PDCN: 88940000145

Hoechst Celanese

(1)

ORIGINAL

8EHQ-1194-12903

Department of
Environmental, Health &
Safety Affairs (DEHSA)

(B)

Hoechst Celanese Corporation
Route 202-206
PO Box 2500
Somerville, NJ 08876-1258
908 231 2000
Telex 833 449
Fax 908 231 4554

October 31, 1994
MRS-172-94

Attn: TSCA Section 8(e) Coordinator
Document Processing Center (TS-790)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Contains No CBI

Dear Sir or Madam:

RE: 8EHQ-0294-12955, 8EHQ-94-12903

As promised in our earlier communication, Hoechst Celanese is now providing the final report of a 2-week inhalation range-finding study of acetic anhydride vapor (CAS No. 108-24-7) in pregnant and non-pregnant rats. Dose levels were 400, 100 and 25 ppm.

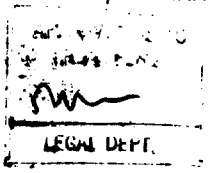
Severe lesions were detected throughout the respiratory tract of rats exposed to 100 and 400 ppm. Changes seen at 25 ppm were less severe. Histophathological examination of the nasal passages and lungs of male rats, revealed microscopic findings which included rhinitis, ulcertion and/or hyperplastic changes to varying degrees with macrophages present in the alveoli. Microscopic examination of the enlarged lymph nodes revealed lymphoid proliferation.

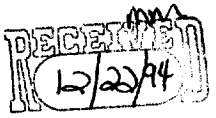
This submission contains no confidential business information.

If any further information is required, do not hesitate to contact Dr. Michele R. Sullivan, Director, Product Stewardship at 908-231-4480.

Sincerely,

Susan P. Engelman
Vice President, Environmental, Health & Safety Affairs





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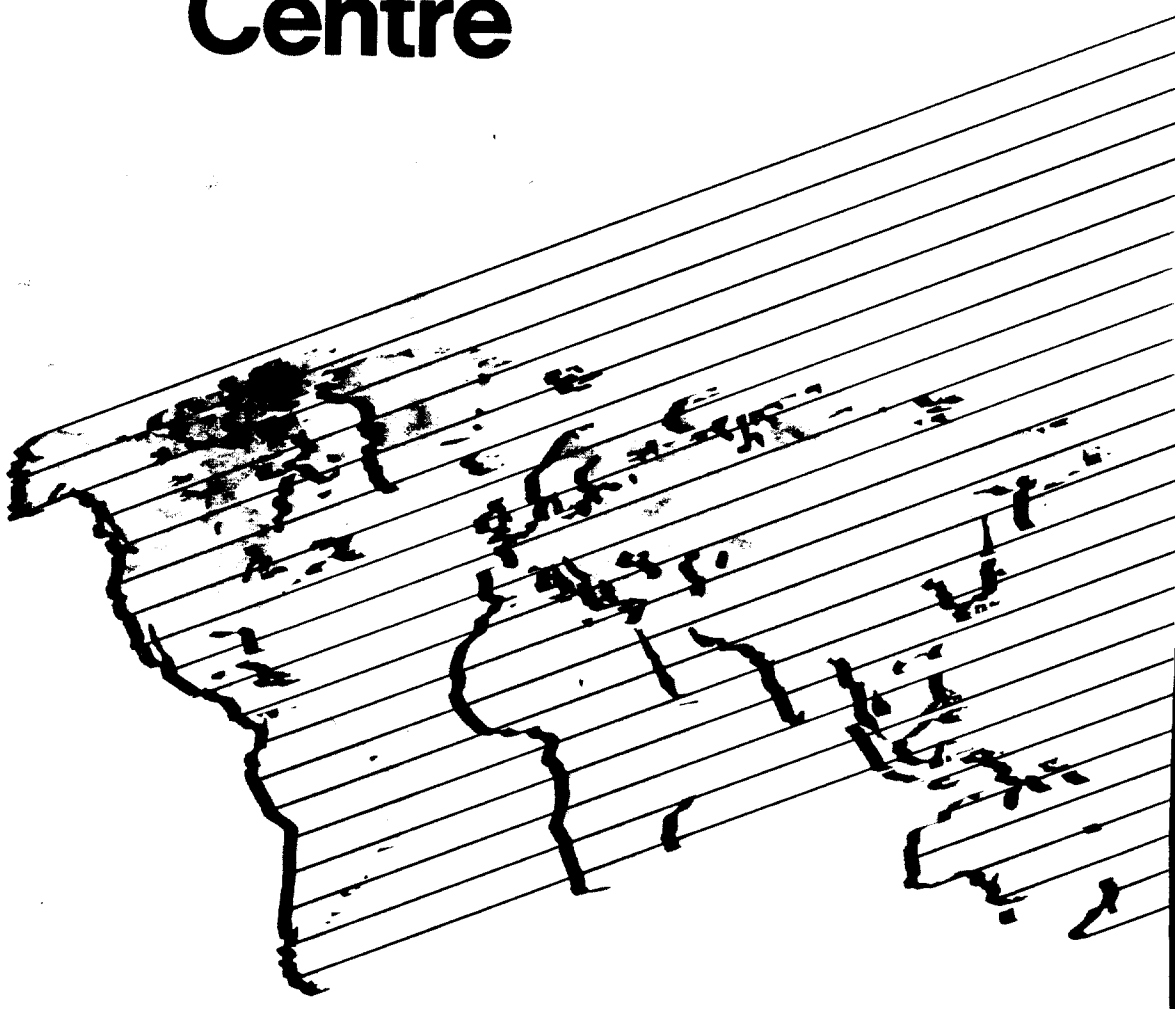
REC'D OCT 18 1994

HRC Report

ACETIC ANHYDRIDE

2 WEEKS REPEAT DOSE INHALATION TOXICITY STUDY IN MALE AND TIME-MATED FEMALE RATS

Huntingdon Research Centre



REC'D OCT 18 1994

ACETIC ANHYDRIDE

2 WEEKS REPEAT DOSE INHALATION TOXICITY STUDY IN MALE AND TIME-MATED FEMALE RATS

Sponsor

Hoechst Celanese Corporation,
Route 202-206, Bedminster 1,
PO Box 2500,
Somerville,
NJ 08876-1258,
U.S.A.

Testing facility

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued 13 October 1994

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.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

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.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

Jones

13th October 1994

Keith Jones, B.Sc., M.Sc., C.Biol., M.I.Biol.,
Study Director,
Huntingdon Research Centre Ltd.

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at HRC and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	4 January 94
Pre-experimental Period	12-14 January 94	14 January 94
Experimental Period	26 January 94	27 January 94
	31 January - 1 February 94	1 February 94
Date of reporting audit findings to the Study Director and HRC Management		13 October 94

G.R. Keeble

13 October 1994

G.R. Keeble,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

Date

RESPONSIBLE PERSONNEL

STUDY MANAGEMENT

Keith Jones, B.Sc. (Hons), M.Sc., C.Biol., M.I.Biol.
Study Director

Mario Bannerman, H.N.D.,
Head,
Department of Inhalation Toxicology.

Terence J. Kenny, B.Sc. (Hons.),
Study Supervisor

TOXICOLOGY

Colin J. Hardy, B.Sc., Ph.D., M.I.Biol., C.Biol.,
Dip.R.C.Path. (Toxicology),
Senior Toxicologist.

PATHOLOGY

Chirukandath Gopinath, B.V.Sc., M.V.Sc., Ph.D., F.R.C.Path.,
Director of Research and Principal Pathologist.

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SUMMARY

Test substance

Acetic anhydride.

Test animals

Albino rats CD (Sprague-Dawley). Five males and five time-mated females per group.

Route of administration

Inhalation, whole-body exposure to the vapour of the test substance.

Duration of exposure

Males - 6 hours per day, 5 days per week (Monday - Friday) for 2 consecutive weeks.
Females - 6 hours per day, Days 6 to 15 post coitum.

Exposure levels

Group	Designation	Acetic anhydride vapour concentration (ppm)				
		Target	Analysed		% Target concentration	
			♂	♀	♂	♀
1	Air control	Air only				
2	Low exposure	25	24	24	96	96
3	Intermediate exposure	100	103	104	103	104
4	High exposure [ó]	400	407	-	102	-

ó Males exposed on one occasion only

Results

Principal treatment-related findings are described in summary as follows:

Exposure at 400 ppm - Only 1 exposure at this level, in males only, was possible, due to a severe acute reaction to treatment; as a result of this, females allocated to this exposure level were not treated.

Clinical signs during exposure included eyes half-closed/closed, lachrymation, prone posture and exaggerated respiration. Immediately after exposure all animals showed noisy respiration.

Bodyweight loss after the exposure was marked whilst food and water consumption were almost zero.

Death occurred in two animals within 24 hours.

Sacrifice of the remaining three animals was necessary on the day after the first exposure, all three showing gasping respiration and two showing lethargy.

Macroscopic findings at necropsy included gaseous distension of the gastrointestinal tract.

Microscopic changes (males), usually in the epithelium (hyperplasia and/or ulceration) were often severe, with the nasal passages, larynx, trachea/tracheal bifurcation and lungs affected.

Exposure at 100 ppm - Although not nearly as an acute response compared with treatment at 400 ppm, there was a progressive and adverse reaction to treatment in both sexes, which resulted in cessation of this level before scheduled termination. Males received 6 exposures and females 7 exposures in total.

Clinical signs during exposure included eyes half-closed/closed, lachrymation, licking inside mouth, gasping respiration and occasional twitching of the head. Post exposure clinical signs included noisy respiration, occasional gasping and sneezing.

Marked bodyweight loss, reduced food and water consumption.

Total resorption of the litter in 2/4 pregnant females.

Macroscopic findings at necropsy included enlarged lymph nodes (cervical and/or tracheobronchial) in males and gaseous distension of the gastrointestinal tract in both sexes.

Similar, though less severe microscopic changes (males) were noted compared with the 400 ppm level.

Exposure at 25 ppm - Clinical signs during exposure included eyes half-closed/closed, licking inside of mouth, occasional twitching of head. Post exposure, noisy respiration was observed in both sexes, persisting after cessation of exposures up to termination.

Lower bodyweight compared with controls was seen for males during the second week of exposure and for females during most of the exposure period.

Reduced food and water consumption.

Macroscopic findings at necropsy included enlarged lymph nodes (cervical and/or tracheobronchial) increased lung weight and decreased liver and kidney weights in males.

As at the two higher exposure levels, microscopic changes were evident in the nasal passages, larynx, trachea/trcheal bifurcation and lungs, which although much less pronounced, were certainly treatment-related.

There was no evidence of an effect of exposure at 25 ppm on incidence and pattern of embryofoetal loss, litter size or litter weight and there were no gross foetal abnormalities.

Conclusion

In consideration of the marked acute toxic treatment-related effects (clinical, macroscopic and microscopic) seen among males exposed at 400 ppm, and the less marked but clear toxic effects seen in both sexes exposed at 100 ppm, neither of these levels would be suitable for further investigation in studies with a longer period of treatment.

At an exposure level of 25 ppm, there was clear evidence of toxicity in both sexes, and although tolerable for 10 days of exposure, changes were similar to, but less severe than, at higher exposure levels. Thus, in a longer period of exposure, this exposure level may prove too high and, as such, it should only serve as an upper level from which dosages can be selected.

INTRODUCTION

This report describes a preliminary study performed to determine appropriate exposure concentrations of the chemical, acetic anhydride for a subsequent OECD screen, whereby assessment would be made of toxicity in males and pregnant females.

The test substance was administered by the inhalation route, 6 hours per day for 2 periods of 5 days to males (Monday - Friday) and Days 6 to 15 post coitum for time-mated females. Inhalation exposure was chosen by the Sponsor as a possible route of accidental human exposure. The exposure concentrations of 0 (Control), 25, 100 and 400 ppm were selected by the Sponsor, on the basis of information available to them. The rat is 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 pathology parameters, reproductive performance and foetal pathology, at these laboratories.

RELEVANT STUDY DATES

Protocol approval by:

Study Director	26 August 1993
HRC Management	26 August 1993
Sponsor	13 September 1993

Animals arrived at HRC

♂	5 January 1994
♀ (Day 1 post coitum)	13 January 1994

Treatment commenced:

♂	17 January 1994
♀ (Day 6 post coitum)	18 January 1994

Terminal kill

♂ (After 2 weeks of treatment)	31 January 1994
♀ (Day 20 post coitum)	1 February 1994

ANIMALS AND MANAGEMENT

ANIMALS

Thirty-one male rats aged approximately 6 weeks and twenty-seven time-mated female rats aged approximately 8 to 10 weeks of the Crl:CD BR strain were obtained from Charles River (UK) Ltd, Manston Road, Margate, Kent on 5 and 13 January 1994 respectively.

All animals were examined upon receipt and were considered satisfactory for allocation to the study. Each animal was assigned a temporary number and weighed. The animals were then randomly assigned to cages using a computer program, stratified by bodyweight in such a way that the cage means were approximately equal. The appropriate cages were then allocated to the experimental group. Time-mated females were allocated on the day of arrival at HRC (Day 1 post coitum) and adjustments were made to ensure an acceptable distribution of the males to which the females were mated.

The identification of individual rats in the 4 groups, together with the group designation and group mean bodyweights at allocation were as follows:

Group	Designation/dose	Animal numbers		Group mean bodyweights (g)	
		♂	♀	♂	♀
1	Air control (0)	1 - 5	21 - 25	205	213
2	Low exposure (25 ppm)	6 - 10	26 - 30	206	213
3	Intermediate exposure (100 ppm)	11 - 15	31 - 35	205	214
4	High exposure (400 ppm)	16 - 20	36 - 40	205	213
HC	Health check	41 - 45	46 - 50		
R	Reserve	A - E	F & G		

Each rat was individually identified by a number tatooed on the ear pinnae.

Rats allocated to the reserve group were held as potential replacements during the pre-exposure period. They were killed when exposure commenced (17 January 1994 for males and 18 January 1994 for females).

The males were aged approximately 8 weeks and the females 9 - 11 weeks at the start of exposure.

HEALTH MONITORING

On the day of animal delivery, five males (numbered 41 - 45) and five females (numbered 46 - 50) were selected, killed and subjected to macroscopic examination. No macroscopic changes associated with infectious disease were apparent in any of these animals. Lungs, liver, kidneys, spleen and heart were preserved in buffered 10% formalin.

ACCOMMODATION

The rats were housed 5 of the same sex to a cage, in suspended cages with stainless steel sides and stainless steel mesh floors. Each cage was 35 cm wide, 53 cm long and 25 cm high. Plastic trays, lined with absorbent paper, were placed below each cage to collect animal excreta. The paper was changed daily. Clean cages were introduced once during the study. 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 (rack). 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 fur of rats in other groups. Exposure took place in the same room.

The temperature and relative humidity of the holding room (room 10, building Y14) were recorded continuously using a Kent Clearspan M105 chart recorder. The study holding room conditions were maintained within rages of:

Temperature:	18 to 24 °C
Relative humidity:	40 to 70%

Lighting was controlled to give 12 hours light (08.00 - 20.00 hours) and 12 hours dark per 24 hours.

DIET

While in their cages, all male 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) and time-mated female rats had access to SDS Laboratory Animal Diet No. 1.

Analyses were made on all batches of diet used to establish levels of basic nutrients and of specified substances and micro-organisms likely to have been present in the feed components and which, if in excess of specified amounts, might have had an undesirable effect on the test system. All batches of diet used conformed with the acceptable standards agreed between the Study Director and the Head of the Quality Assurance Department (see Appendix 1). 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 HRC Archives.

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

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 HRC. Additionally, levels of specified substances known to be present from time to time in local water and which, if in excess of the maxima recommended for (human) drinking water, might have an undesirable effect on the test system, are determined in the tap water at approximately 6-monthly intervals. A list of the principal determinands is given in Appendix 1.

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

The analytical data have been lodged in HRC Archives.

The actual diet batches used during the study were as follows:

Batch no.	Dates of use
9906	5 January 1994[1] to 31 January 1994[2] - males
9623	13 January 1994[1] to 1 February 1994[2] - females

[1] Arrival of rats
[2] Last day of terminal kill

TEST SUBSTANCE AND ADMINISTRATION

TEST SUBSTANCE

Name:	Acetic anhydride
Chemical name:	Acetic anhydride
Action:	For use as an industrial chemical
Received from:	Celanese Canada Inc 195 The West Mall, 10th Floor, Etobicoke, Ontario, M9C 5K1
Date received:	24 November 1993
Batch number:	Not supplied to HRC
Expiry date:	8 October 1994
Purity:	99.05% (Appendix 2)
Appearance:	Clear, colourless, mobile liquid
Storage conditions:	Dry at 4°C
Stability:	Adequate for the study

ADMINISTRATION

Rats were exposed to the vapour of acetic anhydride using whole-body inhalation exposure chambers for 6 hours per day. For males the planned exposure period was 5 days a week (Monday - Friday) for two consecutive weeks. This was only possible at the low exposure level, since, due to treatment-related findings, exposure occurred on only 6 occasions at the intermediate exposure level (100 ppm) and 1 occasion at the high exposure level (400 ppm). Time-mated female rats were exposed on 10 consecutive days (Days 6 to 15 post coitum inclusive), although restricted to only 7 occasions for females in the intermediate group (100 ppm), again due to treatment-related findings. Due to the adverse effect of exposure at 400 ppm, in males, females allocated to this group were not exposed but retained as concurrent controls.

Control rats received air only.

Target concentration

The target concentration for each group was as follows:

Group	Designation	Target concentration (ppm)
1	Air control	Air only
2	Low dose	25
3	Intermediate dose	100
4	High dose	400

Chamber spatial distribution

The arrangement of sample ports around the chamber walls is indicated in Figure 3. Normally during exposure, samples were removed from an upper middle port.

Spatial distribution was assessed for each concentration during preliminary work before the study commenced. The data are presented in Appendix 9.

Spatial variation did not differ significantly from the temporal variation, indicating that the distribution of acetic anhydride within the exposure chambers was satisfactory.

EXPOSURE CHAMBER CONDITIONS

Measurement of chamber concentration of acetic anhydride

The concentration of acetic anhydride within the exposure chambers was determined at least 6 times during exposure, usually at hourly intervals. Samples were concurrently analysed for the presence of acetic acid.

Samples of chamber air were withdrawn from each test group chamber, with a glass gas-tight syringe. The samples were injected via a gas loop into a gas chromatograph and the concentration of acetic anhydride and acetic acid, if present, determined by external standards.

Details of the sampling methods and methods of analysis are presented in Appendix 3.

Aerosol content of chamber atmosphere

One one occasion during the study, air samples from the control, low and intermediate dose chambers were withdrawn through a Royco Particle Monitor (Model 218, Royco Instruments Inc, USA) to determine the presence or absence of test substance aerosol. No aerosol was considered to be present in the chamber. The results are presented in Table 1.

Nominal concentration

The volume of test substance used at each level during each exposure was recorded. Using the relative density of the material the weight was calculated and divided by the total airflow through the chamber per exposure. The mg/l figure derived was converted to ppm according to the following expression:

$$ppm = \frac{mg/l \times Vm}{M.W.}$$

where Vm = molar volume (ml) of an ideal gas at 25°C and 760 mm Hg
M.W. = molecular weight of acetic anhydride (102.05)

Airflow

The airflow into each chamber was monitored continuously using tapered-tube rotometers and recorded at 30-minute intervals throughout each exposure.

Temperature

The air temperature in each chamber was monitored continuously with a mercury bulb thermometer and recorded at 30-minute intervals throughout each exposure.

Pressure

The air pressure in each exposure chamber, relative to that in the exposure room, was monitored using a magnehelic gauge and recorded at 30-minute intervals throughout exposure.

Relative humidity

The humidity in each exposure chamber was monitored continuously with a wet and dry bulb hygrometer and recorded at 30-minute intervals throughout each exposure.

CLINICAL OBSERVATIONS

CLINICAL SIGNS AND MORTALITY CHECKS

During exposure

Clinical signs during exposure were recorded as a group response where all visible animals appeared to be responding similarly. For reporting purposes, "Day", in relation to clinical signs, refers to the day of exposure for males, day of pregnancy/post coitum for females.

At other times

Animals were examined at least twice each day and any signs were recorded on the individual sheets.

Any animals found dead or dying were examined macroscopically on the day of death and a full spectrum of tissues preserved for further examination (see **TERMINAL PROCEDURES**).

BODYWEIGHT

Each male rat was weighed for allocation to groups, one week before exposures commenced (Week -1), on the day of the first exposure (immediately prior to exposure) and daily until the end of the study.

Time-mated female rats were weighed initially (assumed Day 1 of pregnancy) and on Days 2, 3, 6, 8, 10, 12, 14, 16, 18 and 20) post coitum.

FOOD CONSUMPTION

The quantity of food consumed by each cage of male rats was recorded daily, commencing one week before exposures commenced and continuing daily until the end of the study.

For time-mated female rats, food consumption was measured from weighday to weighday, up to termination.

WATER CONSUMPTION

Water consumption was measured daily from Week -1 in males and Day 1 in females to termination.

The amount of water consumed by each cage of rats was determined daily by weight loss from the water bottles.

TERMINAL PROCEDURES

MALES

Following 2 weeks of exposure, the surviving rats were killed. The terminal kill took place on 31 January 1994.

The rats were anaesthetised by intraperitoneal injection of pentobarbitone sodium and killed by exsanguination (incision of the brachial blood vessels).

Macroscopic pathology and organ weighing

At necropsy a detailed macroscopic examination of all male rats was performed. The following organs/tissues were dissected free from each rat and weighed:

adrenals	liver	testes (with epididymides)
kidneys	lungs	

Fixation of tissues

Samples, or the whole of the following organs/tissues, together with any macroscopically abnormal entities were preserved in buffered 10% formalin. The eyes were preserved in Davidson's fixative. The lungs were distended with fixative prior to immersion.

adrenal
animal identification mark
aorta
brain (medulla/pons, cerebellar, cortex, cerebral cortex)
eyes
femur (with joint)
gross abnormalities[a]
head (for paranasal sinuses, oral cavity, nasopharnynx, middle ear, teeth, eyelids, Harderian gland, exorbital lachrymal gland and Zymbal's gland)
heart
kidneys
larynx[a]
liver
lungs[a] (all lobes and mainstem bronchi)
lymph nodes (tracheobronchial, cervical and mesenteric)
mammary gland
nasal passages (4 levels as described by Young (1981))[a]
pancreas
pituitary
pharynx
prostate
salivary gland
sciatic nerve
spleen
sternum
testes (with epididymides)
tongue[a]
thymus
thyroid (with parathyroid)
trachea (including bifurcation)[a]
urinary bladder
ureter

[a] Initially examined from all groups

Microscopic examination

Light microscopic examination was performed on 4 μm thick sections, stained with haematoxylin and eosin, of tissues in the list overleaf marked [a].

FEMALES

Litter data and foetal examination

On Day 20 of pregnancy, Group 1 and 2 animals were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined immediately to determine:

Number of corpora lutea
Number and distribution of live young
Number and distribution of embryofoetal deaths
Individual foetal weight from which the litter weight was calculated
Foetal abnormalities

Embryofoetal deaths were classified as:

Early: Only placenta visible at termination
Late: Both placental and embryonic remnants visible at termination

Uteri without visible implantations were examined for evidence of implantation using a modified Salewski technique (Salewski 1964).

Live young were examined externally, and then discarded.

Dams sacrificed on Day 13 post coitum (Groups 3 and 4) were examined in a similar manner although embryos were not examined or weighed because they were too immature.

RESULTS

Individual litter values

In assessing litter parameters, pre-implantation loss was calculated as a percentage from the formula:

$$\frac{\text{No. of corpora lutea - no. of implantations}}{\text{No. of corpora lutea}} \times 100$$

Post implantation loss was calculated from the formula:

$$\frac{\text{No. of implantations - no. of live young}}{\text{No. of implantations}} \times 100$$

Litter weight and mean foetal weight were calculated from individual foetal weight.

Group values

Group values have generally been presented for all females with live young at termination (Day 20). However, in some instances, where appropriate, group values have also been presented to include all animals or just those which were pregnant including those which resorbed their litter.

All derived values (eg means and percentages) were first calculated within each litter and the group values derived as a mean of the individual litter values.

Statistical analysis

No statistical analyses were performed in view of the small group sizes.

ARCHIVING

All specimens, raw data and other documents generated at HRC during the course of the study, together with a copy of this Final Report, have been lodged in the Huntingdon Research Centre Ltd Archives, Huntingdon, England.

Such specimens and records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's knowledge.

STUDY PROTOCOL

The protocol for the study, together with protocol amendments and a copy of the protocol approval form are maintained with all other study records in the Huntingdon Research Centre Ltd Archives. There were no deviations from the protocol.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Chamber vapour concentration of acetic anhydride

The results are presented in Table 2 and Appendix 4 and are summarised as follows:

Group	Study mean concentration (ppm)		
	Target	Analysed	
		♂	♀
2 (Low dose)	25	24	24
3 (Intermediate dose)	100	103	104
4 (High dose)	400	407[1]	-[2]

[1] Single exposure
[2] Females not exposed at the High dose

The results indicate close agreement between target and analysed levels.

Nominal concentration

The data are presented in Table 3 and are summarised as follows:

Group	Study mean			
	Nominal (ppm)		A/N (%)	
	♂	♀	♂	♀
2 (Low dose)	23	23	104.1	105.7
3 (Intermediate dose)	83	85	124.4	123.6
4 (High dose)	359[1]	-[2]	113.4[1]	-[2]

[1] Single exposure
[2] Females not exposed at the High dose

The analysed to nominal concentration ratios (A/N) indicate that the actual chamber airflows were probably slightly lower than the 150 l/min indicated by the flowmeters. This was considered to be a consequence of heating the air in the water bath. Since the lower airflow was noted only at the start of the exposure period, given that the chamber concentrations were very similar to the targets, it was considered prudent not to make any alterations to the generation conditions.

Chamber temperature and relative humidity

The data are presented in Table 4 and are summarised below:

Group	Study mean	
	T (°C)	RH (%)
1 (Air control)	23.0	33
2 (Low dose)	23.1	29
3 (Intermediate dose)	23.2	23
4 (High dose)[1]	22.5	26

[1] Single exposure, males only

The small differences between groups are considered not to have affected the outcome of the study.

Clinical observations

Due to adverse treatment-related clinical findings among males after one exposure at 400 ppm, further examination of this level was discontinued in males, and was not performed at all in females. For males and females receiving 100 ppm, there was a progressive and marked adverse reaction to treatment which resulted in discontinuation of treatment before scheduled termination. On the basis of these findings, for clarity of reporting, each exposure level is in general described separately, although microscopic findings are presented as a whole.

400 ppm

Male animals only; received 1 exposure (Day 1).

Clinical signs and mortality (Tables 5, 6, 11 and 13 Appendix 8)

During exposure, all males showed prone posture, eyes half-closed/closed, lachrymation and exaggerated breathing. After exposure, all showed noisy respiration.

On Day 2, at the first check of the day, 2 animals were found dead (Nos. 18 and 19). Of the remaining 3, 2 were in a lethargic condition, and all 3 were showing gasping respiration. On the basis of poor general condition, these animals were sacrificed that day, with no further exposure.

Bodyweight (Figures 4 and 5, Table 7, Appendix 5)

All animals showed weight loss after the first exposure (between Day 1 and 2) of approximately 50 g each.

Food and water consumption (Figures 6 and 7, Tables 8, 9)

Group mean cage values for food and water consumption after the first exposure were almost zero. These values were obviously markedly lower compared with concurrent controls and pre-exposure values.

Necropsy (Table 11, Appendix 8)

Macroscopic examination revealed gaseous distension in the gastrointestinal tract, particularly the stomach, of all animals. This finding is consistent with the effects seen on respiration. In addition, the majority of animals showed brown perinasal staining.

100 ppm

Clinical signs and mortality (Tables 5, 6, 11 and 13 Appendix 8)

During the first exposure, males and females showed half-closed/closed eyes, whilst females also showed licking of the inside of the mouth and lachrymation. After further exposures, males also showed these latter clinical signs and, additionally, both sexes showed gasping and/or exaggerated respiration and occasional twitching of the head.

In the post exposure periods, all animals, both sexes, showed noisy respiration throughout, and occasionally, again in all animals, sneezing and gasping respiration. In females, arching of the back and head twitching was also observed.

Although there were no deaths in either sex, the severity of the clinical signs and poor condition resulted in termination of this level, males having received 6 exposures and females 7 exposures.

Bodyweight (Figures 4 and 5, Table 7, Appendix 5)

All animals, both sexes, showed marked weight loss following the first exposure and, thereafter, there was further progressive weight loss for all individuals. Males showed bodyweight gain during the off-treatment period of 2 days which followed the first 5 exposures, but soon returned to weight loss after the 6th exposure.

This bodyweight loss was particularly marked in females and was severe, considering that the majority were pregnant and, as such, marked bodyweight gain is usually the norm.

As a result of these bodyweight losses, both sexes showed markedly lower group mean bodyweight compared with the respective concurrent controls (this included comparison with the unexposed Group 4 females).

Food and water consumption (Figures 6 and 7, Tables 8 and 9)

After the first exposure, group mean food and water consumption values were noticeably lower for both sexes compared with the respective control groups (approximately 40 - 50% for food and 25% for water) and pre-exposure values. Thereafter, there was a further reduction for both sexes, resulting in values which were approximately 80% lower compared with controls.

During the 2 days without exposure which followed the first 5 exposures, males showed noticeable recovery in food and water consumption, although group mean values still remained lower compared with concurrent controls.

Necropsy (Tables 10 and 11, Appendices 6 and 8)

Macroscopic examination revealed gaseous distension at one or more levels of the gastrointestinal tract of all males and two females compared with no such finding in concurrent controls. Also, among all males, enlarged lymph nodes (cervical and/or tracheobronchial) were noted, and adipose tissue was minimal in the majority.

Among females, macroscopic examination concentrated on the pregnancy status. Comparison was made with the Group 4 (unexposed) females also killed at Day 13 post coitum. Four out of five females were pregnant in each group. Two females which received 100 ppm showed total resorption of their litters, compared to none in the concurrent control group. However, the other two pregnant females in this group were supporting live litters of comparable size with those of the unexposed concurrent control group. Examination of foetuses for visual abnormalities was not fully possible given the age at sacrifice.

25 ppm

The scheduled number of exposures was achieved.

Clinical signs and mortality (Tables 5, 6, 11 and 13 Appendix 8)

During the first exposure, no clinical signs were observed among males, whilst for females only half-closed/closed eyes was noted. On each day of exposure thereafter, the latter finding was observed for both sexes. The only other treatment-related clinical sign, for both sexes, was licking of the inside of the mouth and occasional twitching of the head, these signs being evident predominantly on the last 3 - 4 days of exposure.

The only clinical sign noted post exposure, and considered to be treatment-related, was noisy respiration, observed for all animals. This clinical sign persisted after completion of the final exposure, for females in general, up to the day before sacrifice (ie 4 days after completion of the last exposure).

There were no deaths at this exposure level among males or females, during the study.

Bodyweight (Figures 4 and 5, Table 7, Appendix 5)

Males receiving this exposure level had similar bodyweight gain during the first week of exposure compared with the concurrent controls. However, after the first exposure of the second week, all males showed a consistent period of bodyweight loss, followed by depressed weight gain compared with controls. After completion of the last exposure, bodyweight gain showed a marked improvement, but group mean bodyweight still remained lower than controls by termination.

All females at this exposure level were pregnant, as were the concurrent controls. Up to Day 8 of pregnancy (after 2 exposures), group mean bodyweight for treated females was similar to the controls. Thereafter, however, to completion of the exposures, group mean bodyweight gain was depressed compared with controls. On cessation of exposure, there was a marked recovery in bodyweight gain, although not of sufficient magnitude to attain parity with the controls in terms of absolute group mean bodyweight at Day 20 of pregnancy.

Food and water consumption (Figures 6 and 7, Tables 8 and 9)

Group mean food and water consumption were, in general, consistently lower for both sexes compared with the respective concurrent control groups, throughout the exposure periods. A return to parity with controls was seen for both sexes after cessation of exposures. The extent of differences compared with controls were not comparable with those seen at the higher exposure levels.

Necropsy (Tables 10, 11 and 12, Appendices 6, 7 and 8)

Males - Macroscopic examination revealed that all animals had enlarged lymph nodes (cervical and/or tracheobronchial), and two also had some congestion in the anterior lobe of the right lung.

Organ weight analysis (absolute values) revealed higher lung weight, and lower liver and kidney weights compared with the concurrent controls.

Females - There were no treatment-related macroscopic abnormalities among females at this exposure level. Organ weights were not recorded.

All females at this level had a live litter at termination (Day 20 of pregnancy). The incidence and pattern of embryofoetal loss, litter size, litter weight and foetal weight were similar compared with the concurrent controls and there were no gross foetal abnormalities.

MICROSCOPIC PATHOLOGY (Table 13, Appendix 8)

Nasal passages

In the rats from the 100 and 400 ppm dosage groups (decedents), severe changes were detected, these included: rhinitis with atrophy/degeneration or ulceration of the olfactory epithelium; ulceration and/or replacement of the respiratory epithelium with undifferentiated type (also seen in nasopharynx) and squamous metaplasia; and hyperplasia of transitional epithelium.

In rats from the 25 ppm dosage group (killed at termination), these changes were less severe and included rhinitis with atrophy/degeneration of the olfactory epithelium; and hyperplasia of the respiratory (also seen in the nasopharynx) and transitional epithelia.

Larynx

Severe degenerative changes were detected in decedent rats from both the 100 and 400 ppm dosage groups. The major change was epithelial ulceration associated with a luminal inflammatory exudate.

In rats from the 25 ppm group (at termination) the changes were less severe and of a more proliferative nature ie epithelial hyperplasia and squamous metaplasia.

Trachea and tracheal bifurcation

Severe degenerative changes were detected at 400 ppm comprising epithelial ulceration with a luminal inflammatory exudate. The changes at 100 ppm were marginally less severe, with more evidence of a proliferative response ie epithelial hyperplasia.

In rats from the 25 ppm group examined at termination, the changes were considerably less severe and comprised low degrees of epithelial hyperplasia.

Lungs

Severe degenerative changes were detected in the bronchiolar epithelium at 400 ppm (decedents). These comprised epithelial ulceration with associated inflammatory exudation and evidence of epithelial regenerative hyperplasia. The changes at 100 ppm were less severe and predominantly characterised regenerative hyperplasia and epithelial hypertrophy with goblet cells.

At 25 ppm, changes in the bronchiolar epithelium were considerably less severe and comprised epithelial hypertrophy with goblet cells.

In the alveoli, macrophages and macrophages associated with intra-alveolar mucus were detected in some rats at 100 ppm and alveolitis at 400 ppm.

At 25 ppm, macrophages in alveoli and macrophages associated with intra-alveolar mucus were detected in some rats.

Other findings

Tracheobronchial lymph nodes - Lymphoid proliferation was detected in male rats receiving 25 ppm or 100 ppm from which these lymph nodes (and also in the deep cervical lymph nodes) were examined as macroscopic abnormalities. However, as these nodes were not examined from control rats, it is difficult to assess the toxicological significance of this finding.

Incidental findings

All other lesions were considered spontaneous in origin and therefore of no toxicological significance.

Conclusion

Severe lesions were detected throughout the respiratory tract of rats exposed to 100 and 400 ppm. Changes seen at 25 ppm were less severe.

DISCUSSION

The effects of exposure to this test substance, at 25, 100 or 400 ppm for 6 hours per day were consistent with those effects often seen with exposure to an irritant vapour. These effects included noisy respiration, eyes half-closed/closed and lachrymation, and marked effects upon bodyweight gain, food consumption and water consumption. Histopathological examination of the nasal passages and lungs of the males, revealed microscopic findings which included rhinitis, ulceration and/or hyperplastic changes to varying degrees with macrophages present in the alveoli. Enlarged deep cervical and/or tracheobronchial lymph nodes were seen in some male animals exposed at 25 ppm or 100 ppm but not in control animals. Microscopic examination of the enlarged lymph nodes revealed lymphoid proliferation.

Among pregnant females, the effects seen upon *in utero* survival of the conceptus at 100 ppm, (two females which showed total resorption), was probably a consequence of the marked maternal response to the exposure rather than being a direct effect.

CONCLUSION

In consideration of the marked acute toxic treatment-related effects (clinical, macroscopic and microscopic) seen among males exposed at 400 ppm, and the less marked but clear toxic effects seen in both sexes exposed at 100 ppm, neither of these levels would be suitable for further investigation in studies with a longer period of treatment.

At an exposure level of 25 ppm, there was clear evidence of toxicity in both sexes, and although tolerable for 10 days of exposure, changes were similar to, but less severe than, at higher exposure levels. Thus, in a longer period of exposure, this exposure level may prove too high and, as such, it should only serve as an upper level from which dosages can be selected.

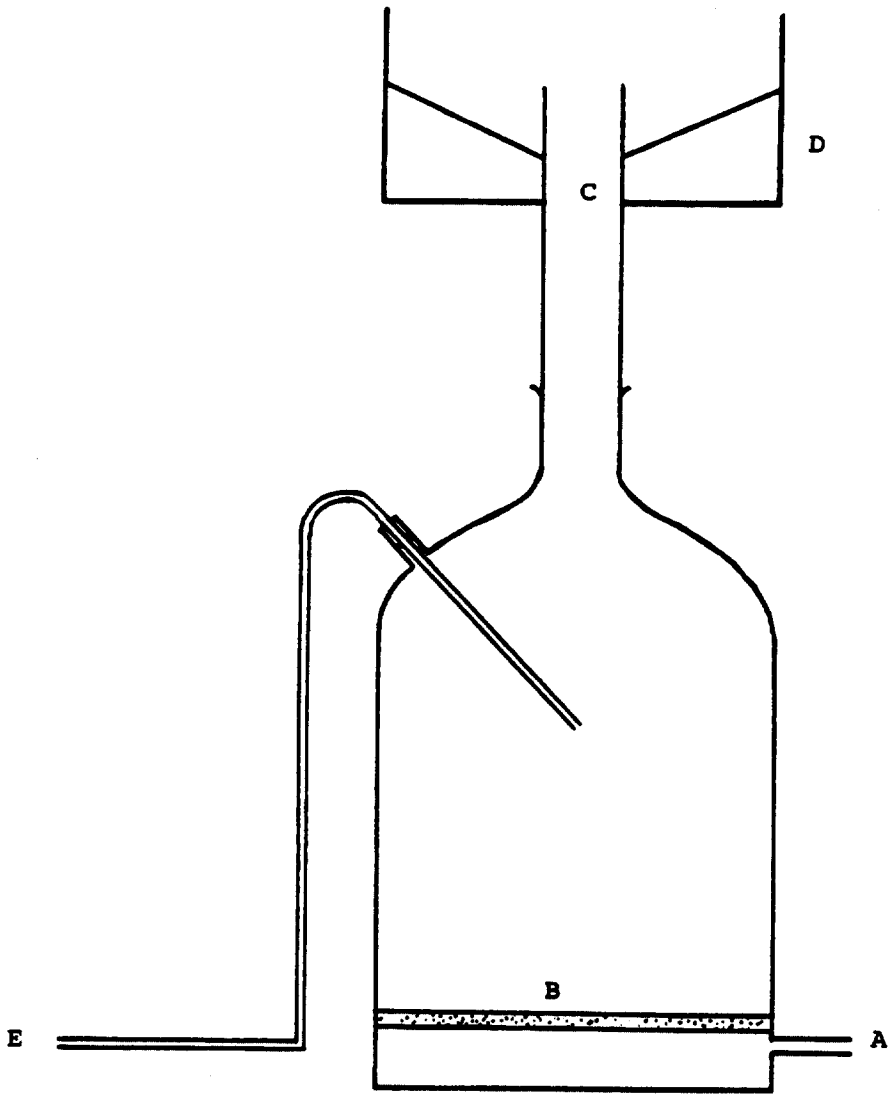
REFERENCES

SALEWSKI, E. (1964) Farbemethode zum makroscopischen Nachweis von Implantationsstellen am Uterus der Ratte. *Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmacol.*, **247**, 367.

YOUNG, J.T. (1981) Histopathological examination of the rats nasal cavity. *Fundamental and Appl. Toxicol.* **1**: 309 - 312.

