration (ppm)} = \frac{\text{Concentration (ppm)} \times \text{"Time weighting" (min)}}{\text{Exposure duration (min)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

Exposures 10 to 20 were monitored by automated sampling