FIGURE 1

Vapour generator



Key

A. Air line

B. Fritted glass disc

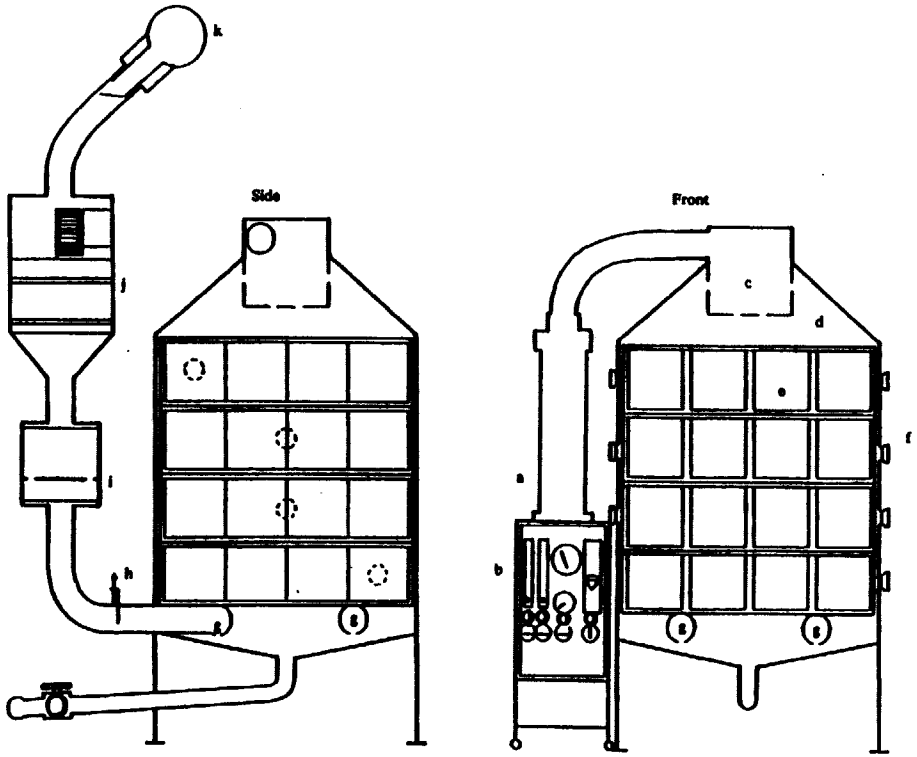
C. Vapour inlet

D. Stainless steel and glass elutriator

E. Test substance supply line

FIGURE 2

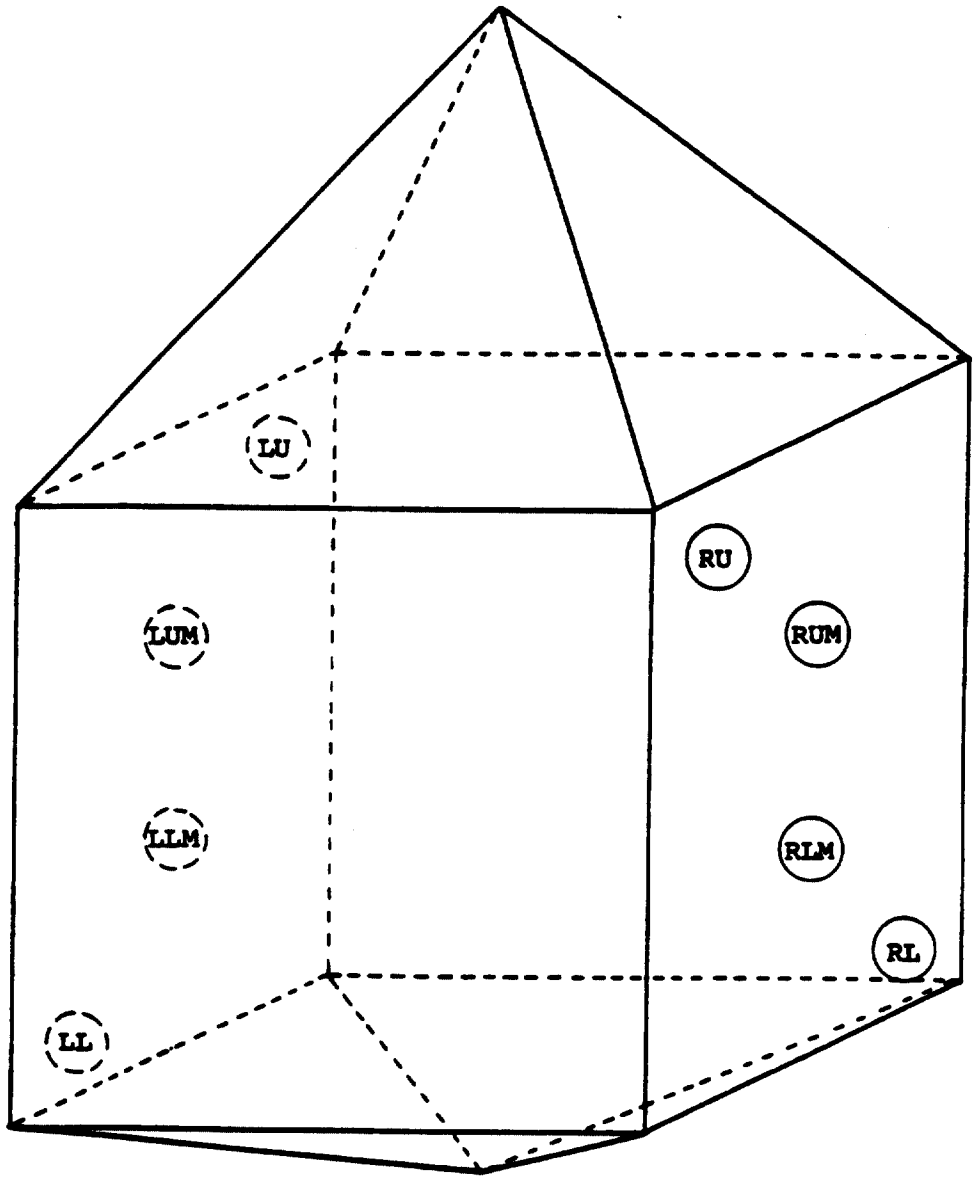
Exposure system



a Glass elutriation column
b Airflow control and chamber pressure monitoring
c Dispersion device
d Exposure chamber
e Animal exposure cages
f Sampling port
g Exhaust plenum
h Gate valve
i Pre-filter
j Powered extract filter
k Main exhaust

FIGURE 3

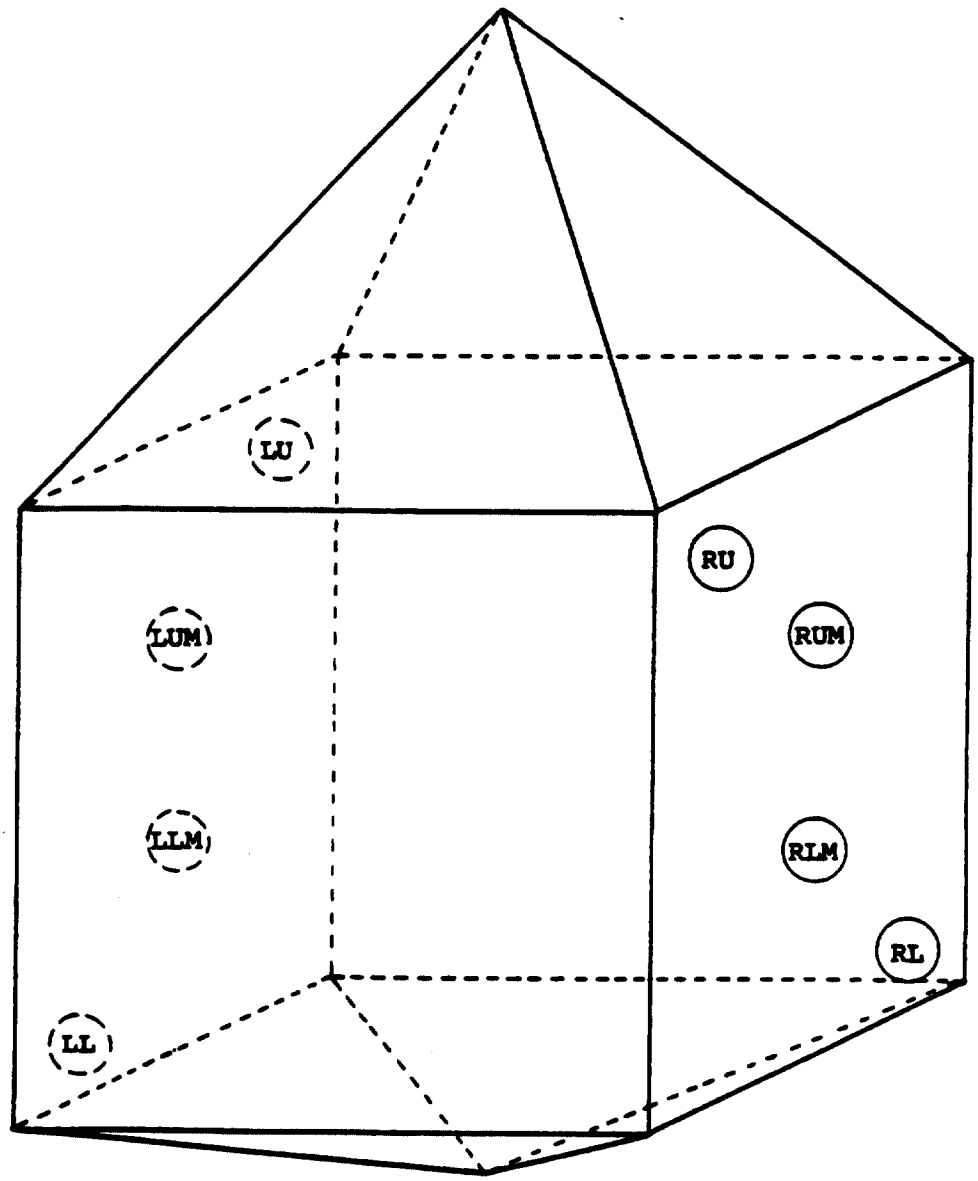
Location of sample ports on exposure chambers



Key			
LU	Left upper	RU	Right upper
LUM	Left upper middle	RUM	Right upper middle
LLM	Left lower middle	RLM	Right lower middle
LL	Left lower	RL	Right lower

FIGURE 3

Location of sample ports on exposure chambers



Key			
LU	Left upper	RU	Right upper
LUM	Left upper middle	RUM	Right upper middle
LLM	Left lower middle	RLM	Right lower middle
LL	Left lower	RL	Right lower

FIGURE 4

Bodyweight - group mean values - Males

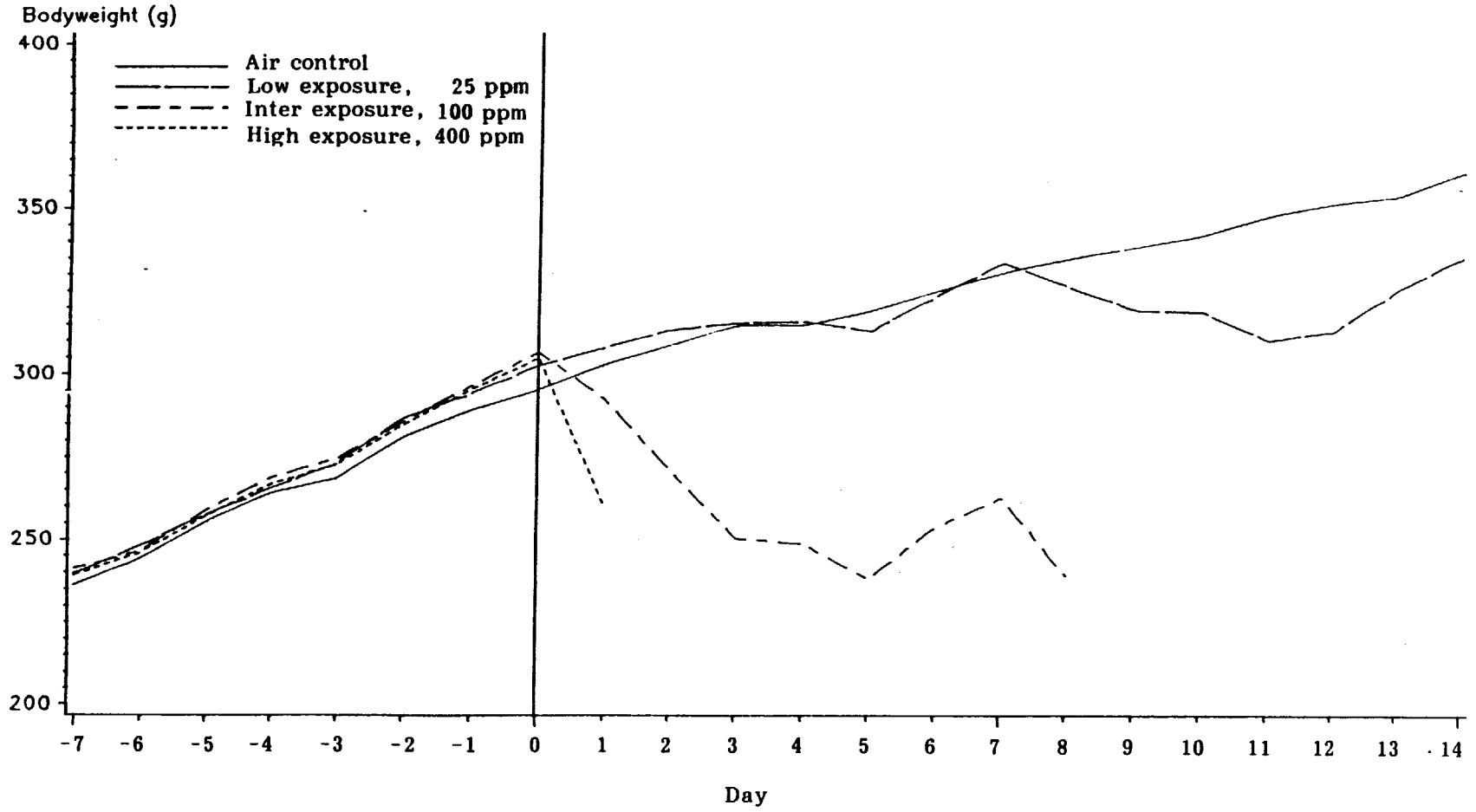


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FIGURE 5

Bodyweight of pregnant females - group mean values

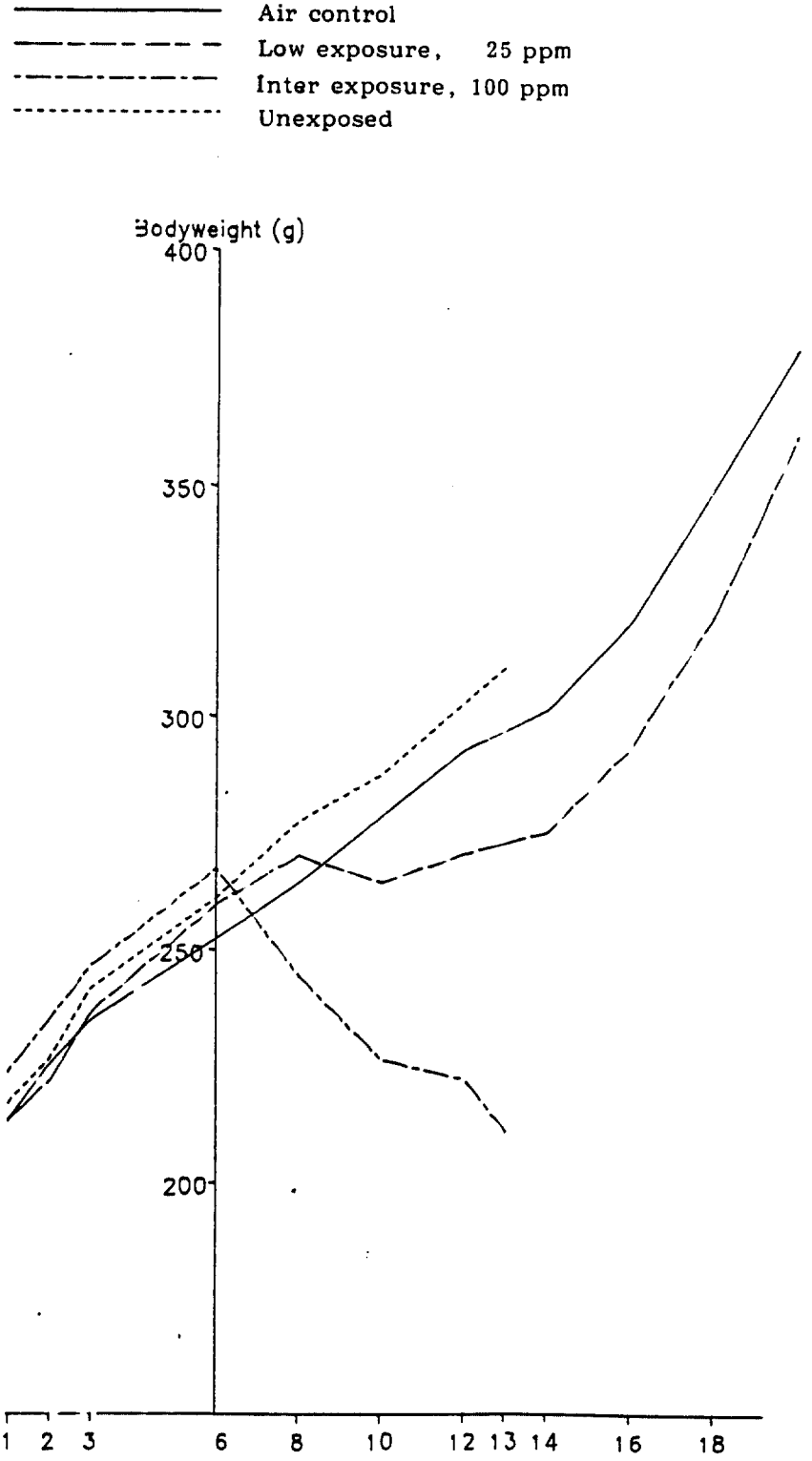

FIGURE 6

Food consumption - group mean values

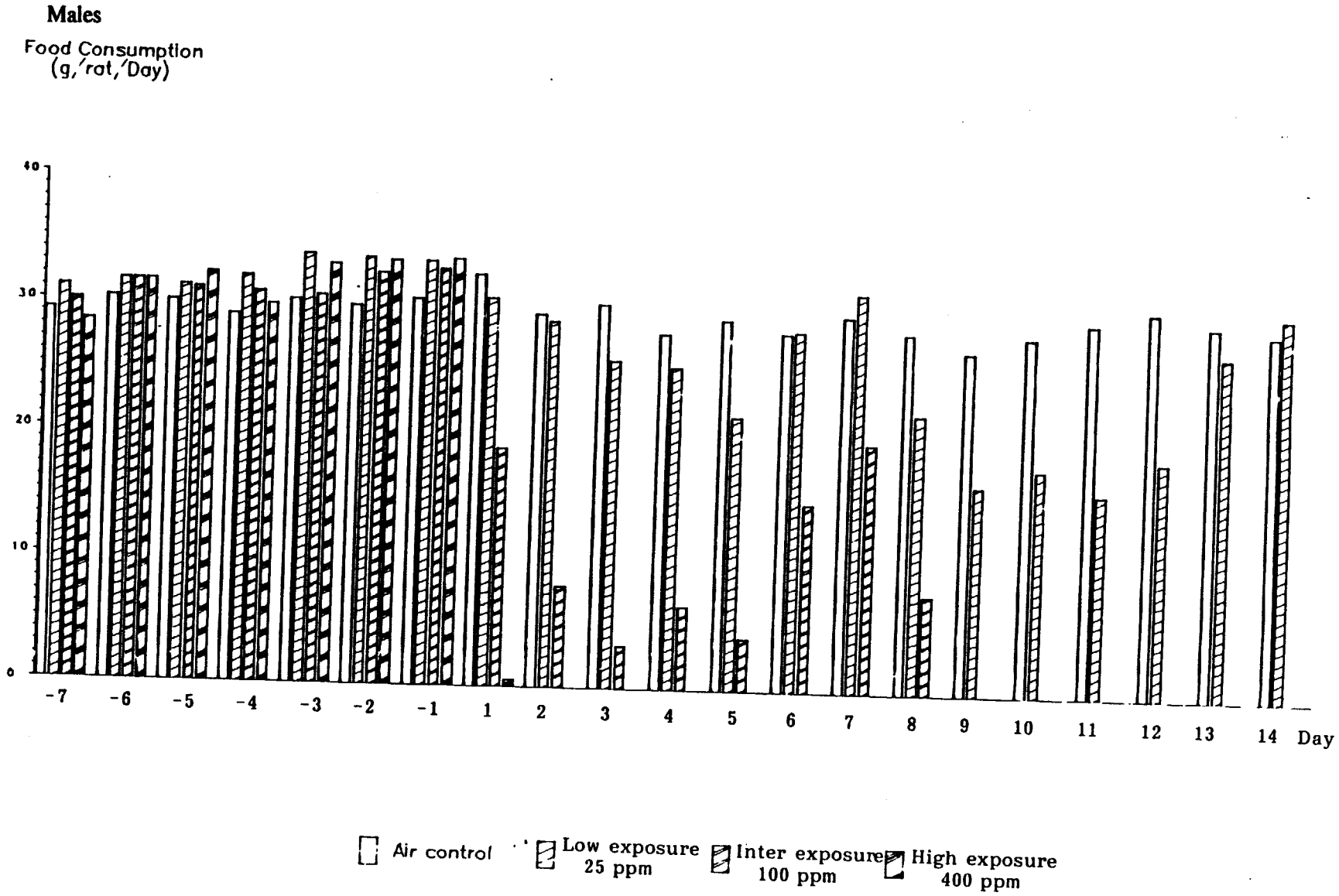

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FIGURE 6

(Food consumption - continued)

Females

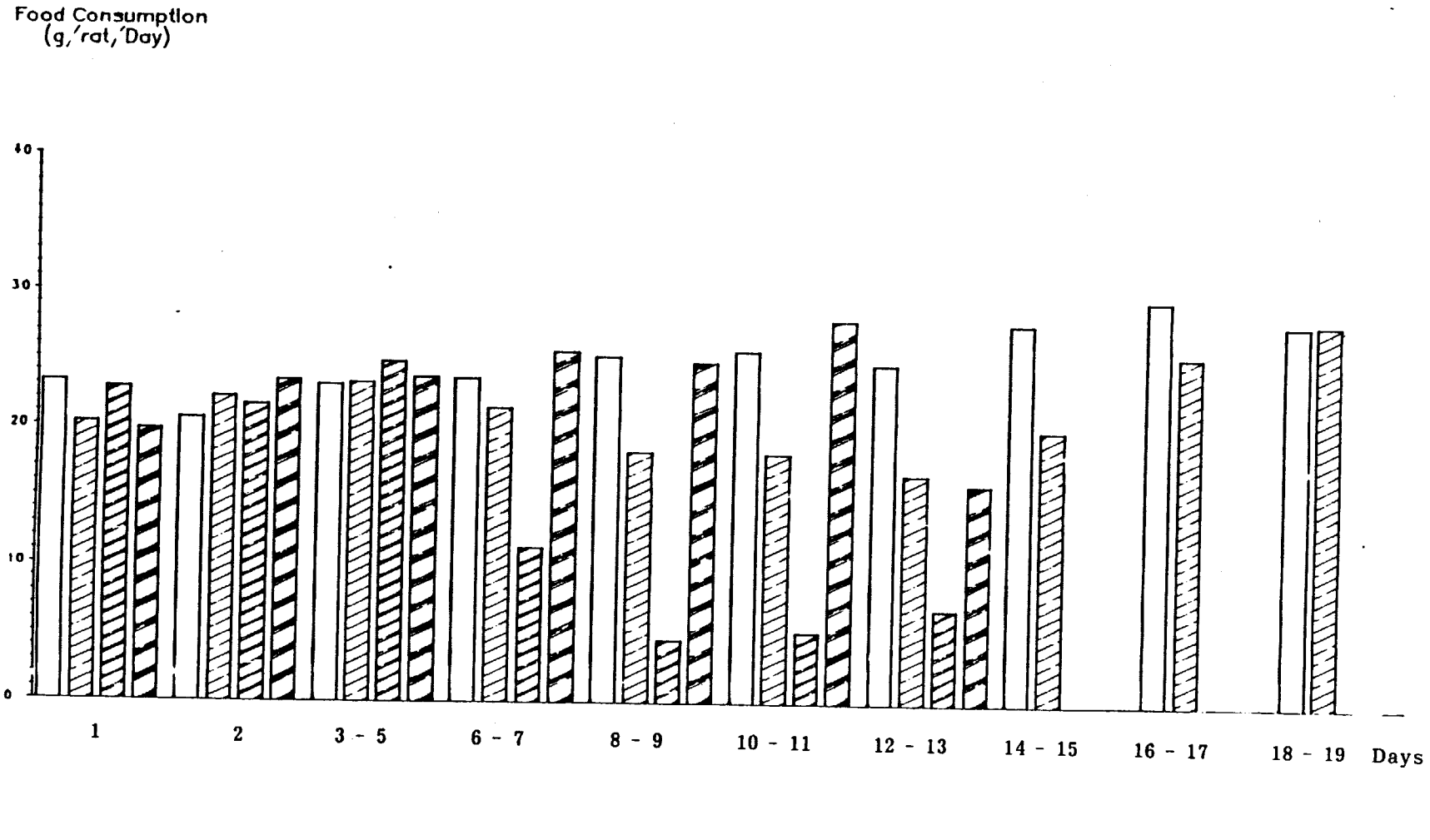

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FIGURE 7

Water consumption - group mean values

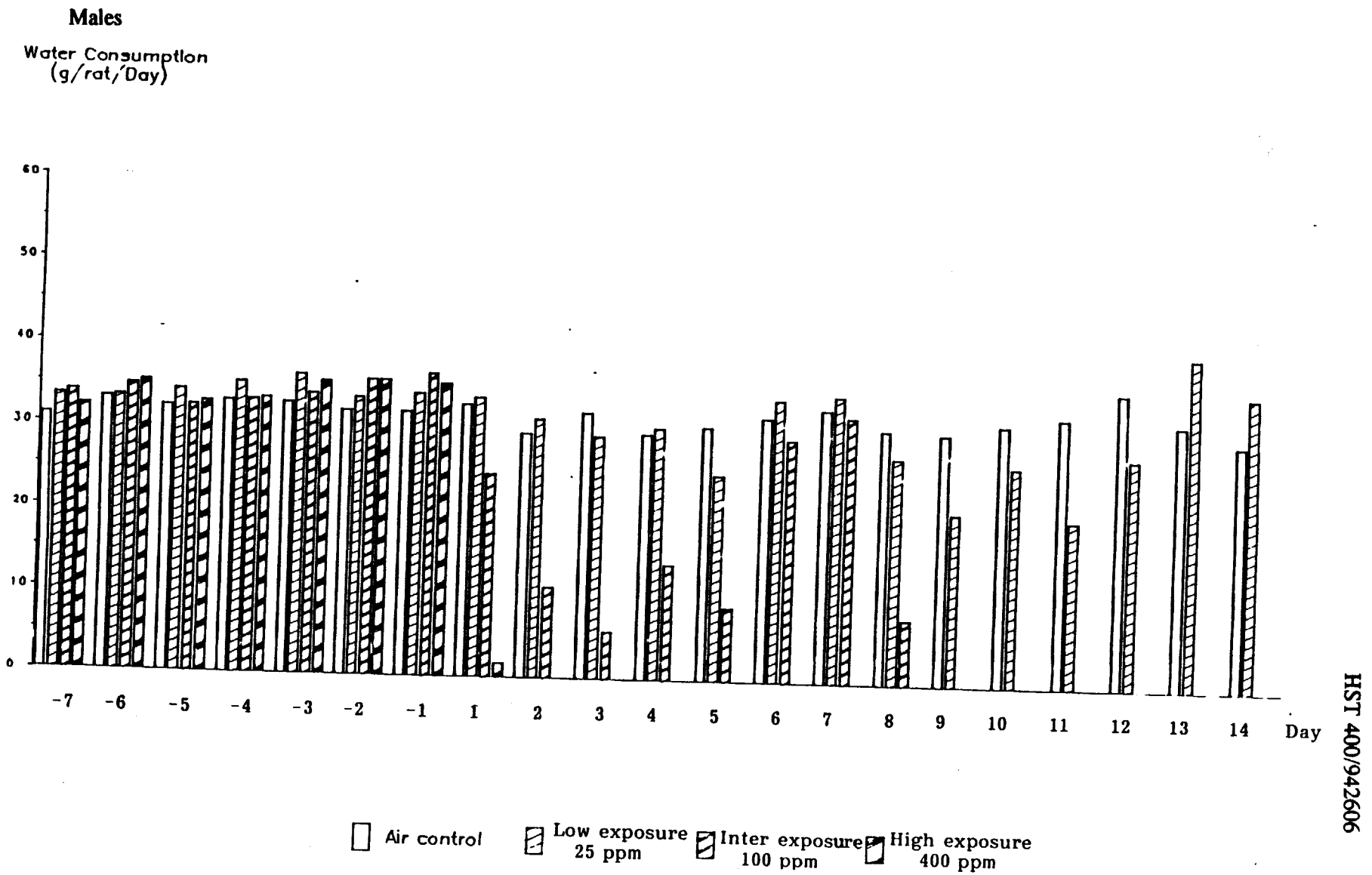

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FIGURE 7

(Water consumption - continued)

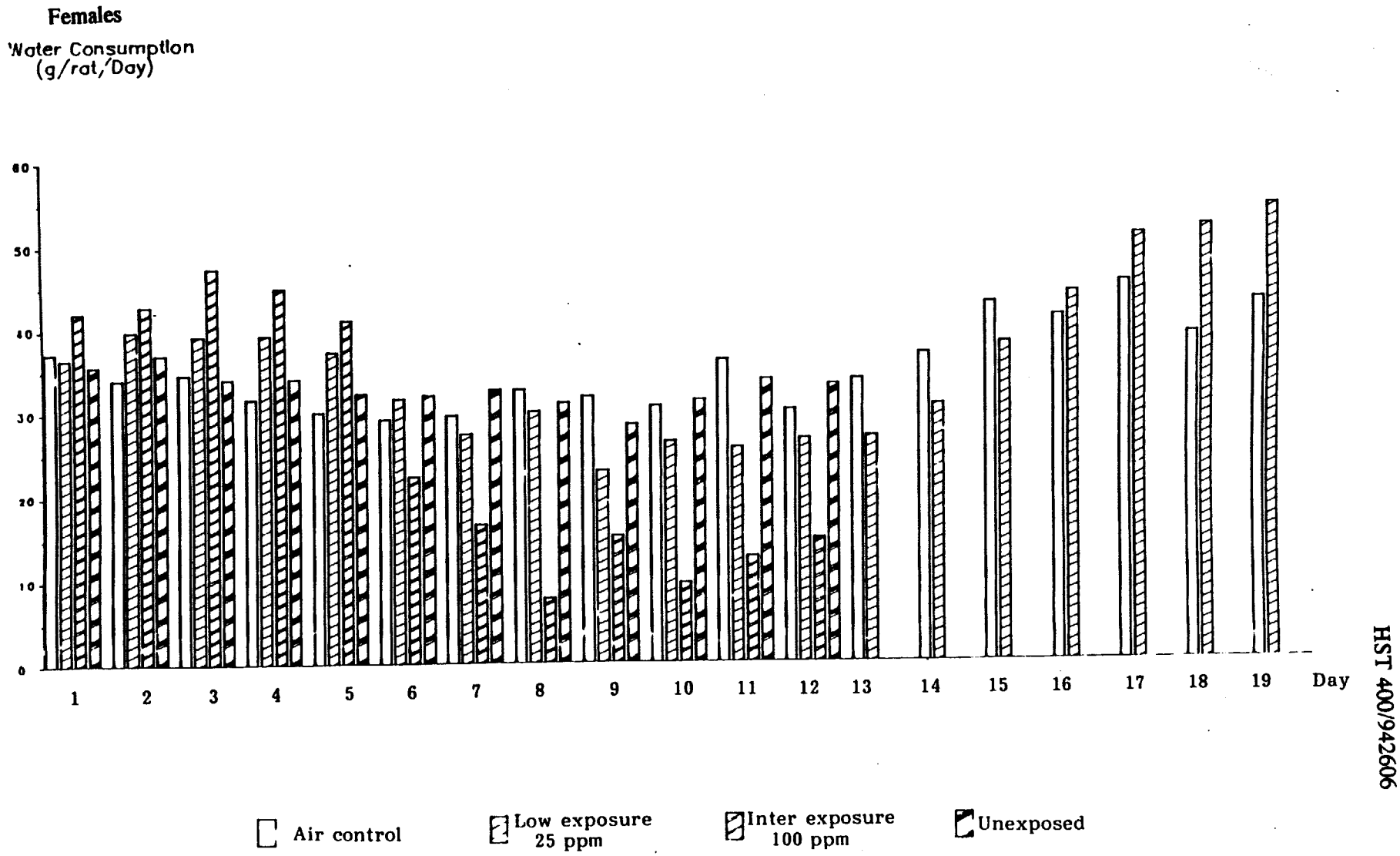

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TABLE 1

Concentration of aerosol in exposure chambers

	Number of particles greater than 0.5 μm diameter in 1 minute			
	Sample origin			
Count number	Holding room	Control[1] chamber	Low dose[1] (25 ppm)	Inter dose[2] (100 ppm)
1	1965	94	123	81
2	2123	131	76	103
3		32	94	47
4		129	88	102

[1] Sampled during exposure
[2] Exposures terminated. Chamber sampled without rats

The holding room count exceeds that of the chambers because the chambers are supplied with filtered air from a compressor

TABLE 2

Exposure mean concentration of acetic anhydride (ppm)

Exposure	Group		
	2 (Low dose)	3 (Inter dose)	4 (High dose)
1[2]	22	104	407
2	22	100	
3	24	98	
4	24	102	
5	24	105	
6[2]	26	108	
7[2]	26	110	
8	25	108	
9	24		
10	24		
11	24		
12[1]	22		
Mean ♂	24	103	407
♀	24	104	-

[1] Male rats only exposed
[2] Female rats only exposed

TABLE 3

Nominal concentration of acetic anhydride (ppm)

Exposure	Group 2 Low dose		Group 3 Inter dose		Group 4 High dose	
	Nominal	A/N × 100	Nominal	A/N × 100	Nominal	A/N × 100
1[1]	24	91.7	77	135.1	359	113.4
2	24	91.7	77	131.2		
3	24	100.0	86	114.0		
4	22	109.1	86	118.6		
5	22	109.1	86	122.1		
6[2]	24	108.3	86	125.6		
7[2]	24	108.3	86	127.9		
8	24	104.2	86	125.6		
9	24	100.0				
10	22	109.1				
11	24	100.0				
12[1]	24	91.7				
Mean ♂	23.4	100.7	83	124.4	359	113.4
♀	23.4	104.0	85	123.6	-	-

$$\text{A/N} \quad \frac{\text{Exposure mean analysed concentration}}{\text{Nominal concentration}} \times 100$$

[1] Male rats only exposed

[2] Female rats only exposed

NB High dose exposures terminated after 1 exposure and the Intermediate dose after 8 exposures due to bodyweight losses and severity of clinical signs

TABLE 4

Temperature and relative humidity during exposures (exposure mean values)

Exposure	Group							
	1 (Air control)		2		3		4	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
1[1]	21.2	27	22.8	41	21.4	21	22.5	26
2	23.2	39	23.5	38	22.5	19		
3	23.5	36	23.7	42	22.6	23		
4	22.7	33	23.5	41	22.5	20		
5	24.1	26	23.5	24	25.0	29		
6[2]	22.1	31	22.5	21	23.5	25		
7[2]	23.7	25	22.9	19	23.9	24		
8	23.4	45	23.4	21	24.3	26		
9	23.8	36	23.2	26				
10	24.1	32	23.1	23				
11	22.7	37	22.8	27				
12[1]	21.7	30	21.8	21				
Study mean	23.0	33	23.1	29	23.2	23	22.5	26

1 Male rats exposed only
2 Female rats exposed only

TABLE 5

Summary of performance

Group: Exposure (ppm):	1♂ Control	2♂ 25	3♂ 100	4♂ 400	1♀ Control	2♀ 25	3♀ 100	4♀ U
Category	Number of animals							
Allocated	5	5	5	5	5	5	5	5
Exposed	5	5	5	5	5	5	5	0
Dead	0	0	0	2	0	0	0	0
Killed	0	0	5	3	0	0	5	5
Completing study	5	5	0	0	5	5	0	0
Total no. exposures	10	10	6	1	10	10	7	0
Non-pregnant	-	-	-	-	0	0	1	1
Total resorption	-	-	-	-	0	0	2	0
With live young at Day 13	-	-	-	-	-	-	2	4
With live young at Day 20	-	-	-	-	5	5	-	-

U Unexposed

TABLE 6

Clinical signs during exposure

Group/ exposure ppm	Observation	Exposure number																			
		Male										Female									
		1	2	3	4	5	6	7	8	9	10	1 (6)	2 (7)	3 (8)	4 (9)	5 (10)	6 (11)	7 (12)	8 (13)	9 (14)	10 (15)
1 Control	No abnormality detected	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2 25	No abnormality detected	✓																			
	Eyes half-closed/closed		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Licking inside mouth							✓	✓	✓	✓					✓			✓	✓	✓
	Occasional twitching of head						✓	✓	✓	✓	✓							✓	✓	✓	✓
3 100	Eyes half-closed/closed	✓	✓	✓	✓	✓	✓					✓	✓	✓	✓	✓	✓	✓			
	Licking inside mouth		✓	✓	✓	✓	✓					✓	✓	✓	✓	✓	✓	✓			
	Lachrymation		✓	✓	✓	✓						✓	✓	✓	✓						
	Gasping			✓	✓	✓							✓	✓	✓	✓	✓				
	Exaggerated respiration					✓	✓								✓	✓	✓	✓			
	Occasional twitching of head						✓									✓	✓	✓			
4 400	Prone posture	✓																			
	Eyes half-closed/closed	✓																			
	Lachrymation	✓																			
	Exaggerated respiration	✓																			

Females allocated to the high dose group were not exposed on the basis of the adverse response seen in males after one exposure. The remaining high exposure males were sacrificed prior to exposure no. 2

Intermediate dose animals were sacrificed prior to exposure no. 7 for males and exposure no. 8 for females, due to poor condition (treatment-related)

( ) Day post coitum

TABLE 7

Bodyweights - group mean values (g)

Males

Day	Group and exposure (ppm)			
	1♂ Air control	2♂ Low dose 25	3♂ Inter dose 100	4♂ High dose 400
-7	236	239	241	239
-6	244	248	247	246
-5	255	258	259	257
-4	264	266	269	267
-3	269	273	275	273
-2	281	287	286	285
-1	289	294	296	295
0E	295	303	307	305
1	303	308	292	260
2	309	313	270	
3	315	315	250	
4	315	316	248	
5X	319	313	238	
6X	325	324	254	
7	331	334	263	
8	335	326	239	
9	338	319		
10	342	319		
11	348	310		
12X	352	313		
13X	354	325		
14X	361	335		

E First day of exposure
X Animals withdrawn from exposure

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TABLE 7

(Bodyweights - continued)

Females

Absolute values

Group/ exposure ppm	No. of animals	Bodyweight (g) at Day post coitum											
		1	2	3	6E	8	10	12	13	14	16	18	20
1 Air control	A=B=5	213.2	225.4	235.0	252.6	264.4	279.0	293.2		301.8	321.0	350.0	379.6
2 25	A=B=5	213.2	221.6	236.6	259.8	270.4	264.8	271.2		276.0	293.8	322.6	361.4
3 100	A=4	216.5	231.3	242.5	264.0	245.8	221.3	212.5	204.5				
	B=2	223.5	235.0	246.5	267.5	244.5	226.5	222.5	211.5				
4 Unexposed	A=B=4	216.8	226.5	241.8	261.3	277.8	287.8	303.5	311.3				

E First day of exposure

Change values (relative to Day 6 post coitum)

Group/ exposure ppm	No. of animals	Bodyweight change (g) at Day post coitum											
		1	2	3	6	8	10	12	13	14	16	18	20
1 Air control	A=B=5	-39.4	-27.2	-17.6	0.0	11.8	26.4	40.6	0	49.2	68.4	97.4	127.0
2 25	A=B=5	-46.6	-38.2	-23.2	0.0	10.6	5.0	11.4	0	16.2	34.0	62.8	101.6
3 100	A=4	-47.5	-32.8	-21.5	0.0	-18.3	-42.8	-51.5	-59.5				
	B=2	-44.0	-32.5	-21.0	0.0	-23.0	-41.0	-45.0	-56.0				
4 Unexposed	A=B=4	-44.5	-34.8	-19.5	0.0	16.5	26.5	42.3	50.0				

A All pregnant animals
B Dams with live young at termination (Day 20 Groups 1 and 2, Day 13 Groups 3 and 4)

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TABLE 8

Food consumption - group mean values (g/rat/day)

Males

Day	Group and exposure (ppm)			
	1♂ Air control	2♂ Low dose 25	3♂ Inter dose 100	4♂ High dose 400
-7	29	31	30	28
-6	30	32	32	32
-5	30	31	31	32
-4	29	32	31	30
-3	30	34	31	33
-2	30	34	32	33
-1	30	33	33	34
1E	32	31	19	1
2	29	29	8	
3	30	26	3	
4	28	25	7	
5	29	22	4	
6X	28	28	15	
7X	30	31	20	
8	28	22	8	
9	27	16		
10	28	18		
11	29	16		
12	30	19		
13X	29	27		
14X	29	30		

E First day of exposure
X Animals withdrawn from exposure

Females

Day (post coitum)	Group and exposure (ppm)			
	1♀ Air control	2♀ Low dose 25	3♀ Inter dose 100	4♀ Unexposed
1	23	20	23	20
2	21	22	22	23
3- 5	23	23	25	24
6- 7E	24	22	11	26
8- 9	25	18	5	25
10-11	26	18	5	28
12-13	25	17	7ø	16ø
14-15	28	20		
16-17	30	26		
18-19	28	28		

ø Animals sacrificed Day 13, value to Day 12 only
E Exposure commenced Day 6

TABLE 9

Water consumption - group mean values (g/rat/day)

Males

Day	Group and exposure (ppm)			
	1♂ Air control	2♂ Low dose 25	3♂ Inter dose 100	4♂ High dose 400
-7	31.0	33.4	33.8	32.2
-6	33.2	33.4	34.8	35.2
-5	32.2	34.2	32.4	32.8
-4	33.0	35.2	33.2	33.4
-3	32.8	36.2	34.0	35.4
-2	32.0	33.6	35.8	35.8
-1	32.0	34.2	36.6	35.4
1E	33.0	33.8	24.6	3.0
2	29.6	31.4	11.0	
3	32.2	29.4	5.8	
4	29.8	30.6	14.0	
5	30.8	25.0	9.0	
6X	32.0	34.2	29.4	
7X	33.2	34.8	32.2	
8	30.8	27.4	8.0	
9	30.4	20.8		
10	31.6	26.6		
11	32.6	20.2		
12	35.8	27.8		
13X	32.0	40.2		
14X	29.8	35.6		

E First day of exposure
X Animals withdrawn from exposure

Females

Day (post coitum)	Group and exposure (ppm)			
	1♀ Air control	2♀ Low dose 25	3♀ Inter dose 100	4♀ Unexposed
1	37.2	36.4	42.0	35.6
2	34.0	39.8	42.8	37.0
3	34.6	39.2	47.2	34.0
4	31.6	39.2	44.8	34.0
5	30.0	37.2	41.0	32.2
6E	29.2	31.6	22.4	32.0
7	29.6	27.4	16.6	32.6
8	32.6	30.0	7.8	31.0
9	31.8	23.0	15.2	28.4
10	30.6	26.4	9.6	31.2
11	36.0	25.6	12.6	33.6
12	30.0	26.6	14.8	33.0
13	33.6	26.8		
14	36.6	30.6		
15	42.6	37.8		
16	41.0	43.8		
17	45.0	50.6		
18	38.8	51.6		
19	42.8	54.0		

E First day of exposure

TABLE 10

Litter data - group mean values

Day 13 sacrifice

Category		Group and exposure (ppm)		
		3 100		4 Untreated
With live young		2	(4)	4
Corpora lutea/Dam		15.0	(14.5)	15.3
Implantations/Dam		14.5	(14.3)	14.5
Pre-implantation loss %		3.0	(1.5)	4.4
Early embryonic death		1.0	(7.5)	2.0
Post implantation loss %		7.7	(53.9)	12.0
Live young/Dam		13.5	(6.8)	12.5
Litter weight		NR		NR
Mean foetal weight		NR		NR
EMBRYONIC DEATHS				
Total (early)	0	1	(-)	2
	1	-	(-)	1
	2	1	(-)	-
	3	-	(-)	-
	= >4	-	(2)	1

NR Not recordable
( ) Data from all pregnant animals, inclusive of 2 showing total resorption

TABLE 10

(Litter data - continued)

Day 20 sacrifice

Category		Group and exposure (ppm)	
		1 Control	2 25
With live young		5	5
Corpora lutea/Dam		14.6	14.6
Implantations/Dam		14.0	14.4
Pre-implantation loss %		3.8	1.3
Early embryonic death		1.4	0.6
Late embryonic death		0.0	0.0
Total embryonic death		1.4	0.6
Post-implantation loss %		9.9	4.2
Live young/Dam		12.6	13.8
Litter weight		50.31	52.09
Mean foetal weight		3.99	3.78
EMBRYONIC DEATHS			
Total	0	-	2
	1	3	3
	2	2	-
	3	-	-
	= >4	-	-
Early	0	-	2
	1	3	3
	2	2	-
	3	-	-
	= >4	-	-
Late	0	5	5
	1	-	-
	2	-	-
	3	-	-
	= >4	-	-

TABLE 11

Macroscopic pathology incidence summary

Males

Males on study	Group 1 Air Control 5		Group 2 25 ppm 5		Group 3 100 ppm 5		Group 4 400 ppm 5	
Animals completed	Decedent 0	Terminal 5	Decedent 0	Terminal 5	Decedent 5	Terminal 0	Decedent 5	Terminal 0
Fur								
Stained	0	0	0	0	0	0	4	0
Skin								
Alopecia	0	0	0	0	1	0	0	0
Subcutis								
Congested	0	0	0	0	0	0	1	0
Lymph Nodes - Cervical								
Enlarged	0	4	0	4	5	0	1	0
Lymph Nodes - Deep Cervical								
Enlarged	0	0	0	4	5	0	0	0
Lungs								
Not collapsed	0	0	0	0	4	0	0	0
Congested	0	0	0	2	0	0	1	0
Lymph Nodes - Tracheobronchial								
Enlarged	0	0	0	4	2	0	0	0
Adipose Tissue								
Minimal	0	0	0	0	4	0	0	0
Liver								
Pale subcapsular area/s - median cleft	0	0	0	0	1	0	0	0
Pale subcapsular area/s	0	0	0	0	1	0	0	0

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TABLE 11

(Macroscopic pathology - continued)

Males (continued)

Males on study	Group 1 Air Control 5		Group 2 25 ppm 5		Group 3 100 ppm 5		Group 4 400 ppm 5	
Animals completed	Decedent 0	Terminal 5	Decedent 0	Terminal 5	Decedent 5	Terminal 0	Decedent 5	Terminal 0
Liver (continued)								
Swollen	0	0	0	0	1	0	0	0
Small	0	0	0	0	1	0	0	0
Pale	0	0	0	0	1	0	0	0
Haemorrhagic	0	0	0	0	1	0	0	0
Gastrointestinal Tract								
Gaseous distension	0	0	0	0	1	0	0	0
Stomach								
Contents minimal	0	0	0	0	3	0	0	0
Gaseous distension	0	0	0	0	3	0	3	0
Empty	0	0	0	0	0	0	2	0
Stomach Antrum Mucosa								
White nodule, near to limiting ridge	0	0	0	1	0	0	0	0
Small Intestine								
Gaseous distension	0	0	0	0	4	0	1	0
Caecum								
Gaseous distension	0	0	0	0	4	0	3	0

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TABLE 11

(Macroscopic pathology - continued)

Females

Animals completed	Group 1		Group 2		Group 3		Group 4	
	Air control		25 ppm		100 ppm		Unexposed	
	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
	0	5	0	5	5	0	5	0
Fur								
brown stained		-		-	1		-	
yellow stained		-		-	1		-	
Skin								
hair loss		1		1	-		1	
Urogenital region								
brown stained		-		-	1		-	
Lungs								
haemorrhagic areas		-		-	2		1	
a few small red fluid-filled cysts		-		-	3		-	
several pale cysts		-		-	-		1	
Abdomen								
distended		-		-	1		-	
Stomach								
gaseous distension		-		-	1		-	
Gastrointestinal tract								
gaseous distension		-		-	1		-	
Ileum								
gaseous distension		-		-	1		-	
Caecum								
gaseous distension		-		-	1		-	
Colon								
gaseous distension		-		-	1		-	

TABLE 12

Organ weights (at scheduled termination) - group mean values

Group and exposure (ppm)	Body wt g	Lungs g	Liver g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Air control	358	1.28	15.1	2.48	48.1	3.88
2♂ 25	332	1.49	13.8	2.19	48.3	3.89

TABLE 13

Microscopic pathology incidence summary

Removal Reason: Intercurrent	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	0	0	5	5
Nasal Passages					
Examined		0	0	5	5
No abnormalities detected		0	0	0	2
Rhinitis (Total)		0	0	4	3
Trace		0	0	1	0
Minimal		0	0	2	0
Moderate		0	0	1	2
Marked		0	0	0	1
Atrophy/degeneration of olfactory epithelium (Total)		0	0	5	0
Minimal		0	0	1	0
Moderate		0	0	1	0
Marked		0	0	3	0
Ulceration of olfactory epithelium (Total)		0	0	0	3
Minimal		0	0	0	1
Moderate		0	0	0	2
Replacement of respiratory epithelium by undifferentiated type (Total)		0	0	2	3
Minimal		0	0	2	2
Moderate		0	0	0	1
Ulceration of respiratory epithelium (Total)		0	0	3	0
Minimal		0	0	2	0
Moderate		0	0	1	0
Squamous metaplasia in nasopharynx (Total)		0	0	3	0
Moderate		0	0	3	0
Epithelial ulceration in nasopharynx (Total)		0	0	3	0
Minimal		0	0	3	0
Hyperplasia of transitional epithelium (Total)		0	0	2	2
Minimal		0	0	1	1
Moderate		0	0	1	1
Larynx					
Examined		0	0	5	5

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TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Intercurrent	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	0	0	5	5
Larynx (Continued)					
Epithelial ulceration (Total)		0	0	5	5
Minimal		0	0	1	0
Moderate		0	0	1	0
Marked		0	0	3	0
Severe		0	0	0	5
Inflammatory exudate in lumen (Total)		0	0	5	5
Moderate		0	0	4	1
Marked		0	0	1	2
Severe		0	0	0	2
Secondary bacterial colonisation		0	0	0	3
Epithelial hyperplasia and squamous metaplasia (Total)		0	0	1	0
Moderate		0	0	1	0
Trachea					
Examined		0	0	5	5
Tracheitis (Total)		0	0	0	1
Minimal		0	0	0	1
Epithelial hyperplasia (Total)		0	0	5	2
Minimal		0	0	0	1
Moderate		0	0	4	1
Marked		0	0	1	0
Epithelial ulceration (Total)		0	0	0	4
Minimal		0	0	0	1
Severe		0	0	0	3
Inflammatory exudate in lumen (Total)		0	0	0	3
Marked		0	0	0	2
Severe		0	0	0	1
Tracheal Bifurcation					
Examined		0	0	5	5
Epithelial ulceration (Total)		0	0	0	5
Minimal		0	0	0	1
Moderate		0	0	0	1
Marked		0	0	0	1
Severe		0	0	0	2

TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Intercurrent	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	0	0	5	5
Tracheal Bifurcation (Continued)					
Epithelial hyperplasia (Total)		0	0	5	2
Minimal		0	0	2	0
Moderate		0	0	2	1
Marked		0	0	1	1
Inflammatory exudate in lumen (Total)		0	0	0	3
Minimal		0	0	0	1
Moderate		0	0	0	1
Severe		0	0	0	1
Lungs					
Examined		0	0	5	5
Bronchiolar epithelial hypertrophy with goblet cells (Total)		0	0	4	0
Minimal		0	0	3	0
Moderate		0	0	1	0
Bronchiolar epithelial regenerative hyperplasia (Total)		0	0	5	4
Minimal		0	0	2	1
Moderate		0	0	3	3
Bronchiolar epithelial ulceration (Total)		0	0	0	4
Trace		0	0	0	1
Moderate		0	0	0	2
Marked		0	0	0	1
Inflammatory exudate in bronchiolar lumina (Total)		0	0	0	1
Trace		0	0	0	1
Alveolitis in alveolar ducts (Total)		0	0	0	2
Trace		0	0	0	1
Minimal		0	0	0	1
Macrophages in alveolar ducts (Total)		0	0	2	0
Trace		0	0	1	0
Minimal		0	0	1	0
Macrophages and mucus in alveoli (Total)		0	0	2	0
Minimal		0	0	2	0

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TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Intercurrent	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	0	0	5	5
Lungs (Continued)					
Vascular congestion (Total)		0	0	0	2
Moderate		0	0	0	1
Marked		0	0	0	1
Lymph Nodes - Cervical					
Examined		0	0	5	1
Lymphoid proliferation (Total)		0	0	5	1
Minimal		0	0	2	0
Moderate		0	0	2	1
Marked		0	0	1	0
Lymph Nodes - Deep Cervical					
Examined		0	0	5	0
Lymphoid proliferation (Total)		0	0	5	0
Minimal		0	0	3	0
Moderate		0	0	2	0
Erythrophagocytosis (Total)		0	0	1	0
Moderate		0	0	1	0
Lymph Nodes - Tracheobronchial					
Examined		0	0	2	0
No abnormalities detected		0	0	1	0
Lymphoid proliferation (Total)		0	0	1	0
Moderate		0	0	1	0
Liver					
Examined		0	0	2	0
Fibrosis mineralisation bile duct proliferation siderocytes (Total)		0	0	1	0
Moderate		0	0	1	0
Hepatocyte necrosis (Total)		0	0	1	0
Moderate		0	0	1	0

TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Intercurrent	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	0	0	5	5
Liver (Continued)					
Lobar infarction		0	0	1	0
Subcutis					
Examined		0	0	0	1
Congestion		0	0	0	1

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TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Terminal	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	5	5	0	0
Nasal Passages					
Examined		5	5	0	0
No abnormalities detected		5	0	0	0
Rhinitis (Total)		0	2	0	0
Minimal		0	2	0	0
Atrophy/degeneration of olfactory epithelium (Total)		0	3	0	0
Trace		0	1	0	0
Minimal		0	2	0	0
Hyperplasia of respiratory epithelium (Total)		0	5	0	0
Minimal		0	5	0	0
Epithelial hyperplasia in nasopharynx (Total)		0	5	0	0
Trace		0	1	0	0
Minimal		0	4	0	0
Hyperplasia of transitional epithelium (Total)		0	3	0	0
Minimal		0	3	0	0
Larynx					
Examined		5	5	0	0
No abnormalities detected		5	0	0	0
Epithelial hyperplasia and squamous metaplasia (Total)		0	5	0	0
Minimal		0	1	0	0
Moderate		0	4	0	0
Trachea					
Examined		5	5	0	0
No abnormalities detected		5	0	0	0
Epithelial hyperplasia (Total)		0	5	0	0
Trace		0	3	0	0
Minimal		0	2	0	0
Tracheal Bifurcation					
Examined		5	5	0	0
No abnormalities detected		5	2	0	0

TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Terminal	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	5	5	0	0
Tracheal Bifurcation (Continued)					
Epithelial hyperplasia (Total)		0	3	0	0
Trace		0	3	0	0
Lungs					
Examined		5	5	0	0
No abnormalities detected		4	1	0	0
Bronchiolar epithelial hypertrophy with goblet cells (Total)		0	4	0	0
Trace		0	1	0	0
Minimal		0	3	0	0
Macrophages in alveolar ducts (Total)		0	2	0	0
Minimal		0	2	0	0
Macrophages and mucus in alveoli (Total)		0	1	0	0
Minimal		0	1	0	0
Pneumonitis (Total)		0	2	0	0
Minimal		0	2	0	0
Subpleural aggregation of alveolar macrophages (Total)		1	0	0	0
Minimal		1	0	0	0
Extravasation of neutrophils (Total)		0	1	0	0
Minimal		0	1	0	0
Lymph Nodes - Cervical					
Examined		4	4	0	0
Lymphoid proliferation (Total)		4	4	0	0
Minimal		2	1	0	0
Moderate		0	2	0	0
Marked		2	1	0	0
Lymph Nodes - Deep Cervical					
Examined		0	4	0	0
Lymphoid proliferation (Total)		0	4	0	0
Minimal		0	1	0	0
Moderate		0	3	0	0
Lymph Nodes - Tracheobronchial					
Examined		0	4	0	0

TABLE 13

(Microscopic pathology incidence summary - continued)

Removal Reason: Terminal	Sex: Males	Group 1 0 ppm	Group 2 25 ppm	Group 3 100 ppm	Group 4 400 ppm
	Males on study	5	5	5	5
	Animals completed	5	5	0	0
Lymph Nodes - Tracheobronchial (Continued)					
Lymphoid proliferation (Total)		0	4	0	0
Minimal		0	3	0	0
Moderate		0	1	0	0
Stomach					
Examined		0	1	0	0
Focus of ectopic non-glandular epithelium within the glandular mucosa		0	1	0	0

APPENDICES

APPENDIX 1

Composition and quality assurance aspects of diet and drinking water

Special Diets Services Rat and Mouse Maintenance Diet

SDS Rat and Mouse No. 1 SQC modified maintenance diet is a fixed formula diet. Each batch of diet is analysed for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess, may have an undesirable effect on the test system.

Prior to release of diet for use HRC Quality Assurance Department checks each certificate of analysis for conformity with the specification detailed below. Occasional slight deviations to this specification may be permitted.

Nutrients	Target level	Tolerance %	Acceptable range			
Moisture	10.0	+25			12.5	% max
Crude fat	3.0	±30	2.0	-	4.0	%
Crude protein	14.5	±15	12.0	-	16.5	%
Crude fibre	4.0	±50	2.0	-	6.0	%
Ash	5.0	±25	3.7	-	6.2	%
Calcium	0.9	±30	0.6	-	1.2	%
Phosphorus	0.6	±20	0.5	-	0.75	%
Sodium	0.25	±40	0.15	-	0.35	%
Chloride	0.5	±40	0.3	-	0.7	%
Potassium	0.9	±50	0.45	-	1.35	%
Magnesium	0.2	±50	0.1	-	0.3	%
Iron	200	±50	100	-	300	mg/kg
Copper	15	±60	6	-	24	mg/kg
Manganese	60	+60-40	36	-	100	mg/kg
Zinc	60	±50	30	-	90	mg/kg
Vitamin A	6	-50			3	iu/g min.
Vitamin E	70	-50			35	mg/kg min.

Contaminants	Maximum concentration	
Fluoride	20	mg/kg
Nitrate (as $NaNO_3$)	30	mg/kg
Nitrite (as $NaNO_2$)	10	mg/kg
Lead	2.0	mg/kg
Arsenic	1.0	mg/kg
Cadmium	0.7	mg/kg
Mercury	0.1	mg/kg
Selenium	0.6	mg/kg
Total Aflatoxins	5.0	mcg/kg
Total P.C.B.	50	mcg/kg
Total D.D.T.	250	mcg/kg
Dieldrin	50	mcg/kg
Lindane	300	mcg/kg
Heptachlor	20	mcg/kg
Malathion	5000	mcg/kg

APPENDIX 1

(Aspects of diet and drinking water - continued)

Microbiological contents	Maximum concentration	
Total viable organisms	25000	per g diet
Mesophilic spores	25000	per g diet
Salmonellae species	0	per g diet
Presumptive E. coli	0	per g diet
E. coli type 1	0	per g diet
Fungal units	300	per g diet
Antibiotic activity	0	

APPENDIX 1

(Aspects of diet and drinking water - continued)

Biosure Laboratory Animal Diet No. 1

Biosure LAD is a fixed formula diet suitable for normal health, growth and reproduction of laboratory rats and mice. Each batch of diet is analysed for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess, may have an undesirable effect on the test system.

Prior to release of diet for use HRC Quality Assurance Department checks each certificate of analysis for conformity with the specification detailed below. Occasional slight deviations to this specification may be permitted.

Nutrients	**Target level**	**Tolerance %**	**Acceptable range**
Moisture	9.5	+10	10.5 % max
Crude fat	3.7	±15	3.1 - 4.3 %
Crude protein	21.5	±10	19.4 - 23.7 %
Crude fibre	2.0	±40	1.2 - 2.8 %
Ash	5.5	±15	4.7 - 6.3 %
Calcium	1.0	±20	0.8 - 1.2 %
Phosphorus	0.9	±20	0.7 - 1.1 %
Sodium	0.3	+100-50	0.15 - 0.60 %
Chloride	0.5	+100-50	0.25 - 1.0 %
Potassium	0.8	+100-50	0.4 - 1.6 %
Magnesium	0.15	±50	0.08 - 0.23 %
Iron	220	±50	110.0 - 330 mg/kg
Copper	15	±50	8.0 - 23 mg/kg
Manganese	70	±50	35.0 - 105 mg/kg
Zinc	60	±50	30.0 - 90 mg/kg
Vitamin A	12	+50-20	9.5 - 18 iu/g
Vitamin E	35	+150-20	28 - 88 mg/kg

Contaminants	**Maximum concentration**
Fluoride	40 mg/kg
Nitrate (as $NaNO_3$)	200 mg/kg
Nitrite (as $NaNO_2$)	10 mg/kg
Lead	2.5 mg/kg
Arsenic	1.5 mg/kg
Cadmium	0.5 mg/kg
Mercury	0.1 mg/kg
Selenium	0.6 mg/kg
Total Aflatoxins	5 mcg/kg
Total P.C.B.	50 mcg/kg
Total D.D.T.	150 mcg/kg
Dieldrin	50 mcg/kg
Lindane	150 mcg/kg
Heptachlor	50 mcg/kg
Malathion	5000 mcg/kg

APPENDIX 1

(Aspects of diet and drinking water - continued)

Microbiological contents	Maximum concentration
	LAD 1 (powder)
Total viable organisms	100,000 per g diet
Mesophilic spores	30,000 per g diet
Salmonellae species	0 per g diet
Presumptive E. coli	0 per g diet
E. coli type 1	0 per g diet
Fungal units	1,200 per g diet
Antibiotic activity	0

APPENDIX 1

(Aspects of diet and drinking water - continued)

Quality assurance aspects of drinking water

The water supplied to HRC, by Anglian Water, is potable water for human consumption. Anglian Water takes its guidelines on water quality from the EEC directive relating to water for human consumption, viz: Council Directive 80/778/EEC.

Results of routine physical and chemical examination of drinking water at source as conducted, usually weekly by the supplier, are made available to HRC as quarterly summaries.

These results include levels of:

Nitrites	Potassium	Chloride
Nitrates	Silicon	Iron
Calcium	Arsenic	Selenium
Magnesium	Barium	Silver
Sodium	Antimony	Phosphorus

as well as concentrations of pesticides, related products, polycyclic aromatic hydrocarbons, haloforms, chlorophenols and polychlorinated biphenyls.

APPENDIX 2

Chemical analysis of acetic anhydride

** TOTAL PAGE.002

CELANESE CANADA INC.
EDMONTON, ALBERTA

PRODUCT QUALITY REPORT
Acetic Anhydride

HCCG Order No...
Cust. Order No..
Container No.... Sample bottle
Preload #'s..... 1061,1064,1070
Date Oct 8, 1993

		MIN.	MAX.	RESULT(1)
Acetic Anhydride,	wt%	99.0		99.05
Color,	APHA		10.	5.
Permanganate time,	min	5.		PASS
Iron,	ppm		1.	0.2
Aluminum,	ppm		1.	< 1
Non-volatiles,	g/100mL		0.003	0.0002
Acetic acid,	wt%			0.81
Acetic-Propionic-anhydride,	wt%		0.35	0.14

QA Contact T. Brundage
Tel: (403) 471-0161
Fax: (403) 471-0134

(1) Unless otherwise indicated as shipment specific (*), the results in this report are representative of the shipping inventory. If you have any questions regarding this shipment, please contact your nearest Celanese District Sales Office.

NOV 12'93 18:23 FR CELANESE PC LAB 403 471 0134 TO 9-011444808906893 P.02

APPENDIX 3

Method of analysis for acetic anhydride

Analysis conditions and instrumentation

Instruments:	Pye series 304 chromatograph fitted with a 1000 μl gas loop Pye Unicam PU4700 autosampler Spectra-Physics SP4200 integrator	
Detector:	FID range: 100 atten: 1	
Column:	DB-1701, 15 m × 0.53 mm id, 1 μm film	
Gases (ml/min):	He (carrier) H_2 Air	= 15 = 30 = 280
Temperatures (°C):	Column Injector Detector Gas loop & valve	= 40 = 100 = 200 = Ambient
Apparatus:	Tedlar® gas sample bags (SKC Inc.) All polypropylene gas tight syringes (Sigma Chemical Co.)	

Materials

Acetic anhydride:	Supplied by Sponsor
Acetic acid (A.R. grade)	Fisons PLC
Acetone (glass distilled)	Rathbourn Chemicals

Standardisation

Wet type gas meter (Models DM3B and DM3D, Alexander Wright and Co. (Westminster) Ltd).

Acetic anhydride

Prior to the commencement of exposures 2 vapour standards of acetic anhydride were prepared by injecting 20 μl and 5 μl of liquid acetic anhydride into two Tedlar® gas sample bags each containing 10 l and 25 l of air respectively. The air was metered into each bag through a wet-type gas meter. The respective concentrations of the two gas standards were 519 ppm and 52 ppm.

One ml of each vapour standard was injected onto the G.C. column. Dilution of each standard were prepared by drawing either 25 ml or 10 ml of each standard into a 50 ml gas tight syringe and diluting to 50 ml with air. In this manner a 6 point calibration line was constructed comprising standard concentration of 519, 260, 104, 52, 26 and 10 ppm acetic anhydride in air.

APPENDIX 3

(Method of analysis - continued)

A log-log linear regression analysis of the resultant peak areas for each standard was used to construc the calibration line.

Throughout the study at least two of the standard concentrations were injected on the G.C. at severa points during each exposure to act as check standards confirming the calibration.

Acetic acid

Approximately 25 mg of acetic acid was accurately weighed into a 50 ml volumetric flask and dilute to volume with acetone, to give a stock standard of concentration 0.5 mg/ml. Aliquots of the stoc were diluted with acetone to give 6 calibration standards in the range approximately 8 to 500 μg/ml

A 2 μl aliquot of each standard was injected onto the G.C. and a calibration line was calculated usin linear regression analysis of the peak areas resultant from each standard.

The standard amounts injected onto the G.C. were converted to equivalent ppm in air concentration using the following expression:

$$\text{ppm} = \frac{2 \times \text{standard concentration } (\mu\text{g/ml}) \times \text{Vm}}{1000 \times \text{M.W.}}$$

Where Vm = molar volume of an ideal gas (24450 at 25°C and 760 mmHg)

M.W. = molecular weight of acetic acid (60.05)

The equivalent concentrations were in the range of approximately 7 to 417 ppm in air.

In addition a single gas standard was prepared by injecting 5 μl of acetic acid into a gas sample ba containing 5 l of air to give a standard concentration of 427 ppm. The peak area for the gas standar was in good agreement with the area predicted from the calibration line.

Sampling

Using a gas tight syringe the following procedure was performed for each sample. The needle wa pushed through a septum located on the side of the exposure chamber and a sample of chambe atmosphere was withdrawn.

The syringe was attached to the gas sampling valve and injected into the sample loop. The sampl was allowed to attain equilibrium before injection to the head of the column.

APPENDIX 3

(Method of analysis - continued)

The concentration of acetic anhydride in each sample was calculated using the following expression:

$$\log Cx = \log \frac{Ax - I}{As}$$

Where Cx = concentration of acetic anyhydride
Ax = peak area due to acetic anhydride
I = intercept of calibration line
As = response factor (slope of calibration line)

No acetic acid was detected.

APPENDIX 4

Chamber concentration of acetic anhydride (ppm)

Exposure number	Group		
	2 (Low dose)	3 (Inter dose)	4 (High dose)
1 Males only	20	102	410
	20	102	397
	21	103	391
	23	104	420
	23	105	411
	23	107	410
Mean	22	104	407
2	22	96	
	19	101	
	20	98	
	23	103	
	22	101	
	23	104	
Mean	22	100	
3	23	99	
	21	91	
	26	96	
	22	100	
	25	102	
	25	(56)[1]	
	25		
Mean	24	98	
4	21	99	
	23	99	
	25	101	
	24	101	
	27	103	
	25	106	
Mean	24	102	

[1] Injection error - value excluded from mean

APPENDIX 4

(Chamber concentration of acetic anhydride (ppm) - continued)

Exposure number	Group		
	2 (Low dose)	3 (Inter dose)	4 (High dose)
5	21	103	
	21	101	
	26	107	
	23		
	26	102	
	25	108	
	24	108	
Mean	24	105	
6 Females only	26	108	
	27	110	
	26	108	
	27	105	
	24	108	
	24	107	
Mean	26	108	
7 Females only	25	106	
	24	108	
	26	110	
	24	111	
	30	111	
	27	112	
Mean	26	110	
8	25	109	
	23	107	
	25	106	
	24	108	
	27	112	
	25	104	
Mean	25	108	

APPENDIX 4

(Chamber concentration of acetic anhydride (ppm) - continued)

Exposure number	Group		
	2 (Low dose)	3 (Inter dose)	4 (High dose)
9	24 24 24 24 23 25		
Mean	24		
10	28 32 21 22 21 23 23 24		
Mean	24		
11	20 [2] 27 28 25 23 23		
Mean	24		
12 Males only	21 24 24 22 21 23 22 21 23		
Mean	22		
Mean: Females	24	104	
Mean: Males	24	103	407

[2] Sample lost through GC fault

APPENDIX 5

Bodyweight - individual values (g)

Group 1♂: Air control

Cage number	Animal number	Day										
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3
1	1	237	246	258	265	267	281	288	293	302	310	316
	2	235	235	250	261	264	278	286	295	294	303	314
	3	228	249	262	272	276	291	300	306	318	325	329
	4	241	237	248	254	261	272	279	284	294	296	298
	5	240	254	259	269	275	283	293	298	307	310	317

Cage number	Animal number	Day										
		4	5	6	7	8	9	10	11	12	13	14
1	1	312	312	317	322	323	326	332	339	343	347	355
	2	312	317	324	330	337	344	348	352	361	359	368
	3	337	341	351	357	362	368	370	376	381	381	390
	4	296	301	306	310	312	312	313	320	319	325	326
	5	317	324	327	335	340	342	347	353	355	358	367

APPENDIX 5

(Bodyweight - continued)

Group 2♂: 25 ppm

Cage number	Animal number	Day										
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3
2	6	234	243	253	263	273	284	292	300	307	314	319
	7	233	243	253	259	267	281	288	295	300	306	311
	8	240	251	256	264	270	285	292	300	302	311	311
	9	238	247	258	267	275	286	297	305	306	312	309
	10	252	257	268	275	281	298	302	313	326	324	327

Cage number	Animal number	Day										
		4	5	6	7	8	9	10	11	12	13	14
2	6	322	320	336	343	332	339	341	338	344	347	360
	7	314	313	323	333	333	325	325	324	327	340	346
	8	308	307	319	327	322	311	307	285	293	313	317
	9	308	305	313	326	309	296	290	282	278	298	313
	10	327	320	327	339	335	326	331	323	324	329	341

APPENDIX 5

(Bodyweight - continued)

Group 3♂: 100 ppm

Cage number	Animal number	Day										
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3
3	11	248	253	267	278	281	290	304	317	305	293	264
	12	239	251	263	270	279	291	299	308	296	258	239
	13	236	241	254	264	272	285	295	305	291	282	262
	14	252	254	267	278	282	294	304	315	292	259	242
	15	229	234	244	254	259	269	280	288	277	260	243

Cage number	Animal number	Day				
		4	5	6	7	8
3	11	277	252	282	283	252
	12	241	222	217	251	221
	13	255	253	267	256	246
	14	231	222	236	258	230
	15	237	240	267	265	244

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APPENDIX 5

(Bodyweight - continued)

Group 4♂: 400 ppm

Cage number	Animal number	Day								
		-7	-6	-5	-4	-3	-2	-1	0	1
4	16	234	242	254	263	271	283	297	307	262
	17	246	253	267	275	283	289	301	308	257
	18	241	244	257	267	272	284	294	302	259ø
	19	231	238	246	257	260	274	284	295	248ø
	20	244	253	262	272	278	294	301	312	261

ø Bodyweight at death

APPENDIX 5

(Bodyweight - continued)

Group 1♀: Air control

Animal number	Bodyweight (g) at Day post coitum										
	1	2	3	6	8	10	12	14	16	18	20
	With live young at Day 20										
21	207	219	223	238	249	264	279	290	302	325	350
22	202	206	217	233	247	263	273	280	309	340	371
23	223	239	250	274	284	299	309	322	335	365	396
24	207	217	229	244	255	268	284	286	310	340	374
25	227	246	256	274	287	301	321	331	349	380	407

Group 2♀: 25 ppm

Animal number	Bodyweight (g) at Day post coitum										
	1	2	3	6	8	10	12	14	16	18	20
	With live young at Day 20										
26	213	221	229	257	259	259	261	276	292	323	354
27	218	232	248	271	279	285	289	292	315	343	375
28	200	208	230	257	279	237	247	249	271	304	342
29	207	210	225	243	253	263	272	281	294	333	371
30	228	237	251	271	282	280	287	282	297	310	365

HST 400/942606

APPENDIX 5

(Bodyweights - continued)

Group 3♀: 100 ppm

Animal number	Bodyweight (g) at Day post coitum							
	1	2	3	6	8	10	12	13
	With live young at Day 13							
32	218	231	244	266	241	225	235	227
33	229	239	249	269	248	228	210	196
	Total resorption							
34	204	223	233	259	248	203	190	192
35	215	232	244	262	246	229	215	203
	Non-pregnant							
31	202	208	218	231	223	205	185	198

Group 4♀: Unexposed

Animal number	Bodyweight (g) at Day post coitum							
	1	2	3	6	8	10	12	13
	With live young at Day 13							
36	205	216	232	256	268	271	282	289
38	216	221	240	256	273	283	301	305
39	226	240	252	270	287	297	315	323
40	220	229	243	263	283	300	316	328
	Non-pregnant							
37	199	207	221	242	253	267	281	279

HST 400/942606

APPENDIX 6

Litter data - individual values

Group 1: Air control

Animal number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Live young	Litter wt (g)	Mean foetal wt (g)
				Early	Late	Total				
	With live young at Day 20									
21	14	13	7.1	1	0	1	7.7	12	45.73	3.81
22	15	15	0.0	1	0	1	6.7	14	60.06	4.29
23	13	13	0.0	1	0	1	7.7	12	47.47	3.96
24	17	15	11.8	2	0	2	13.3	13	50.80	3.91
25	14	14	0.0	2	0	2	14.3	12	47.48	3.96

Group 2: 25 ppm

Animal number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Live young	Litter wt (g)	Mean foetal wt (g)
				Early	Late	Total				
	With live young at Day 20									
26	15	15	0.0	1	0	1	6.7	14	53.86	3.85
27	14	14	0.0	0	0	0	0.0	14	49.44	3.53
28	13	13	0.0	1	0	1	7.7	12	47.19	3.93
29	16	15	6.3	0	0	0	0.0	15	60.85	4.06
30	15	15	0.0	1	0	1	6.7	14	49.10	3.51

APPENDIX 6

(Litter data - continued)

Group 3: 100 ppm

Animal number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Live young
				Early	Late	Total		
	With live young at Day 13							
32	13	13	0.0	2	0	2	15.4	11
33	17	16	5.9	0	0	0	0.0	16
	Total resorption							
34	14	14	0.0	14	0	14	100.0	0
35	14	14	0.0	14	0	14	100.0	0
	Non-pregnant							
31								

Group 4: Unexposed

Animal number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Live young
				Early	Late	Total		
	With live young at Day 13							
36	13	13	0.0	0	0	0	0.0	13
38	17	15	11.8	1	0	1	6.7	14
39	18	17	5.6	7	0	7	41.2	10
40	13	13	0.0	0	0	0	0.0	13
	Non-pregnant							
37								

APPENDIX 7

Organ weights (at scheduled termination) - individual values

Group and exposure (ppm)	Animal no.	Body wt g	Lungs g	Liver g	Kidneys g	Adrenals mg	Testes+ Epididymides g
1♂ Air control	1	352	1.26	15.5	2.36	45.6	3.78
	2	364	1.27	14.6	2.65	44.3	4.02
	3	385	1.23	17.5	2.86	61.5	4.04
	4	326	1.22	11.0	2.24	40.1	3.56
	5	365	1.42	16.7	2.28	48.9	4.01
	Mean	358	1.28	15.1	2.48	48.1	3.88
	SD	21.7	0.082	2.51	0.265	8.14	0.209
2♂ 25	6	355	1.40	15.2	2.28	43.3	3.87
	7	341	1.44	13.8	2.22	43.9	4.04
	8	315	1.55	13.7	2.12	54.1	3.66
	9	310	1.60	12.8	2.10	60.2	3.83
	10	337	1.44	13.4	2.21	40.2	4.07
	Mean	332	1.49	13.8	2.19	48.3	3.89
	SD	19.0	0.082	0.89	0.073	8.44	0.168

SD Standard deviation

APPENDIX 8

Clinical signs, macroscopic and microscopic pathology - individual animals

Compound: Air

Dosage Level: Control

Rat No/Sex: 1♂ (Terminal)

CLINICAL FINDINGS

No abnormality detected.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea; Tracheal Bifurcation; Lungs

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage Level: Control

Rat No/Sex: 2♂ (Terminal)

CLINICAL FINDINGS

No abnormality detected.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Subpleural aggregation of alveolar macrophages: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea; Tracheal Bifurcation

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage Level: Control

Rat No/Sex: 3♂ (Terminal)

CLINICAL FINDINGS

No abnormality detected.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea; Tracheal Bifurcation; Lungs

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage Level: Control

Rat No/Sex: 4♂ (Terminal)

CLINICAL FINDINGS

No abnormality detected.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Lymphoid proliferation: (Marked)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea; Tracheal Bifurcation; Lungs

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage Level: Control

Rat No/Sex: 5♂ (Terminal)

CLINICAL FINDINGS

No abnormality detected.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Lymphoid proliferation: (Marked)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea; Tracheal Bifurcation; Lungs

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 25 ppm

Rat No/Sex: 6♂ (Terminal)

CLINICAL FINDINGS

Noisy respiration Days 3 to 14.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

Lymph Nodes - Deep Cervical
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Atrophy/degeneration of olfactory epithelium: (Minimal)
Hyperplasia of respiratory epithelium: (Minimal)
Epithelial hyperplasia in nasopharynx: (Minimal)

Larynx
Epithelial hyperplasia and squamous metaplasia: (Moderate)

Trachea
Epithelial hyperplasia: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Marked)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Moderate)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 6♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Tracheal Bifurcation; Lungs

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 25 ppm

Rat No/Sex: 7♂ (Terminal)

CLINICAL FINDINGS

Noisy respiration Days 3 to 14.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

Lymph Nodes - Deep Cervical
Enlarged

Lymph Nodes - Tracheobronchial
Enlarged: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Minimal)
Hyperplasia of respiratory epithelium: (Minimal)
Epithelial hyperplasia in nasopharynx: (Minimal)

Larynx
Epithelial hyperplasia and squamous metaplasia: (Moderate)

Trachea
Epithelial hyperplasia: (Trace)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Minimal)
Pneumonitis: (Minimal)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 7♂ - continued

MICROSCOPIC FINDINGS - continued

Lymph Nodes - Cervical
Lymphoid proliferation: (Moderate)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Moderate)

Lymph Nodes - Tracheobronchial
Lymphoid proliferation: (Minimal)

The following tissues were considered normal:

Tracheal Bifurcation

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 25 ppm

Rat No/Sex: 8♂ (Terminal)

CLINICAL FINDINGS

Noisy respiration Days 3 to 14.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

Lymph Nodes - Deep Cervical
Enlarged

Lungs
Congested: Right anterior lobe

Lymph Nodes - Tracheobronchial
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Atrophy/degeneration of olfactory epithelium: (Trace)
Hyperplasia of respiratory epithelium: (Minimal)
Epithelial hyperplasia in nasopharynx: (Minimal)
Hyperplasia of transitional epithelium: (Minimal)

Larynx
Epithelial hyperplasia and squamous metaplasia: (Minimal)

Trachea
Epithelial hyperplasia: (Minimal)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 8♂ - continued

MICROSCOPIC FINDINGS - continued

Tracheal Bifurcation
Epithelial hyperplasia: (Trace)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Minimal)
Macrophages and mucus in alveoli: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Moderate)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Moderate)

Lymph Nodes - Tracheobronchial
Lymphoid proliferation: (Moderate)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 25 ppm

Rat No/Sex: 9♂ (Terminal)

CLINICAL FINDINGS

Noisy respiration Days 3 to 14.

MACROSCOPIC FINDINGS

Lymph Nodes - Tracheobronchial
Enlarged

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Hyperplasia of respiratory epithelium: (Minimal)
Epithelial hyperplasia in nasopharynx: (Minimal)
Hyperplasia of transitional epithelium: (Minimal)

Larynx
Epithelial hyperplasia and squamous metaplasia: (Moderate)

Trachea
Epithelial hyperplasia: (Trace)

Tracheal Bifurcation
Epithelial hyperplasia: (Trace)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Minimal)
Macrophages in alveolar ducts: (Minimal)
Extravasation of neutrophils: (Minimal)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 9♂ - continued

MICROSCOPIC FINDINGS - continued

Lymph Nodes - Tracheobronchial
Lymphoid proliferation: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 25 ppm

Rat No/Sex: 10♂ (Terminal)

CLINICAL FINDINGS

Noisy respiration Days 3 to 14.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

Lymph Nodes - Deep Cervical
Enlarged

Lungs
Congested: (Patchy) Right anterior lobe

Lymph Nodes - Tracheobronchial
Enlarged: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Minimal)
Atrophy/degeneration of olfactory epithelium: (Minimal)
Hyperplasia of respiratory epithelium: (Minimal)
Epithelial hyperplasia in nasopharynx: (Trace)
Hyperplasia of transitional epithelium: (Minimal)

Larynx
Epithelial hyperplasia and squamous metaplasia: (Moderate)

Trachea
Epithelial hyperplasia: (Trace)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 10♂ - continued

MICROSCOPIC FINDINGS - continued

Tracheal Bifurcation
Epithelial hyperplasia: (Trace)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Trace)
Macrophages in alveolar ducts: (Minimal)
Pneumonitis: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Minimal)

Lymph Nodes - Tracheobronchial
Lymphoid proliferation: (Minimal)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 100 ppm

Rat No/Sex: 11♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Days 1 to 8, sneezing Day 4, gasping Days 4,5 and 9 and brown staining around snout Day 5.

Killed Day 9.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lymph Nodes - Deep Cervical
Enlarged: 6mm

Lungs
Not collapsed

Lymph Nodes - Tracheobronchial
Enlarged: 3mm

Adipose Tissue
Minimal

Small Intestine
Gaseous distension: (Minimal)

Caecum
Gaseous distension

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 11♂ - continued

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Minimal)
Atrophy/degeneration of olfactory epithelium: (Moderate)
Squamous metaplasia in nasopharynx: (Moderate)
Hyperplasia of transitional epithelium: (Moderate)

Larynx
Epithelial ulceration: (Marked)
Inflammatory exudate in lumen: (Marked)

Trachea
Epithelial hyperplasia: (Marked)

Tracheal Bifurcation
Epithelial hyperplasia: (Marked)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Moderate)
Bronchiolar epithelial regenerative hyperplasia: (Moderate)
Macrophages and mucus in alveoli: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Marked)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Minimal)

Lymph Nodes - Tracheobronchial
Lymphoid proliferation: (Moderate)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 100 ppm

Rat No/Sex: 12♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Days 1 to 8, sneezing Day 4 and gasping Days 4,5 and 9.

Killed Day 9.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 3mm

Adipose Tissue
Minimal

Stomach
Contents minimal
Gaseous distension

Small Intestine
Gaseous distension

Caecum
Gaseous distension: (Severe)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 12♂ - continued

MICROSCOPIC FINDINGS - continued

Nasal Passages
Rhinitis: (Trace)
Atrophy/degeneration of olfactory epithelium: (Marked)
Replacement of respiratory epithelium by undifferentiated type: (Minimal)
Epithelial ulceration in nasopharynx: (Minimal)

Larynx
Epithelial ulceration: (Marked)
Inflammatory exudate in lumen: (Moderate)

Trachea
Epithelial hyperplasia: (Moderate)

Tracheal Bifurcation
Epithelial hyperplasia: (Moderate)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Minimal)
Bronchiolar epithelial regenerative hyperplasia: (Moderate)
Macrophages in alveolar ducts: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Moderate)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 100 ppm

Rat No/Sex: 13♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Days 1 to 9, sneezing Day 4, gasping Day 5 and brown staining around snout Day 5.

Killed Day 9.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 5mm

Lungs
Not collapsed

Adipose Tissue
Minimal

Liver
Pale subcapsular area/s: (One) Left lobe 2mm

Stomach
Contents minimal
Gaseous distension

Small Intestine
Gaseous distension

Caecum
Gaseous distension

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 13♂ - continued

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Minimal)
Atrophy/degeneration of olfactory epithelium: (Marked)
Ulceration of respiratory epithelium: (Minimal)
Epithelial ulceration in nasopharynx: (Minimal)
Hyperplasia of transitional epithelium: (Minimal)

Larynx
Epithelial ulceration: (Marked)
Inflammatory exudate in lumen: (Moderate)

Trachea
Epithelial hyperplasia: (Moderate)

Tracheal Bifurcation
Epithelial hyperplasia: (Minimal)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Minimal)
Bronchiolar epithelial regenerative hyperplasia: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Moderate)

Liver
Fibrosis,mineralisation,bile duct proliferation,siderocytes: (Moderate , Areas , One lobe)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 100 ppm

Rat No/Sex: 14♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Days 1 to 8, sneezing Days 4 and 5 and gasping Days 4,5 and 9.

Killed Day 9.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lymph Nodes - Deep Cervical
Enlarged: 6mm

Lungs
Not collapsed

Adipose Tissue
Minimal

Liver
Pale subcapsular area/s - median cleft: (One) 2mm
Swollen: Anterior caudate lobe
Small: Posterior caudate lobe
Pale: Posterior caudate lobe
Haemorrhagic: Anterior caudate lobe

Stomach
Contents minimal
Gaseous distension

Small Intestine
Gaseous distension

Caecum
Gaseous distension

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 14♂ - continued

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Atrophy/degeneration of olfactory epithelium: (Marked)
Ulceration of respiratory epithelium: (Minimal)
Squamous metaplasia in nasopharynx: (Moderate)

Larynx
Epithelial ulceration: (Moderate)
Inflammatory exudate in lumen: (Moderate)

Trachea
Epithelial hyperplasia: (Moderate)

Tracheal Bifurcation
Epithelial hyperplasia: (Moderate)

Lungs
Bronchiolar epithelial regenerative hyperplasia: (Moderate)

Lymph Nodes - Cervical
Lymphoid proliferation: (Moderate)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Minimal)

Liver
Hepatocyte necrosis: (Moderate , Areas , Some lobes)
Lobar infarction

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 100 ppm

Rat No/Sex: 15♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Days 1 to 8, sneezing Day 4 and gasping Days 5 and 9.

Killed Day 9.

MACROSCOPIC FINDINGS

Skin Alopecia
Hindlimb/s: (Minimal , Diffuse)

Lymph Nodes - Cervical
Enlarged: 7mm

Lymph Nodes - Deep Cervical
Enlarged: 4mm

Lungs
Not collapsed

Lymph Nodes - Tracheobronchial
Enlarged: 3mm

Gastro-intestinal Tract
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 15♂ - continued

MICROSCOPIC FINDINGS - continued

Nasal Passages
Rhinitis: (Moderate)
Atrophy/degeneration of olfactory epithelium: (Minimal)
Replacement of respiratory epithelium by undifferentiated type: (Minimal)
Ulceration of respiratory epithelium: (Moderate)
Squamous metaplasia in nasopharynx: (Moderate)
Epithelial ulceration in nasopharynx: (Minimal)

Larynx
Epithelial ulceration: (Minimal)
Inflammatory exudate in lumen: (Moderate)
Epithelial hyperplasia and squamous metaplasia: (Moderate)

Trachea
Epithelial hyperplasia: (Moderate)

Tracheal Bifurcation
Epithelial hyperplasia: (Minimal)

Lungs
Bronchiolar epithelial hypertrophy with goblet cells: (Minimal)
Bronchiolar epithelial regenerative hyperplasia: (Minimal)
Macrophages in alveolar ducts: (Trace)
Macrophages and mucus in alveoli: (Minimal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Moderate)

Lymph Nodes - Deep Cervical
Lymphoid proliferation: (Minimal)
Erythrophagocytosis: (Moderate)

The following tissues were considered normal:

Lymph Nodes - Tracheobronchial : (W.N.L.)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 400 ppm

Rat No/Sex: 16♂ (Intercurrent)

CLINICAL FINDINGS

Brown staining around snout Days 1 and 2, yellow staining urogenital region Day 1, noisy respiration Day 1, gasping Day 2, brown staining under chin Day 2, brown staining front paws Day 2 and lethargic Day 2.

Killed Day 2.

MACROSCOPIC FINDINGS

Fur
Stained - perinasal region: (Red , Brown)
Stained - perioral region: (Red , Brown)

Stomach
Empty

Caecum
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Moderate)
Ulceration of olfactory epithelium: (Moderate)
Replacement of respiratory epithelium by undifferentiated type: (Moderate)
Hyperplasia of transitional epithelium: (Moderate)

Larynx
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Severe)
Secondary bacterial colonisation

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 16♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Severe)

Tracheal Bifurcation
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Severe)

Lungs
Bronchiolar epithelial regenerative hyperplasia: (Moderate)
Bronchiolar epithelial ulceration: (Moderate)
Inflammatory exudate in bronchiolar lumina: (Trace)
Alveolitis in alveolar ducts: (Minimal)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 400 ppm

Rat No/Sex: 17♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Day 1, brown staining around snout Day 2, gasping Day 2, brown staining under chin Day 2, brown staining on forepaws Day 2 and lethargic Day 2.

Killed Day 2.

MACROSCOPIC FINDINGS

Fur
Stained - perinasal region: (Brown)

Stomach
Empty

Caecum
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Moderate)
Ulceration of olfactory epithelium: (Moderate)
Replacement of respiratory epithelium by undifferentiated type: (Minimal)
Hyperplasia of transitional epithelium: (Minimal)

Larynx
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Severe)
Secondary bacterial colonisation

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 17♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea
Tracheitis: (Minimal)
Epithelial hyperplasia: (Minimal)
Epithelial ulceration: (Minimal)

Tracheal Bifurcation
Epithelial ulceration: (Moderate)
Epithelial hyperplasia: (Moderate)
Inflammatory exudate in lumen: (Minimal)

Lungs
Bronchiolar epithelial regenerative hyperplasia: (Moderate)
Bronchiolar epithelial ulceration: (Trace)
Alveolitis in alveolar ducts: (Trace)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 400 ppm

Rat No/Sex: 18♂ (Intercurrent)

CLINICAL FINDINGS

Noisy respiration Day 1.

Dead Day 2.

MACROSCOPIC FINDINGS

Found dead

Fur
Stained - perinasal region: (Minimal , Red , Brown)

Subcutis
Congested

Stomach
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

General Autolysis

Larynx
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Marked)

Trachea
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Marked)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 18♂ - continued

MICROSCOPIC FINDINGS - continued

Tracheal Bifurcation
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Moderate)

Lungs
Bronchiolar epithelial ulceration: (Marked)
Vascular congestion: (Moderate)

Subcutis
Congestion

The following tissues were considered normal:

Nasal Passages

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 400 ppm

Rat No/Sex: 19♂ (Intercurrent)

CLINICAL FINDINGS

Brown staining around snout Day 1 and noisy respiration Day 1.

Dead Day 2.

MACROSCOPIC FINDINGS

Found dead

Fur
Stained - perinasal region: (Brown)
Stained - perioral region: (Brown)
Stained - forepaw/s: (Brown)

Lungs
Congested

Stomach
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

General Autolysis

Larynx
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Moderate)
Secondary bacterial colonisation

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 19♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Marked)

Tracheal Bifurcation
Epithelial ulceration: (Marked)

Lungs
Bronchiolar epithelial regenerative hyperplasia: (Minimal)
Bronchiolar epithelial ulceration: (Moderate)
Vascular congestion: (Marked)

The following tissues were considered normal:

Nasal Passages

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: 400 ppm

Rat No/Sex: 20♂ (Intercurrent)

CLINICAL FINDINGS

Brown staining around snout Days 1 and 2, noisy respiration Day 1, gasping Day 2, brown staining under chin and on forepaws Day 2.

Killed Day 2.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Stomach
Gaseous distension

Small Intestine
Gaseous distension

Caecum
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Rhinitis: (Marked)
Ulceration of olfactory epithelium: (Minimal)
Replacement of respiratory epithelium by undifferentiated type: (Minimal)

Larynx
Epithelial ulceration: (Severe)
Inflammatory exudate in lumen: (Marked)

APPENDIX 8

(Pathology - continued)

Rat No/Sex: 20♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea
Epithelial hyperplasia: (Moderate)

Tracheal Bifurcation
Epithelial ulceration: (Minimal)
Epithelial hyperplasia: (Marked)

Lungs
Bronchiolar epithelial regenerative hyperplasia: (Moderate)

Lymph Nodes - Cervical
Lymphoid proliferation: (Moderate)

Pathologist: D.J.Lewis

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Control

Rat No/Sex: 21♀ (Terminal)

CLINICAL SIGNS

Hair loss on face Day 20

MACROSCOPIC FINDINGS

Skin

Hair loss between pinnae and orbits (minimal)

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Control

Rat No/Sex: 22♀ (Terminal)

CLINICAL SIGNS

No abnormality detected

MACROSCOPIC FINDINGS

No abnormality detected
[One foetus with marked subcutaneous oedema - probable malformation]

[ ] Foetal finding

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Control

Rat No/Sex: 23♀ (Terminal)

CLINICAL SIGNS

No abnormality detected

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Control

Rat No/Sex: 24♀ (Terminal)

CLINICAL SIGNS

No abnormality detected

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Control

Rat No/Sex: 25♀ (Terminal)

CLINICAL SIGNS

No abnormality detected

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 25 ppm

Rat No/Sex: 26♀ (Terminal)

CLINICAL SIGNS

Noisy respiration Days 7 to 19

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 25 ppm

Rat No/Sex: 27♀ (Terminal)

CLINICAL SIGNS

Noisy respiration Days 7 to 19
Hair loss on back Days 10 to 20

MACROSCOPIC FINDINGS

Skin

Dorsal thoracic region thinning of hair (minimal)

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 25 ppm

Rat No/Sex: 28♀ (Terminal)

CLINICAL SIGNS

Noisy respiration Days 7 to 19

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 25 ppm

Rat No/Sex: 29♀ (Terminal)

CLINICAL SIGNS

Noisy respiration Days 7 to 19

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 25 ppm

Rat No/Sex: 30♀ (Terminal)

CLINICAL SIGNS

Noisy respiration Days 7 to 20

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 100 ppm

Rat No/Sex: 31♀ (Intercurrent)

CLINICAL SIGNS

Noisy respiration Days 6 to 13
Sneezing Day 8
Gasping Days 8, 9 and 10
Walking with arched back Days 12 and 13
Occasionally twitching head Days 12 and 13

KILLED DAY 13 POST COITUM

MACROSCOPIC FINDINGS

Lungs

A few haemorrhagic areas (minimal) (2 × 2 mm)

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 100 ppm

Rat No/Sex: 32♀ (Intercurrent)

CLINICAL SIGNS

Noisy respiration Days 6 to 13
Sneezing Day 8
Gasping Days 9 and 10
Brown staining under chin Day 9
Brown staining around snout Days 9 and 10
Walking with arched back Days 12 and 13
Occasionally twitching head Days 12 and 13

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

Lungs

A few small red fluid-filled cysts (1 × 1 mm)

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 100 ppm

Rat No/Sex: 33♀ (Intercurrent)

CLINICAL SIGNS

Noisy respiration Days 6 to 12
Sneezing Day 8
Brown staining under chin Days 8 and 9
Gasping Days 9, 10, 11 and 13
Brown staining around snout Days 9, 10, 11 and 13
Walking with arched back Days 12 and 13
Occasionally twitching head Days 12 and 13
Yellow staining urogenital region Day 12
Swollen abdomen Day 13

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

Fur
Brown staining nasal region
Yellow staining urogenital region

Abdomen
Distended

Lungs
A few haemorrhagic areas (1 mm)

Stomach
Gaseous distension

Gastrointestinal tract
Gaseous distension

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 100 ppm

Rat No/Sex: 34♀ (Intercurrent)

CLINICAL SIGNS

Noisy respiration Days 6 to 13
Sneezing Day 8
Gasping Days 8, 9 and 10
Brown staining under chin Day 9
Brown staining around snout Day 9
Walking with arched back Days 12 and 13
Occasionally twitching head Days 12 and 13

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

Lungs
A few small red fluid-filled cysts (2 × 1 mm)

Ileum
Gaseous distension

Caecum
Gaseous distension

Colon
Gaseous distension

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Compound: Acetic anhydride

Dosage level: 100 ppm

Rat No/Sex: 35♀ (Intercurrent)

CLINICAL SIGNS

Noisy respiration Days 6 to 13
Sneezing Day 8
Gasping Days 9 and 10
Red staining urogenital region Day 12
Brown staining urogenital region Day 13
Walking with arched back Days 12 and 13
Occasionally twitching head Days 12 and 13

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

Lungs
A few small red fluid-filled cysts (2 × 1 mm)

Urogenital
Brown staining

All the other organs and tissues appeared normal.

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Untreated

Rat No/Sex: 36♀ (Intercurrent)

CLINICAL SIGNS

Hair loss on neck Days 12 and 13

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

Skin

Dorsal cervical region, hair loss (minimal)

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Untreated

Rat No/Sex: 37♀ (Intercurrent)

CLINICAL SIGNS

No abnormality detected

KILLED DAY 13 POST COITUM

MACROSCOPIC FINDINGS

Lungs

A few haemorrhagic areas (minimal) (3 × 2 mm)
Several pale cysts (1 × 1 mm)

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Untreated

Rat No/Sex: 38♀ (Intercurrent)

CLINICAL SIGNS

No abnormality detected

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Untreated

Rat No/Sex: 39♀ (Intercurrent)

CLINICAL SIGNS

No abnormality detected

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

No abnormality detected

APPENDIX 8

(Pathology - continued)

Compound: Air

Dosage level: Untreated

Rat No/Sex: 40♀ (Intercurrent)

CLINICAL SIGNS

No abnormality detected

KILLED DAY 13 OF PREGNANCY

MACROSCOPIC FINDINGS

No abnormality detected

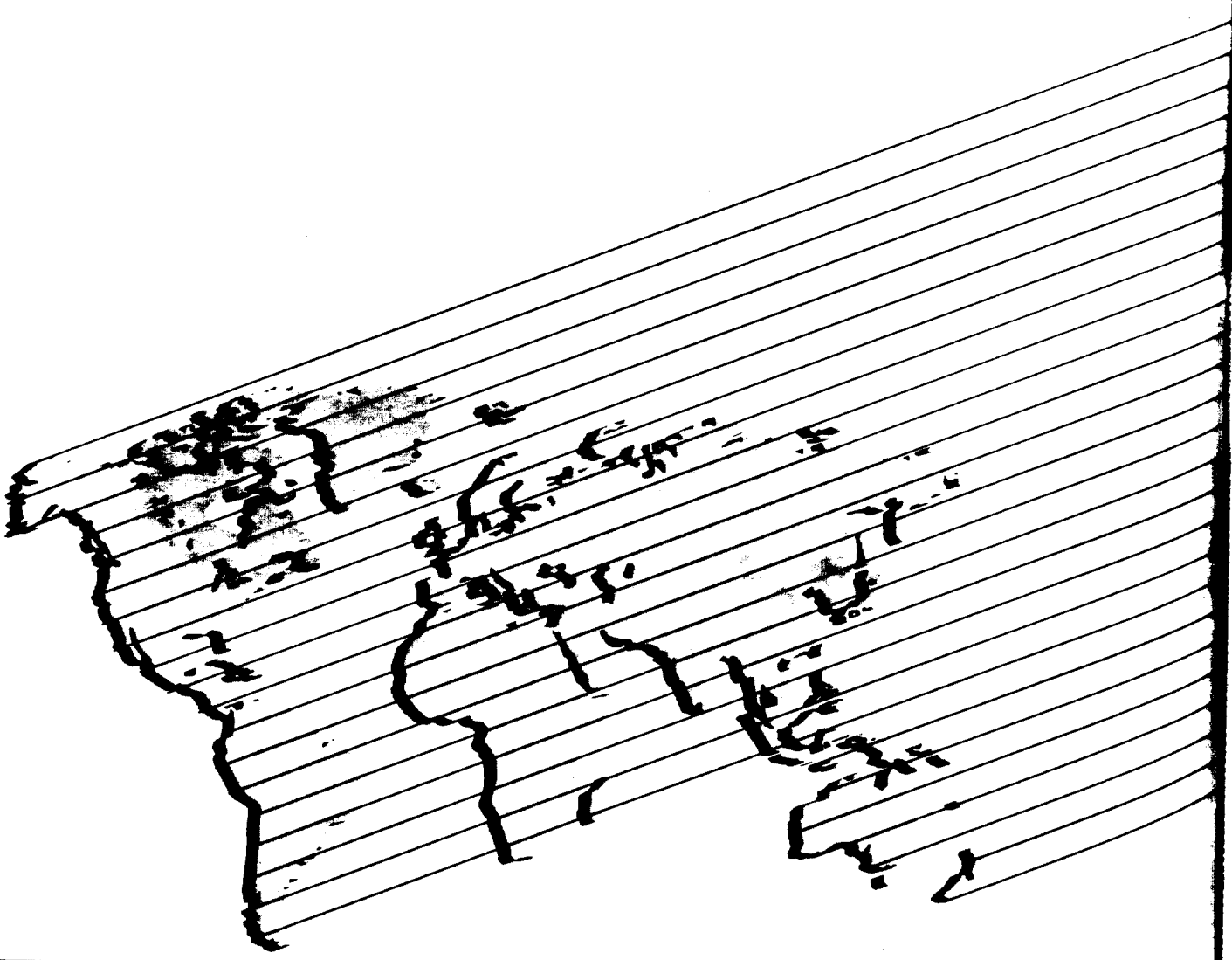
APPENDIX 9

Chamber spatial distribution of acetic anhydride

Group	Port	Chamber concentration (ppm)
2 Low dose	LU	22
	RL	25
	LLM	27
	RUM	23
	LL	23
	RU	24
	Mean	24
	Variation	20.8%
3 Inter dose	LU	120
	RL	129
	LUM	122
	RLM	123
	LL	122
	RU	124
	Mean	123
	Variation	7.3%
4 High dose	LU	470
	RL	396
	LUM	428
	RLM	438
	LL	463
	RU	433
	Mean	438
	Variation	16.9%

Key LU Left upper
LUM Left upper middle
LLM Left lower middle
LL Left lower
RU Right upper
RUM Right upper middle
RLM Right lower middle
RL Right lower

Huntingdon Research Centre Ltd.
Huntingdon
Cambridgeshire PE18 6ES
England
Telephone
Huntingdon (0480) 890431
International +44 480 890 431
Telex
32100 HRCUK G
Cable
Research Huntingdon
Fax
(0480) 890693 Gp 2 & Gp 3
International +44 480 890 693



Triage of 8(e) Submissions

Date sent to triage: APR 04 1995

NON-CAP CAP

Submission number: 12903B

TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX	CTOX	EPI	RTOX	GTOX
STOX/ONCO	CTOX/ONCO	IMMUNO	CYTO	NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: 0 1 2 **pages** — **pages** 1, tabs

Notes: SS

Contractor reviewer : LPS **Date:** 1/20/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 1194 -12903 SEQ. B

TYPE: INT (SUPP) FLWP

SUBMITTER NAME: Hoechst Celanese Corporation

SUB. DATE: 10/31/94 [redacted] 11/09/94 CSRAD DATE: 12/22/94

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
0401 NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME:

CAS#
108 - 24 - 7

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
0243	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATE:

NON-CBI INVENTORY
(YES)
NO
UNKNOWN

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REVIEW

SPECIES
RAT

TOXICOLOGICAL CONCERN:
LOW
MED
(HIGH)

USE: industrial

PRODUCTION:

CAS SR

COMMENTS Non-CAP, SS

-CPSS-

> <ID NUMBER>
8(E)-12903B

> <TOX CONCERN>
H

> <COMMENT>
SUBCHRONIC INHALATION TOXICITY IN PREGNANT AND NON-PREGNANT RATS IS OF HIGH CONCERN. EXPOSURES OF 400, 100 AND 25 PPM 5 DAYS/WEEK ADMINISTERED TO GROUPS OF 5 MALE CD SPRAGUE-DAWLEY ALBINO RATS PRODUCED TOXIC EFFECTS SO SEVERE AT 400 PPM AND 100 PPM TO PREVENT COMPLETION OF THE 2-WEEK REPEAT EXPOSURE PERIODS AND REQUIRING REDESIGN OF PROTOCOL FOR PREGNANT FEMALE GROUPS: A SINGLE 6-HOUR EXPOSURE AT 400 PPM RESULTED IN DEATH OF 2/5 MALE RATS WITHIN 24 HOURS. PROGRESSIVE EXPOSURE-RELATED EFFECTS OF SIX-HOUR DAILY EXPOSURES AT 100 PPM RESULTED IN TERMINATION OF THE EXPOSURE PERIOD AFTER ONLY SIX OF TEN DAYS IN MALES AND SEVEN OF TEN DAYS IN FEMALES. DOSE-RELATED CLINICAL OBSERVATIONS DURING EXPOSURE WERE EVIDENT IN 25 PPM GROUPS AND INCLUDED HALF-CLOSED EYES, LACHRYMATION, LICKING OF INSIDE MOUTH, GASPING RESPIRATION AND OCCASIONAL TWITCHING OF THE HEAD. NOISY RESPIRATION, OCCASIONAL GASPING AND SNEEZING CHARACTERIZED POST-EXPOSURE OBSERVATION. BOTH MALE AND FEMALE GROUPS SHOWED MARKED BODYWEIGHT LOSS WITH TOTAL RESORPTION OF LITTERS IN 2/4 FEMALES OF THE 100 PPM GROUP. NECROPSY OF MALES GIVEN A SINGLE 6-HOUR EXPOSURE AT 400 PPM SHOWED DISTENTION OF THE GASTROINTESTINAL TRACT WITH MICROSCOPIC CHANGES INCLUDING HYPERPLASIA AND/OR ULCERATION OF THE EPITHELIUM OF NASAL PASSAGES, LARYNX, TRACHEA/TRACHEAL BIFURCATION AND LUNGS WHICH WAS ALSO EVIDENT AT LOWER EXPOSURE LEVELS. MALE AND FEMALE GROUPS UPON 100, 25 PPM EXPOSURES SHOWED ENLARGED LYMPH NODES WITH GASEOUS DISTENSION OF THE GASTROINTESTINAL TRACT WITH SIMILAR, ALTHOUGH MILDER, MICROSCOPIC CHANGES. WITH COMPLETION OF THE 2-WEEK EXPOSURE PERIOD, THERE WAS NO EVIDENCE OF EFFECT FROM REPEATED 6-HOUR 25 PPM EXPOSURE ON EMBRYOFETAL LOSS, LITTER SIZE OR LITTER WEIGHT; THERE WERE NO OVERT FETAL ABNORMALITIES.

$$$$

PDCN, 88 ...

Hoechst Celanese

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96 FEB 13 AM 11:47

8EHQ-0296-12903

Department of
Environmental, Health &
Safety Affairs (DEHSA)

Hoechst Celanese Corporation
Route 202-206
PO Box 2500
Somerville, NJ 08876-1258
908 231 2000
Telex 833 449
Fax 908 231 4554

96 FEB 26 AM 9:04

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OPPT NCIC

February 2, 1996
RAJ-010-96

C

Attn: TSCA Section 8(e) Coordinator
Document Processing Center (TS-790)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

ORIGINAL

Subject: **8EHQ-94-12903**

Contains No CBI

Dear Sir or Madam:

With this letter, Hoechst Celanese Corporation is providing additional inhalation toxicity information for acetic anhydride (CAS no. 108-24-7).

A draft histopathology report (enclosed) on a 13-week inhalation/toxicity mutagenicity study in rats indicates respiratory tract effects at both the 5 and 20 ppm exposure levels. No effects were detected at 1 ppm. No mutagenic effects were observed.

This submission contains no confidential business information.

If any further information is required, do not hesitate to contact Dr. Richard A. Jourdenais, Corporate Manager, Product Stewardship at 908-231-3746.

Sincerely,

Susan Engelman Volkert

Susan Engelman Volkert
Vice President, Environmental, Health &
Safety Affairs

8EHQ-94-12903
SP002 02/13/96

RAJ/mcs
Attachment

CERTIFIED MAIL/
RETURN RECEIPT REQUESTED

File: Log No. 171

89960000039

RECEIVED 3/25/96 mm

QCP 2/5/96

Hoechst

HUNTINGDON LIFE SCIENCES Ltd.,
HUNTINGDON, CAMBS, PE18 6ES, ENGLAND.
FACSIMILE NO: International +44 1480 890693: National (01480) 890693

Huntingdon Life Sciences

TOTAL NUMBER OF PAGES TO THIS FAX MESSAGE INCLUDING THIS PAGE 5
If this transmission is unsatisfactory please contact us on Telephone no: International +44 1480 892000: National (01480) 892000 extn 1022.

ATTENTION OF: **Dr Tito Cascieri**

FROM: **Terry Kenny**

COMPANY: **Hoechst Celanese Corporation USA**

DATE: **11 January 1996**

FAX NUMBER: **908 231 4554**

MESSAGE:

13-week inhalation toxicity/mutagenicity study in rats with acetic anhydride (Study No HST/411)

Please find attached a draft copy of the histopathology summary report for those animals killed after 13 weeks of exposure in the above named study.

Please contact us if you should have any comments or queries.

With kind regards

Terence J Kenny
Study Director
Division of Toxicology

Telephone direct dial/voice mail +44 1480 892070

also, Reviewed previously

FAX CENTRE OUTGOING
12 JAN 1996

LOG	SHEETS	1	2	3	4
DC		5	6	7	8

FOR HUNTINGDON LIFE SCIENCES USE ONLY

CC: FILES, C K Shepherd, C J Hardy, M Bannerman, USA office.

DRAFT

HISTOPATHOLOGY SUMMARY

Treatment-related findings

A range of treatment related changes were seen in the respiratory tract of both male and female animals from the high and intermediate dosage levels, the incidence of which is shown in the table below. For the majority of these lesions a statistically significant increase in incidence compared to controls was demonstrated.

Nasal passages

Lesions were primarily seen in the anterior portion of the nose and comprised varying degrees of hyperplasia of the respiratory epithelium, frequently with increased goblet cell prominence; inflammation associated with the respiratory epithelium and the presence of granular eosinophilic inclusions within respiratory epithelium, possibly representative of globule leucocytes. In the transitional epithelium lining anterior portions of the nasal turbinates hyperplasia, squamous metaplasia and inflammation, occasionally with areas of erosion, was seen together with the presence of granular eosinophilic inclusions which may represent the presence of globule leucocytes. These latter inclusions were more evident in animals from the intermediate dosage group than in those from the high dosage group where transitional epithelial squamous metaplasia was more prominent.

In general olfactory epithelium was unaffected by the acetic anhydride inhalation.

Various amounts of exudative inflammatory cell accumulation were seen in the nasal chambers of high dosage group animals and of a single male intermediate group animal.

	Male				Female			
	Control	Low	Inter	High	Control	Low	Inter	High
Exudative inflammation	0	0	1	9**	0	0	0	8**
Respiratory epithelium								
Inflammation	0	0	1	9**	0	0	0	8**
Granular eosinophilic inclusions	0	0	1	5*	0	0	0	3
Prominent goblet cells	0	0	1	10**	0	0	1	10**
Squamous metaplasia	0	0	0	6**	0	0	0	4*
Transitional epithelium								
Inflammation	0	0	3	8**	0	0	0	8**
Erosion	0	0	0	3	0	0	0	6**
Granular eosinophilic inclusions	0	0	7**	1	0	0	9**	3
Hyperplasia	0	0	9**	8**	0	0	8**	9**
Squamous metaplasia	0	0	1	9**	0	0	0	9**
Olfactory epithelium								
Hyperplasia	0	0	0	0	0	0	0	1
Total number of animals examined	10	10	10	10	10	10	10	10

** $p < 0.01$ with Fisher's Exact test

DRAFT

Larynx

Inflammatory infiltration, squamous metaplasia of the ventrolateral epithelium and hyperplasia of the epithelium covering the arytenoid processes, frequently with varying degrees of erosion or ulceration, were seen in the majority of animals from the high dosage group as well as occasional animals from the intermediate dosage group.

	Male				Female			
	Control	Low	Inter	High	Control	Low	Inter	High
Inflammation	0	0	1	9**	0	0	3	4*
Ventrolateral squamous metaplasia	0	0	2	10**	0	0	1	10**
Ventrolateral hyperplasia	0	0	0	2	0	0	0	2
Ventrolateral erosion/ulceration	0	0	0	1	0	0	0	2
Arytenoid hyperplasia	0	1	5*	10**	0	0	0	10**
Arytenoid erosion/ulceration	0	0	0	9**	0	0	0	8**
Total number of animals examined	10	10	10	10	10	10	10	10

* $p<0.05$ ** $p<0.01$ with Fisher's Exact test

Trachea

Epithelial hyperplasia was seen at various sites including at the carina was seen in the majority of animals from the high dosage group and in a proportion of intermediate dosage group animals. Also seen were epithelial squamous metaplasia at the carina, eosinophilic epithelial inclusions and inflammatory infiltration of the lamina propria.

	Male				Female			
	Control	Low	Inter	High	Control	Low	Inter	High
Inflammation	0	0	0	9**	0	0	0	10**
Eosinophilic inclusions	0	0	0	3	0	0	0	0
Hyperplasia	0	0	0	9**	0	0	0	10**
Squamous metaplasia	0	0	0	1	0	0	0	1
Hyperplasia (carina)	0	0	0	8**	0	0	0	6**
Squamous hyperplasia (carina)	0	0	0	6**	0	0	1	4*
Total number of animals examined	10	10	10	10	10	10	10	10

* $p<0.05$ ** $p<0.01$ with Fisher's Exact test

DRAFT

Lungs

Various changes were seen in a proportion of animals primarily from the high dosage group. These included the presence of occasional perivascular inflammatory cells, increased prominence of bronchus-associated lymphoid tissue (BALT), small foci of fibrosis in the walls of the alveolar ducts and increased prominence of alveolar macrophages.

	Male				Female			
	Control	Low	Inter	High	Control	Low	Inter	High
Perivascular inflammatory cells	0	0	0	6**	0	0	0	7**
Prominent BALT	0	0	1	4*	0	0	0	2
Alveolar duct fibrosis	0	0	0	5*	0	0	0	9**
Increased alveolar macrophages	0	0	0	3	0	0	0	5*
Total number of animals examined	10	10	10	10	10	10	10	10

* $p<0.05$ ** $p<0.01$ with Fisher's Exact test

Cervical lymph nodes

An increased severity, but not incidence of occurrence, was seen in high dosage group animals in respect of parameters indicative of overall activity - specifically an increase in the degree of medullary plasmacytosis. This change is probably associated with the inflammatory lesions of the respiratory tract.

		Male				Female			
		Control	Low	Inter	High	Control	Low	Inter	High
Plasmacytosis	Total	6	5	7	9	8	2	6	8
	Minimal	2	1	5	0	3	1	3	2
	Moderate	1	3	2	8**	3	0	2	6
	Marked	3	1	0	1	2	1	1	0
Total number of animals examined		10	10	10	10	10	10	10	10

** $p<0.01$ with Fisher's Exact test

DRAFT

Incidental changes

The various other histological changes that were seen in animals from this study were within the normal range of spontaneous lesions commonly encountered in animals of this species and age range and were, consequently, not considered to be of any toxicological significance.

Some minor perturbations of the splenic white pulp and of the thymic cortex predominantly seen in occasional high dosage group animals were considered to be fortuitous and not associated with treatment.

No histological changes were seen to correlate with, or to account for the treatment related ophthalmoscopical or haematological observations. No changes were seen that might account for the increased testicular weights at necropsy.

Conclusion

Exposure of rats to acetic anhydride by inhalation at the high, and to a lesser extent at the intermediate, dosage levels produced treatment related inflammatory, hyperplastic and metaplastic lesions at all levels of the respiratory tract. These changes are characteristic of the lesions that are produced by irritant compounds.

No treatment related effects were discerned at the low dosage level.

R Gregson
Senior Pathologist

D J Lewis
Head, Department of Pathology
10 January 1996

H

Triage of 8(e) Submissions

Date sent to triage: 7/17/96 (NON-CAP) CAP

Submission number: 12903C TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Gordon Cash (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 -HERD (1 copy each)

(STOX) CTOX EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.):_____

Notes:

☒ This is the **original** 8(e) submission; refile after triage evaluation.

☐ This **original** submission has been **split**; rejoin after triage evaluation.

☐ Other:

Photocopies Needed for Triage Evaluation

entire document: (0) 1 2 3

front section and CECATS: (0) 1 2 3

Initials: JW **Date:** 7/18/96

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ-0296-12903 SEQ. C

TYPE: INT. (SUPP) FLWP

SUBMITTER NAME: Hoechst Celanese Corporation

SUB. DATE: 02/02/96 OTS DATE: 02/26/96 CSRAD DATE: 03/25/96

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME: CAS#

108-24-7

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
(0205)	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
0209	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	0299	OTHER	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
0212	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
0213	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
(0214)	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
(illegible)	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

TRIAGE DATA:

NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
(YES (CONTINUE))	YES (DROP/REFER)	RAT	LOW		
NO (DROP)	NO (CONTINUE)		MED		
DETERMINE	REFER:		[HIGH]		

COMMENTS: N-C, SS
Rats were exposed to acetic anhydride via inhalation at concentrations of 0, 1, 5 and 20 ppm for 13 weeks. Treatment-related inflammatory, hyperplastic and metaplastic lesions occurred at all levels of the respiratory tract. Details of lesions are excellent. There was no NOAEL achieved.

H

Triage of 8(e) Submissions

Date sent to triage: 12/18/96 (NON-CAP) CAP

Submission number: 12903 D TSCA Inventory: Y (N) D

Study type (circle appropriate):

Group 1 - Gordon Cash (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 -HERD (1 copy each)

STOX (CTOX) EPI RTOX GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.):_____

Notes:

☒ This is the **original** 8(e) submission; refile after triage evaluation.

☐ This **original** submission has been split; rejoin after triage evaluation.

☐ Other:

Photocopies Needed for Triage Evaluation

entire document: 0 1 2 3

front section and CECATS: 0 1 2 3

Initials:_____ Date:_____

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ-0996-12903 SEQ. D

TYPE: INT. (SUPP) FLWP

SUBMITTER NAME:
Hoechst Celanese Corporation

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 9-19-96 OTS DATE: 9-24-96 CSRAD DATE: 11-4-96

CHEMICAL NAME: acetic acid anhydride

CAS# 108-24-7

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	(0243)	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE RBQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
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0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
0212	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
0213	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
(0214)	SUB CHRONIC TOX (ANIMAL)	01 (02) 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	YES	YES (DROP/REFER)	Rat	LOW		
CAS SR	(NO)	NO (CONTINUE)		MED		
	IN TRAINING	REFER		[HIGH]		

COMMENTS: Rats were exposed to acetic anhydride vapor via inhalation for 6 hr/day, 5 days/week to 0, 1, 5 or 20 ppm for 13 weeks. The respiratory tract was the target for the chemical. The NOAEL was 1 ppm.

Hoechst Celanese

8EHQ-0996-12903

RECEIVED
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96 SEP 24 PM 2:40

Department of
Environmental, Health &
Safety Affairs (DEHSA)

8EHQ-94-12903

Hoechst Celanese Corporation
Route 202-206
PO Box 2500
Somerville, NJ 08876-1258
908 231 2000
Telex 833 449
Fax 908 231 4554

September 19, 1996

US Environmental Protection Agency
Document Processing Center (TS-790)
401 M. Street, SW
Washington, DC 20460

Attention: TSCA Section 8(e) Coordinator

S. Corley
RECEIVED
11/4/96
CSEAD/OPPT

Subject: 8EHQ-94-12903

Dear Sir or Madam:

In accordance with the reporting requirements of TSCA Section 8(e), Hoechst Celanese Corporation hereby submits the final report of a 13 week inhalation toxicity study in rats involving acetic acid anhydride (CAS no. 108-24-7)

In the 13-week inhalation study, rats were exposed for 6 hrs/day, 5 days/week to 0, 1, 5 or 20 ppm acetic anhydride vapor. The respiratory tract was the target for this chemical. The effects were consistent with those typical of substances which act as local/site-of-contact irritants. In animals exposed to the same levels of acetic anhydride for 13 weeks and then allowed a 13-week period without exposure, significant recovery from irritation effects was reported. Based on bone marrow analysis for micronuclei, no mutagenic effects were detected. In conclusion, the subchronic NOEL for acetic anhydride in this study was 1 ppm.

RECEIVED
OPPT NCIC
96 SEP 26 AM 12:16

This submission contains no confidential business information

If any further information is required, please contact Dr. Richard A. Jourdenais, Corporate Manager Product Stewardship at 908-231-3746.

Sincerely,

Richard A. Jourdenais

Richard A. Jourdenais, Ph.D.
Corporate Manager
Product Stewardship

89960000214

Attachments
Certified Mail/Return Receipt Requested
File log no. 171

raj061.doc

Hoechst

TSCA HEALTH & SAFETY STUDY COVER SHEET - revised 6/25/96

TSCA CBI STATUS:

☐ **CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)**

Clearly mark the confidential information with bracketing and check the box in the appropriate section (☐ *Contains CBI*).
Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

1.0 SUBMISSION TYPE ☐ *Contains CBI*

☐ 8(d) ☒ 8(e) ☐ FYI ☐ 4 ☐ OTHER: Specify_____

☐ Intial Submission ☐ Follow-up Submission ☒ Final Report Submission

Previous EPA Submission Number or Title if update or follow-up: 8EHQ-94-12903 Docket Number, if any: #

☐ continuation sheet attached

2.1 SUMMARY/ABSTRACT ATTACHED
(may be required for 8(e): optional for §4, 8(d) & FYI)
☒ YES ☐ NO

2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID
Log No. 171

2.3 FOR EPA USE ONLY

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY ☐ *Contains CBI*

CAS# 108 - 24-7

Reported Chemical Name (specify nomenclature if other than CAS name):
Acetic Acid Anhydride

Purity 99+ %

☒ Single Ingredient
☐ Commerical/Tech Grade
☐ Mixture

Trade Name: *Common Name:* ACETIC ANHYDRIDE

Other chemical(s) present in tested mixture

CAS Number	*NAME*	*% WEIGHT*

☐ continuation sheet attached

96 SEP 24 PM 2:40 RECEIVED OPPT CBIC

4.0 REPORT/STUDY TITLE ☐ *Contains CBI*

13 WEEK INHALATION TOXICITY STUDY IN RATS

continuation sheet attached

5.1 STUDY/TSCATS INDEXING TERMS
[CHECK ONE]
HEALTH EFFECTS (HE): X ENVIRONMENTAL EFFECTS (EE):_____ ENVIRONMENTAL FATE (EF):_____

5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes)

STUDY TYPE: STOX	SUBJECT ORGANISM (HE, EE only): RATS	ROUTE OF EXPOSURE (HE only): INHL	VEHICLE OF EXPOSURE (HE only)_____
Other:	*Other:*	*Other:*	*Other:*

6.0 REPORT/STUDY INFORMATION ☐ *Contains CBI* ☐ Study is GLP

Laboratory HUNTINGDON LIFE SCIENCES Report/Study Date 8/27/96

Source of Data/Study Sponsor (if different than submitter) HOECHST CELANESE CORP Number of pages 334

☐ continuation sheet attached

7.0 SUBMITTER INFORMATION ☐ *Contains CBI*

Submitter: Richard A. Jourdenais Title MGR Prod.Stewardshi Phone: (908) 231-3746

Company Name: HOECHST CELANESE CORP Company Address: RT 202-206 PO Box 2500

Somerville, NJ 08876-1258 Submitter Address (if different): _____

Technical Contact: Richard Jourdenais Phone: (908) 231-3746

☐ continuation sheet attached

8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS ☐ *Contains CBI*

In the 13-week inhalation study, rats were exposed for 6 hrs/day, 5 days/week to 0, 1, 5 or 20 ppm acetic anhydride vapor. The respiratory tract was the target for this chemical. The effects were consistent with those typical of substances which act as local/sit-of-contact irritants. In animals exposed to the same

☒ continuation sheet attached

Submitter Signature: Richard A. Jourdenais **Date:** 9/19/96

9.0 CONTINUATION SHEET

TSCA CBI STATUS:

☐ **CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)**

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Submitter Tracking Number/Internal ID

CONTINUED FROM COVER SHEET SECTION # 8.0

☐ Contains CBI

level of acetic anhydride for 13 weeks and then allowed a 13-week period without exposure, significant recovery from irritation effects was reported. Based on bone marrow analysis for micronuclei, no mutagenic effects were detected. In conclusion, the subchronic NOEL for acetic anhydride in this study was 1 ppm.

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ACETIC ANHYDRIDE

13-WEEK INHALATION TOXICITY STUDY IN RATS

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ACETIC ANHYDRIDE

13-WEEK INHALATION TOXICITY STUDY IN RATS

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited with effect from 21 November 1995

Sponsor

Department of Environment,
Health and Safety Affairs,
Hoechst Celanese Corporation,
Route 202-206,
P.O. Box 2500,
Somerville, N.J. 08876-1258,
U.S.A.

Sponsor's representative

Dr. T. Cascieri

Testing facility

Huntingdon Life Sciences Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued: 27 August 1996

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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.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

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.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

14 August 1996

Terence J. Kenny, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to Management, Huntingdon Life Sciences are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	14 July 1995
Pre-experimental Period	24 July 1995	24 July 1995
Experimental Period	16-17 August 1995	17 August 1995
	4 October 1995	4 October 1995
	2 November 1995	2 November 1995
	6 & 9 November 1995	9 November 1995
	28 November 1995	28 November 1995
	7 February 1996	7 February 1996
Date of reporting audit findings to the Study Director and Management		8 July 1996

Mark Somerset,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

23 July 1996
Date

RESPONSIBLE PERSONNEL

STUDY MANAGEMENT

Terence J. Kenny, B.Sc. (Hons.),
Study Director.

Craig K. Shepherd, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol.,
Study Supervisor.

Mario Bannerman, H.N.D.,
Head, Inhalation Toxicology.

TOXICOLOGY

Colin J. Hardy, B.Sc., Ph.D., M.I.Biol., C.Biol.,
Dip.R.C.Path. (Toxicology),
Senior Toxicologist.

ANALYSIS

Ian S. Gilkison, M.A., Ph.D.,
Section Head, Aerosol Technology and Analysis.

CLINICAL PATHOLOGY

David Crook, B.Sc., Ph.D.,
Head, Department of Clinical Pathology.

PATHOLOGY

Chirukandath Gopinath, B.V.Sc., M.V.Sc., Ph.D., F.R.C.Path.,
Director of Pathology.

SUMMARY

This study was designed to assess the response in rats to exposure, by inhalation, to the vapour of acetic anhydride.

Groups of rats were exposed for 6 hours a day, 5 days a week for 13 consecutive weeks. The groups of rats, Low, Intermediate and High dose were exposed to 0.98, 4.96 and 20.0 ppm acetic anhydride in air respectively. A fourth group of rats, acting as a control, was exposed to air only. In addition, one third of each group of animals exposed were retained, unexposed, for a further 13 weeks in order to assess any regression of treatment-related effects.

The following comments in relation to principal findings are made in summary.

Clinical signs

Partially closed eyes were seen in High dose animals during the first two exposures. At other times noisy breathing was recorded throughout the 13-week exposure period in High dose rats, this progressively diminished over the course of the withdrawal period. An occasional incidence of noisy breathing was also recorded in Intermediate dose rats during the 13-week exposure period only.

Bodyweight and food consumption

Weight gain and food consumption were lower in High dose rats compared with controls during the 13-week exposure period. A degree of recovery was seen during the withdrawal period.

Ophthalmic examination

During Week 13 of exposure treatment-related corneal changes were observed in a number of animals primarily from the High dose group. The changes were not observed histologically. Treatment-related changes were no longer present by Week 26.

Laboratory investigations

Treatment-related changes were observed in High dose animals only during Week 13. Packed cell volume, haemoglobin concentration and total red cell numbers were greater than controls. This was probably an adaptive response to impaired gas exchange resulting from respiratory tract lesions. Blood cholesterol levels were lower.

***Post mortem* investigations**

After 13 weeks of exposure the lung weights were greater than controls in High dose rats. The difference was smaller following 13 weeks of recovery.

In rats sacrificed after 13 weeks of exposure the lungs remained inflated when the thoracic cavity was opened in a proportion of High dose rats. A reduction in adipose tissue was seen in a proportion of High dose rats.

No treatment-related macroscopic changes were seen in rats killed after the withdrawal period.

Histopathology

The histological changes reported in the respiratory tract of rats from this study that were exposed to acetic anhydride over a 13-week period were seen to be dose-related and consistent with changes typical of a substance acting as a local/contact irritant. The changes seen were predominantly localised inflammatory lesions with subsequent areas of epithelial hyperplasia and/or squamous metaplasia. In general the changes were of minimal to moderate severity in the majority of High dose animals. Changes seen in occasional animals from the Intermediate dose group, were, with a few exceptions, minimal in degree. Changes seen in cervical lymph nodes were associated with the normal processes of an inflammatory response and not directly caused by the inhaled material.

On cessation of exposure to the irritant material the changes were all seen to regress, indicating that long term damage had not been caused within the respiratory tract.

The changes reported were similar in character, but considerably less severe than, those reported in a previous inhalation study with this substance (Report number HST 400/942606) in which rats were exposed to doses of up to 400 ppm for a two week period. Although the period of exposure in the current study was longer (13 weeks) the maximum dose administered was only 20 ppm.

Micronucleus test

The test substance did not show any evidence of causing chromosome damage or bone marrow cell toxicity.

Conclusion

The no-effect-level for this study is considered to be 1.0 ppm.

INTRODUCTION

The purpose of this study performed at Huntingdon Life Sciences Ltd, Huntingdon, England, was to assess the response in rats to repeat administration, whole-body by inhalation, of the test substance for 13 consecutive weeks. In addition, withdrawal rats in all groups were kept for a further 13 weeks in order to assess the reversibility of any effects due to exposure.

The study was designed with reference to national and international guidelines to meet the requirements of regulatory authorities for the toxicity testing of industrial chemicals, in particular OECD Guidelines for testing of Chemicals No. 413.

The test substance was to be 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 in consultation with the Sponsor following review of the results of a 2-week preliminary study in pregnant and non-pregnant rats (Report no. HST 400/942606).

Bone marrow samples were analysed to assess the mutagenic potential of the test substance in an *in vivo* cytogenic system. The methods and experimental design complied with EEC Annex to Directive 92/69/EEC (Method B12).

This report contains all the relevant data collected during the study. All specimens, raw data and other documents generated at Huntingdon Life Sciences Ltd pertaining to the study, together with a copy of this final report, have been lodged in the Huntingdon Life Sciences Archive, Huntingdon, England.

RELEVANT STUDY DATES

Approved by:

Study Director:	24 July 1995
HRC Management:	12 July 1995
Study Sponsor:	14 July 1995

Animals arrived at HRC: 12 July 1995

Exposures commenced:
♂ - 9 August 1995
♀ - 10 August 1995

Haematology/biochemistry:

Week 13:	6 November 1995
Week 26:	2 February 1996

Terminal kill:

Main groups:	♂ - 9 November 1995
	♀ - 10 November 1995
Withdrawal:	7 February 1996

TEST SUBSTANCE

Identity:	Acetic anhydride
Appearance:	Liquid
Storage conditions:	Ambient temperature, dry
Batch no.:	50218009
Expiry date:	Responsibility of the Sponsor
Purity:	99% +
Date received:	3 August 1995
Supplier:	Sigma-Aldrich Co Ltd

EXPERIMENTAL PROCEDURE

ANIMALS

One hundred and fifty-six (78 male and 78 female) rats, aged approximately 6 - 7 weeks, of the CD® (Sprague-Dawley) strain were obtained from Charles River (UK) Limited, Manston Road Margate, Kent, on 12 July 1995.

All of the rats were examined upon receipt and appeared to be in good health.

Five male (131 - 135) and five female (136 - 140) rats were selected at random and sacrificed as health check on the day of arrival.

A detailed macroscopic examination at necropsy revealed no abnormalities.

Allocation took place on 18 July 1995. Individual bodyweights were processed using a computer program which selected 130 rats (65 male and 65 female) for allocation to 5 groups such that the group mean bodyweights were approximately equalised. These animals were then uniquely identified by numbers tattooed into the ear pinnae and where appropriate on the foot.

The identification of individual rats in the groups together with the initial group mean weights at allocation were as follows:

Group	Rat numbers				Group mean bodyweights (g)	
	Main		Withdrawal			
	♂	♀	♂	♀	♂	♀
1 (Air control)	1 - 10	61 - 70	11 - 15	71 - 75	227	184
2 (Low dose)	16 - 25	76 - 85	26 - 30	86 - 90	227	184
3 (Inter dose)	31 - 40	91 - 100	41 - 45	101 - 105	227	184
4 (High dose)	46 - 55	106 - 115	51 - 60	116 - 120	227	184
5 (Positive control for micronucleus test)	121 - 125	126 - 130	-	-	226	184
Reserve	A - E	F - J				

Five male and 5 female were assigned to the reserve group. These were retained as potential replacements during the acclimatisation period. Following the commencement of exposures the reserves were killed.

Group 5 animals were not subjected to inhalation exposures. The sole purpose of this group of animals was to act as a positive control for the micronucleus test used to assess the mutagenic potential of acetic anhydride. Details are presented in Appendix 1.

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. Each cage was 35 cm wide, 53 cm long and 25 cm high. Plastic trays lined with absorbent paper, were placed below each cage to collect animal excreta. The paper was changed daily. Clean cages were introduced at intervals throughout the study. 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 fur of 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 TH105 chart recorder. The study holding room conditions were maintained within the limits 20 ± 3°C and 55 ± 15%. Occasional deviations outside the stated protocol range were minor and are considered not to have affected the integrity of the study. The data are presented in Appendix 2.

Lighting was controlled to give 12 hours light (0730 - 1930 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. Analyses were made on all batches of diet used to establish levels of basic nutrients and of specific substances and micro-organism likely to have been present in the feed components and which, if in excess of specified amounts, might have an undesirable effect on the test system.

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 rinsed 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. Additionally, levels of specified substances known to be present from time to time in local water and which, if in excess of the maxima recommended for (human) drinking water, might have an undesirable effect on the test system, are determined in the tap water at intervals.

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

TEST ATMOSPHERE GENERATION (Figure 1)

The vapour was generated by metering the test substance from a Fortuna® all polypropylene syringe mounted on an infusion pump (Precidor® type 5003) to a glass frit contained in a glass vessel. Air was passed through the vapouriser at a rate of 80 l/min. In order to facilitate production of the vapour, the air to the vapouriser passed through a copper coil immersed in a water bath, maintained at 60 ± 1°C. The vapour/air mixture passed out of the vapouriser into the chamber inlet ducting. The vapour generating equipment was housed in an extracted cabinet.

Different chamber concentrations were achieved by varying the syringe pump infusion rate.

For all groups the vapour/air mixture produced passed along the chamber inlet ducting to a tangential inlet mounted at the apex of the chamber where it was mixed with additional diluent air at a rate (approximately 570 l/min) sufficient to maintain the total chamber airflow at approximately 650 l/min.

The air control group received clean air only at a rate of approximately 650 l/min.

EXPOSURE CHAMBERS (Figure 2)

The exposure chambers were of stainless steel and glass construction, consisting of a cuboidal main body fitted with pyramidal base and top. Each chamber was approximately 2.43 m^3 internal volume.

At the apex of the upper pyramidal figure was the tangentially mounted air inlet. Immediately below the air inlet was a perforated canister that ensured even distribution of the test atmosphere within the chamber.

The animal exposure cages were of the suspended basket type, constructed of stainless steel mesh. Each basket had a capacity to hold ten rats, individually housed during exposure, and 4 such baskets were suspended on the middle levels in the chamber. Above each basket was a mesh sheet to prevent the rats from leaving the individual compartments during exposure.

Projecting through the rear wall of each chamber were 4 × 0.25 inch diameter stainless steel tubes. Each of the tubes was of a different length. These were used for collection of chamber atmosphere samples.

A three-inch diameter, square-shaped exhaust plenum, perforated ventrally was positioned in the lower pyramidal figure, connecting with the chamber extract system.

A wet and dry thermistor device, used to monitor chamber temperature and produce data in order to derive chamber humidity, was situated in the extract duct outside the chamber body.

The pyramidal base sloped from the front to the rear of the chamber where a 2 inch diameter drain was sited. This chamber drain connected with a common drainage duct through a ball-valve.

Access to the interior of the chamber was by use of a hinged, glass panelled stainless steel framed door at the front of the main body of the chamber. The door was sealed using moulded rubber sealing strip and four screw clamps.

A wall-mounted magnehelic gauge (0 to 25 mm water gauge) was connected through the rear wall of the chamber and monitored the chamber pressure relative to the exposure room.

The operational parameters of the chambers during generation are summarised below:

Vapouriser (generation) airflow:	80 litres/minute.
Water bath thermostirrer unit setting:	60°C.
Chamber (diluent) airflow:	Approximately 570 litres/minute (Test chambers) and 650 litres/minute (Control chamber).
Chamber pressure:	1 to 10 mm water gauge below ambient.

PROCEDURE

The rats were transferred from the holding cages and placed into the individual compartments of the exposure cages. During this study, four exposure baskets were used positioned centrally within the chamber. Male and female rats were alternated weekly between the left and right side of the chambers.

The chamber doors were closed and secured. The generator airflow was turned on and the magnehelic gauge checked to ensure that operation of the chamber took place with internal pressure below that of the room.

The syringes were filled with test substance, the volume of liquid was recorded and the syringes were mounted on the infusion pumps. The syringes were connected to the vapourisers by PTFE tubing. The pumps were turned on briefly in order to engage the gearing and to move the liquid along the PTFE tubing almost to the surface of the glass frit. The injection rates to be used were then selected.

Generation commenced as the pumps were turned on and simultaneously the chamber environmental monitoring system was activated (see below). At intervals of 30 minutes, any reactions by the rats to exposure, together with checks of generation and chamber operational parameters were recorded. Chamber performance in terms of airflow, temperature and relative humidity was monitored automatically and sequentially during 6-minute intervals for each chamber by a computer driven monitoring system throughout each exposure.

Samples for the determination of acetic anhydride in the chamber atmosphere were removed from each chamber at approximately hourly intervals throughout each exposure. Each sample was removed from a different sampling point within each chamber.

At the end of six hours generation, the infusion pumps were turned off and the volume of the remaining syringe contents were recorded. Generation airflow was turned off and the chamber extract allowed to clear the vapour for 15 to 30 minutes.

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.

During loading, unloading and washing the chambers, protective gloves and respiratory protection were worn.

CHAMBER MONITORING SYSTEM

Information of the environment within each inhalation chamber was collected at intervals of 6 minutes during each 6-hour exposure period. The data collection sequence and display were controlled by a microprocessor and all information collected was displayed on a monitor and printed as hard copy. Simultaneously, the data was transferred and stored on a 3.5 inch floppy disk. At the end of each day, all data were reformatted and presented as separate records for each chamber monitored.

A Tulip AT Compact 3 computer screen connected with an NEC pinwriter P20 printer were used to monitor and record the system performance during exposure.

Monitoring of the chamber environment was organised by a program written at Huntingdon Life Sciences. This program was composed of three basic stages of operation: an initial setting up, pre-exposure phase, an exposure monitoring phase and the post exposure data collation and presentation phase.

Setting-up phase

The initial phase comprises entry of study identification, dates, times and other relevant study details together with airflow transducer calibration and actual chamber airflow data sets.

Exposure monitoring phase

This phase was started coincident with the commencement of generation. Each chamber's environment was monitored during a 6-minute cycle, during which airflow and temperatures were recorded, and relative humidity calculated. The data were displayed on screen, printed and stored on disc. This cycle of monitoring took place in the following sequence: Air control, Low dose, Intermediate dose and High dose, and was repeated throughout the six-hour exposure period. A total of twelve cycles were recorded. During each 6-minute cycle the data collected from each chamber were as follows:

Temperature	(°C)
Relative humidity	(%)
Airflow	(litres/minute)

Post exposure phase

At the end of 6 hours, the data collected during exposure were collated into separate groups. The mean values, together with standard deviation were calculated for each parameter recorded. These data were printed and stored on disc. The first set of data was excluded from calculation of the mean because chamber temperatures did not stabilise until approximately 15 - 20 minutes from the start of exposure (equilibration time, t_{99} was 17 minutes).

The monitoring systems are described in more detail as follows:

Temperature and relative humidity - Temperature and relative humidity measurements were based on the wet and dry bulb principle using a device manufactured at Huntingdon Life Sciences, Huntingdon. Two matched platinum resistance probes were mounted in thermally isolated fittings in a perforated plate, located in a tubular body approximately 2.5 cm in diameter. One of the probes was covered by a close fitting wick which dipped into a distilled water reservoir attached to the upper end of the device. Relative humidity was calculated from psychrometric data stored in a look-up table in the memory of the microprocessor.

Airflow - The volume flow of air to the exposure chambers was measured using brass-bodied Venturi nozzles and associated differential transducers and transmitters([1]). The Venturi nozzles were installed in the air supply ductwork leading to each chamber, in accordance with recommendations for such devices.

Each Venturi nozzle used was supplied with a calibration of pressure against airflow. Mathematical descriptions of the calibrations were derived and incorporated into the system program, enabling flows to be calculated from the signals provided by the transmitters. The integrated operation of the flow measuring system was checked by applying a range of pressure signals to the transducer and comparing the flows indicated by the system with the calibration data supplied by the manufacturer. Correction factors for the installation were applied, to allow for actual, compared with calibration conditions.

([1]) Venturi nozzles, Type VN 32/P/B; Transducers, Type TR 7; Transmitters, Type TR 2 - all supplied by Tekflo Ltd, Albany Road, Granby Industrial Estate, Weymouth, Dorset, England

TARGET CONCENTRATIONS

The target concentrations of acetic anhydride were:

Group	Designation	Concentration (ppm)
2	Low dose	1
3	Inter dose	5
4	High dose	20

The target concentrations in this study were selected following review of available data supplied by the Sponsor together with effects seen in a 2-week inhalation study performed at Huntingdon Life Sciences, Huntingdon using the same test substance (Report no. HST 400/942606).

EXPOSURE CHAMBER CONDITIONS

Chamber analysed concentration of acetic anhydride

Samples of the test chamber air were removed at approximately hourly intervals throughout each exposure.

Samples were withdrawn *via* sample ports on the back of the exposure chamber into a gas-tight 1 ml syringe. The samples were analysed by gas chromatography. The methodology is presented in Appendix 12.

Chamber spatial distribution

During exposures, each of the samples removed was taken from a separate sample port to the one previous. In this way a measure of 'spatial' distribution was made to confirm uniform distribution of test atmospheres within the chamber.

Nominal concentration of chamber atmospheres

The chamber nominal concentrations were calculated from the amount of liquid used over the six-hour exposure period, the density of the liquid and the exposure mean airflow. The formula used was as follows:

$$\text{ppm} = \frac{\text{Usage (ml)} \times 1.083 \times 24450}{\text{Airflow (m}^3\text{/mm)} \times \text{exposure duration (mins)} \times 10^2}$$

where 1.083 = relative density of acetic anhydride
24450 = molar volume (ml) of an ideal gas at 25°C and 760 mmHg
102 = molecular weight of acetic anhydride

Airflow, temperature and relative humidity

These parameters were recorded automatically by the chamber monitoring system, the details of which have been described previously.

CLINICAL SIGNS

During exposure

Clinical signs during exposure were recorded as a group response where all visible animals appeared to be responding similarly or a proportion were affected. Group responses seen during exposure were not transferred to the individual clinical signs sheets, and are reported in the text of this report.

At other times

Animals were examined twice each day, usually prior to loading and immediately following unloading from the chambers on exposure days, and in the morning and afternoon of non-exposure days. An entry was made in the individual clinical signs sheets twice daily, even if abnormalities were not seen.

BODYWEIGHT

Each rat was weighed for allocation to groups, then on each week commencing 3 weeks before the start of exposures and continuing throughout the study.

In addition, the weight of each rat at necropsy was recorded.

FOOD CONSUMPTION

The quantity of food consumed by each cage of rats was recorded weekly commencing 3 weeks prior to the start of exposures until the end of the study.

OPHTHALMIC EXAMINATION

The eyes (anterior structure, vitreous and fundus) of all rats allocated to experimental groups were examined once before the start of dosing using Keeler indirect ophthalmoscope. Similarly, all main study rats, were examined during Week 13 of dosing (prior to dosing on the day) and all withdrawal rats were examined during Week 26.

Prior to examination, the pupils of each animal were dilated using a Tropicamide ophthalmic solution (0.5% Mydriacyl, Alcon Laboratories).

LABORATORY INVESTIGATIONS

Sample collection

Blood samples were collected from all main study animals on one occasion during Week 13 (following ophthalmoscopic examination) and from all withdrawal rats during Week 26.

Samples of venous blood were obtained from the orbital sinus using sterile glass pipettes while the rats were held under light ether anaesthesia. Food was withheld from the animals overnight prior to sampling.

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

EDTA	for haematological investigations
Citrate	for coagulation tests
Heparin	for biochemical investigations

A limited range of parameters were measured at Week 26 and are indicated with an asterisk (*) in the following lists of investigations.

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

Haematology	**Units**
The following estimations were performed on samples withdrawn during Week 13 using a Bayer-Technicon H1E haematology analyser:	
*Packed cell volume (PCV)	%
*Haemoglobin (Hb)	g/dl
*Red cell count (RBC)	$\times 10^6/mm^3$
Absolute indices calculated as follows:	
Mean corpuscular haemoglobin concentration (MCHC) Hb (g/dl) × 100 ÷ PCV (%)	g/dl
Mean corpuscular volume (MCV) PCV (%) × 10 ÷ RBC ($\times 10^{12}$/l)	fl
Mean corpuscular haemoglobin (MCH) Hb (g/dl) × 10 ÷ RBC ($\times 10^{12}$/l)	pg
Total white cell count (WBC Total)	$\times 10^9$/l
Differential WBC count (Diff)	
Neutrophils (N) Lymphocytes (L) Eosinophils (E) Basophils (B) Monocytes (M) Large unstained cells (LUC)	$\times 10^9$/l

Cell morphology: the most common morphological changes (anisocytosis, micro/macrocytosis, variation in colour, hypo/hyperchromasia, left shift, atypical/blast cells) were recorded as follows:

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

	Units
In the case of atypical/blast cells, or other abnormalities, confirmation or a written description from a blood film was made.	
Platelet count (Plts)	$\times 10^9$/l
*Thrombotest (TT) - Owren, P.A. (1959)	s
Reticulocyte count (retic) - Dacie, J.V. and Lewis, S.M. (1966)	% (of red cells)

Biochemistry

The following parameter was analysed with a Roche Cobas centrifugal analyser, using the appropriate BCL test kit:

Creatinine phosphokinase (CPK), also known as 'creatinine kinase' - reaction temperature 30°C	mU/ml

The following parameters were analysed with a Hitachi 737 clinical chemistry analyser, using standard Hitachi 737 methodology:

Total protein	g/dl
Albumin (Alb)	g/dl
Globulin (Glob) - by subtraction, total protein (g/dl) minus albumin (g/dl)	g/dl
*Urea nitrogen (Urea Nitr)	mg/dl
Alkaline phosphatase (AP) - reaction temperature 30°C	mU/ml
Total bilirubin	mg/dl
*Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic phosphorus (P)	mEq/l
Chloride (Cl)	mEq/l

	Units
*Cholesterol (Chol)	mg/dl
Glucose - hexokinase mediated	mg/dl
*Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase' - reaction temperature 30°C	mU/ml
*Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase' - reaction temperature 30°C	mU/ml
γ-Glutamyltransferase (γGT), - reaction temperature 30°C	mU/ml

TERMINAL SACRIFICE

Sacrifice

All main group animals were killed on the day following completion of 13 weeks of dosing. Withdrawal group animals were killed following 13 weeks withdrawal from exposures.

Animals were killed by intraperitoneal injection of pentobarbitone sodium and exsanguination from the brachial blood vessels. Immediately prior to exsanguination, the terminal bodyweight of each animal was recorded.

Macroscopic examination and organ weights

All rats were subjected to a detailed macroscopic *post mortem* examination.

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

adrenals	lungs	seminal vesicles
brain	ovaries	spleen
kidneys	prostate	testes*
liver		

* Testes weighed together with epididymides

Paired organs were weighed together.

Fixation of tissues

Samples, or the whole, of the following organs/tissues, together with any macroscopically abnormal entities were preserved in buffered 10% formalin. The eyes were preserved in Davidson's fixative and the testes in Bouin's fixative. The testes were transferred to 70% alcohol after 24 hours. The lungs were infused with fixative prior to immersion.

[a]adrenals
alimentary tract
animal identification mark
[a]aorta
[a]brain
[a]caecum
[a]colon
[a]duodenum
[a]epididymides
[a]eyes
femur with joint (for bone and marrow *in situ*)
[b]gross abnormalities
head (paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, eyelids, lachrymal gland, Harderian gland and Zymbal's gland)
[a]heart
[a]ileum
[a]jejunum
[a]kidneys
[b]larynx (2 levels) (Figures 4 and 5)
[a]liver
[b]lungs (all lobes and mainstem bronchi)
lymph nodes ([b]cervical, [a]mesenteric and [a]tracheobronchial)
[a]mammary gland
[b]nasal passages (3 levels) (Figure 3)
[a]oesphagus
optic nerve
[a]ovaries
oviduct
[a]pancreas
[a]pharynx
[a]pituitary
[a]prostate
[a]salivary gland
[a]sciatic nerve
[a]seminal vesicles
[a]skeletal muscle (thigh)
[a]skin
spinal column
[a]spinal cord (cervical, thoracic and lumbar)
[a]spleen
[a]sternum
[a]stomach (glandular and non-glandular)
[a]rectum
[a]testes
[a]thymus
[a]thyroids (with parathyroids)
tongue
[b]trachea (including bifurcation)
ureter
[a]urinary bladder
[a]uterus (corpus and cervix)
vagina

Microscopic examination

Light microscopic examination was performed on 4 μm thick sections, stained with haematoxylin and eosin, of those tissues in the table above. Testes and epididymides were stained with PAS/haematoxylin. The testes were cut TS and epididymides LS at approximately 2 μm.

The tissues examined are marked in the table above as follows:

[a] Rats from Main groups 1 (Air control) and 4 (High dose)

[b] All main group and withdrawal group rats.

Bone marrow sampling for micronucleus test

Bone marrow samples were collected from all rats in the main Groups 1 to 4 and from Group 5 (Positive control).

Details of the collection and treatment are presented in Appendix 1.

STATISTICAL ANALYSIS

All statistical analyses were carried out separately for males and females.

Data relating to food were analysed on a cage basis. For all other parameters the analyses were carried out using the individual animal as the experimental unit. Food consumption data were analysed using cumulative cage weekly average intake per group. Bodyweight data were analysed using weight gains. The following sequence of statistical tests was used for food consumption, bodyweight, organ weight and clinical pathology data:

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

Bartlett's test (1937) 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), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (1952/3) was used.

Except for pre-exposure data, analyses of variance were followed by Student's *t* test and Williams' test (1971/2) for a dose-related response, although only Williams' test was reported. The Kruskal-Wallis analyses were followed by Shirley's test (1977), the non-parametric equivalent of the *t* and Williams' tests.

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 affect the organ weights.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Chamber analysed concentration of acetic anhydride

The data are presented as follows:

Table 1 - Exposure mean chamber concentration of acetic anhydride
Appendix 3 - chamber concentration of acetic anhydride

The data are summarised below:

Group	Study mean concentration (ppm) (standard deviation)	
	Target	Analysed
2	1	0.98 (0.11)
3	5	4.96 (0.38)
4	20	20.0 (1.63)

The analysed concentrations were close to the target concentrations throughout the study.

Spatial distribution of the atmosphere within the chamber was considered to be uniform.

Nominal concentration of acetic anhydride

The data are presented in Table 2 and are summarised as follows:

Group	Study mean nominal conc. (ppm)	A/N (%)
2 (Low dose)	1.23	80
3 (Inter dose)	6.5	76
4 (High dose)	26.3	76

$$\text{A/N} \frac{\text{Analysed concentration}}{\text{Nominal concentration}} \times 100$$

The differences between analysed and nominal concentration are considered to be due to a degree of vapour condensation within the generation system and ductwork leading to the exposure chambers.

Temperature and relative humidity

The data are presented in Table 2 and are summarised as follows:

Group	Study mean	
	Temperature (°C)	Relative humidity (%)
1 (Air control)	20.8	66.7
2 (Low dose)	20.4	58.8
3 (Inter dose)	20.5	59.4
4 (High dose)	20.7	56.1

The differences between groups are considered not to have affected the outcome of the study.

CLINICAL SIGNS

Individual clinical signs at times other than during exposure are presented in Appendix 4.

During exposure signs were confined to the High dose. Partially closed eyes were observed during the first two exposures. This is a general, non-specific response to an irritant atmosphere. Subsequent to this no other clinical signs were seen during exposure.

At other times treatment-related signs were seen in all High dose animals. Signs were principally noisy breathing with occasional instances of red staining around the snout. The signs were observed throughout most of the 13-week exposure period.

Noisy breathing was also recorded in 1 male and 3 females from the Intermediate dose each on a single occasion.

During the withdrawal period noisy breathing was observed in a proportion of High dose animals only. The incidence progressively diminished through the withdrawal period and was no longer observed during Week 26.

BODYWEIGHT

The data are presented as follows:

Figure 6 - group mean values
Table 3 - group mean values
Appendix 5 - individual values

During the 13-week exposure period weight gain by High dose rats was significantly lower than that of controls. A small reduction in rate of gain was also seen in Intermediate dose rats, however, this did not achieve statistical significance. Weight gain by Low dose rats was similar to controls.

During the withdrawal period, weight gain by Intermediate dose males and High dose males and females exceeded that of controls indicating a regression of effect.

FOOD CONSUMPTION

The data are presented as follows:

Table 4 - group mean values
Appendix 6 - cage mean values

During the 13-week exposure period consumption by High dose rats was significantly lower than that of controls. Consumption by Intermediate and Low dose rats was comparable to that of controls.

During the withdrawal period consumption by all groups was similar.

OPHTHALMOSCOPIC EXAMINATION

The findings are presented in Appendix 7.

During Week 13 corneal changes were evident in a number of animals in the Intermediate (5 ppm) and High (20 ppm) dose level groups. Corneal lacklustre was recorded in three animals of each sex in Group 3 and one male and one female in Group 4. Corneal opacities were observed in one male animal in Group 3 and in six males and nine females in Group 4. Nasal quadrant keratitis was seen in one female animal in Group 4.

The corneal opacities were mostly pinpoint in size, and close to, or just below, the centre of the cornea. They were observed bilaterally in less than half of the cases. The incidence and distribution of the corneal changes is indicative of a treatment-related effect.

Other ophthalmoscopic findings were within normal limits for animals of this age and strain and were considered to be unrelated to treatment.

There were no treatment-related findings during the examination at Week 26.

LABORATORY INVESTIGATIONS

Haematology

The data are presented as follows:

Table 5 - group mean values
Appendix 8 - individual values

Packed cell volume, haemoglobin concentration and total red cell numbers were significantly greater in High dose rats of both sexes in comparison with controls, during Week 13 of exposure. The differences are considered to be treatment-related and were probably due to impaired gas exchange caused by respiratory tract lesions.

Other statistically significant differences from controls were lower lymphocyte and large unstained cell numbers and a higher thrombotest time in High dose females. These differences were small, inconsistent between the sexes and considered unlikely to be of toxicological importance.

During Week 26 differences between groups were small and considered to be of no toxicological importance.

Biochemistry

The data are presented as follows:

Table 6 - group mean values
Appendix 9 - individual values

During Week 13 of exposure blood cholesterol levels were lower in male and female High dose animals compared with controls. The difference was statistically significant in males only. The difference is considered to be treatment-related in that it probably reflects the reduced food consumption by these animals.

Other significant differences from control were lower albumin in High dose males, higher urea nitrogen and creatinine in the High dose of both sexes; higher alkaline phosphatase in High dose females; higher GPT in High dose males; higher sodium levels in High dose females; lower calcium levels in High dose males and higher chloride levels in High dose females. All of these differences were minimal in scale and are considered unlikely to be of any toxicological importance.

During Week 26 GPT and GOT levels were significantly greater in High dose females compared with controls. This finding is considered to be incidental. The values of all other parameters measured at Week 26 were similar in all groups.

TERMINAL STUDIES

Organ weights

The data are presented as follows:

Table 7 - group mean values
Appendix 10 - individual values

After 13 weeks of exposure the lung weights of High dose rats of both sexes were significantly ($p < 0.01$) greater than those of controls. This is considered to be a consequence of exposure to acetic anhydride.

Testicular weights in High dose males killed after 13 weeks of exposure were also significantly greater than controls, however, this occurred in the right testis only and since no abnormalities were seen during histopathological examination this finding is probably incidental.

Following 13 weeks of withdrawal the lung weights of male High dose rats were still significantly ($p < 0.05$) greater than controls, however, the difference was smaller than that seen at Week 14 and the difference was no longer present in females.

Macroscopic pathology

The data are presented as follows:

Table 8 - incidence summary
Appendix 11 - individual findings

The macroscopic examination performed at termination revealed the following changes:

Lungs - The lungs remained inflated when the thoracic cavity was opened (reported as 'not collapsed') in 5/10 male and 9/10 female rats treated with 20 ppm compared with 0/10 male and female control rats. Areas of consolidation were seen in 2/10 male and 1/10 female rats treated with 20 ppm compared with 0/10 male and female control rats.

Pale foci were observed in 5/10 female rats treated with 20 ppm compared with 1/10 female control rats.

Adipose tissue - A reduction in adipose tissue was seen in 9/10 male and 7/10 female rats treated with 20 ppm compared with 0/10 male and female control rats.

The incidence and distribution of all other findings were considered to fall within the expected background range of macroscopic changes.

Macroscopic examination performed after 13 weeks of withdrawal revealed no changes associated with acetic anhydride.

Microscopic examination

The data are presented as follows:

Table 9 - incidence summary
Appendix 10 - individual findings

Terminal kill

Treatment-related findings

A range of treatment-related changes were seen in the respiratory tract of both male and female animals from the high and intermediate dosage levels, the incidence of which is shown in the table below. For the majority of these lesions a statistically significant increase in incidence compared to controls was demonstrated.

Nasal passages - Lesions were primarily located in the anterior portion of the nose and comprised varying, predominantly minimal, degrees of hyperplasia of the respiratory epithelium, frequently with increased goblet cell prominence; inflammation associated with the respiratory epithelium and the presence of granular eosinophilic inclusions within the respiratory epithelium, possibly representative of globule leucocytes. In the transitional epithelium lining anterior portions of the nasal turbinates, hyperplasia, squamous metaplasia and inflammation, occasionally with areas of erosion, was seen together with the presence of granular eosinophilic inclusions which may represent the presence of globule leucocytes. These latter inclusions were more evident in animals from the intermediate dosage group than in those from the high dosage group where transitional epithelial squamous metaplasia was more prominent.

In general olfactory epithelium was unaffected by the acetic anhydride inhalation.

Various amounts of exudative inflammatory cell accumulation, predominantly minimal in degree, were seen in the nasal chambers of high dosage group animals and of a single male intermediate group animals. These changes achieved statistical significance as indicated below.

	Male				Female			
Group	**Control**	**Low**	**Inter.**	**High**	**Control**	**Low**	**Inter.**	**High**
Exudative inflammation								
Total	0	0	1	9**	0	0	0	8**
Minimal	0	0	1	8**	0	0	0	7**
Moderate	0	0	0	1	0	0	0	1
Respiratory epithelium Inflammation								
Total	0	0	1	9**	0	0	0	8**
Minimal	0	0	1	8**	0	0	0	6**
Moderate	0	0	0	1	0	0	0	2
Granular eosinophilic inclusions								
Total	0	0	1	5*	0	0	0	3
Minimal	0	0	1	1	0	0	0	1
Moderate	0	0	0	4*	0	0	0	2
Hyperplasia/prominent goblet cells								
Total	0	0	1	10**	0	0	1	10**
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	1	7**	0	0	0	7**
Moderate	0	0	0	3	0	0	0	3
Number of animals examined	10	10	10	10	10	10	10	10

(continued)	Male				Feale			
Group	**Control**	**Low**	**Inter.**	**High**	**Control**	**Low**	**Inter.**	**High**
Squamous metaplasia								
Total	0	0	0	6**	0	0	0	4*
Minimal	0	0	0	6**	0	0	0	3
Moderate	0	0	0	0	0	0	0	1
Transitional epithelium								
Inflammation								
Total	0	0	3	8**	0	0	0	8**
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	3	2	0	0	0	4*
Moderate	0	0	0	6**	0	0	0	3
Erosion								
Total	0	0	0	3	0	0	0	6**
Minimal	0	0	0	1	0	0	0	6**
Moderate	0	0	0	2	0	0	0	0
Granular eosinophilic inclusions								
Total	0	0	7**	1	0	0	9**	3
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	3	0	0	0	7**	0
Moderate	0	0	4*	1	0	0	1	0
Hyperplasia								
Total	0	0	9**	8**	0	0	8**	9**
Minimal	0	0	7**	8**	0	0	6**	7**
Moderate	0	0	2	0	0	0	2	2
Squamous metaplasia								
Total	0	0	1	9**	0	0	0	9**
Minimal	0	0	1	4*	0	0	0	4*
Moderate	0	0	0	5*	0	0	0	5*
Olfactory epithelium								
Hyperplasia								
Total	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Number of animals examined	10	10	10	10	10	10	10	10

* $p < 0.05$ ** $p < 0.01$ with Fisher's Exact Test

Larynx - Inflammatory infiltration, squamous metaplasia of the ventral epithelium and hyperplasia of the epithelium covering the arytenoid processes, frequently with varying degree of erosion or ulceration, were seen in the majority of animals from the high dosage group as well as occasional animals from the intermediate dosage group. In general these changes were of minimal to moderate severity and achieved statistical significance as shown in the table overleaf.

Group	Male				Female			
	Control	Low	Inter.	High	Control	Low	Inter.	High
Subepithelial inflammatory cell infiltration								
Total	0	0	1	9**	0	0	3	4*
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	1	7**	0	0	1	2
Moderate	0	0	0	2	0	0	1	2
Ventrolateral squamous metaplasia								
Total	0	0	2	10**	0	0	1	10**
Trace	0	0	2	0	0	0	1	0
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	0	9**	0	0	0	9**
Marked	0	0	0	1	0	0	0	0
Ventrolateral hyperplasia								
Total	0	0	0	2	0	0	0	2
Minimal	0	0	0	2	0	0	0	2
Ventrolateral erosion								
Total	0	0	0	1	0	0	0	2
Minimal	0	0	0	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Arytenoid hyperplasia								
Total	0	1	5*	10**	0	0	0	10**
Trace	0	1	0	0	0	0	0	0
Minimal	0	0	5*	1	0	0	0	0
Moderate	0	0	0	9**	0	0	0	10**
Arytenoid erosion/ulceration								
Total	0	0	0	9**	0	0	0	8**
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	0	5*	0	0	0	3
Moderate	0	0	0	4*	0	0	0	4*
Number of animals examined	10	10	10	10	10	10	10	10

* $p < 0.05$ ** $p < 0.01$ with Fisher's Exact Test

Trachea - Epithelial hyperplasia was seen at various sites, including at the carina, in the majority of animals from the high dosage group and in a proportion of intermediate dosage group animals. Also seen were epithelial squamous metaplasia at the carina; eosinophilic epithelial inclusions and inflammatory infiltration of the lamina propria. These changes were predominantly of a minimal degree of severity and achieved statistical significance as shown in the table overleaf.

	Male				Female			
Group	**Control**	**Low**	**Inter.**	**High**	**Control**	**Low**	**Inter.**	**High**
Inflammation								
Total	0	0	0	9**	0	0	0	10**
Minimal	0	0	0	6**	0	0	0	10**
Moderate	0	0	0	3	0	0	0	0
Eosinophilic epithelial inclusions								
Total	0	0	0	3	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Moderate	0	0	0	2	0	0	0	0
Epithelial hyperplasia								
Total	0	0	1	9**	0	0	0	10**
Minimal	0	0	1	7**	0	0	0	9**
Moderate	0	0	0	2	0	0	0	1
Squamous metaplasia								
Total	0	0	0	1	0	0	0	1
Minimal	0	0	0	1	0	0	0	1
Hyperplasia (carina)								
Total	0	0	0	8**	0	0	0	6**
Minimal	0	0	0	8**	0	0	0	5*
Moderate	0	0	0	0	0	0	0	1
Squamous metaplasia (carina)	0	0	0	6**	0	0	1	4*
Total	0	0	0	0	0	0	1	0
Trace	0	0	0	3	0	0	0	4*
Minimal	0	0	0	3	0	0	0	0
Moderate								
Number of animals examined	10	10	10	10	10	10	10	10

* $p < 0.05$ ** $p < 0.01$ with Fisher's Exact Test

Lungs - Various changes were seen in a proportion of animals primarily from the high dosage group. These included the presence of occasional perivascular inflammatory cells, increased prominence of bronchus-associated lymphoid tissue (BALT), small foci of fibrosis in the walls of the alveolar ducts and increased prominence of alveolar macrophages. Changes were predominantly of minimal severity and achieved statistical significance as shown in the table overleaf.

Group	Male				Female			
	Control	Low	Inter.	High	Control	Low	Inter.	High
Perivascular inflammatory cells								
Total	0	0	0	6**	0	0	0	7**
Trace	0	0	0	0	0	0	0	2
Minimal	0	0	0	6**	0	0	0	4*
Moderate	0	0	0	0	0	0	0	1
Prominent BALT								
Total	0	0	0	4*	0	0	0	2
Minimal	0	0	0	3	0	0	0	2
Moderate	0	0	0	1	0	0	0	0
Alveolar duct fibrosis								
Total	0	0	0	5*	0	0	0	9**
Trace	0	0	0	1	0	0	0	2
Minimal	0	0	0	4*	0	0	0	7**
Increased alveolar macrophages								
Total	0	0	0	3	0	0	0	5*
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	0	3	0	0	0	4
Number of animals examined	10	10	10	10	10	10	10	10

* $p < 0.05$ ** $p < 0.01$ with Fisher's Exact Test

Cervical lymph nodes - An increased severity, primarily from minimal to moderate, but not incidence of occurrence, was seen in high dosage group animals in respect of parameters indicative of overall activity - specifically an increase in the degree of medullary plasmacytosis. Statistical significance was only achieved in male high dosage group animals.

This change is probably associated with the inflammatory lesions of the respiratory tract.

Group	Male				Female			
	Control	Low	Inter.	High	Control	Low	Inter.	High
Plasmacytosis								
Total	6	5	7	9	8	2	6	8
Minimal	2	1	5	0	3	1	3	2
Moderate	1	3	2	8**	3	0	2	6
Marked	3	1	0	1	2	1	1	0
Number of animals examined	10	10	10	10	10	9	9	10

* $p < 0.05$ ** $p < 0.01$ with Fisher's Exact Test

Incidental changes (Termination)

The various other histological changes that were seen in animals from this study killed at termination were within the normal range of spontaneous lesions commonly encountered in animals of this species and age range and were, consequently, not considered to be of any toxicological significance.

Some minor perturbation of the splenic white pulp and of the thymic cortex, predominantly seen in occasional high dosage group animals, were considered to be fortuitous and not associated with treatment.

No histological changes were seen to correlate with, or to account for the treatment-related ophthalmoscopical or haematological observations. No changes were seen that might account for the increased testicular weights at necropsy.

Recovery kill

Treatment-related findings

The treatment-related changes that were seen in the respiratory tract of both male and female animals from the high and intermediate dosage levels at termination of dosing (see above) were diminished in both incidence and severity in the high dosage level group animals after the recovery period. With the exception of minor nasal passage lesions in a single male animal at the intermediate dosage level, regression was complete in this group. The principal findings are described below.

Nasal passages - After a period of recovery nasal passage lesions seen were reduced in both extent and severity and consisted of minor degrees of respiratory epithelium hyperplasia/goblet cell prominence with some transitional epithelial inflammation and localised hyperplasia, statistically significant only in male, high dosage group animals. Of the remaining lesions only respiratory epithelial hyperplasia/goblet cell prominence in males receiving the high dosage level was statistically significantly increased with relation to the control incidence.

	Male				Female			
Group	**Control**	**Low**	**Inter.**	**High**	**Control**	**Low**	**Inter.**	**High**
Respiratory epithelium								
Hyperplasia/prominent goblet cells								
Total	0	0	0	4*	0	0	0	1
Minimal	0	0	0	3	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Transitional epithelium								
Inflammation								
Total	0	0	1	2	0	0	0	2
Trace	0	0	0	2	0	0	0	0
Minimal	0	0	1	0	0	0	0	2
Hyperplasia								
Total	0	0	1	2	0	0	0	2
Trace	0	0	0	1	0	0	0	0
Minimal	0	0	1	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Number of animals examined	5	5	5	5	4	5	5	5

* $p < 0.05$ with Fisher's Exact Test

Larynx - After a period of recovery ventrolateral epithelial squamous metaplasia was seen in a single high dosage group male animal only. All other lesions had resolved.

	Male				Female			
Group	**Control**	**Low**	**Inter.**	**High**	**Control**	**Low**	**Inter.**	**High**
Ventrolateral squamous metaplasia								
Total	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Number of animals examined	5	5	5	5	4	5	5	5

Trachea - After a recovery period the treatment-related changes recorded in the trachea at termination were not detected.

Lungs - After a recovery period the lung lesions appeared to have resolved with the sole exception of an area of minor perivascular inflammatory cells in a single male animal previously receiving the high dosage level.

	Male				Female			
Group	**Control**	**Low**	**Inter.**	**High**	**Control**	**Low**	**Inter.**	**High**
Perivascular inflammatory cells								
Total	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Number of animals examined	5	5	5	5	4	5	5	5

Cervical lymph nodes - After a recovery period there was no evidence of any maintenance of the increased incidence of plasmacytosis seen at termination.

Incidental changes (Recovery)

The various other histological changes that were seen in animals from this study killed after a period of recovery were within the normal range of spontaneous lesions commonly encountered in animals of this species and age range and were, consequently, not considered to be of any toxicological significance.

Conclusion

Exposure of rats to acetic anyhdride by inhalation over a 13 week period at the high, and to a lesser extent at the intermediate dosage levels, produced treatment-related inflammatory, hyperplastic and metaplastic lesions at all levels of the respiratory tract. These changes are characteristic of the lesions that are produced by irritant compounds.

No treatment-related effects were discerned at the low dosage level.

After a period of recovery/withdrawal the majority of respiratory trace lesions were seen to have resolved with only some minor lesions still present in localised areas of occasional animals previously exposed at the highest dosage level.

DISCUSSION

In this study rats were exposed to acetic anhydride by inhalation for 6 hours a day, 5 days a week for 13 weeks. The mean exposure levels were 1.0, 5.0 and 20.0 ppm in air. Groups of rats were held unexposed for a subsequent 13-week withdrawal period.

Clinical signs, principally noisy breathing, consistent with exposure to an irritant atmosphere were observed in all rats exposed to 20 ppm. Bodyweight and food consumption were reduced in rats exposed to 20 ppm. A progressive recovery from treatment-related effects was observed over the withdrawal period. Corneal lesions were observed in most of the animals exposed to 20 ppm and in a few exposed to 5 ppm after 13 weeks of exposure, these findings are consistent with exposure to an irritant or mildly corrosive atmosphere. The changes were not observed histologically. No treatment-related effects on the eyes were present after 13 weeks of withdrawal.

Treatment-related changes in red cell parameters, that is increased PCV, Hb and RBCs, were probably an adaptive response to impaired gas exchange resulting from respiratory tract lesions. A reduction in blood cholesterol levels seen in rats exposed to 20 ppm probably reflects the reduced food intake by these animals. Again all treatment-related changes had regressed after 13 weeks of withdrawal.

At *post mortem* the lungs did not collapse on opening the thoracic cavity in a proportion of rats exposed to 20 ppm. Lung weights of rats exposed to 20 ppm were significantly greater than control rats. A degree of regression of effect was observed after 13 weeks of withdrawal. A reduction in adipose tissue seen in rats exposed to 20 ppm probably reflects the reduced food intake by these animals.

The histological changes reported in the respiratory tract of rats from this study that were exposed to acetic anhydride over a 13-week period were seen to be dose-related and consistent with changes typical of a substance acting as a local/contact irritant. The changes seen were predominantly localised inflammatory lesions with subsequent areas of epithelial hyperplasia and/or squamous metaplasia. In general the changes were of minimal to moderate severity in the majority of High dose animals. Changes seen in occasional Intermediate dose animals were, with a few exceptions, minimal in degree. Changes seen in cervical lymph nodes were associated with the normal processes of an inflammatory response and not directly caused by the inhaled material.

On cessation of exposure to the irritant material the changes were all seen to regress, indicating that long term damage had not been caused within the respiratory tract.

The changes reported were similar in character, but considerably less severe than, those reported in a previous inhalation study with this substance (Report number HST 400/942606) in which rats were exposed to doses of up to 400 ppm for a two week period. Although the period of exposure in the current study was longer (13 weeks) the maximum dose administered was only 20 ppm.

Acetic anhydride did not cause any substantial increase in the incidence of micronucleated immature erythrocytes or any substantial decrease in the proportion of immature erythrocytes. It is concluded that there was no evidence of chromosome damage or bone marrow cell toxicity after 13 weeks of exposure to acetic anhydride.

The no-effect-level for this study is considered to be 1.0 ppm acetic anhydride in air.

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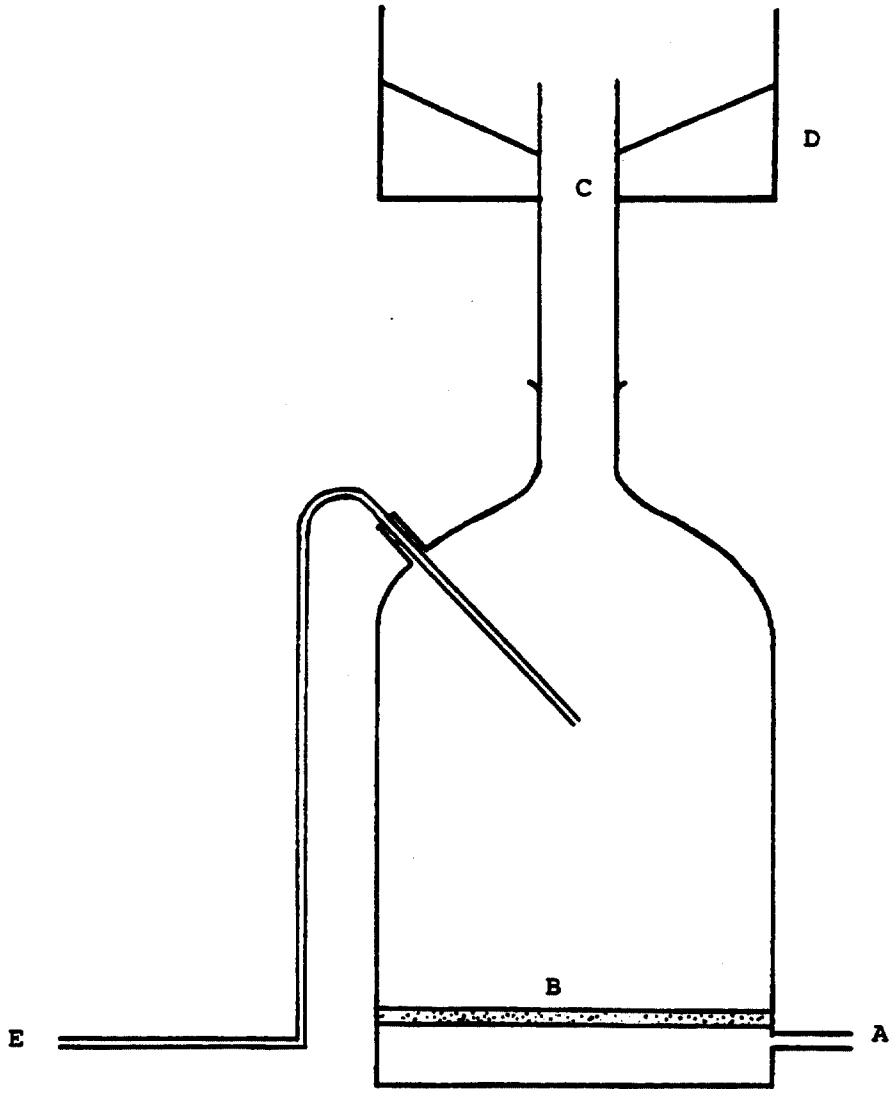
FIGURE 1

Vapour generator



Key

A Air line

B Fritted glass disc

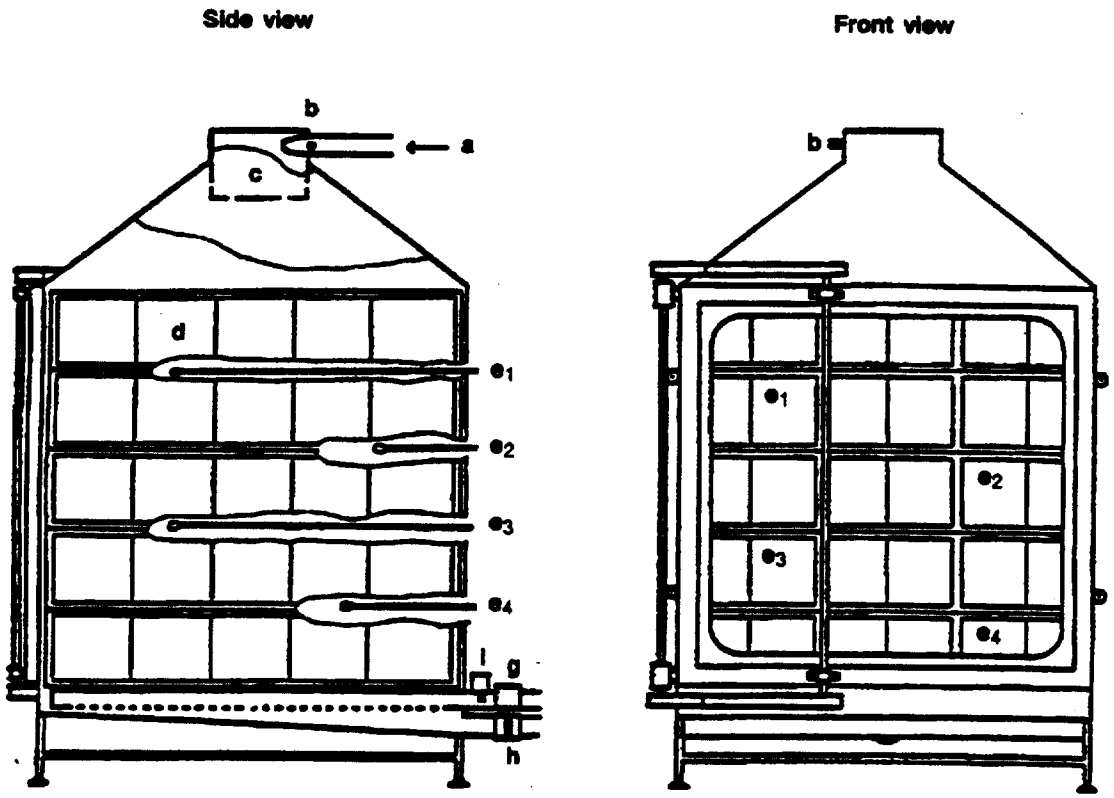
C Vapour outlet

D Stainless steel and glass elutriator

E Test substance supply line

FIGURE 2

Exposure chamber



Key

a	Chamber air inlet	f	Extract plenum
b	Vapour line	g	Extract duct
c	Dispersion device	h	Drain valve
d	Exposure cage	i	Temperature (relative humidity) probe
e_{1-4}	Sample lines		

FIGURE 3

Rodent nasal turbinates - sectioning levels

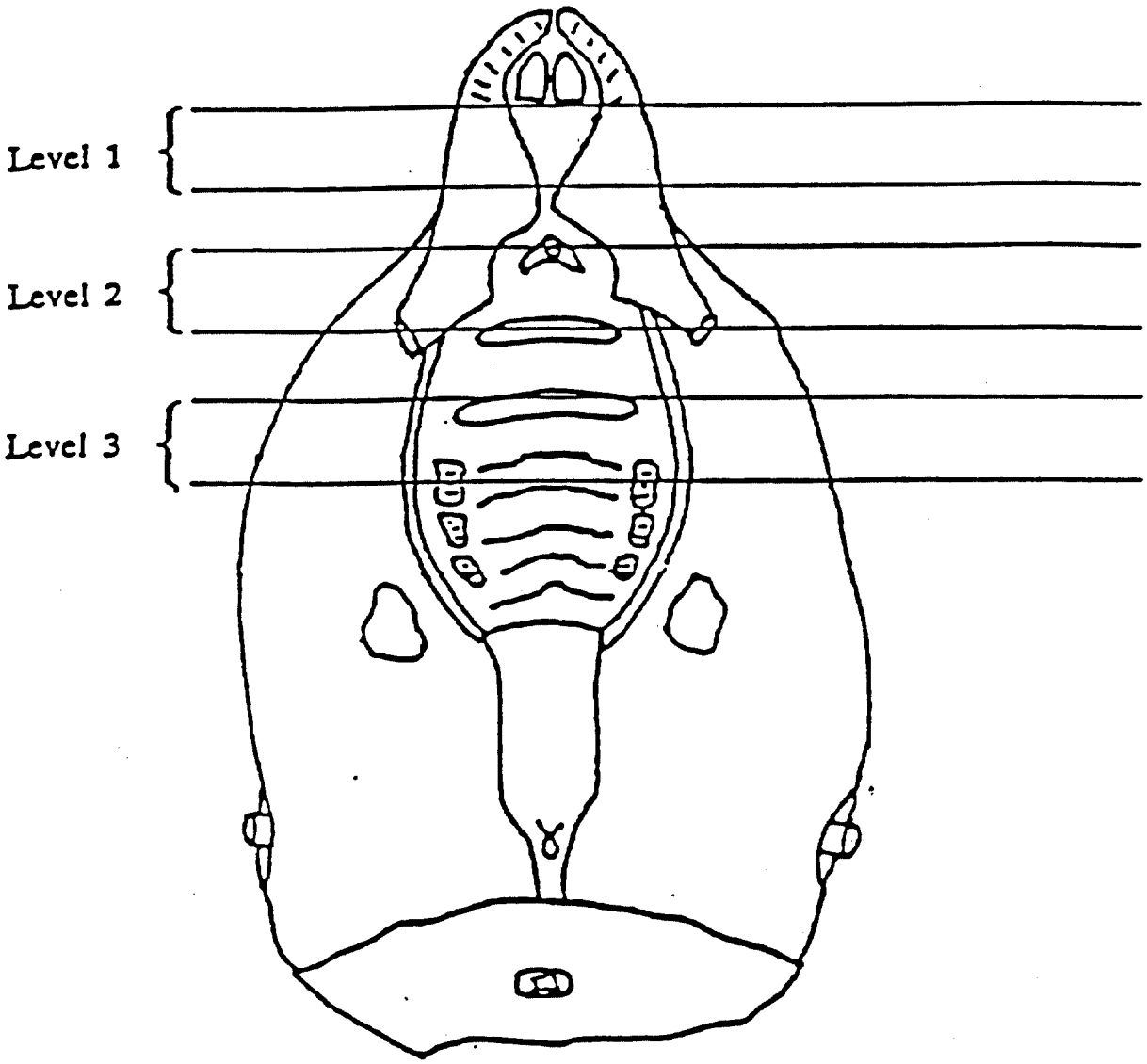


FIGURE 4

Levels of larynx processed for histology

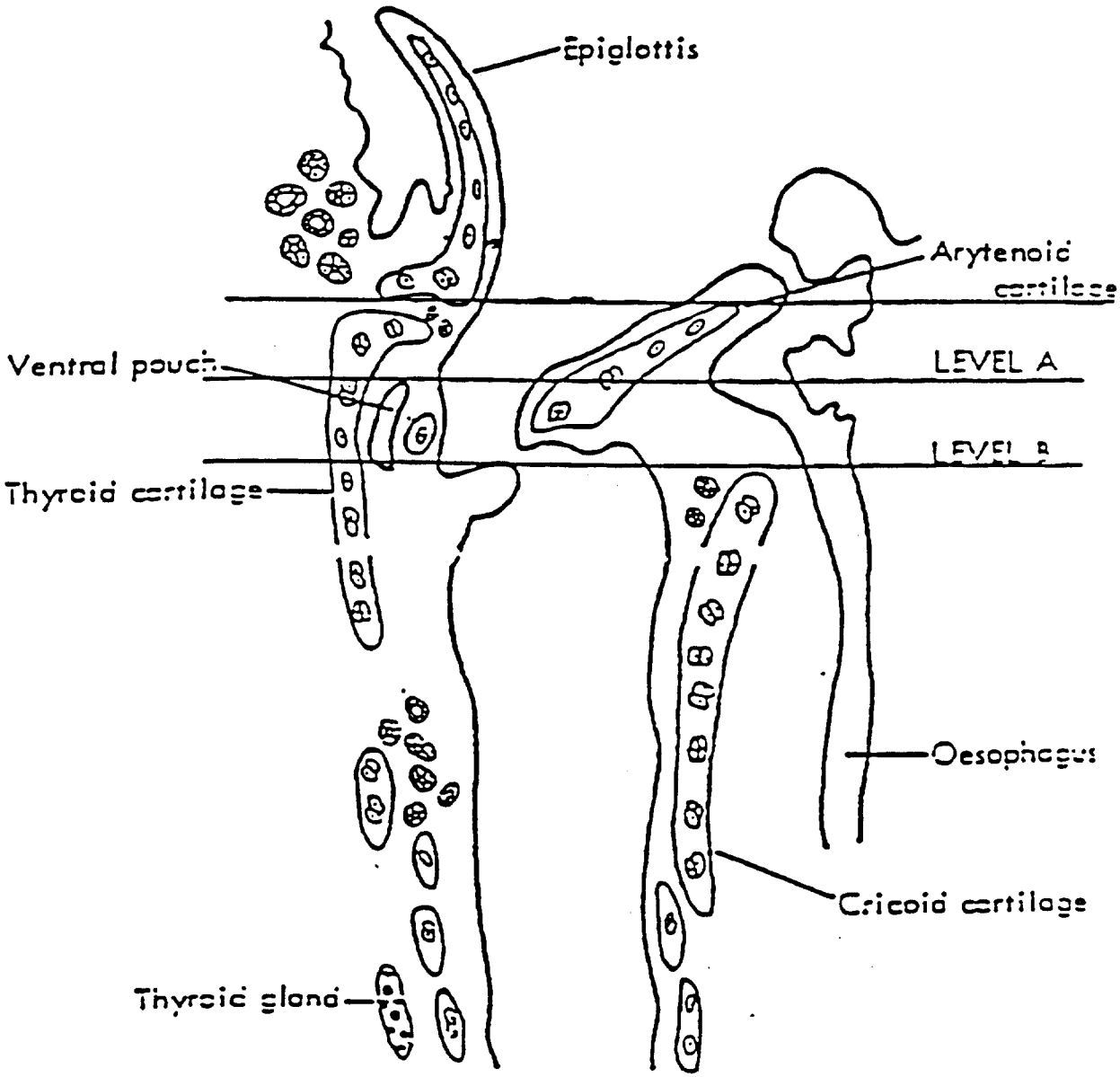


FIGURE 5

Rodent larynx - sectioning for inhalation studies

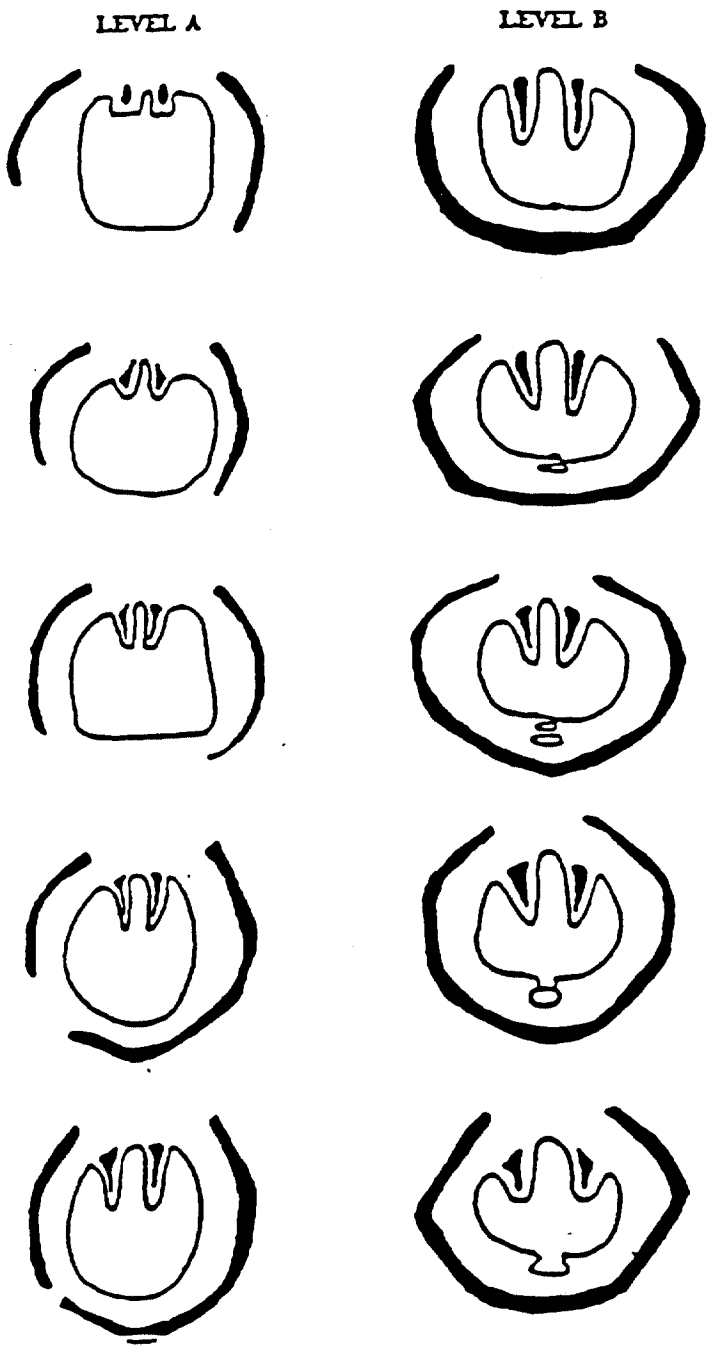


FIGURE 6

Bodyweights - group mean values

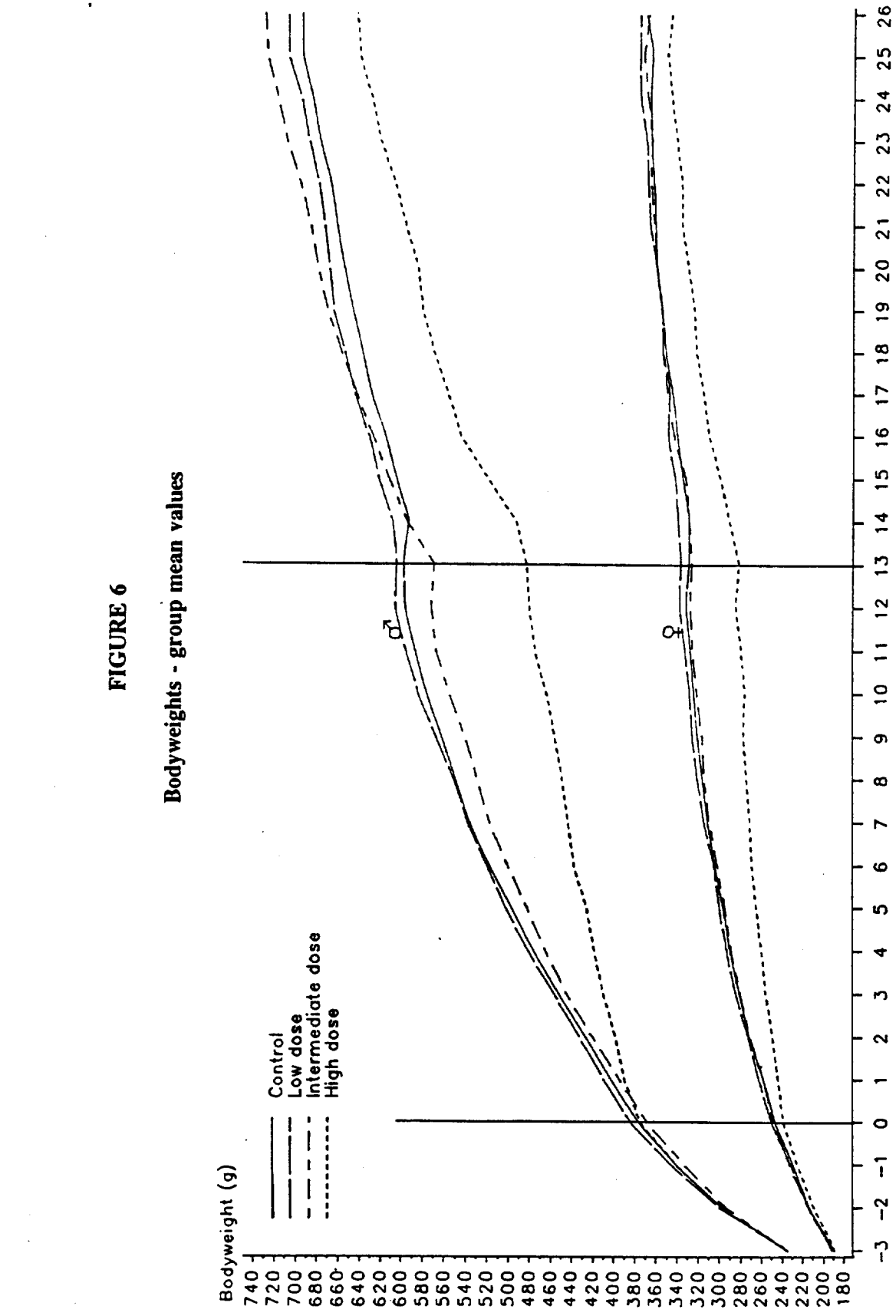


TABLE 1

Chamber concentrations of acetic anhydride

Exposure no.	Exposure mean concentration (ppm)		
	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
1	1.2	6.0	21.8
2	1.1	4.9	19.2
3	1.1	5.1	19.4
4	1.0	4.9	19.3
5	1.0	5.1	19.2
6	1.1	5.0	19.3
7	1.0	4.6	18.3
8	1.0	4.7	18.5
9	0.9	4.6	19.2
10	1.0	5.1	19.6
11	0.9	5.1	19.7
12	1.0	5.1	19.6
13	1.0	5.1	20.1
14	1.0	5.1	18.9
15	1.1	4.9	19.9
16	1.0	4.9	18.8
17	1.0	4.8	19.7
18	1.0	5.0	20.2
19	1.1	5.1	20.8
20	1.0	4.5	17.1

TABLE 1

(Chamber concentrations of acetic anhydride - continued)

Exposure no.	Exposure mean concentration (ppm)		
	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
21	0.8	4.3	17.0
22	0.8	4.6	15.8
23	0.8	4.3	16.6
24	0.9	4.1	16.1
25	0.9	3.4	13.9
26	0.7	4.1	18.4
27	0.8	4.6	20.1
28	0.8	4.5	17.3
29	0.8	4.9	19.7
30	0.8	4.9	21.0
31	0.8	4.6	20.0
32	0.9	4.9	21.0
33	0.8	4.8	20.5
34	0.9	4.8	20.3
35	1.0	4.9	19.4
36	0.9	5.3	21.4
37	1.0	5.3	20.9
38	0.9	5.1	19.9
39	1.0	5.4	21.7
40	1.0	5.0	20.6

TABLE 1

(Chamber concentrations of acetic anhydride - continued)

Exposure no.	Exposure mean concentration (ppm)		
	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
41	0.9	5.3	20.8
42	1.0	5.0	20.8
43	1.0	5.2	21.0
44	0.9	4.7	19.8
45	0.9	5.4	21.4
46	1.1	5.2	22.3
47	1.0	5.4	20.9
48	1.1	5.4	22.0
49	1.0	5.2	21.3
50	1.0	5.1	21.7
51	1.0	5.2	19.4
52	0.9	5.2	19.6
53	1.0	5.2	19.9
54	1.0	4.8	21.2
55	1.0	5.3	21.2
56	1.0	5.0	21.6
57	1.1	5.0	20.8
58	1.0	4.9	20.7
59	1.0	4.8	20.7
60	1.2	5.1	20.7
61	1.1	5.3	21.7
62	1.1	4.8	22.0
63	1.1	5.1	22.4
64	1.1	5.1	21.8
65	1.1	5.3	21.4
66	1.1	5.4	20.5
67	1.1	5.4	22.5
Mean	0.98	4.96	20.0
sd	0.109	0.382	1.63

sd Standard deviation

TABLE 2

Chamber conditions - daily mean values

Group 1: Control

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)	
1	20.3	(0.5)	65	(3)	647	(2)
2	20.9	(0.4)	66	(3)	653	(2)
3	20.5	(0.5)	68	(3)	652	(2)
4	20.9	(0.5)	63	(3)	656	(1)
5	20.6	(0.6)	65	(2)	646	(2)
6	20.9	(0.5)	65	(3)	655	(1)
7	20.6	(0.4)	67	(3)	655	(1)
8	20.7	(0.4)	67	(4)	655	(3)
9	20.9	(0.4)	65	(3)	654	(2)
10	20.9	(0.5)	67	(3)	650	(3)
11	20.9	(0.4)	69	(3)	664	(3)
12	20.5	(0.4)	72	(3)	656	(2)
13	20.8	(0.4)	71	(2)	658	(3)
14	20.9	(0.4)	70	(2)	661	(1)
15	20.7	(0.5)	70	(3)	645	(2)
16	20.6	(0.4)	71	(3)	646	(3)
17	20.8	(0.4)	70	(2)	659	(1)
18	20.6	(0.3)	71	(2)	658	(2)
19	20.8	(0.5)	69	(5)	657	(1)
20	20.7	(0.3)	66	(2)	655	(2)
21	20.6	(0.4)	67	(4)	659	(6)
22	20.8	(0.3)	70	(4)	664	(1)
23	20.6	(0.4)	66	(3)	671	(2)
24	21.0	(0.3)	65	(2)	662	(2)
25	20.8	(0.5)	66	(3)	660	(2)
26	20.7	(0.5)	66	(4)	655	(2)
27	20.8	(0.4)	66	(5)	669	(7)
28	20.6	(0.4)	68	(4)	655	(1)
29	20.9	(0.4)	68	(6)	646	(2)
30	20.7	(0.3)	68	(3)	647	(3)
31	20.7	(0.3)	68	(2)	659	(1)
32	21.1	(0.7)	66	(3)	644	(2)
33	21.4	(1.0)	65	(5)	649	(3)
34	20.8	(0.5)	64	(3)	656	(3)
35	20.6	(0.4)	67	(4)	663	(2)
36	20.5	(0.4)	56	(9)	650	(2)
37	20.8	(0.4)	67	(3)	638	(2)
38	20.8	(0.4)	66	(3)	648	(2)
39	20.9	(0.3)	66	(2)	655	(4)
40	20.6	(0.4)	71	(2)	650	(3)

Figures in parentheses denote standard deviations

TABLE 2

(Chamber conditions - continued)

Group 1: Control (continued)

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)	
41	20.9	(0.2)	67	(4)	657	(2)
42	21.2	(0.7)	67	(4)	660	(1)
43	20.9	(0.4)	68	(2)	657	(1)
44	21.2	(0.5)	65	(4)	655	(2)
45	21.0	(0.3)	68	(3)	651	(2)
46	21.0	(0.4)	68	(3)	656	(2)
47	20.9	(0.5)	68	(3)	662	(2)
48	20.9	(0.5)	69	(4)	663	(2)
49	21.0	(0.4)	67	(4)	665	(5)
50	20.7	(0.5)	68	(4)	657	(2)
51	20.9	(0.5)	67	(3)	659	(3)
52	21.0	(0.4)	67	(2)	652	(3)
53	20.9	(0.5)	66	(3)	659	(2)
54	21.1	(0.4)	63	(3)	659	(1)
55	20.9	(0.6)	66	(4)	664	(1)
56	21.0	(0.4)	66	(3)	660	(3)
57	20.9	(0.4)	66	(2)	653	(2)
58	20.8	(0.5)	67	(3)	661	(1)
59	21.1	(0.4)	64	(2)	652	(2)
60	20.8	(0.7)	66	(3)	664	(1)
61	21.1	(0.3)	64	(2)	666	(2)
62	21.3	(0.1)	63	(1)	664	(2)
63	20.8	(0.4)	67	(3)	666	(1)
64	21.1	(0.2)	65	(2)	665	(4)
65	20.7	(0.8)	66	(2)	664	(2)
66	21.0	(0.4)	65	(2)	667	(2)
67	20.2	(0.3)	66	(2)	659	(1)
Mean	20.8		66.7		656.7	
sd	0.22		2.43		6.68	

Figures in parentheses denote standard deviations
sd Standard deviation

TABLE 2

(Chamber conditions - continued)

Group 2: Low dose

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)		Concentration (ppm)		A/N %
							Nominal	Analysed	
1	20.1	(0.3)	55	(3)	643	(3)	1.23	1.2 (0.13)	98
2	20.4	(0.4)	59	(5)	650	(2)	1.22	1.1 (0.12)	90
3	19.6	(0.4)	63	(3)	650	(2)	1.44	1.1 (0.14)	76
4	20.5	(0.3)	57	(3)	656	(2)	0.99	1.0 (0.15)	101
5	20.3	(0.5)	58	(4)	646	(1)	1.12	1.0 (0.17)	89
6	20.6	(0.3)	57	(4)	655	(2)	0.99	1.1 (0.14)	111
7	20.5	(0.3)	57	(2)	655	(1)	1.10	1.0 (0.15)	91
8	20.4	(0.4)	58	(2)	654	(2)	1.11	1.0 (0.08)	90
9	20.7	(0.4)	55	(5)	653	(2)	1.11	1.0 (0.23)	90
10	20.6	(0.4)	57	(3)	648	(3)	1.11	1.0 (0.23)	90
11	20.6	(0.3)	60	(2)	662	(3)	0.98	1.0 (0.23)	102
12	20.3	(0.3)	62	(2)	655	(2)	0.99	1.0 (0.17)	101
13	20.4	(0.3)	63	(2)	656	(2)	0.99	1.0 (0.38)	101
14	20.5	(0.3)	61	(2)	659	(1)	1.09	1.0 (0.13)	92
15	20.2	(0.4)	62	(2)	645	(3)	1.01	1.1 (0.10)	109
16	20.3	(0.3)	61	(2)	646	(3)	1.12	1.0 (0.21)	89
17	20.4	(0.3)	62	(2)	657	(1)	1.10	1.0 (0.15)	91
18	20.3	(0.2)	62	(3)	657	(1)	1.10	1.0 (0.26)	91
19	20.4	(0.3)	59	(2)	656	(1)	1.10	1.1 (0.24)	100
20	20.4	(0.3)	61	(2)	654	(1)	1.22	1.0 (0.00)	82
21	20.2	(0.3)	62	(4)	656	(7)	1.10	0.8 (0.28)	73
22	20.4	(0.2)	59	(2)	663	(2)	1.09	0.8 (0.26)	73
23	20.2	(0.3)	60	(3)	669	(1)	0.97	0.8 (0.22)	82
24	20.6	(0.2)	56	(2)	660	(1)	1.09	0.9 (0.22)	83
25	20.4	(0.4)	58	(3)	659	(1)	0.98	0.8 (0.14)	82
26	20.2	(0.4)	60	(2)	654	(2)	0.99	0.7 (0.10)	71
27	20.4	(0.4)	59	(2)	669	(7)	1.08	0.8 (0.14)	74
28	20.3	(0.3)	61	(3)	654	(2)	1.10	0.8 (0.15)	73
29	20.2	(0.2)	60	(3)	647	(3)	1.23	0.8 (0.12)	65
30	20.3	(0.2)	60	(3)	646	(2)	1.12	0.8 (0.12)	71
31	20.3	(0.2)	60	(3)	656	(1)	1.21	0.8 (0.10)	66
32	20.8	(0.7)	58	(2)	641	(1)	1.24	0.9 (0.14)	73
33	21.1	(1.0)	58	(5)	648	(2)	1.34	0.8 (0.10)	60
34	20.5	(0.3)	57	(1)	653	(2)	1.33	0.9 (0.14)	68
35	20.2	(0.3)	58	(3)	667	(3)	1.30	1.0 (0.18)	77
36	20.1	(0.3)	46	(8)	649	(2)	1.44	0.9 (0.08)	63
37	20.3	(0.3)	59	(2)	636	(2)	1.47	1.0 (0.13)	68
38	20.4	(0.4)	59	(2)	645	((2)	1.45	0.9 (0.17)	62
39	20.5	(0.3)	58	(2)	653	(3)	1.44	1.0 (0.15)	69
40	20.3	(0.3)	61	(3)	649	(2)	1.33	1.0 (0.21)	75

Figures in parentheses denote standard deviations
A/N Analysed × 100 ÷ Nominal

TABLE 2

(Chamber conditions - continued)

Group 2: Low dose (continued)

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)		Concentration (ppm)		A/N %
							Nominal	Analysed	
41	20.4	(0.1)	59	(2)	656	(2)	1.32	0.9 (0.15)	68
42	20.7	(0.7)	60	(5)	657	(1)	1.32	1.0 (0.13)	76
43	20.5	(0.3)	59	(2)	656	(1)	1.32	1.0 (0.18)	76
44	20.7	(0.3)	56	(3)	654	(2)	1.32	0.9 (0.13)	68
45	20.6	(0.2)	60	(4)	650	(1)	1.33	0.9 (0.20)	68
46	20.4	(0.3)	61	(3)	656	(2)	1.32	1.1 (0.12)	83
47	20.5	(0.4)	59	(3)	661	(2)	1.31	1.0 (0.15)	76
48	20.4	(0.4)	59	(5)	662	(1)	1.31	1.1 (0.09)	84
49	20.6	(0.3)	56	(3)	662	(4)	1.31	1.0 (0.17)	76
50	20.3	(0.3)	60	(4)	655	(2)	1.32	1.0 (0.23)	76
51	20.4	(0.4)	61	(3)	658	(2)	1.32	1.0 (0.08)	76
52	20.6	(0.3)	59	(4)	648	(2)	1.34	0.9 (0.12)	67
53	20.5	(0.4)	59	(3)	656	(2)	1.32	1.0 (0.14)	76
54	20.7	(0.3)	55	(2)	656	(1)	1.32	1.0 (0.17)	76
55	20.5	(0.5)	58	(3)	660	(1)	1.42	1.0 (0.10)	70
56	20.5	(0.3)	59	(4)	657	(3)	1.32	1.0 (0.12)	76
57	20.4	(0.4)	60	(2)	650	(2)	1.33	1.1 (0.19)	83
58	20.3	(0.4)	61	(2)	659	(1)	1.31	1.0 (0.10)	76
59	20.7	(0.2)	55	(3)	647	(2)	1.34	1.0 (0.16)	75
60	20.5	(0.6)	57	(3)	659	(2)	1.31	1.2 (0.18)	92
61	20.7	(0.3)	57	(3)	662	(3)	1.31	1.1 (0.27)	84
62	20.5	(0.5)	54	(5)	660	(2)	1.31	1.1 (0.13)	84
63	20.3	(0.3)	60	(2)	664	(1)	1.30	1.1 (0.15)	85
64	20.6	(0.2)	56	(3)	661	(5)	1.31	1.1 (0.06)	84
65	20.2	(0.7)	63	(2)	662	(1)	1.31	1.1 (0.15)	84
66	20.5	(0.3)	61	(2)	664	(2)	1.30	1.1 (0.14)	85
67	19.8	(0.3)	58	(3)	659	(1)	1.31	1.1 (0.20)	84
Mean	20.4		58.8		655.0		1.23	0.98	
sd	0.22		2.66		6.60		0.140	0.109	

Figures in parentheses denote standard deviations
A/N Analysed × 100 ÷ Nominal
sd Standard deviation

TABLE 2

(Chamber conditions - continued)

Group 3: Inter dose

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)		Concentration (ppm)		A/N %
							Nominal	Analysed	
1	20.1	(0.3)	57	(4)	640	(2)	6.4	6.0 (0.90)	94
2	20.5	(0.3)	59	(4)	646	(2)	5.6	4.9 (0.46)	88
3	20.4	(0.4)	60	(3)	646	(2)	5.2	5.1 (0.24)	98
4	20.6	(0.3)	57	(3)	650	(2)	5.2	4.9 (0.12)	94
5	20.2	(0.6)	61	(2)	642	(1)	5.3	5.1 (0.30)	96
6	20.6	(0.3)	58	(4)	649	(2)	5.3	5.0 (0.28)	94
7	19.7	(0.3)	66	(4)	651	(1)	5.5	4.7 (0.34)	85
8	20.4	(0.3)	61	(4)	648	(2)	5.6	4.7 (0.26)	84
9	20.7	(0.3)	59	(3)	647	(1)	5.6	4.6 (0.39)	82
10	20.6	(0.4)	60	(3)	644	(2)	5.6	5.0 (0.46)	89
11	20.6	(0.2)	62	(2)	657	(3)	5.5	5.1 (0.28)	93
12	20.3	(0.3)	61	(3)	650	(3)	5.5	5.0 (0.39)	91
13	20.5	(0.2)	60	(2)	651	(2)	5.5	5.1 (0.30)	93
14	20.6	(0.2)	61	(2)	653	(1)	5.2	5.1 (0.27)	98
15	20.3	(0.4)	61	(2)	640	(2)	5.6	4.9 (0.56)	88
16	20.4	(0.3)	61	(3)	641	(3)	5.6	4.9 (0.40)	88
17	20.4	(0.3)	59	(2)	653	(1)	5.5	4.8 (0.29)	87
18	20.3	(0.2)	62	(2)	652	(2)	5.5	5.0 (0.47)	91
19	20.6	(0.3)	57	(3)	650	(1)	5.5	5.1 (0.51)	93
20	20.5	(0.2)	61	(1)	650	(2)	5.5	4.5 (0.10)	82
21	20.2	(0.3)	60	(5)	651	(6)	5.5	4.3 (0.31)	78
22	20.5	(0.2)	60	(3)	658	(1)	5.5	4.6 (0.22)	84
23	20.3	(0.3)	62	(2)	664	(1)	5.4	4.3 (0.35)	80
24	20.7	(0.2)	56	(4)	657	(2)	5.5	4.1 (0.38)	75
25	20.5	(0.3)	61	(2)	654	(1)	5.5	3.4 (0.70)	62
26	20.3	(0.4)	62	(2)	650	(2)	5.8	4.1 (0.34)	71
27	20.4	(0.3)	62	(3)	662	(7)	6.0	4.6 (0.33)	77
28	20.3	(0.3)	62	(3)	650	(2)	6.4	4.5 (0.46)	70
29	20.6	(0.2)	58	(3)	643	(2)	7.0	4.9 (0.51)	70
30	20.5	(0.2)	60	(3)	644	(2)	7.0	4.9 (0.21)	70
31	20.4	(0.2)	62	(1)	653	(1)	7.2	4.6 (0.23)	64
32	20.9	(0.7)	59	(3)	640	(1)	7.3	4.9 (0.30)	67
33	21.3	(0.9)	59	(4)	645	(2)	7.0	4.8 (0.27)	69
34	20.7	(0.2)	57	(4)	651	(2)	6.9	4.8 (0.16)	70
35	20.3	(0.3)	61	(3)	662	(2)	7.1	4.9 (0.29)	69
36	20.3	(0.3)	54	(12)	646	(2)	7.8	5.3 (0.25)	68
37	20.4	(0.3)	60	(2)	635	(2)	7.9	5.3 (0.38)	67
38	20.5	(0.3)	60	(2)	644	(2)	7.8	5.1 (0.81)	65
39	20.7	(0.2)	56	(2)	649	(3)	7.8	5.4 (0.28)	69
40	20.3	(0.2)	62	(2)	646	(2)	7.5	5.0 (0.28)	67

Figures in parentheses denote standard deviations
A/N Analysed × 100 ÷ Nominal

TABLE 2

(Chamber conditions - continued)

Group 3: Inter dose (continued)

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)		Concentration (ppm)		A/N %
							Nominal	Analysed	
41	20.5	(0.1)	60	(2)	651	(2)	7.5	5.3 (0.33)	71
42	20.9	(0.6)	59	(3)	654	(1)	7.4	5.0 (0.13)	68
43	20.6	(0.3)	60	(3)	652	(1)	7.5	5.2 (0.27)	69
44	20.8	(0.3)	56	(3)	650	(1)	7.5	4.7 (0.74)	63
45	20.6	(0.2)	61	(2)	648	(1)	7.5	5.4 (0.36)	72
46	20.6	(0.3)	59	(4)	652	(2)	7.5	5.2 (0.34)	69
47	20.7	(0.3)	58	(3)	657	(2)	7.4	5.4 (0.39)	73
48	20.6	(0.3)	61	(3)	659	(2)	7.4	5.4 (0.40)	73
49	20.7	(0.3)	57	(3)	660	(4)	7.4	5.2 (0.22)	70
50	20.5	(0.3)	61	(2)	653	(2)	7.5	5.1 (0.36)	68
51	20.5	(0.4)	60	(5)	655	(2)	7.4	5.2 (0.28)	70
52	20.7	(0.3)	58	(3)	648	(2)	7.5	5.2 (0.29)	69
53	20.5	(0.3)	61	(2)	655	(1)	7.4	5.2 (0.30)	70
54	20.8	(0.3)	55	(2)	654	(2)	7.2	4.8 (0.85)	67
55	20.6	(0.4)	57	(3)	658	(1)	6.8	5.3 (0.64)	78
56	20.6	(0.3)	60	(3)	655	(3)	6.6	5.0 (0.44)	76
57	20.4	(0.4)	62	(2)	649	(2)	6.7	5.0 (0.31)	75
58	20.5	(0.4)	60	(3)	656	(1)	6.9	4.9 (0.41)	71
59	20.8	(0.2)	55	(2)	647	(2)	6.7	4.8 (0.42)	72
60	20.6	(0.4)	57	(3)	657	(2)	6.6	5.1 (0.57)	77
61	20.7	(0.2)	57	(3)	660	(2)	6.6	5.3 (0.38)	80
62	20.7	(0.3)	54	(2)	660	(2)	6.6	4.8 (0.92)	73
63	20.4	(0.3)	60	(2)	661	(2)	6.5	5.1 (0.30)	78
64	20.6	(0.2)	56	(1)	661	(1)	6.5	5.1 (0.35)	78
65	20.4	(0.6)	60	(3)	658	(1)	6.6	5.3 (0.27)	80
66	20.6	(0.3)	59	(2)	661	(2)	6.5	5.4 (0.37)	83
67	19.9	(0.2)	58	(2)	654	(1)	6.6	5.4 (0.18)	82
Mean	20.5		59.4		651.5		6.5	4.96	
sd	0.23		2.28		6.33		0.87	0.382	

Figures in parentheses denote standard deviations
A/N Analysed × 100 ÷ Nominal
sd Standard deviation

TABLE 2

(Chamber conditions - continued)

Group 4: High dose

Exposure no.	Temperature (°C)	Relative humidity (%)	Airflow (L/min)	Concentration (ppm)		A/N %
				Nominal	Analysed	
1	20.4 (0.2)	54 (1)	640 (2)	23.7	21.8 (3.84)	92
2	20.8 (0.2)	54 (3)	645 (2)	21.2	19.2 (0.46)	91
3	20.4 (0.3)	59 (3)	646 (1)	20.1	19.4 (0.43)	97
4	20.9 (0.2)	54 (2)	650 (1)	21.1	19.3 (0.81)	91
5	20.5 (0.5)	57 (4)	642 (1)	21.3	19.2 (1.29)	90
6	20.7 (0.3)	56 (2)	649 (2)	21.2	19.3 (0.67)	91
7	20.6 (0.3)	57 (3)	650 (2)	21.7	18.3 (1.24)	84
8	20.6 (0.2)	58 (2)	648 (3)	21.2	18.5 (0.91)	87
9	20.9 (0.2)	54 (2)	647 (1)	22.3	19.2 (1.25)	86
10	20.7 (0.3)	55 (2)	643 (3)	22.5	19.6 (0.65)	87
11	20.7 (0.1)	55 (2)	656 (3)	22.0	19.8 (0.98)	90
12	20.5 (0.3)	57 (1)	649 (3)	22.3	19.6 (1.90)	88
13	20.6 (0.2)	56 (3)	651 (2)	22.2	20.1 (1.08)	91
14	20.8 (0.2)	54 (2)	653 (1)	22.1	18.9 (0.63)	86
15	20.5 (0.3)	59 (3)	639 (2)	21.4	19.9 (1.17)	93
16	20.6 (0.2)	58 (3)	639 (3)	22.6	18.9 (0.71)	84
17	20.6 (0.3)	56 (3)	651 (1)	24.4	19.7 (0.59)	81
18	20.6 (0.2)	57 (3)	652 (2)	23.2	20.2 (1.10)	87
19	20.8 (0.2)	54 (2)	650 (1)	23.3	20.8 (0.28)	89
20	20.6 (0.2)	56 (2)	649 (2)	23.3	17.1 (0.06)	73
21	20.4 (0.2)	57 (3)	648 (6)	23.4	17.0 (1.18)	73
22	20.7 (0.2)	54 (2)	657 (1)	24.1	16.2 (2.91)	67
23	20.6 (0.3)	54 (3)	662 (1)	21.8	16.6 (1.34)	76
24	20.9 (0.1)	53 (1)	655 (2)	23.1	16.1 (1.04)	70
25	20.7 (0.3)	56 (2)	653 (1)	23.2	14.4 (2.10)	62
26	20.5 (0.3)	56 (2)	649 (2)	24.4	18.4 (0.78)	75
27	20.6 (0.3)	56 (4)	662 (7)	24.0	20.1 (4.15)	84
28	20.6 (0.2)	57 (2)	649 (2)	24.4	17.3 (1.83)	71
29	20.5 (0.1)	57 (2)	639 (2)	28.2	19.7 (1.89)	70
30	20.6 (0.2)	57 (2)	642 (2)	30.3	21.0 (1.35)	69
31	20.6 (0.1)	58 (2)	651 (1)	31.0	20.0 (1.87)	65
32	21.2 (0.7)	54 (4)	637 (1)	30.6	21.0 (2.05)	69
33	21.5 (0.9)	55 (5)	642 (2)	29.2	20.5 (1.29)	70
34	20.9 (0.2)	54 (2)	648 (2)	28.9	20.3 (0.91)	70
35	20.5 (0.2)	60 (2)	660 (2)	29.5	19.4 (2.24)	66
36	20.6 (0.3)	46 (9)	643 (2)	30.3	21.4 (1.59)	71
37	20.7 (0.2)	57 (3)	632 (2)	33.1	20.9 (2.92)	63
38	20.7 (0.2)	57 (3)	641 (2)	30.4	19.9 (1.09)	65
39	20.9 (0.2)	54 (2)	647 (2)	31.2	21.7 (1.28)	70
40	20.6 (0.2)	59 (2)	642 (2)	31.5	20.6 (1.12)	65

Figures in parentheses denote standard deviations
A/N Analysed × 100 ÷ Nominal

TABLE 2

(Chamber conditions - continued)

Group 4: High dose (continued)

Exposure no.	Temperature (°C)		Relative humidity (%)		Airflow (L/min)		Concentration (ppm)		A/N %
							Nominal	Analysed	
41	20.7	(0.1)	57	(3)	650	(2)	31.1	20.8 (1.20)	67
42	21.1	(0.6)	57	(3)	652	(1)	31.0	20.8 (0.90)	67
43	20.8	(0.2)	57	(3)	650	(2)	30.0	21.0 (0.85)	70
44	21.1	(0.2)	54	(2)	649	(2)	28.9	19.8 (2.19)	69
45	20.7	(0.2)	58	(3)	647	(2)	31.2	21.4 (1.34)	69
46	20.8	(0.2)	58	(3)	649	(2)	31.1	22.3 (0.77)	72
47	20.8	(0.3)	58	(3)	657	(2)	29.6	20.9 (0.96)	71
48	20.7	(0.3)	59	(3)	658	(2)	30.7	22.0 (0.74)	72
49	20.8	(0.2)	55	(3)	658	(4)	29.6	21.3 (0.64)	72
50	20.6	(0.2)	59	(3)	651	(3)	31.0	21.7 (1.27)	70
51	20.7	(0.3)	59	(3)	651	(2)	28.8	19.4 (0.75)	67
52	20.8	(0.3)	56	(3)	646	(3)	27.9	19.6 (0.96)	70
53	20.8	(0.2)	57	(3)	651	(1)	28.8	19.9 (0.83)	69
54	21.0	(0.2)	55	(3)	651	(2)	27.7	21.2 (2.36)	77
55	20.8	(0.4)	55	(2)	654	(1)	28.7	21.2 (1.14)	74
56	20.8	(0.2)	59	(2)	653	(2)	27.6	21.6 (1.27)	78
57	20.6	(0.3)	61	(2)	647	(2)	27.9	20.8 (1.17)	75
58	20.6	(0.3)	59	(2)	654	(1)	26.5	20.7 (0.86)	78
59	21.0	(0.1)	54	(2)	643	(2)	26.9	20.7 (1.24)	77
60	20.8	(0.3)	54	(2)	656	(1)	27.5	20.7 (1.89)	75
61	20.9	(0.1)	54	(2)	656	(2)	27.5	21.7 (0.84)	79
62	20.8	(0.3)	54	(3)	658	(2)	27.9	22.0 (1.03)	79
63	20.5	(0.3)	57	(2)	658	(3)	27.4	22.4 (0.23)	82
64	20.8	(0.2)	53	(2)	658	(2)	26.3	21.8 (2.89)	83
65	20.6	(0.6)	57	(3)	658	(1)	26.3	21.4 (1.56)	81
66	20.6	(0.2)	57	(3)	658	(1)	24.1	20.5 (0.92)	85
67	20.1	(0.2)	55	(2)	653	(1)	26.5	22.5 (0.82)	85
Mean	20.7		56.1		649.8		26.3	20.0	
sd	0.21		2.28		6.39		3.61	1.63	

Figures in parentheses denote standard deviations
A/N Analysed × 100 ÷ Nominal
sd Standard deviation

TABLE 3

Bodyweights - group mean values (g)

Week	Group and dosage ( )							
	1♂ Air control	2♂ Low dose	3♂ Inter dose	4♂ High dose	1♀ Air control	2♀ Low dose	3♀ Inter dose	4♀ High dose
-3	233	234	233	234	188	191	189	188
-2	297	299	293	298	214	215	215	209
-1	340	345	332	340	229	231	232	225
0	376	384	368	375	246	250	248	238
1	402	409	394	390	257	262	257	244
2	426	432	419	398	271	273	270	249
3	451	456	444	410	281	284	280	255
4	475	480	464	419	290	293	288	259
5	496	501	482	426	296	299	295	264
6	517	520	500	438	304	305	301	269
7	536	538	517	443	310	314	309	271
8	549	551	529	449	315	320	314	274
9	562	567	540	456	320	325	316	277
10	576	584	555	463	324	328	321	275
11	587	596	567	475	327	332	325	279
12	597	606	572	480	331	337	327	284
13	598	604	569	483	328	336	325	281
13	581	595	578	465	327	336	327	280
14	593	609	595	493	328	339	328	290
15	605	622	612	519	334	342	331	298
16	616	632	628	545	340	349	343	310
17	630	646	644	557	344	347	350	315
18	638	655	658	571	351	354	354	321
19	648	666	672	581	354	355	355	323
20	656	670	680	586	360	360	360	329
21	663	675	688	598	361	367	363	336
22	669	680	697	608	363	369	366	336
23	679	690	710	622	366	370	364	341
24	686	697	719	630	366	376	369	346
25	696	709	729	642	364	377	373	350
26	697	710	731	645	371	377	368	345
Gain								
0 - 13	222	221	201	108**	82	86	77	42**
13 - 26	116	115	153	180**	45	41	41	65

** $p < 0.01$ compared with controls

TABLE 4

Food consumption - group mean values (g)

Week	Group and dosage ( )							
	1♂ Air control	2♂ Low dose	3♂ Inter dose	4♂ High dose	1♀ Air control	2♀ Low dose	3♀ Inter dose	4♀ High dose
-3	231	238	232	233	160	161	159	155
-2	234	242	229	237	164	165	162	161
-1	230	240	226	233	171	171	167	165
1	227	237	225	200	171	169	166	154
2	228	229	227	185	175	175	169	145
3	237	239	234	183	176	174	171	139
4	239	244	235	180	173	177	167	137
5	244	246	235	184	174	173	170	145
6	236	240	226	181	171	170	169	139
7	239	240	229	172	173	177	166	137
8	237	241	229	172	169	172	162	131
9	232	240	227	176	169	172	163	133
10	230	240	228	183	168	173	165	132
11	231	237	229	192	166	171	161	138
12	228	235	220	186	169	175	161	140
13	213	216	207	169	143	150	136	127
14	225	241	247	236	169	181	176	168
15	226	235	249	243	172	173	181	190
16	225	229	245	246	169	173	184	187
17	228	231	250	238	170	173	183	186
18	230	238	250	232	177	175	178	183
19	228	238	250	239	172	177	184	181
20	230	234	241	230	172	173	179	181
21	238	238	254	235	178	181	186	186
22	232	236	253	234	172	181	187	179
23	237	246	251	240	167	178	177	179
24	228	241	250	238	162	182	186	189
25	231	243	252	241	163	174	181	185
26	209	228	235	223	125	129	132	131
Cumulative								
1 - 13	3717	3804	3639	3066**	2695	2726	2614	2278**
14 - 26	2968	3078	3226	3074	2169	2250	2312	2326

** $p < 0.01$ compared with controls

Note: Statistical analysis of withdrawal data was not possible since there was one cage of rats per sex per group only

TABLE 5

Haematology - group mean values

Week 13

Group	PCV	Hb	RBC	MCHC	MCV	MCH	Retic	WBC Total	N	L	E	B	M	LUC	Plt	TT
	%	g/dl	10^{12}/l	g/dl	fl	pg	%	10^9/l	10^9/l	10^9/l	10^9/l	10^9/l	10^9/l	10^9/l	10^9/l	s
1♂ Air control	46.2	LT 16.2	8.93	35.1	51.7	18.2	2.1	14.29	2.69	10.74	0.20	0.04	0.29	0.33	1058	25
2♂ Low dose	46.1	16.1	8.78	34.9	52.5	18.3	2.1	15.50	3.29	11.21	0.18	0.04	0.35	0.43	1090	25
3♂ Inter dose	45.8	16.0	8.91	35.0	51.5	18.0	3.2	14.40	2.78	10.71	0.20	0.04	0.32	0.35	1129	26
4♂ High dose	** 49.5	** 17.4	** 9.61	35.1	51.6	18.1	1.8	14.73	2.70	11.08	0.24	0.04	0.29	0.37	996	26
1♀ Air control	44.5	15.6	8.18	35.1	54.4	19.1	1.6	8.97	1.57	6.85	0.14	0.02	0.19	0.21	1018	21
2♀ Low dose	45.3	15.9	8.42	35.1	53.9	18.9	1.6	8.47	1.58	6.27	0.17	0.02	0.22	0.21	1032	21
3♀ Inter dose	45.0	15.8	8.33	35.2	54.1	19.0	1.8	8.79	1.51	6.67	0.18	0.02	0.21	0.20	985	21
4♀ High dose	** 46.7	** 16.5	** 8.63	35.3	54.2	19.1	1.7	7.42	1.59	* 5.45	0.11	0.01	0.13	* 0.13	1006	** 23

LT Log-transformed data used in the analysis

* $p < 0.05$, ** $p < 0.01$ compared with controls

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TABLE 5

(Haematology - continued)

Week 26

Group	PCV %	Hb g/dl	RBC 10^{12}/l	TT s
1♂ Air control	44.0	14.8	8.91	23
2♂ Low dose	43.1	14.6	8.38	23
3♂ Inter dose	45.7	15.3	8.56	22
4♂ High dose	44.9	15.3	8.81	* 25
1♀ Air control	42.5	14.6	7.77	20
2♀ Low dose	43.4	14.9	8.00	21
3♀ Inter dose	42.5	14.5	7.94	19
4♀ High dose	41.9	14.3	7.64	20

* $p < 0.05$ compared with controls

TABLE 6

Biochemistry - group mean values

Week 13

Group	Glu-cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat-inine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml
		Total	Alb	Glob					
1♂ Air control	128	6.8	2.8	4.0	12	0.5	151	30	57
2♂ Low dose	125	6.8	2.8	4.0	12	0.5	145	30	57
3♂ Inter dose	137	6.8	2.8	4.0	13	0.5	137	31	60
4♂ High dose	117	6.7	2.7 *	4.0	15 **	0.6 **	155	37 **	70 **
1♀ Air control	115	7.2	3.4	3.9	16	0.6	72	28 LT	56
2♀ Low dose	122	7.1	3.4	3.8	19	0.6	85	32	61
3♀ Inter dose	123	7.3	3.3	4.0	18	0.7	78	38	67
4♀ High dose	115	7.1	3.2	3.9	22 **	0.7 **	98 *	36	67

* $p < 0.05$, ** $p < 0.01$ compared with controls
LT Log-transformed data used in the analysis

TABLE 6

(Biochemistry - continued)

Week 13

Group	γGT mU/ ml	CPK mU/ ml	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Air control	<1	72	FA <0.1	145	3.6	5.4	3.4	103	72
2♂ Low dose	<2	82	<0.1	145	3.5	5.4	3.4	103	74
3♂ Inter dose	<2	81	<0.1	144	3.5	5.3	3.2	103	76
4♂ High dose	<2	88	<0.1	145	3.6	** 5.2	3.4	104	* 57
1♀ Air control	<2	85	0.1	143	NP 3.3 (3.4)	5.5	2.8	104	79
2♀ Low dose	<2	79	<0.2	144	3.6 (3.7)	5.5	2.7	105	80
3♀ Inter dose	<1	85	<0.2	143	3.5 (3.4)	5.4	2.5	104	87
4♀ High dose	<1	52	<0.2	* 145	3.4 (3.4)	5.3	2.7	* 106	70

* $p < 0.05$, ** $p < 0.01$ compared with controls
FA Frequency analysis applied to the data
NP Non-parametric analysis employed, median values in parentheses

TABLE 6

(Biochemistry - continued)

Week 26

Group	Urea Nitr mg/dl	Creat-inine mg/dl	GPT mU/ml	GOT mU/ml	Chol mg/dl
1♂ Air control	12	0.5	27	57	96
2♂ Low dose	11	0.6	34	68	106
3♂ Inter dose	12	0.6	32	71	104
4♂ High dose	13	0.6	33	63	69
1♀ Air control	16	0.7	LT 32	LT 65	98
2♀ Low dose	18	0.7	65	143	86
3♀ Inter dose	17	0.7	55	114	110
4♀ High dose	17	0.6	* 102	* 215	111

* $p < 0.05$ compared with controls
LT Log-transformed data used in the analysis

TABLE 7

Organ weights - group mean values

Week 14

Group	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Prostate g	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g
1♂ Air control	605	A 2.13 (2.10)	A 1.75 (1.69)	A 19.3 (18.0)	A 0.93 (0.87)	A 3.10 (2.96)	A 56.6 (54.4)	1.052	A 1.776 (1.709)	A 1.822 (1.755)	A 1.80 (1.73)	A 0.670 (0.651)	A 0.702 (0.681)
2♂ Low dose	605	2.10 (2.07)	1.75 (1.69)	19.8 (18.5)	1.07 (1.01)	3.23 (3.10)	52.1 (49.9)	1.112	1.810 (1.744)	1.793 (1.728)	1.86 (1.78)	0.711 (0.692)	0.703 (0.683)
3♂ Inter dose	566	2.06 (2.06)	1.75 (1.75)	17.6 (17.5)	0.85 (0.85)	3.12 (3.12)	56.7 (56.6)	0.975	1.803 (1.801)	1.802 (1.799)	1.83 (1.83)	0.686 (0.686)	0.704 (0.704)
4♂ High dose	484	2.02 (2.08)	** 2.08 (2.21)	14.9 (17.5)	0.81 (0.93)	2.76 (3.04)	53.0 (57.4)	0.977	1.787 (1.923)	** 1.866 (2.001)	1.65 (1.80)	0.646 (0.686)	0.696 (0.737)

** $p < 0.01$ compared with controls

A Analysis conducted using mean values adjusted for bodyweight as covariate. Adjusted mean values in parentheses

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TABLE 7

(Organ weights - continued)

Week 14

Group	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Air control	324	A 1.95 (1.95)	A 1.30 (1.27)	A 10.6 (10.4)	0.59	A 1.86 (1.82)	66.7	A 91.3 (88.8)
2♀ Low dose	331	1.97 (1.96)	1.34 (1.28)	11.0 (10.5)	0.55	1.93 (1.86)	65.2	94.8 (90.2)
3♀ Inter dose	323	1.93 (1.92)	1.28 (1.26)	10.7 (10.4)	0.61	1.82 (1.78)	60.5	90.9 (88.5)
4♀ High dose	281	1.93 (1.96)	** 1.49 (1.60)	9.1 (10.0)	0.55	1.72 (1.87)	62.6	78.4 (87.8)

** $p < 0.01$ compared with controls

A Analysis conducted using mean values adjusted for bodyweight as covariate. Adjusted mean values in parentheses

HST 411/961219

TABLE 7

(Organ weights - continued)

Week 27

Group	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Prostate g	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g
1♂ Air control	691	2.13	A 1.85 (1.84)	A 20.1 (20.0)	0.98	A 3.31 (3.29)	54.6	1.119	A 1.952 (1.949)	A 1.949 (1.946)	2.49	0.698	0.783
2♂ Low dose	701	2.16	1.81 (1.80)	20.2 (19.8)	1.09	3.69 (3.64)	53.2	1.004	1.816 (1.801)	1.827 (1.813)	2.19	0.736	0.768
3♂ Inter dose	722	2.19	1.92 (1.90)	21.4 (20.2)	1.09	3.39 (3.25)	52.2	1.165	1.883 (1.845)	1.880 (1.843)	2.05	0.728	0.740
4♂ High dose	638	2.13	* 2.02 (2.06)	17.8 (19.5)	0.98	3.11 (3.33)	55.3	0.982	1.834 (1.890)	1.849 (1.903)	2.15	0.704	0.742

* $p < 0.05$ compared with controls

A Analysis conducted using mean values adjusted for bodyweight as covariate. Adjusted mean values in parentheses

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TABLE 7

(Organ weights - continued)

Week 27

Group	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Air control	365	A 1.91 (1.91)	A 1.44 (1.44)	A 10.9 (10.9)	0.67	1.99	A 62.6 (62.0)	A 86.9 (85.7)
2♀ Low dose	372	1.95 (1.94)	1.33 (1.31)	10.4 (10.2)	0.64	1.91	67.5 (66.2)	90.9 (88.2)
3♀ Inter dose	361	1.94 (1.93)	1.37 (1.37)	11.8 (11.8)	0.58	2.11	76.3 (76.2)	69.6 (69.3)
4♀ High dose	345	1.89 (1.90)	1.37 (1.40)	10.3 (10.5)	0.55	1.75	57.4 (59.2)	69.4 (73.4)

A Analysis conducted using mean values adjusted for bodyweight as covariate. Adjusted mean values in parentheses

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TABLE 8

Macroscopic pathology incidence summary

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Skin								
Scab/s	1	1	1	0	1	1	0	0
Skin								
Alopecia	2	1	1	0	8	2	0	2
Tail								
Pustule/s	0	0	0	1	0	0	0	0
Lymph Nodes - Cervical								
Enlarged	10	10	10	10	10	9	9	9
Congested	1	2	3	0	0	0	0	0
Lymph Nodes - Deep Cervical								
Enlarged	0	1	1	3	0	0	0	1
Lungs								
Not collapsed	0	0	0	5	0	0	0	9
Consolidated	0	0	0	2	0	0	0	1
Pale subpleural foci	1	0	1	1	1	0	2	5
Dark subpleural focus	0	0	0	0	0	0	0	1
Lymph Nodes - Tracheobronchial								
Enlarged	0	0	0	0	0	0	0	1
Adipose Tissue								
Torsioned nodule/s	0	0	0	0	0	0	1	0
Minimal	0	0	0	9	0	0	0	7

TABLE 8

(Macroscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Liver								
Median cleft, pale subcapsular area	0	0	0	0	1	0	0	0
Pale subcapsular area/s	1	0	0	0	0	0	0	0
Spleen								
Clear fluid-filled cyst/s	0	0	1	0	0	0	0	0
Enlarged	0	1	0	0	0	0	0	0
Stomach								
A caseous cyst	2	0	2	0	0	0	0	0
Stomach Antrum Mucosa								
White nodule/s	3	1	1	1	1	1	0	1
Small Intestine								
Contents minimal	0	0	0	0	0	0	0	1
Gaseous distension	0	0	0	0	0	0	0	1
Adrenals								
Dark subcapsular foci	0	0	0	0	0	1	0	0
Kidneys								
Irregular cortical scarring	0	0	0	0	0	0	0	1
Pale cortical foci	0	0	0	0	0	1	0	0
Small	0	0	0	0	0	0	0	1
Testes								
Small	0	0	0	1	0	0	0	0

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TABLE 8

(Macroscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Epididymides								
Small	0	0	0	1	0	0	0	0
Uterus								
Fluid distension	0	0	0	0	2	4	0	3
Bone								
Sternum misshapen	0	0	0	0	1	0	0	0

TABLE 8

(Macroscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Fur								
Coarse - sacral region	0	0	0	0	0	0	0	1
Skin								
Scab/s	0	1	0	0	0	0	0	0
Skin								
Alopecia	0	1	0	0	0	0	0	0
Extra-orbital Lachrymal Glands								
Pale subcapsular foci	0	0	1	0	0	0	0	0
Tail								
Tip missing	0	0	0	1	0	0	0	0
Lymph Nodes - Cervical								
Enlarged	5	5	5	5	4	5	5	3
Congested	2	1	0	1	1	0	0	0
Mammary Glands								
Creamy fluid-filled cysts	0	0	0	0	0	0	1	0
Lungs								
Not collapsed	0	0	0	0	1	0	0	0
Firm	0	0	0	0	1	0	0	0
Congested	0	0	1	0	0	0	0	0
Dark subpleural foci	0	0	1	0	1	0	0	0
Lobe atelectatic	0	0	0	1	0	0	0	0

TABLE 8

(Macroscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Adipose Tissue								
Torsioned nodule/s	0	0	0	0	0	0	0	1
Splenulus - omental	0	0	0	0	0	1	0	0
Minimal	1	0	0	1	0	0	0	0
Excessive	0	0	0	0	0	0	1	0
Liver								
Median cleft, pale subcapsular area	1	1	0	0	0	0	1	0
Spleen								
Clear fluid-filled cyst/s	0	0	0	0	0	0	0	1
Forestomach								
A depression	0	0	0	0	1	0	0	0
Stomach Antrum Mucosa								
White nodule/s	0	0	0	1	0	0	0	1
Kidneys								
Irregular cortical scarring	1	0	0	0	0	0	0	0
Testes								
White subtunical striae	1	0	0	0	0	0	0	0
Oedematous	1	0	0	0	0	0	0	0
Epididymides								
Small	1	0	0	0	0	0	0	0

TABLE 8

(Macroscopic pathology incidence summary - continued)

	Group	Group	Group	Group	Group	Group	Group	Group
Removal reason: Recovery	1	2	3	4	1	2	3	4
	---------- Males ----------				---------- Females ----------			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Ovaries								
No corpora lutea visible	0	0	0	0	0	0	2	1
Uterus								
Fluid distension	0	0	0	0	2	1	1	1

TABLE 9

Microscopic pathology incidence summary

Removal reason: Intercurrent	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	0	0	0	0	1	0	0	0
Nasal Passages								
Examined	0	0	0	0	1	0	0	0
No abnormalities detected	0	0	0	0	1	0	0	0
Larynx								
Examined	0	0	0	0	1	0	0	0
No abnormalities detected	0	0	0	0	1	0	0	0
Trachea (including Bifurcation)								
Examined	0	0	0	0	1	0	0	0
No abnormalities detected	0	0	0	0	1	0	0	0
Lungs								
Examined	0	0	0	0	1	0	0	0
No abnormalities detected	0	0	0	0	1	0	0	0
Lymph Nodes - Cervical								
Examined	0	0	0	0	1	0	0	0
Sinus erythrocytosis (Total)	0	0	0	0	1	0	0	0
Minimal	0	0	0	0	1	0	0	0
Skeletal Muscle								
Examined	0	0	0	0	1	0	0	0
Periorbital haemorrhage	0	0	0	0	1	0	0	0
Eyes								
Examined	0	0	0	0	1	0	0	0
Traumatic disruption	0	0	0	0	1	0	0	0

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Nasal Passages								
Examined	10	10	10	10	10	10	10	10
No abnormalities detected	10	10	1	0	10	10	0	0
Respiratory epithelium - inflammation (Total)	0	0	1	9	0	0	0	8
Minimal	0	0	1	8	0	0	0	6
Moderate	0	0	0	1	0	0	0	2
Respiratory epithelium - granular eosinophilic inclusions (Total)	0	0	1	5	0	0	0	3
Minimal	0	0	1	1	0	0	0	1
Moderate	0	0	0	4	0	0	0	2
Respiratory epithelium - hyperplasia/prominent goblet cells (Total)	0	0	1	10	0	0	1	10
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	1	7	0	0	0	7
Moderate	0	0	0	3	0	0	0	3
Respiratory epithelium - squamous metaplasia (Total)	0	0	0	6	0	0	0	4
Minimal	0	0	0	6	0	0	0	3
Moderate	0	0	0	0	0	0	0	1
Transitional epithelium - inflammation (Total)	0	0	3	8	0	0	0	8
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	2	2	0	0	0	4
Moderate	0	0	0	6	0	0	0	3
Transitional epithelium - erosion (Total)	0	0	0	3	0	0	0	6
Minimal	0	0	0	1	0	0	0	6
Moderate	0	0	0	2	0	0	0	0
Transitional epithelium - granular eosinophilic inclusions (Total)	0	0	7	1	0	0	9	3
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	3	0	0	0	7	0
Moderate	0	0	4	1	0	0	1	3

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Nasal Passages	(Continued)							
Transitional epithelium - hyperplasia (Total)	0	0	9	8	0	0	8	9
Minimal	0	0	7	8	0	0	6	7
Moderate	0	0	2	0	0	0	2	2
Transitional epithelium - squamous metaplasia (Total)	0	0	1	9	0	0	0	9
Minimal	0	0	1	4	0	0	0	4
Moderate	0	0	0	5	0	0	0	5
Exudative inflammatory cells (sinusitis) (Total)	0	0	1	9	0	0	0	8
Minimal	0	0	1	8	0	0	0	7
Moderate	0	0	0	1	0	0	0	1
Olfactory epithelium - hyperplasia (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Pharynx								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Larynx								
Examined	10	10	10	10	10	10	10	10
No abnormalities detected	10	9	4	0	10	10	6	0
Ventrolateral epithelial squamous metaplasia (Total)	0	0	2	10	0	0	1	10
Trace	0	0	2	0	0	0	1	0
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	0	9	0	0	0	9
Marked	0	0	0	1	0	0	0	0
Ventrolateral epithelial hyperplasia (Total)	0	0	0	2	0	0	0	2
Minimal	0	0	0	2	0	0	0	2

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Larynx	(Continued)							
Ventrolateral epithelial erosion (Total)	0	0	0	1	0	0	0	2
Minimal	0	0	0	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Arytenoid - epithelial keratinisation (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Arytenoid - epithelial erosion/ulceration (Total)	0	0	0	9	0	0	0	8
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	0	5	0	0	0	3
Moderate	0	0	0	4	0	0	0	4
Arytenoid - squamous epithelial hyperplasia (Total)	0	1	5	10	0	0	0	10
Trace	0	1	0	0	0	0	0	0
Minimal	0	0	5	1	0	0	0	0
Moderate	0	0	0	9	0	0	0	10
Subepithelial inflammatory infiltration (Total)	0	0	1	9	0	0	3	4
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	1	7	0	0	1	2
Moderate	0	0	0	2	0	0	1	2
Arytenoid - subepithelial inflammation (Total)	0	1	2	0	0	0	1	0
Minimal	0	1	2	0	0	0	1	0
Trachea (including Bifurcation)								
Examined	10	10	10	10	10	10	10	10
No abnormalities detected	10	10	9	0	10	10	9	0
Tracheitis (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Trachea (including Bifurcation)	(Continued)							
Epithelial hyperplasia (Total)	0	0	1	9	0	0	0	10
Minimal	0	0	1	7	0	0	0	9
Moderate	0	0	0	2	0	0	0	1
Epitheial squamous metaplasia (Total)	0	0	0	1	0	0	0	1
Minimal	0	0	0	1	0	0	0	1
Inflammatory cells in the lamina propria (Total)	0	0	0	9	0	0	0	10
Minimal	0	0	0	6	0	0	0	10
Moderate	0	0	0	3	0	0	0	0
Epithelial hyperplasia at the carina (Total)	0	0	0	8	0	0	0	6
Minimal	0	0	0	8	0	0	0	5
Moderate	0	0	0	0	0	0	0	1
Eosinophilic epithelial inclusions (Total)	0	0	0	3	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Moderate	0	0	0	2	0	0	0	0
Epithelial squamous metaplasia at the carina (Total)	0	0	0	6	0	0	1	4
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	0	3	0	0	0	4
Moderate	0	0	0	3	0	0	0	0
Lungs								
Examined	10	10	10	10	10	10	10	10
No abnormalities detected	9	10	9	1	10	10	10	1
Pneumonitis (Total)	1	0	1	0	0	0	0	0
Trace	1	0	0	0	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Bronchiolitis (Total)	0	0	1	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	1	0	0	0	0	0

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Lungs	(Continued)							
Inflammatory cell foci at terminal bronchioles (Total)	0	0	0	2	0	0	0	0
Trace	0	0	0	2	0	0	0	0
Perivascular inflammatory cells (Total)	0	0	0	6	0	0	0	7
Trace	0	0	0	0	0	0	0	2
Minimal	0	0	0	6	0	0	0	4
Moderate	0	0	0	0	0	0	0	1
Prominent BALT (Total)	0	0	1	4	0	0	0	2
Minimal	0	0	1	3	0	0	0	2
Moderate	0	0	0	1	0	0	0	0
Alveolar ducts - fibrosis (Total)	0	0	0	5	0	0	0	9
Trace	0	0	0	1	0	0	0	2
Minimal	0	0	0	4	0	0	0	6
Alveolar ducts - epithelial ciliated metaplasia (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Increased numbers of alveolar macrophages (Total)	0	0	0	3	0	0	0	5
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	0	3	0	0	0	4
Bronchiolar squamous cell metaplasia (Total)	0	0	0	1	0	0	0	1
Minimal	0	0	0	1	0	0	0	1
Alveolitis (Total)	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Aorta								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Heart								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	9	0	0	9	10	0	0	10

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Heart	(Continued)							
Myocardial fibrosis (Total)	1	0	0	1	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Thymus								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	8	10	0	0	10
Reduced cellularity of cortex (Total)	0	0	0	2	0	0	0	0
Minimal	0	0	0	2	0	0	0	0
Lymph Nodes - Cervical								
Examined	10	10	10	10	10	9	9	10
No abnormalities detected	0	0	0	0	0	1	0	1
Increased cellularity - generalised (Total)	10	10	9	10	10	8	9	9
Minimal	2	1	5	5	1	4	4	3
Moderate	8	8	3	5	8	4	4	6
Marked	0	1	0	0	1	0	1	0
Medullary plasmacytosis (Total)	6	5	7	9	8	2	6	8
Minimal	2	1	5	0	3	1	3	2
Moderate	1	3	2	8	3	0	2	6
Marked	3	1	0	1	2	1	1	0
Lymph Nodes - Deep Cervical								
Examined	0	1	1	3	0	0	0	1
No abnormalities detected	0	1	0	0	0	0	0	0
Increased cellularity - generalised (Total)	0	0	1	2	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	1	2	0	0	0	0
Medullary plasmacytosis (Total)	0	0	0	2	0	0	0	0
Minimal	0	0	0	2	0	0	0	0
Lymph Nodes - Mesenteric								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Lymph Nodes - Tracheobronchial								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	8
Increased cellularity - generalised (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Increased cellularity - paracortex (Total)	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Spleen								
Examined	10	1	1	10	10	0	0	10
No abnormalities detected	10	0	0	5	9	0	0	6
Reduced cellularity of the white pulp - generalised (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Reduced cellularity of the white pulp - PALS (Total)	0	0	0	2	1	0	0	3
Minimal	0	0	0	2	1	0	0	2
Moderate	0	0	0	0	0	0	0	1
Reduced cellularity of the white pulp - marginal zone (Total)	0	0	0	2	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Increased cellularity of the white pulp - marginal zone (Total)	0	0	0	1	0	0	0	3
Minimal	0	0	0	1	0	0	0	3
Red pulp congestion	0	1	0	0	0	0	0	0
Mesothelial capsular cyst (s)	0	0	1	0	0	0	0	0
Liver								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	9	0	0	10	10	0	0	10

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Liver	(Continued)							
Subpleural area of rarefied hepatocytes (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Pancreas								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Kidneys								
Examined	10	0	0	10	10	1	0	10
No abnormalities detected	9	0	0	8	6	1	0	5
Tubular basophilia (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Tubular dilatation (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Cortical scarring (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Dystrophic mineralisation (Total)	0	0	0	0	4	0	0	4
Trace	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	3	0	0	3
Moderate	0	0	0	0	1	0	0	0
Dilatation of the renal pelvis (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Urinary Bladder								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Uterus								
Examined	0	0	0	0	10	4	0	10
No abnormalities detected	0	0	0	0	9	0	0	8

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Uterus	(Continued)							
Luminal dilatation (Total)	0	0	0	0	1	4	0	2
Minimal	0	0	0	0	1	3	0	2
Moderate	0	0	0	0	0	1	0	0
Cervix								
Examined	0	0	0	0	10	0	0	10
No abnormalities detected	0	0	0	0	10	0	0	10
Oviduct								
Examined	0	0	0	0	10	0	0	10
No abnormalities detected	0	0	0	0	10	0	0	10
Ovaries								
Examined	0	0	0	0	10	0	0	10
No abnormalities detected	0	0	0	0	10	0	0	10
Prostate								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	10	0	0	0	0
Seminal Vesicles								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	10	0	0	0	0
Epididymides								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	9	0	0	0	0
Spermatozoa absent - unilateral	0	0	0	1	0	0	0	0
Testes								
Examined	10	0	0	10	0	0	0	0
No abnormalities detected	10	0	0	9	0	0	0	0

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Testes	(Continued)							
Unilateral tubular atrophy (Total)	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Unilateral intratubular oedema (Total)	0	0	0	1	0	0	0	0
Marked	0	0	0	1	0	0	0	0
Thyroids								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Parathyroids								
Examined	10	0	0	10	9	0	0	8
Missing	0	0	0	0	1	0	0	2
No abnormalities detected	10	0	0	10	9	0	0	8
Adrenals								
Examined	10	0	0	10	10	1	0	10
No abnormalities detected	10	0	0	9	10	0	0	10
Vacuolation of cells of the zona fasciculata (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Area (s) of cortical congestion (Total)	0	0	0	0	0	1	0	0
Minimal	0	0	0	0	0	1	0	0
Pituitary								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Salivary Glands								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Skeletal Muscle								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Oesophagus								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Stomach								
Examined	10	1	3	10	10	1	0	10
No abnormalities detected	6	0	0	9	9	0	0	9
Focus of ectopic non-glandular epithelium within the glandular mucosa	3	1	1	1	1	1	0	1
Cystic structure at limiting ridge	2	0	2	0	0	0	0	0
Duodenum								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Jejunum								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Ileum								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Caecum								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Colon								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Rectum								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Mammary Glands								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Skin								
Examined	10	1	1	10	10	1	0	10
No abnormalities detected	9	0	0	10	9	0	0	10
Scab	0	0	1	0	0	1	0	0
Epidermal erosion (Total)	1	1	0	0	1	0	0	0
Minimal	1	1	0	0	1	0	0	0
Epidermal hyperplasia (Total)	0	0	1	0	0	1	0	0
Minimal	0	0	1	0	0	1	0	0
Eyes								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	9	10	0	0	10
Keratitis (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Spinal Cord								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Sciatic Nerve								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Brain								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	10	0	0	10
Bone Marrow/sternum								
Examined	10	0	0	10	10	0	0	10
No abnormalities detected	10	0	0	10	9	0	0	10

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	10	10	10	10	10	10	10	10
Bone Marrow/sternum	(Continued)							
Dislocation	0	0	0	0	1	0	0	0
Adipose Tissue								
Examined	0	0	0	0	0	0	1	0
Fat necrosis	0	0	0	0	0	0	1	0
Tail								
Examined	0	0	0	1	0	0	0	0
Pustule	0	0	0	1	0	0	0	0

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Nasal Passages								
Examined	5	5	5	5	4	5	5	5
No abnormalities detected	5	5	4	0	4	5	5	2
Respiratory epithelium - granular eosinophilic inclusions (Total)	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Respiratory epithelium - hyperplasia/prominent goblet cells (Total)	0	0	0	4	0	0	0	1
Minimal	0	0	0	3	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Transitional epithelium - inflammation (Total)	0	0	1	2	0	0	0	2
Trace	0	0	0	2	0	0	0	0
Minimal	0	0	1	0	0	0	0	2
Transitional epithelium - hyperplasia (Total)	0	0	1	2	0	0	0	2
Trace	0	0	0	1	0	0	0	0
Minimal	0	0	1	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Larynx								
Examined	5	5	5	5	4	5	5	5
No abnormalities detected	5	5	5	4	4	5	5	5
Ventral epithelial squamous metaplasia (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Trachea (including Bifurcation)								
Examined	5	5	5	5	4	5	5	5
No abnormalities detected	5	5	5	5	4	5	5	5
Lungs								
Examined	5	5	5	5	4	5	5	5
No abnormalities detected	5	5	5	4	3	4	4	5

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Lungs	(Continued)							
Pneumonitis (Total)	0	0	0	0	1	1	1	0
Trace	0	0	0	0	1	0	1	0
Minimal	0	0	0	0	0	1	0	0
Perivascular inflammatory cells (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Aggregations of brown pigmented alveolar macrophages (Total)	0	0	0	0	1	0	0	0
Moderate	0	0	0	0	1	0	0	0
Lymph Nodes - Cervical								
Examined	5	5	5	5	4	5	5	3
Prominent distended venules containing small lymphocytes (Total)	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Increased cellularity - generalised (Total)	4	4	4	4	4	4	4	1
Minimal	3	4	3	4	3	2	4	1
Moderate	1	0	1	0	1	2	0	0
Medullary plasmacytosis (Total)	4	3	3	4	2	3	4	3
Minimal	0	0	1	3	2	2	2	3
Moderate	3	3	2	1	0	1	1	0
Marked	1	0	0	0	0	0	1	0
Spleen								
Examined	0	0	0	0	0	0	0	1
Mesothelial capsular cyst (s) (Total)	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Liver								
Examined	1	1	0	0	0	0	1	0
No abnormalities detected	1	1	0	0	0	0	1	0
Kidneys								
Examined	1	0	0	0	0	0	0	0

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TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Kidneys	(Continued)							
Subcapsular nephritis (Total)	1	0	0	0	0	0	0	0
Trace	1	0	0	0	0	0	0	0
Uterus								
Examined	0	0	0	0	2	1	1	1
No abnormalities detected	0	0	0	0	0	0	1	0
Luminal dilatation (Total)	0	0	0	0	2	1	0	1
Minimal	0	0	0	0	2	1	0	1
Ovaries								
Examined	0	0	0	0	0	0	2	1
Absence of corpora lutea	0	0	0	0	0	0	2	1
Epididymides								
Examined	1	0	0	0	0	0	0	0
Spermatozoa absent - unilateral	1	0	0	0	0	0	0	0
Testes								
Examined	1	0	0	0	0	0	0	0
Unilateral intratubular oedema	1	0	0	0	0	0	0	0
Unilateral pressure atrophy	1	0	0	0	0	0	0	0
Stomach								
Examined	0	0	0	1	1	0	0	1
No abnormalities detected	0	0	0	0	1	0	0	0
Focus of ectopic non-glandular epithelium within the glandular mucosa	0	0	0	1	0	0	0	1
Mammary Glands								
Examined	0	0	0	0	0	0	1	0

TABLE 9

(Microscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	15	15	15	15	15	15	15	15
Animals completed	5	5	5	5	4	5	5	5
Mammary Glands	(Continued)							
Secretory activity (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Skin								
Examined	0	1	0	0	0	0	0	0
Scab (Total)	0	1	0	0	0	0	0	0
Trace	0	1	0	0	0	0	0	0
Extra-orbital Lachrymal Glands								
Examined	0	0	1	0	0	0	0	0
Vacuolation of groups of acinar cells (Total)	0	0	1	0	0	0	0	0
Minimal	0	0	1	0	0	0	0	0
Adipose Tissue								
Examined	0	0	0	0	0	1	0	1
Fat necrosis	0	0	0	0	0	0	0	1
Splenulus	0	0	0	0	0	1	0	0

APPENDIX 1

Acetic anhydride rat micronucleus test

Authors

R.J. Proudlock
J. Statham

Mutagenesis and Cell Biology

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

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APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

SUMMARY

This study was designed to assess the potential induction of micronuclei by acetic anhydride in bone marrow cells of rats. Rats were treated by six hour exposure on 5 days of each week for 13 weeks by inhalation at a dose levels of 1, 5 and 20 ppm. The exposure levels were selected in consultation with the Sponsor following review of data from a 2-week preliminary study (HRC Report Number HST 400/942606).

The bone marrow smears were obtained from ten male and ten female animals in the negative control and test substance groups 24 hours after the final exposure. Bone marrow smears were obtained from the positive control group (five male and five females) 24 hours after oral administration of cyclophosphamide. One smear from each animal in the negative, high level and positive control groups were examined for the presence of micronuclei in 1000 immature erythrocytes. The percentage of immature erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated mature erythrocytes was also kept.

Rats treated with the high level acetic anhydride did not show any significant increase in the frequency of micronucleated immature erythrocytes. Since no response was obtained for the high level group, slides prepared from animals exposed at lower levels were not examined.

There was no significant decrease in the proportion of immature erythrocytes after treatment of the animals with acetic anhydride, compared with the concurrent control group.

The positive control compound, cyclophosphamide, produced large, highly significant increases in the frequency of micronucleated immature erythrocytes together with decreases in the proportion of immature erythrocytes.

It is concluded that acetic anhydride has not shown any evidence of causing chromosome damage in this *in vivo* test.

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

INTRODUCTION

The purpose of this study was to assess the potential of acetic anhydride to induce mutagenic effects in rats following subchronic inhalation exposure (Boller and Schmid 1970, Heddle *et al* 1983, MacGregor *et al* 1987, Mavournin *et al* 1990).

Although the micronucleus test is normally performed following acute exposure, the test may be used to assess chromosome damage following longer term exposure (MacGregor *et al* 1990, Henderson *et al* 1993, Garriott *et al* 1995). This has the advantage that the number of animals used in the experimental procedures is minimised since samples can be taken from animals on a dedicated toxicity test and there is no requirement for multiple sampling times since the bone marrow will be expected to have achieved a steady state. In addition subchronic exposure allows possible induction of metabolising enzymes and accumulation of the test substance.

The procedures used were based on the recommendations of EEC Annex to Directive 92/69/EEC (Method B12).

In mitotic cells in which chromosomal damage has been caused by the test substance or its metabolites, acentric fragments of the chromosomes do not separate at the anaphase stage of cell division. After telophase these fragments may not be included in the nuclei of the daughter cells and hence will form single or multiple micronuclei (Howell-Jolly bodies) in the cytoplasm of these cells. Micronuclei are seen in a wide variety of cells, but erythrocytes are chosen for examination since micronuclei are easily detected in this cell type.

A few hours after the last mitosis is completed, erythroblasts expel their nucleus. Young erythrocytes, less than 24 hours old, stain blue with Haemalum due to the presence of ribonucleic acid which gradually disappears so that more mature erythrocytes stain orange. If scoring is restricted to immature erythrocytes, virtually all the chromosome damage detected will have been caused during the recent exposure to the test substance.

Substances which interfere with the mitotic spindle apparatus will cause non-disjunction (unequal separation of the chromosomes at anaphase resulting in aneuploidy) or lagging chromosomes at anaphase which may not be incorporated into the daughter nuclei. These lagging chromosomes are not excluded from the erythroblast at the same time as the nucleus and hence also give rise to micronuclei.

Mature erythrocytes may also be examined for the presence of micronuclei (Schmid 1976).

Any toxic effects of the test substance on the immature nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in cell numbers and to compensate for this, peripheral blood is shunted into the bone marrow (von Ledebur and Schmid 1973). If the percentage of the immature erythrocytes is scored and found to be significantly less than the control value, this is taken as being indicative of bone marrow toxicity.

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

EXPERIMENTAL PROCEDURE

ANIMALS, ANIMAL HUSBANDRY AND TREATMENT

Full details can be found in the main body of this report. Briefly all animals in this study were Crl:CD® BR rats, a Caesarean-derived strain of Sprague-Dawley origin (obtained from Charles River UK Limited, Margate, Kent, England) approximately 6 weeks old on delivery were treated with the test agent by inhalation exposure. Identically specified positive control animals were obtained from the same source and were housed alongside animals in the main inhalation toxicity test.

POSITIVE CONTROL COMPOUND

Cyclophosphamide, obtained from Sigma, batch number 73H0846, was used as the positive control compound. It was prepared as a solution in purified water at a concentration of 4 mg/ml just prior to administration.

EXPOSURE PROCEDURE

(See Main Report)

The exposure levels 1, 5 and 20 ppm acetic anhydride were selected in consultation with the Sponsor following review of data from a 2-week preliminary study (HRC Report Number HST 400/942606).

Cyclophosphamide, the positive control compound, was given as a single oral administration (by gastric intubation) using a standard dose volume of 10 ml/kg bodyweight 24 hours prior to sampling.

CRITICAL DATES

Date of first exposure - Males:	9 August 1995
Date of first exposure - Females:	10 August 1995
Date of dosing positive control:	8 November 1995
Dates of bone marrow sampling:	9 and 10 November 1995

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

MICRONUCLEUS TEST

Bone marrow samples were taken from ten male and ten female animals in each group (except the positive control group where only five male and five female animals were treated and sampled), 24 hours after completion of the final exposure. The experimental design is shown below:

Experimental design

Group	Treatment	Exposure level	Number of rats sampled	
			♂	♀
1	Air control	-	10	10
2	Acetic anhydride	1 ppm	10	10
3		5 ppm	10	10
4		20 ppm	10	10
5	Cyclophosphamide (positive control)	40 mg/kg	5	5

Sampled animals in the negative control and test substance groups were sacrificed 24 hours after completion of the final exposure. The positive control group was sacrificed 24 hours after dosing. The negative control and test group animals were killed by an intraperitoneal injection of sodium pentobarbitone and exsanguination from the brachial arteries, positive control animals were killed by cervical dislocation. One femur was dissected out from each animal. The femurs were cleared of tissue and the contents of each femur were eluted in 10 ml Hank's balanced salt solution by aspiration through a 21 g needle fitted to a plastic syringe. The resulting cell suspension were spun at 1000 rpm for 5 minutes using a MSE Centaur 2 swing-out centrifuge. Each resulting cell pellet was resuspended in 2 ml of filtered foetal calf serum before being sedimented out using the MSE centrifuge. The final cell pellet was resuspended in a small volume of foetal calf serum to facilitate smearing in the conventional manner on a glass microscope slide. Several smears were made from each animal in case of technical problems with staining *etc*, but only one slide per animal from groups 1, 4 and 5 was subjected to microscopic analysis.

Due to the presence of mast cell granules in rat bone marrow smears, which appear identical to micronuclei when stained using the Romanowsky methods, a modified Feulgen staining method is employed for the rat micronucleus test in this laboratory. This method specifically stains DNA-containing bodies deep purple while leaving mast cell granules unstained. This method also allows reasonable differentiation of mature and immature erythrocytes and produces permanent preparations.

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

Staining method

1. Fixed for 10 minutes in SLR grade methanol (Fisons product number M/3950/17).
2. Hydrolysed in Bouin's fluid supplemented with 10% methanol at room temperature for 30 hours.
3. Washed three times in distilled water (5 minutes per wash).
4. Stained in Schiff's reagent (BDH product number 19120) for 1 hour at room temperature.
5. Washed three times in distilled water (5 minutes per wash).
6. Counter-stained for 1 minute in aqueous 0.002% Eosin yellowish (BDH product number 34197). The dilution and staining time in Eosin were adjusted to give optimum differentiation between mature and immature erythrocytes.
7. Washed for 5 minutes in distilled water.
8. Stained for 30 minutes in Mayer's Haemalum (BDH product number 35060) diluted 2 volumes: 1 volume distilled water.
9. Briefly rinsed in distilled water.
10. Briefly rinsed in running tap water.
11. Washed for 5 minutes in distilled water.
12. Air-dried.
13. Slides were mounted with coverslips using DPX mountant.
14. The mountant was allowed to harden at approximately 37°C.

NB All stains and Bouin's fluid were filtered immediately prior to use to remove particulate material.

The stained smears were examined (under code) by light microscopy to determine the incidence of micronucleated cells per 1000 immature erythrocytes per animal.

Micronuclei are identified by the following criteria:

(i) Large enough to discern morphological characteristics
(ii) Should possess a generally rounded shape with a clearly defined outline
(iii) Should be deeply stained and similar in colour to the nuclei of other cells - not black
(iv) Should lie in the same focal plane as the cell
(v) Lack internal structure *ie* they are pyknotic
(vi) There should be no micronucleus-like debris in the area surrounding the cell

The proportion of immature erythrocytes for each animal was assessed by examination of at least 1000 erythrocytes. A record of the number of micronucleated mature erythrocytes observed during assessment of this percentage was also kept as recommended by Schmid (Schmid 1976).

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

ASSESSMENT OF RESULTS BY STATISTICAL ANALYSIS

Non-parametric statistical methods, based on rank are chosen for analysis of results because:

(a) They are suited to analysis of data consisting of discrete/integer values such as the incidence of micronucleated immature erythrocytes.

(b) The methods make few assumptions about the underlying distribution of data and therefore the values do not require transformation to fit a theoretical distribution (where data can be approximately fitted to a normal distribution, the results of non-parametric analysis and classical analysis of variance are very similar).

(c) 'Outliers' are frequently found in the immature erythrocyte to mature erythrocyte percentages for both control and treated animals; non-parametric analysis does not give such values an undue weighting.

Unless there is a substantial difference in response between sexes (which is rare) results for the two sexes are combined to facilitate interpretation and maximise the power of statistical analysis. For a comparison of an individual treated group with a concurrent control group, Wilcoxon's sum of ranks test is used (Hollander and Wolfe 1973, Langley 1979).

EVALUATION CRITERIA

A positive response is normally indicated by a substantial, statistically significant increase ($p < 0.01$) in the incidence of micronucleated immature erythrocytes compared to the incidence for the concurrent vehicle control group; individual and/or group mean values should exceed the laboratory historical control range. A negative result is indicated where individual and group mean incidence of micronucleated immature erythrocytes for animals treated with the test substance are not significantly greater than incidence for the concurrent control group and where these values fall within the historical control range. An equivocal response is obtained when the results cannot be adequately classified using the criteria for a positive or negative response.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial, statistically significant decrease ($p < 0.01$) in the proportion of immature erythrocytes. A very large decrease in this percentage would be indicative of a cytotoxic effect.

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

RESULTS

MICRONUCLEUS TEST

Table 1 gives a summary of the results for the rat micronucleus test on acetic anhydride and the results of statistical analysis. The results of individual animals are presented in Tables 2. Table 3 summarises the vehicle control micronucleated immature erythrocyte counts obtained in previous, unrelated experiments. Effects on bodyweight and histopathological changes indicate that significant toxicity occurred in rats exposed to 20 ppm acetic anhydride.

Micronucleated immature erythrocyte counts (mie)

Acetic anhydride did not cause any statistically significant increases in the number of micronucleated immature erythrocytes [$p > 0.01$ using Wilcoxon's sum of ranks test (Hollander and Wolfe 1973, Langley 1979)].

Cyclophosphamide caused large, highly significant increases ($P < 0.001$) in the frequency of micronucleated immature erythrocytes.

Micronucleated mature erythrocytes (mme)

Acetic anhydride did not cause any substantial increases in the incidence of micronucleated mature erythrocytes.

Proportion of immature erythrocytes (%ie)

Acetic anhydride failed to cause any significant decreases in the proportion of immature erythrocytes [$p > 0.01$ using Wilcoxon's sum of ranks test (Hollander and Wolfe 1973, Langley 1979)]. cyclophosphamide caused statistically significant decreases in the percentage [$p < 0.001$].

CONCLUSION

Since the test substance did not cause any substantial increase in the incidence of micronucleated immature erythrocytes or any substantial decrease in the proportion immature erythrocytes, it is concluded that acetic anhydride did not show any evidence of causing chromosome damage or bone marrow cell toxicity following subchronic inhalation exposure of rats in this *in vivo* test procedure.

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

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APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

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APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

TABLE 1

Micronucleus test

Group totals/means and results of statistical analysis

Treatment	Exposure level	ie/ie+me (mean %)†	Incidence mie (mean)†	Incidence mme (total)
Negative control	-	44	1.9	0.6
Acetic anhydride	20 ppm	41 ns	1.5 ns	0.4
Cyclophosphamide	40 mg/kg	18 **	14.1 **	0.0

ie/ie+me % Percentage immature erythrocytes
mie Number of micronucleated cells observed in 1000 immature erythrocytes examined
mme Number of micronucleated cells observed in 1000 mature erythrocytes examined
† Results of statistical analysis using Wilcoxon's sum of ranks test:

ns $p > 0.01$
* $p < 0.01$
** $p < 0.001$

} one-sided probabilities

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

TABLE 2

Micronucleus test - results for individual animals

Treatment	Exposure level	Animal number	ie/ie+me %	mie	me	mme
Air control						
		1	34	2	666	0
		2	36	2	658	1
		3	34	0	692	0
		4	53	3	473	0
		5	41	1	595	0
		6	42	0	609	2
		7	50	1	509	0
		8	45	1	567	0
		9	36	3	694	0
	-	10	45	4	558	0
		61	55	2	458	0
		62	45	0	567	0
		63	45	0	556	1
		64	45	2	562	2
		65	44	3	583	0
		66	49	5	533	1
		67	39	5	612	0
		68	42	0	580	0
		69	50	2	507	0
		70	52	1	481	0

ie/(ie+me) %	Proportion of immature erythrocytes
mie	Number of micronucleated cells observed in 1000 immature erythrocytes examined
me	Total number of mature erythrocytes examined for micronuclei
mme	Number of micronucleated mature erythrocytes observed

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

TABLE 2

(Micronucleus test - continued)

Treatment	Exposure level	Animal number	ie/ie+me %	mie	me	mme
Acetic anhydride - 20 ppm		46	43	0	582	0
		47	28	1	762	0
		48	45	4	556	0
		49	39	2	621	0
		50	40	1	599	0
		51	25	3	796	1
		52	28	1	750	0
		53	31	0	776	1
		54	16	1	873	0
		106	56	0	462	0
		107	39	1	699	0
		108	52	0	484	1
		109	44	2	563	0
		110	48	1	520	0
		111	50	1	507	0
		112	40	2	609	0
		113	46	1	553	0
		114	56	2	450	0
		115	49	1	522	0
Cyclophosphamide	40 mg/kg	121	26	14	742	0
		122	35	4	725	0
		123	33	15	761	0
		124	11	38	916	0
		125	3	9	1074	0
		126	11	8	907	0
		127	16	7	913	0
		128	20	13	928	0
		129	10	16	908	0
		130	16	17	872	0

ie/(ie+me) % Proportion of immature erythrocytes
mie Number of micronucleated cells observed in 1000 immature erythrocytes examined
me Total number of mature erythrocytes examined for micronuclei
mme Number of micronucleated mature erythrocytes observed

APPENDIX 1

(Acetic anhydride rat micronucleus test - continued)

TABLE 3

Rat micronucleus test

Historical control values

This summary presents a cumulative total of results for vehicle control animals used in previous, unrelated experiments during the period September 1990 to November 1993.

Cumulative results for 90 individual animals

mie	0	1	2	3	4	5	6	7	>7
Frequency	36	33	16	4	1	0	0	0	0

mie The incidence of micronucleated cells per thousand immature erythrocytes
Frequency The number of times that the result has been obtained

The individual animal mean mnp is 0.90

APPENDIX 2

Environmental conditions

Week number	Temperature (°C)		Relative humidity (%)	
	Minimum	Maximum	Minimum	Maximum
-2	20.5	22.0	50	75
-1	21.0	22.0	50	62
1	21.0	22.0	46	62
2	21.0	22.0	48	64
3	21.0	22.0	52	60
4	20.5	22.0	50	59
5	21.0	22.0	52	59
6	20.5	22.0	52	63
7	20.5	24.5	41	65
8	20.5	22.5	40	58
9	21.5	23.5	49	59
10	19.5	22.5	50	70
11	20.0	22.0	48	60
12	17.5	21.5	35	58
13	20.0	22.0	40	52
14	20.0	21.0	50	56
15	20.0	21.0	42	52
16	20.0	21.5	47	53
17	20.0	21.5	24	52
18	20.0	21.5	37	49
19	18.0	21.0	45	53
20	20.0	21.0	37	49
21	20.0	20.5	41	48
22	20.0	20.5	43	49
23	20.0	21.0	37	49
24	20.0	20.5	36	49
25	20.0	21.0	38	52
26	20.0	20.5	44	49
Mean	20.1	21.7	43.7	56.6
sd	0.83	0.90	6.73	7.00

sd Standard deviation

APPENDIX 3

Chamber concentration of acetic anhydride (ppm)

Exposure number	Sample number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
1 (males only)	1	1.2	7.2	26.9
	2	1.3	7.3	27.6
	2a	1.2	5.6	20.7
	3	1.1	5.6	19.0
	4	0.9	5.2	20.3
	5	1.2	5.2	17.9
	6	1.2	5.6	20.3
	Mean	1.2	6.0	21.8
	sd	0.13	0.90	3.84
2	7	0.9	5.6	19.3
	8	1.1	4.7	18.9
	9	1.1	4.9	19.6
	10	0.9	4.3	18.9
	11	1.2	5.1	19.7
	12	1.1	4.5	18.5
	Mean	1.1	4.9	19.2
	sd	0.12	0.46	0.46
3	13	1.1	4.8	19.0
	14	1.1	5.4	19.9
	15	1.2	5.1	18.7
	16	0.9	4.8	19.5
	17	1.3	5.2	19.6
	18	1.2	5.2	19.4
	Mean	1.1	5.1	19.4
	sd	0.14	0.24	0.43
4	19	1.0	4.8	19.9
	20	1.2	5.1	19.4
	21	0.8	4.8	19.4
	22	1.2	4.9	18.6
	23	1.0	4.8	20.3
	24	1.0	4.9	18.1
	Mean	1.0	4.9	19.3
	sd	0.15	0.12	0.81

a Additional sample taken to check level adjustment

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
5	25	1.2	5.1	19.3
	26	1.2	5.2	20.5
	27	0.9	4.8	16.7
	28	0.9	5.6	19.3
	29	1.0	5.2	19.8
	30	0.8	4.8	19.4
	Mean	1.0	5.1	19.2
	sd	0.17	0.30	1.29
6	31	1.1	5.3	20.3
	32	1.2	5.2	19.4
	33	0.9	4.5	18.3
	34	1.0	4.9	19.5
	35	1.2	5.0	19.1
	36	0.9	4.9	18.9
	Mean	1.1	5.0	19.3
	sd	0.14	0.28	0.67
7	37	1.1	5.2	(15.9)
	37b			20.3
	38	1.1	4.9	18.4
	39	0.8	4.5	16.8
	40	1.1	4.2	16.9
	41	1.1	4.8	19.3
	42	0.8	4.8	18.3
	Mean	1.0	4.7	18.3
	sd	0.15	0.34	1.36
8	43	1.0	4.7	19.4
	44	1.0	4.9	18.8
	45	0.9	4.3	18.3
	46	1.0	4.5	17.0
	47	1.1	5.0	18.1
	48	1.1	4.7	19.4
	Mean	1.0	4.7	18.5
	sd	0.08	0.26	0.91

b Additional sample taken. Initial sample, in parentheses, was faulty and is excluded from the mean

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
9	49	1.1	5.2	20.3
	50	1.2	4.7	20.4
	51	0.6	4.1	17.0
	52	1.0	4.5	19.2
	53	1.1	4.9	19.5
	54	0.8	4.4	18.7
	Mean	1.0	4.6	19.2
	sd	0.23	0.39	1.25
10	55	1.3	5.3	19.9
	56	1.2	5.6	20.3
	57	0.8	5.1	19.9
	58	0.9	4.3	18.4
	59	1.0	5.0	19.6
	60	0.7	4.7	19.6
	Mean	1.0	5.0	19.6
	sd	0.23	0.46	0.65
11	61	1.1	5.4	19.6
	62	1.2	5.2	20.5
	63	0.6	5.1	20.4
	64	0.9	4.7	19.9
	65	1.1	5.3	20.3
	66	0.8	4.8	17.9
	Mean	1.0	5.1	19.8
	sd	0.23	0.28	0.98
12	67	1.2	5.7	22.6
	68	1.2	4.8	17.3
	69	0.9	5.2	19.6
	70	0.8	4.6	20.8
	71	1.1	4.9	19.2
	72	0.9	4.9	18.1
	Mean	1.0	5.0	19.6
	sd	0.15	0.38	1.90

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
13	73	0.9	4.7	20.4
	74	1.5	5.2	20.3
	75	0.6	4.8	18.9
	76	0.8	4.9	18.8
	77	1.5	5.5	20.3
	78	0.9	5.2	21.7
	Mean	1.0	5.1	20.1
	sd	0.38	0.30	1.08
14	79	1.1	5.4	19.8
	80	1.0	5.3	19.5
	81	0.9	4.9	18.2
	82	0.8	4.9	18.6
	83	1.1	4.7	18.9
	84	1.1	5.1	18.4
	Mean	1.0	5.1	18.9
	sd	0.13	0.27	0.63
15	85	1.2	5.8	21.9
	86	1.1	5.1	20.0
	87	1.0	4.8	18.3
	88	1.0	4.4	20.1
	89	1.1	5.2	19.8
	90	0.9	4.3	19.4
	Mean	1.1	4.9	19.9
	sd	0.10	0.56	1.17
16	91	1.1	5.4	18.4
	92	0.9	5.1	20.1
	93	0.8	4.7	18.8
	94	0.8	4.5	18.0
	95	1.3	5.3	18.9
	96	0.8	4.5	19.1
	Mean	1.0	4.9	18.9
	sd	0.21	0.40	0.71

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
17	97	1.1	5.3	20.1
	98	1.0	4.5	19.1
	99	0.9	4.8	19.5
	100	1.1	4.7	19.0
	101	1.2	4.7	20.5
	102	0.8	4.5	19.9
	Mean	1.0	4.8	19.7
	sd	0.15	0.29	0.59
18	103	1.1	5.8	19.9
	104	1.4	5.2	20.5
	105	0.9	4.6	19.6
	106	0.7	4.6	20.0
	107	1.2	5.0	19.0
	108	0.8	4.7	22.2
	Mean	1.0	5.0	20.2
	sd	0.26	0.47	1.10
19	109	1.2	6.0	20.7
	110	1.3	4.8	20.7
	111	0.9	4.9	20.6
	112	0.8	4.9	20.5
	113	1.3	5.5	21.3
	114	0.8	4.7	20.7
	Mean	1.1	5.1	20.8
	sd	0.24	0.51	0.28
20	115	NV	NV	NV
	116	NV	NV	NV
	117	NV	NV	NV
	118	NV	4.5	17.0
	119	NV	4.6	17.1
	120	1.0	4.4	17.1
	Mean	1.0	4.5	17.1
	sd	0.0	0.10	0.06

NV No value, fault with Gas chromatagraph

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
21	121	1.0	4.7	18.8
	122	0.9	4.5	17.2
	123	0.5	4.3	15.6
	124	0.4	3.8	16.3
	125	1.1	4.1	17.7
	126	0.8	4.2	16.1
	Mean	0.8	4.3	17.0
	sd	0.28	0.31	1.18
22	127	0.9	4.8	18.3
	128	1.1	4.8	18.3
	129	0.6	4.6	13.0
	130	0.4	4.2	17.3
	131	1.0	4.6	18.3
	132	0.7	4.7	12.0
	Mean	0.8	4.6	16.2
	sd	0.26	0.22	2.91
23	133	0.9	4.6	17.3
	134	1.1	4.6	16.9
	135	0.8	4.5	17.8
	136	0.5	4.1	17.0
	137	1.0	4.2	16.4
	138	0.7	3.7	14.0
	Mean	0.8	4.3	16.6
	sd	0.22	0.35	1.34
24	139	1.2	3.9	16.3
	140	0.9	4.8	17.5
	141	0.8	4.3	16.0
	142	0.6	3.7	14.3
	143	1.0	4.1	16.3
	144	0.7	4.0	16.4
	Mean	0.9	4.1	16.1
	sd	0.22	0.38	1.04

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
25	145	0.9	4.3	17.1
	146	0.8	c	14.6
	147	c	3.3	c
	148	0.9	2.6	12.1
	149	0.6	3.4	13.6
	Mean	0.8	3.4	14.4
	sd	0.14	0.70	2.10
26	150	0.8	4.3	18.7
	151	0.8	4.3	19.6
	152	0.6	3.9	17.9
	153	0.6	3.6	17.7
	154	0.7	4.4	18.0
	Mean	0.7	4.1	18.4
	sd	0.10	0.34	0.78
27	155	0.7	4.6	19.2
	156	0.8	4.6	19.0
	157	0.6	4.6	17.0
	158	0.8	4.2	18.3
	159	1.0	5.2	18.6
	160	0.7	4.4	28.4
	Mean	0.8	4.6	20.1
	sd	0.14	0.33	4.15
28	161	0.8	4.9	19.4
	162	1.0	4.9	19.1
	163	0.6	4.1	16.4
	164	0.7	3.8	17.5
	165	0.9	4.5	14.5
	166	0.7	4.8	16.6
	Mean	0.8	4.5	17.3
	sd	0.15	0.46	1.83

c Damaged needle on sample probe. No meaningful result obtained

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
29	167	1.0	4.7	19.4
	168	0.8	4.6	17.6
	169	0.7	4.6	17.0
	169a			19.8
	170	0.9	5.9	20.5
	171	0.9	5.0	22.5
	172	0.7	4.6	20.8
	Mean	0.8	4.9	19.7
	sd	0.12	0.51	1.89
30	173	0.9	5.1	22.1
	174	1.0	5.2	22.4
	175	0.7	5.0	21.1
	176	0.7	4.9	20.4
	177	0.9	4.7	21.5
	178	0.8	4.7	18.7
	Mean	0.8	4.9	21.0
	sd	0.12	0.21	1.35
31	179	0.8	4.9	20.9
	180	0.8	4.8	20.1
	181	0.8	4.3	18.8
	182	0.6	4.7	19.5
	183	0.9	4.7	23.1
	184	0.7	4.4	17.7
	Mean	0.8	4.6	20.0
	sd	0.10	0.23	1.87
32	185	0.9	5.4	22.6
	186	1.0	4.9	23.1
	187	0.7	4.6	21.1
	188	0.9	4.6	17.4
	189	1.1	5.0	21.5
	190	0.8	4.9	20.1
	Mean	0.9	4.9	21.0
	sd	0.14	0.30	2.05

a Additional sample taken to check level adjustment

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
33	191	0.8	5.0	22.0
	192	0.9	4.9	22.2
	193	0.7	5.0	19.7
	194	0.9	4.3	19.2
	195	0.9	4.7	19.9
	196	0.7	4.6	19.8
	Mean	0.8	4.8	20.5
	sd	0.10	0.27	1.29
34	197	0.9	4.9	20.5
	198	0.8	4.8	21.8
	199	0.7	4.8	20.6
	200	0.9	4.6	19.8
	201	1.1	4.9	20.0
	202	1.0	4.5	19.1
	Mean	0.9	4.8	20.3
	sd	0.14	0.16	0.91
35	203	1.0	5.4	21.8
	204	1.1	5.0	20.2
	205	0.8	4.9	19.4
	206	0.8	4.5	18.4
	207	1.2	5.0	21.0
	208	0.8	4.8	15.5
	Mean	1.0	4.9	19.4
	sd	0.18	0.29	2.24
36	209	1.0	5.6	23.8
	210	0.9	5.3	21.9
	211	0.8	5.1	19.6
	212	0.9	5.0	22.1
	213	1.0	5.6	21.5
	214	0.9	5.2	19.7
	Mean	0.9	5.3	21.4
	sd	0.08	0.25	1.59

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
37	215	1.0	5.3	24.3
	216	1.2	5.9	21.6
	217	0.8	5.2	22.6
	218	1.0	4.8	15.9
	219	1.0	5.0	19.5
	220	0.9	5.4	21.7
	Mean	1.0	5.3	20.9
	sd	0.13	0.38	2.92
38	221	1.1	4.4	21.0
	222	1.1	4.9	19.3
	223	0.7	6.7	20.3
	224	0.9	4.8	20.2
	225	0.8	5.2	20.6
	226	0.8	4.8	18.0
	Mean	0.9	5.1	19.9
	sd	0.17	0.81	1.09
39	227	1.2	5.9	23.6
	228	1.1	5.3	21.2
	229	0.8	5.1	21.5
	230	0.9	5.3	22.2
	231	1.0	5.4	21.7
	232	0.9	5.2	19.7
	Mean	1.0	5.4	21.7
	sd	0.15	0.28	1.28
40	233	1.0	5.4	21.6
	234	1.2	4.8	18.6
	235	0.8	4.7	20.8
	236	1.1	5.1	20.8
	237	1.3	5.3	20.0
	238	0.8	4.9	21.5
	Mean	1.0	5.0	20.6
	sd	0.21	0.28	1.12

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
41	239	1.1	5.2	22.3
	240	0.9	5.1	21.2
	241	0.8	4.8	21.2
	242	0.7	5.4	18.7
	243	0.9	5.6	21.0
	244	0.7	5.7	20.4
	Mean	0.9	5.3	20.8
	sd	0.15	0.33	1.20
42	245	1.2	5.2	19.1
	246	1.0	4.9	21.3
	247	0.9	4.9	21.2
	248	1.0	4.9	20.3
	249	1.0	5.1	21.2
	250	0.8	5.1	21.4
	Mean	1.0	5.0	20.8
	sd	0.13	0.13	0.90
43	251	1.1	5.6	21.8
	252	1.0	5.2	21.0
	253	0.7	5.2	21.2
	254	1.1	5.1	20.4
	255	1.2	4.8	19.6
	256	0.9	5.4	21.8
	Mean	1.0	5.2	21.0
	sd	0.18	0.27	0.85
44	257	1.1	6.1	21.8
	258	1.0	4.9	23.1
	259	0.8	4.3	19.3
	260	0.9	4.4	18.3
	261	0.8	4.6	18.3
	262	0.8	4.0	17.7
	Mean	0.9	4.7	19.8
	sd	0.13	0.74	2.19

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
45	263	0.7	5.0	20.5
	264	1.1	5.7	22.7
	265	0.7	5.0	21.8
	266	0.7	5.2	19.2
	267	1.1	5.6	21.3
	268	0.8	5.8	22.6
	Mean	0.9	5.4	21.4
	sd	0.20	0.36	1.34
46	269	1.1	5.3	23.8
	270	1.2	5.7	22.1
	271	1.0	5.0	22.2
	272	0.9	4.8	21.9
	273	1.2	5.5	22.1
	274	1.1	5.0	21.6
	Mean	1.1	5.2	22.3
	sd	0.12	0.34	0.77
47	275	0.8	5.7	22.2
	276	1.1	5.8	21.8
	277	1.0	5.0	19.8
	278	0.9	4.8	20.6
	279	0.9	5.5	21.1
	280	1.2	5.3	20.0
	Mean	1.0	5.4	20.9
	sd	0.15	0.39	0.96
48	281	1.2	6.1	23.1
	282	1.1	5.6	22.7
	283	1.0	5.0	21.7
	284	1.0	5.1	21.3
	285	1.1	5.4	22.1
	286	1.2	5.3	21.3
	Mean	1.1	5.4	22.0
	sd	0.09	0.40	0.74

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
49	287	1.2	5.0	22.0
	288	1.0	5.5	21.8
	289	0.7	5.2	21.5
	290	1.1	4.9	20.3
	291	1.0	5.3	21.3
	292	1.0	5.3	20.8
	Mean	1.0	5.2	21.3
	sd	0.17	0.22	0.64
50	293	1.3	5.6	23.7
	294	1.2	5.2	22.4
	295	0.9	4.7	21.3
	296	0.7	4.7	20.2
	297	0.8	5.3	21.7
	298	0.9	4.9	20.6
	Mean	1.0	5.1	21.7
	sd	0.23	0.36	1.27
51	299	1.1	5.4	19.4
	300	1.0	5.0	20.5
	301	0.9	5.0	19.2
	302	1.0	4.9	18.9
	303	1.0	5.6	20.1
	304	0.9	5.0	18.5
	Mean	1.0	5.2	19.4
	sd	0.08	0.28	0.75
52	305	1.0	5.4	21.0
	306	1.1	5.4	20.2
	307	0.9	4.9	19.2
	308	0.8	5.3	18.3
	309	0.9	5.2	20.0
	310	0.8	4.7	19.1
	Mean	0.9	5.2	19.6
	sd	0.12	0.29	0.96

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
53	311	1.0	5.5	19.9
	312	1.1	5.6	21.1
	313	1.0	5.0	20.5
	314	0.7	4.8	18.7
	315	0.9	5.2	19.5
	316	1.0	5.1	19.7
	Mean	1.0	5.2	19.9
	sd	0.14	0.30	0.83
54	317	1.2	5.8	25.3
	318	1.1	5.4	21.3
	319	0.9	5.1	20.0
	320	0.9	4.5	20.0
	321	1.2	4.4	10.1
	322	0.8	3.4	
	Mean	1.0	4.8	21.2
	sd	0.17	0.85	2.36
55	323	0.9	5.6	22.3
	324	1.1	5.9	22.1
	325	1.0	5.7	20.7
	326	0.9	4.3	21.6
	327	0.8	5.6	19.2
	328	1.0	4.7	21.0
	Mean	1.0	5.3	21.2
	sd	0.10	0.64	1.14
56	329	1.2	5.4	24.0
	330	1.1	5.2	21.8
	331	0.9	5.1	21.0
	332	0.9	4.3	20.4
	333	1.1	5.2	21.3
	334	1.0	4.5	21.0
	Mean	1.0	5.0	21.6
	sd	0.12	0.44	1.27

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
57	335	1.1	5.6	22.2
	336	1.4	5.0	22.2
	337	0.9	4.7	19.6
	338	0.9	4.8	20.3
	339	1.1	5.0	19.7
	340	1.0	5.0	20.6
	Mean	1.1	5.0	20.8
	sd	0.19	0.31	1.17
58	341	1.0	5.6	21.8
	342	1.1	4.9	21.0
	343	0.9	4.7	20.5
	344	0.9	4.4	19.2
	345	1.1	4.7	20.9
	346	0.9	5.1	21.0
	Mean	1.0	4.9	20.7
	sd	0.10	0.41	0.86
59	347	1.2	5.3	22.3
	348	1.1	5.2	19.9
	349	0.9	4.6	20.2
	350	0.8	4.6	19.1
	351	1.2	5.0	20.9
	352	1.0	4.2	22.0
	Mean	1.0	4.8	20.7
	sd	0.16	0.42	1.24
60	353	1.2	5.5	21.9
	354	1.3	5.1	19.9
	355	0.9	4.2	19.9
	356	1.1	4.7	17.7
	357	1.1	5.1	22.5
	358	1.4	5.8	22.4
	Mean	1.2	5.1	20.7
	sd	0.18	0.57	1.89

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
61	359	1.0	5.4	22.5
	360	1.0	5.9	22.6
	361	0.9	5.0	20.5
	362	0.9	4.8	21.5
	363	1.6	5.1	22.0
	364	1.0	5.3	21.0
	Mean	1.1	5.3	21.7
	sd	0.27	0.38	0.84
62d	365	1.2	5.4	23.4
	366	1.1	5.5	21.9
	367	0.9	4.8	21.9
	368	1.0	3.5	20.9
	Mean	1.1	4.8	22.0
	sd	0.13	0.92	1.03
63	369	1.2	5.4	22.7
	370	1.3	5.3	22.5
	371	1.2	4.9	22.1
	372	1.0	4.7	22.4
	373	1.2	5.4	22.2
	374	0.9	4.9	22.6
	Mean	1.1	5.1	22.4
	sd	0.15	0.30	0.23
64e	375	1.0	5.3	24.9
	376	1.1	5.4	23.6
	377	1.0	4.8	19.6
	378	1.1	4.7	19.1
	Mean	1.1	5.1	21.8
	sd	0.06	0.35	2.89

d Duration of exposure reduced to 4 hours in order to perform ophthalmoscopic examination
e Duration of exposure reduced to 4.5 hours in order to perform blood sampling

APPENDIX 3

(Chamber concentration of acetic anhydride - continued)

Exposure Number	Sample Number	Group 2 (Low dose)	Group 3 (Inter dose)	Group 4 (High dose)
65	379	1.2	5.6	24.4
	380	1.1	5.0	21.1
	381	1.1	5.4	20.7
	382	0.8	5.0	20.0
	383	1.2	5.6	21.7
	384	1.2	5.3	20.7
	Mean	1.1	5.3	21.4
	sd	0.15	0.27	1.56
66	385	1.3	5.9	21.8
	386	1.3	5.7	21.2
	387	1.0	5.0	20.1
	388	1.0	5.2	19.7
	389	1.1	5.6	20.7
	390	1.1	5.1	19.4
	Mean	1.1	5.4	20.5
	sd	0.14	0.37	0.92
67 (females only)	391	1.2	5.5	23.3
	392	1.2	5.4	23.0
	393	1.0	5.1	21.8
	394	0.8	5.6	22.1
	395	1.4	5.3	23.3
	396	1.1	5.5	21.4
	Mean	1.1	5.4	22.5
	sd	0.20	0.18	0.82
Study mean		0.98	4.96	20.0
		0.109	0.382	1.63

APPENDIX 4

Individual clinical signs

Group	Animal no.	Observation
1♂ (Air control)	1 - 5	No abnormalities detected.
	6	Week nos. 11.1 to 13.2: hair loss from head. Week nos. 11.5 to 13.0: scabs on head.
	7 - 13	No abnormalities detected.
	14	Week nos. 11.1 to 11.2: scab on snout.
	15	No abnormalities detected.
1♀ (Air control)	61	No abnormalities detected.
	62	Week nos. 10.3 to 13.2: hair loss from head. Week nos. 11.4 to 13.0: hair loss from body.
	63	Week nos. 9.0 to 13.2: hair loss from body. Week nos. 11.0 to 13.0: hair loss from head.
	64	Week nos. 12.1 to 13.2: hair loss from head.
	65	No abnormalities detected.
	66	Week nos. 10.4 to 13.2: hair loss from body.
	67	Week nos. 11.3 to 13.2: hair loss from body. Week no. 11.2: hair loss from body post exposure only.
	68	Week nos. 11.3 to 13.1: hair loss from body. Week no. 11.2: hair loss from body post exposure only.
	69	Week nos. 5.1 to 13.1: hair loss from body. Week nos. 13.1 to 13.2 hair loss from head.
	70	Week nos. 10.1 to 13.1: hair loss from head. Week nos. 6.1 to 7.0, 10.3 to 12.0: hair loss from body.
	71	Week nos. 20.1 to 20.6, 21.3 to 25.5: hair loss from limbs. Week nos. 25.3 to 25.5: eyeball protruding (result of orbital sinus blood sampling.
	72 - 75	No abnormalities detected.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
2♂ (Low dose)	16 - 21	No abnormalities detected.
	22	Week nos. 8.1 to 9.0, 10.2 to 12.0: scabs on head. Week nos. 8.1 to 13.0: hair loss from head.
	23	No abnormalities detected.
	24	Week nos. 8.1 to 12.3: hair loss from head. Week nos. 10.4 to 11.0: scabs on head.
	25	Week nos. 7.1 to 13.0: scabs on head. Week no 7.1 to 13.2: hair loss from head.
	26	Week no. 10.1 onwards: hair loss from head. Week nos. 10.2 to 13.0, 14.0, 14.6 to 15.2, 24.1 to 24.5: scabs on head. Week no. 10.1: scabs on head pre-exposure only.
	27 - 28	No abnormalities detected.
	29	Week nos. 11.1 to 11.2: scabs on head. Week no. 11.3: scabs on head pre-exposure only.
	30	Week nos. -2 to 1: abrasion on back. Week nos. -1.2 to 1: isolated.
2♀ (Low dose)	76 - 90	No abnormalities detected.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
3♂ (Inter dose)	31 - 33	No abnormalities detected.
	34	Week nos. 11.3 to 13.0: left hind foot swollen.
	35 - 36	No abnormalities detected.
	37	Week nos. 7.1 to 10.3: scab on snout.
	38	Week nos. 9.1 to 10.0: hair loss from head.
	39	Week no. 6.0 onwards: scabs on head. Week no. 7.1 onwards: hair loss from head. Week nos. 11.5 to 13.0: hair loss from body.
	40	No abnormalities detected.
	41	Week no. 3.2: noisy breathing post exposure only. Week no. 10.0: rales.
	42 - 45	No abnormalities detected.
3♀ (Inter dose)	91 - 99	No abnormalities detected.
	100	Week no. 8.2: noisy breathing post exposure only.
	101	No abnormalities detected.
	102	Week no. 8.2: noisy breathing post exposure only.
	103	No abnormalities detected.
	104	Week no. 8.2: noisy breathing post exposure only.
	105	No abnormalities detected.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
4♂ (High dose)	46	Week nos. 2.2, 3.2, 4.4, 5.5 to 5.6, 6.3, 7.0 to 7.4, 8.0 to 8.2, 8.4 to 8.5, 9.1 to 9.5, 10.0 to 10.1, 10.3 to 10.5, 11.0 to 11.2, 11.4 to 11.5, 12.0 to 13.0, 13.2: noisy breathing. Week nos. 2.0, 2.1, 3.1, 5.3, 8.3, 8.6, 9.0, 11.3: noisy breathing pre-exposure only. Week nos. 1.6, 2.3, 3.3, 3.7, 4.3, 5.0 to 5.2, 6.2, 6.6, 7.6, 9.6, 10.2, 10.6, 11.6: noisy breathing post exposure only.
	47	Week nos. 4.1, 5.1, 5.3 to 5.5, 6.0 to 6.2, 6.4 to 6.5, 7.0 to 7.4, 8.0 to 8.5, 9.0, 9.2 to 9.5, 10.0 to 10.4, 10.6 to 11.2, 11.4 to 12.5, 13.0 to 13.2: noisy breathing. Week nos. 1.2, 2.3 to 2.4, 3.0, 4.2, 5.2, 6.3, 8.6, 9.1: noisy breathing pre-exposure only. Week nos. 3.2, 4.3, 6.6, 7.6, 9.6, 12.6: noisy breathing post exposure only. Week no, 1.6: red staining on snout pre-exposure only. Week no. 6.6: red staining on snout post exposure only.
	48	Week nos. 1.3, 2.2, 5.1 to 5.2, 5.5 to 5.6, 6.3, 7.0 to 7.6, 8.1 to 8.3, 8.5 to 8.6, 9.0 to 9.2, 9.4 to 9.5, 10.0, 10.2 to 11.5, 12.0 to 12.5, 13.0 to 13.2: noisy breathing. Week nos. 2.0 to 2.1, 4.3, 9.3: noisy breathing pre-exposure only. Week nos. 1.6, 2.3, 2.6, 3.1, 3.7, 4.2, 5.0, 6.2, 6.6, 8.0, 9.6, 10.1, 11.6, 12.6, 13.1: noisy breathing post exposure only. Week nos. 4.2, 6.2: red staining on snout post exposure only.
	49	Week nos. 2.3, 3.4, 4.4 to 4.5, 5.3 to 5.5, 6.0, 6.2 to 6.4, 7.0 to 7.4, 8.0 to 8.2, 8.5 to 11.5, 12.0 to 13.2: noisy breathing. Week nos. 2.2, 3.0, 4.1, 5.4, 8.3: noisy breathing pre-exposure only. Week nos. 2.0 to 2.1, 5.0 to 5.2, 6.1, 6.6, 7.6, 11.6: noisy breathing post exposure only. Week no. 7.2: red staining on snout pre-exposure only.
	50	Week nos. 4.6, 5.3 to 6.0, 6.3 to 6.4, 7.0 to 7.3, 8.0 to 8.5, 9.2 to 10.1, 10.6, 11.1 to 12.5, 13.0 to 13.2: noisy breathing. Week nos. 2.2, 3.2, 4.3 to 4.4, 7.4, 9.0: noisy breathing pre-exposure only. Week nos. 2.6 to 3.0, 5.0 to 5.2, 6.1 to 6.2, 6.6, 7.6, 8.6, 10.2, 11.0, 12.6: noisy breathing post exposure only. Week no. 12.3: rales. Week no. 4.2: red staining on snout post exposure only.
	51	Week nos. 2.1 to 2.2, 3.7, 4.4, 5.2 to 5.5, 6.0 to 6.4, 6.6 to 7.2, 7.4 to 8.3, 8.5 to 11.5, 12.0 to 13.2: noisy breathing. Week nos. 3.1, 7.3, 8.4: noisy breathing pre-exposure only. Week nos. 1.3, 2.0, 2.3, 3.6, 4.1, 4.3, 5.0 to 5.1, 11.6: noisy breathing post exposure only. Week no. 11.1: rales post exposure only.
	52	Week nos. 1.1, 2.3, 3.0, 3.2, 4.4, 5.4 to 5.6, 6.5, 7.0 to 7.2, 7.4 to 10.2, 10.5 to 13.0, 13.2: noisy breathing. Week nos. 2.0, 2.2, 4.1, 4.3, 5.3, 6.1, 7.3, 10.3: noisy breathing pre-exposure only. Week nos. 3.3, 5.0, 5.2, 6.6, 13.1: noisy breathing post exposure only.
	53	Week nos. 2.3, 4.4, 5.2 to 5.6, 6.3, 7.0 to 9.1, 9.3 to 9.5, 10.0 to 11.5, 12.0 to 13.2: noisy breathing. Week nos. 2.2, 6.2, 9.2: noisy breathing pre-exposure only. Week nos. 1.3, 2.0 to 2.1, 2.6, 3.0, 3.2, to 3.3, 4.2 to 4.3, 5.0 to 5.1, 6.0 to 6.1, 6.6, 9.6, 11.6 : noisy breathing post exposure only. Week no. 8.0: rales. Week nos. 9.1, 13.1: rales pre-exposure only.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
4♂ (continued)	54	Week nos. 2.0 to 2.2, 5.3, 5.5 to 6.0, 7.0 to 7.5, 8.0 to 8.4, 8.6 to 11.2, 11.4 to 11.5, 12.0 to 12.5, 13.0 to 13.2: noisy breathing. Week nos. 4.4, 5.1, 11.3: noisy breathing pre-exposure only. Week nos. 2.3, 3.6, 5.2, 6.2, 6.6, 7.6, 11.6, 12.6: noisy breathing post exposure only.
	55	Week nos. 1.1 to 1.2, 2.4, 3.2, 3.4, 4.0, 4.3, 5.1, 5.3 to 5.5, 6.2 to 6.5, 7.0 to 7.5, 8.0 to 8.2, 8.4 to 8.5, 9.2 to 9.5, 10.0 to 10.2, 10.5 to 11.0, 11.2 to 11.5, 12.0 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 2.2, 3.0, 3.3, 4.2, 6.0, 8.3, 8.6 to 9.0: noisy breathing pre-exposure only. Week nos. 3.7, 5.0, 5.2, 6.1, 6.6, 7.6, 9.1, 9.6, 11.1, 11.6, 12.5: noisy breathing post exposure only. Week no. 5.4: rales. Week no. 12.6: rales pre-exposure only. Week no. 7.5: red staining on snout. Week no. 8.2: Abrasions on back/side post exposure only.
	56	Week nos. 2.0 to 2.1, 4.4, 5.1, 5.4, 5.6 to 6.0, 6.4 to 6.5, 7.1 to 7.4, 8.0 to 8.2, 8.4 to 11.4, 12.0 to 12.5, 13.0 to 13.6, 14.4 to 14.5: noisy breathing. Week nos. 2.2, 3.0, 4.2, 5.3, 6.1, 7.0, 8.3: noisy breathing pre-exposure only. Week nos. 2.6, 5.0, 5.2, 6.2, 6.6, 7.6, 11.6, 12.6: noisy breathing post exposure only. Week nos. 18.2 to 19.3: abrasions on back/side. Week nos. 19.4 to 24.0: hair loss from body. Week no. 2.1: red staining on snout post exposure only.
	57	Week nos. 4.4 to 4.5, 5.1 to 5.6, 6.3, 7.0 to 7.1, 7.4 to 7.5, 8.0 to 8.6, 9.1 to 9.2, 9.4 to 10.5, 11.0 to 13.6, 14.4 to 15.3: noisy breathing. Week nos. 2.3, 4.1, 7.2 to 7.3, 9.3: noisy breathing pre-exposure only. Week nos. 3.1 to 3.2, 4.2, 6.1, 6.6, 10.6: noisy breathing post exposure only. Week nos. 10.1 to 10.2: rales. Week nos. 12.3 to 15.5: End of tail black. Week no. 15.6 onwards: end of tail missing. Week nos. 18.1 to 18.4: abrasions on back/side.
	58	Week nos. 4.4 to 4.5, 5.5 to 5.6, 6.4 to 6.5, 7.0 to 8.3, 8.5 to 9.5, 10.0 to 10.1, 10.3 to 14.0, 14.4 to 14.5: noisy breathing. Week nos. 2.0, 3.0, 5.3: noisy breathing pre-exposure only. Week nos. 2.1, 2.3, 4.2, 5.1 to 5.2, 6.0, 6.6, 9.6, 10.2: noisy breathing post exposure only. Week no. 4.2: red staining on snout post exposure only.
	59	Week nos. 2.4, 5.3 to 5.4, 6.2, 6.4, 7.0 to 7.5, 8.0 to 8.5, 9.0, 9.2 to 9.5, 10.0, 10.3 to 10.4, 10.6 to 11.0, 11.2 to 13.2, 13.5 to 14.0, 14.5 to 15.1: noisy breathing. Week nos. 2.1, 4.4, 6.3, 8.6, 9.1: noisy breathing pre-exposure only. Week nos. 3.1 to 3.2, 3.6, 5.0 to 5.2, 6.0, 6.6, 7.6, 9.6, 10.1 to 10.2, 11.1: noisy breathing post exposure only. Week nos. 4.2, 7.1: red staining around snout post exposure only.
	60	Week nos. 3.0, 5.3 to 5.4, 6.1, 6.3, 7.0 to 8.4, 8.6 to 9.1, 9.3 to 9.5, 10.0 to 10.1, 10.3 to 11.2, 11.4 to 12.6, 13.1 to 13.6, 14.1 to 14.2, 14.5 to 15.1: noisy breathing. Week nos. 4.1, 9.2, 11.3, 13.0: noisy breathing pre-exposure only. Week nos. 1.3, 2.1 to 2.3, 2.6, 3.6, 5.0, 6.0, 6.2, 6.6, 9.6, 10.2: noisy breathing post exposure only. Week no. 8.3: rales pre-exposure only.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
4♀ (High dose)	106	Week nos. 1.3, 4.0 to 4.1, 6.0, 6.6, 7.1 to 7.2, 7.4, 8.0 to 8.1, 8.3 to 8.4, 8.6 to 9.0, 9.3 to 9.4, 9.6 to 10.0, 10.2 to 10.5, 11.0 to 11.1, 11.3 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 6.2, 7.6, 9.2, 11.2: noisy breathing pre-exposure only. Week nos. 1.0, 1.2, 1.6 to 2.1, 3.1 to 3.2, 3.5, 5.2, 6.1, 6.5, 7.0, 8.2, 8.5, 9.1, 9.5, 10.1, 10.6, 12.5: noisy breathing post exposure only. Week no 4.1: red staining on snout post exposure only. Week nos. 13.1 to 13.2: hair loss from body.
	107	Week nos. 2.2, 3.2 to 3.3, 4.1, 4.3, 4.6, 5.1 to 5.4, 6.0, 6.2 to 6.3, 6.5 to 7.4, 7.6, 8.2 to 9.4, 9.6 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 6.1, 8.0: noisy breathing pre-exposure only. Week nos. 1.0 to 1.2, 1.6 to 2.1, 4.0, 4.5, 5.0, 5.6, 7.5, 9.5, 12.5: noisy breathing post exposure only. Week no. 4.5: rales. Week no. 11.2: hair loss from body. Week no. 11.1: hair loss from body post exposure only.
	108	Week nos. 1.3, 2.3, 3.1, 3.4, 4.3, 5.2 to 5.4, 6.1, 6.3 to 6.4, 6.6 to 7.4, 7.6 to 8.4, 8.6, 9.1 to 13.2: noisy breathing. Week nos. 1.6, 2.1, 4.2, 5.1: noisy breathing pre-exposure only. Week nos. 1.0 to 1.1, 2.0, 2.2, 2.6 to 3.0, 3.2, 4.6 to 5.0, 5.6, 6.2, 6.5, 7.5, 8.5, 9.0: noisy breathing post exposure only. Week nos. 11.3 to 11.4, 12.0 to 12.4, 13.0 to 13.2: hair loss from body. Week no. 11.1: hair loss from body pre-exposure only.
	109	Week nos. 2.3, 3.1, 5.3 to 5.4, 6.1, 6.3, 6.6 to 7.3, 7.6 to 8.0, 8.2 to 8.3, 8.6, 9.2 to 9.4, 9.6 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 2.1, 3.0, 5.2, 6.5, 8.5: noisy breathing pre-exposure only. Week nos. 1.1 to 1.2, 1.5, 2.2, 4.1, 4.6 to 5.1, 5.6 to 6.0, 7.5, 8.1, 9.0 to 9.1, 9.5, 12.5: noisy breathing post exposure only.
	110	Week nos. 1.1, 3.2, 3.4, 4.6, 6.1, 6.6 to 7.0, 7.3, 7.5 to 8.2, 8.4, 8.6 to 11.4, 11.6 to 12.1, 12.3 to 12.5, 13.0 to 13.2: noisy breathing. Week nos. 7.1 to 7.2, 8.5, 12.6: noisy breathing pre-exposure only. Week nos. 1.0, 1.2, 1.5, 2.0, 2.2, 2.6 to 3.0, 5.0 to 5.2, 6.2, 11.5, 12.2: noisy breathing post exposure only. Week no. 10.1: rales.
	111	Week nos. 2.2, 5.2, 6.0 to 6.1, 6.3, 6.6 to 7.1, 7.3 to 8.0, 8.2 to 8.3, 8.6, 9.1 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 3.2, 5.6, 7.2: noisy breathing pre-exposure only. Week nos. 1.1 to 1.2, 2.1, 2.6 to 3.0, 4.2, 5.0 to 5.1, 5.5, 6.2, 6.5, 8.1, 8.5, 9.0, 12.5: noisy breathing post exposure only.
	112	Week nos. 6.0 to 6.2, 6.4 to 7.2, 7.6 to 8.0, 8.3 to 9.1, 9.3 to 9.4, 9.6 to 11.3, 11.5 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 5.5, 9.2: noisy breathing pre-exposure only. Week nos. 1.0 to 1.2, 2.1, 3.0, 3.2, 3.5, 4.6 to 5.2, 7.5, 8.1 to 8.2, 9.5, 12.5: noisy breathing post exposure only.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
4♀ (continued)	113	Week nos. 1.3, 2.6, 3.1 to 3.3, 4.3, 5.1 to 5.4, 5.6 to 6.3, 7.0 to 8.2, 8.6 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 1.6, 2.2, 4.1: noisy breathing pre-exposure only. Week nos. 1.0 to 1.2, 1.5, 2.0 to 2.1, 3.5 to 4.0, 4.2, 4.6 to 5.0, 5.5, 6.5 to 6.6, 8.5, 12.5: noisy breathing post exposure only.
	114	Week nos. 3.3, 4.3, 6.1, 6.6, 7.1 to 7.4, 7.6 to 8.1, 8.3, 9.1 to 9.4, 9.6 to 11.4, 11.6 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 1.0, 1.5 to 2.0, 2.5, 3.0 to 3.2, 4.6 to 5.2, 5.5 to 5.6, 6.5, 7.0, 7.5, 8.2, 8.5, 9.0, 9.5, 11.5, 12.5: noisy breathing post exposure only.
	115	Week nos. 2.3, 4.3, 4.6, 5.2 to 5.3, 6.0 to 6.1, 6.3, 6.6 to 7.3, 8.0 to 8.4, 8.6 to 9.4, 9.6 to 10.1, 10.3 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 3.0, 4.1, 9.0: noisy breathing pre-exposure only. Week nos. 2.2, 2.5 to 2.6, 3.2, 4.2, 5.0 to 5.1, 5.5, 6.2, 6.5, 7.5 to 7.6, 8.5, 9.5, 10.2, 12.5: noisy breathing post exposure only.
	116	Week nos. 2.3, 2.6, 3.3, 4.2 to 4.3, 5.3 to 5.4, 6.4, 6.6 to 7.1, 7.3, 7.6 to 8.4, 8.6 to 10.1, 10.3 to 12.3, 12.5 to 14.4: noisy breathing. Week nos. 3.1 to 3.2, 4.1, 5.5, 6.2, 7.2: noisy breathing pre-exposure only. Week nos. 1.6 to 2.0, 3.5 to 4.0, 5.0 to 5.2, 5.6 to 6.1, 6.5, 7.5, 10.2: noisy breathing post exposure only.
	117	Week nos. 1.3, 4.3, 5.0, 5.4, 6.0, 6.3, 6.5, 7.0 to 7.3, 7.6 to 8.4, 8.6 to 9.4, 9.6 to 10.1, 10.3 to 11.4, 11.6 to 12.1, 12.3 to 12.4, 12.6 to 13.2: noisy breathing. Week nos. 4.6, 6.2: noisy breathing pre-exposure only. Week nos. 1.2, 1.5 to 1.6, 2.1, 3.1 to 3.2, 4.5, 5.1, 5.5, 6.1, 6.6, 8.5, 9.5, 10.2, 11.5, 12.2, 12.5: noisy breathing post exposure only.
	118	Week nos. 4.1, 5.2 to 5.4, 6.6 to 7.3, 7.5 to 8.0, 8.4, 8.6 to 11.1, 11.3 to 11.4, 12.0 to 12.1, 12.3 to 12.4, 12.6 to 13.2, 14.1: noisy breathing. Week nos. 5.5, 6.1, 8.2, 11.2: noisy breathing pre-exposure only. Week nos. 1.0, 1.2, 2.0 to 2.2, 2.5, 3.0 to 3.2, 3.5, 5.1, 5.6, 6.5, 8.1, 11.6, 12.2, 12.5: noisy breathing post exosure only. Week no. 4.1: red staining on snout post exposure only.
	119	Week nos. 3.0, 5.1, 5.3 to 5.4, 6.2 to 6.4, 6.6 to 7.0, 7.2 to 7.5, 8.0 to 9.0, 9.2 to 11.4, 11.6 to 14.4: noisy breathing. Week nos. 5.5, 6.1, 7.1, 7.6: noisy breathing pre-exposure only. Week nos. 1.1, 1.6, 2.1 to 2.2, 2.6, 3.1 to 3.2, 4.6 to 5.0, 5.2, 6.0, 6.5, 9.1, 11.5: noisy breathing post exposure only.
	120	Week nos. 2.1, 2.6, 4.3, 5.1, 5.3, 6.2, 6.6 to 7.1, 7.3, 7.6 to 8.4, 8.6 to 11.1, 11.3 to 13.3: noisy breathing. Week nos. 1.0 to 1.1, 1.5 to 2.0, 3.6, 4.1, 4.6 to 5.0, 5.2, 5.6 to 6.0, 6.5, 7.5, 8.5, 11.2: noisy breathing post exposure only.

APPENDIX 4

(Individual clinical signs - continued)

Group	Animal no.	Observation
5♂ (Positive control)	121 - 125	No abnormalities detected.
5♀ (Positive control)	126 - 130	No abnormalities detected.

APPENDIX 5

Bodyweights - individual values (g)

Group 1♂: Air control

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
1	1	221	266	302	337	357	388	410	441	454	474	499	506	524	540	540
	2	230	297	347	392	430	462	495	524	542	567	588	602	621	638	645
	3	244	311	350	382	409	430	458	481	499	516	532	544	559	571	586
	4	222	292	348	387	402	423	445	462	488	517	529	543	554	575	582
	5	246	321	369	412	437	464	492	522	547	577	596	608	619	639	644
2	6	233	292	320	353	376	392	416	442	461	489	509	517	524	536	541
	7	219	275	317	352	369	384	396	415	434	458	467	485	492	501	506
	8	225	293	346	386	405	436	463	481	506	531	543	555	577	585	595
	9	247	309	380	421	461	495	528	558	587	610	638	673	696	709	736
	10	245	321	350	367	393	417	439	473	497	510	531	537	551	570	586
3	11	232	284	312	333	357	372	400	418	442	458	473	491	503	522	545
	12	206	259	283	321	337	356	380	393	405	413	439	439	448	448	458
	13	224	302	356	399	436	457	478	505	531	545	561	571	571	585	590
	14	243	310	363	399	428	448	475	500	520	545	563	583	591	600	619
	15	256	317	360	404	432	466	491	514	531	548	573	585	600	625	639

APPENDIX 5

(Bodyweights - continued)

Group 1♂: Air control

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1	559	550													
	2	660	657													
	3	603	596													
	4	593	606													
	5	658	653													
2	6	550	541													
	7	520	519													
	8	602	602													
	9	760	744													
	10	593	604													
3	11	550	561	571	584	594	603	615	628	631	640	648	660	669	690	696
	12	441	459	461	469	470	487	486	494	500	498	503	503	503	510	507
	13	598	606	626	646	657	668	677	692	700	715	721	726	739	753	753
	14	628	632	647	662	679	698	709	722	731	733	743	766	773	782	788
	15	640	647	658	666	679	693	702	703	716	727	728	742	748	747	741

APPENDIX 5

(Bodyweights - continued)

Group 2♂: Low dose

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
4	16	248	331	369	416	439	463	488	505	529	541	557	565	577	598	610
	17	239	310	362	404	423	448	480	517	525	545	560	568	576	595	618
	18	224	270	298	331	349	367	387	403	421	428	448	456	471	481	494
	19	251	318	369	418	451	475	506	529	553	580	590	614	643	648	669
	20	232	304	359	396	420	451	473	500	524	546	561	581	596	611	630
5	21	243	313	359	395	416	433	447	474	506	524	542	558	567	580	588
	22	218	275	316	353	373	387	414	444	463	482	495	506	519	542	550
	23	228	290	337	381	404	437	450	482	507	526	547	557	584	605	621
	24	228	299	348	383	406	428	447	463	483	502	523	531	545	564	568
	25	248	319	362	407	445	467	509	534	557	578	601	620	638	661	660
6	26	255	320	369	406	441	470	494	516	538	567	583	599	614	632	648
	27	210	262	302	328	352	370	392	407	426	430	448	458	478	480	492
	28	244	311	357	395	424	456	472	517	535	563	590	614	636	656	672
	29	225	289	347	380	411	427	457	472	503	519	543	550	571	593	603
	30	215	275	322	362	378	398	421	441	452	465	478	486	495	513	518

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APPENDIX 5

(Bodyweights - continued)

Group 2♂: Low dose

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
4	16	619	613													
	17	628	623													
	18	500	500													
	19	671	694													
	20	639	644													
5	21	599	590													
	22	554	549													
	23	630	631													
	24	586	576													
	25	674	671													
6	26	662	665	675	694	707	722	738	753	757	754	764	774	787	797	807
	27	499	497	508	518	526	537	544	556	554	561	563	577	584	584	581
	28	690	685	697	714	727	740	753	767	772	785	792	803	812	827	827
	29	615	610	633	642	649	671	667	673	669	673	684	684	687	710	708
	30	527	518	530	543	550	558	572	580	597	600	597	610	614	626	625

APPENDIX 5

(Bodyweights - continued)

Group 3♂: Inter dose

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
7	31	235	311	353	383	414	439	472	501	522	550	577	597	609	617	631
	32	244	309	354	396	422	449	460	481	495	508	521	534	541	554	570
	33	223	281	323	360	388	406	430	449	470	490	509	523	540	555	572
	34	244	314	360	409	445	470	501	525	541	557	584	591	612	636	621
	35	233	292	334	370	400	426	440	454	474	498	509	518	526	546	556
8	36	227	277	305	336	359	381	406	425	435	446	464	471	475	488	505
	37	217	281	313	358	376	404	436	457	475	485	491	500	512	528	546
	38	235	294	327	358	372	401	424	434	450	480	487	500	503	518	524
	39	234	304	350	388	420	443	472	494	514	540	568	570	587	600	617
	40	230	275	304	337	346	367	394	414	429	440	461	480	489	508	516
9	41	231	294	346	385	417	447	474	497	517	540	551	565	593	598	617
	42	251	315	361	399	438	468	496	525	550	565	581	603	605	624	635
	43	219	272	313	351	381	410	440	461	474	495	513	529	548	558	579
	44	235	290	325	354	375	403	422	436	460	476	492	501	515	525	543
	45	235	285	313	337	358	377	392	409	417	430	450	446	452	467	480

APPENDIX 5

(Bodyweights - continued)

Group 3♂: Inter dose

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
7	31	637	631													
	32	578	573													
	33	580	580													
	34	600	594													
	35	565	564													
8	36	506	508													
	37	555	551													
	38	525	523													
	39	620	621													
	40	522	496													
9	41	630	640	660	680	693	726	741	755	772	776	787	800	808	825	831
	42	642	640	658	684	708	721	731	746	768	774	784	797	812	816	818
	43	589	580	598	605	619	627	644	654	656	669	681	697	701	710	711
	44	549	552	572	594	609	621	642	654	661	672	675	690	700	705	702
	45	486	480	489	498	511	524	532	549	544	548	559	567	574	590	594

APPENDIX 5

(Bodyweights - continued)

Group 4♂: High dose

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
10	46	216	282	310	331	345	348	354	371	385	396	411	418	421	419	438
	47	218	272	305	332	342	336	341	344	355	360	365	378	380	396	400
	48	242	315	358	392	405	416	430	443	448	463	474	485	496	498	518
	49	255	323	371	414	444	456	472	486	497	518	519	529	545	549	562
	50	229	282	313	351	376	393	392	402	404	426	433	439	455	463	476
11	51	229	286	320	356	367	384	412	423	442	449	451	456	442	459	461
	52	217	276	322	363	390	373	398	411	417	427	433	423	437	449	462
	53	242	302	347	381	388	405	424	436	445	452	448	446	451	459	477
	54	256	323	370	398	424	429	440	455	463	481	487	499	502	512	519
	55	232	306	360	423	431	438	438	426	430	443	456	456	470	484	500
12	56	218	282	317	346	357	369	382	391	376	381	370	380	403	409	419
	57	222	286	324	350	357	357	366	364	385	382	392	390	398	386	392
	58	235	307	357	383	396	407	423	436	446	451	459	464	468	467	477
	59	258	333	389	434	445	463	470	487	489	510	518	540	539	558	574
	60	238	292	335	366	376	403	406	408	412	429	433	429	431	443	444

APPENDIX 5

(Bodyweights - continued)

Group 4♂: High dose

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
10	46	443	450													
	47	398	405													
	48	528	523													
	49	575	581													
	50	479	480													
11	51	478	493													
	52	463	462													
	53	484	493													
	54	521	527													
	55	509	500													
12	56	423	426	455	475	492	513	523	533	535	547	548	562	567	588	593
	57	386	380	406	446	489	497	507	513	506	526	539	557	568	574	572
	58	484	479	510	532	563	559	576	593	597	605	618	624	635	645	646
	59	584	591	604	634	642	660	677	689	696	707	722	742	745	759	761
	60	450	451	489	509	540	557	570	579	596	603	611	624	637	644	654

APPENDIX 5

(Bodyweights - continued)

Group 1♀: Air control

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
13	61	192	217	235	252	258	270	284	286	301	310	312	316	327	333	334
	62	178	206	215	238	248	256	258	271	280	284	285	294	305	300	299
	63	187	207	226	246	260	263	282	290	294	302	311	321	328	332	336
	64	186	223	236	250	262	274	291	298	300	316	328	324	324	328	328
	65	199	219	228	229	244	258	265	267	281	290	302	298	318	328	341
14	66	193	216	228	241	254	268	269	275	278	292	294	295	301	304	305
	67	207	239	254	278	285	303	299	323	330	335	344	348	360	359	366
	68	175	212	228	240	241	258	266	276	278	289	299	304	299	310	308
	69	187	204	228	253	254	269	283	293	302	304	314	317	322	319	328
	70	179	206	220	244	247	278	282	290	292	304	312	315	313	317	323
15	71	188	215	231	246	265	270	286	299	301	311	313	323	336	327	330
	72	187	222	238	251	267	293	312	314	322	332	341	345	344	358	360
	73	191	211	227	249	263	275	285	296	303	305	313	322	324	322	334
	74	179	199	218	233	253	258	273	284	282	283	293	299	289	301	299
	75	198	210	222	246	250	273	274	284	293	301	292	311	315	317	319

APPENDIX 5

(Bodyweights - continued)

Group 1♀: Air control

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
13	61	341	334													
	62	303	292													
	63	343	348													
	64	345	339													
	65	320	334													
14	66	302	307													
	67	369	362													
	68	319	317													
	69	342	329													
	70	322	323													
15	71	325	330	330	330	340	345	348	345	355	353	352	353	347	347	
	72	363	347	355	359	367	367	384	392	393	400	406	407	415	408	423
	73	344	340	337	349	340	366	368	375	384	386	387	389	395	393	388
	74	307	298	300	309	316	306	318	322	320	314	318	322	311	317	320
	75	325	319	317	325	337	337	339	336	350	354	353	357	363	356	353

APPENDIX 5

(Bodyweights - continued)

Group 2♀: Low dose

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
16	76	195	211	232	247	260	256	269	275	276	280	287	294	298	302	302
	77	173	205	223	235	245	263	271	278	282	292	298	304	305	312	316
	78	199	222	244	266	276	281	300	310	320	318	332	340	340	343	364
	79	189	206	226	246	247	259	260	270	278	278	283	285	289	291	290
	80	183	219	236	253	274	289	294	309	310	314	330	335	352	342	343
17	81	184	210	218	231	245	254	259	269	276	287	290	299	306	305	305
	82	186	200	220	244	251	253	271	283	297	297	307	319	310	318	330
	83	207	222	241	266	279	294	312	321	327	330	340	343	353	350	357
	84	190	209	236	252	266	269	287	285	293	293	303	297	314	312	305
	85	209	245	249	282	297	324	322	339	352	362	373	382	397	403	402
18	86	198	222	237	259	269	275	292	302	307	316	324	339	337	337	343
	87	199	223	232	245	265	284	288	303	317	324	325	338	345	348	348
	88	191	201	214	232	244	255	275	282	285	291	303	308	306	321	329
	89	173	194	204	221	228	240	250	253	256	261	268	270	273	278	285
	90	193	231	256	275	281	293	309	314	316	325	346	348	351	357	362

APPENDIX 5

(Bodyweights - continued)

Group 2♀: Low dose

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
16	76	314	310													
	77	320	306													
	78	361	361													
	79	296	292													
	80	357	362													
17	81	309	308													
	82	342	335													
	83	362	359													
	84	302	314													
	85	415	411													
18	86	336	357	355	359	368	363	371	374	377	394	396	396	403	405	410
	87	355	358	359	362	369	376	377	371	383	386	385	387	396	395	395
	88	334	326	323	332	334	335	346	348	348	353	354	355	362	362	360
	89	289	284	289	286	299	291	298	299	302	311	314	313	319	320	320
	90	370	356	368	370	377	368	378	384	390	390	395	399	402	402	400

APPENDIX 5

(Bodyweights - continued)

Group 3♀: Inter dose

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
19	91	180	198	215	226	240	256	268	271	270	283	298	298	296	313	314
	92	196	225	237	247	260	265	284	285	294	300	305	302	309	316	316
	93	195	203	226	240	254	258	277	284	295	291	301	314	309	308	311
	94	185	202	220	244	255	266	276	285	289	301	313	325	330	328	344
	95	197	233	252	268	274	291	306	315	313	320	332	331	330	342	343
20	96	195	223	243	257	272	282	284	299	308	319	318	324	323	337	337
	97	190	215	248	261	279	276	290	294	303	301	316	322	322	317	317
	98	180	210	226	241	240	256	264	272	273	286	299	295	301	301	313
	99	184	209	225	239	244	264	275	286	291	303	315	322	328	331	341
	100	179	205	226	237	252	268	273	288	293	298	303	304	312	317	325
21	101	185	208	238	247	253	261	273	283	287	287	300	301	297	304	310
	102	190	215	223	237	250	266	269	271	281	286	287	289	292	293	294
	103	176	203	207	233	235	243	251	258	264	271	270	277	280	284	288
	104	209	238	251	279	288	314	314	332	351	360	352	372	380	392	389
	105	195	234	248	267	265	286	291	302	306	312	324	328	325	325	330

APPENDIX 5

(Bodyweights - continued)

Group 3♀: Inter dose

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
19	91	313	321													
	92	314	315													
	93	319	318													
	94	356	341													
	95	333	334													
20	96	338	329													
	97	331	327													
	98	312	306													
	99	338	333													
	100	323	321													
21	101	321	306	316	320	326	334	338	343	341	345	349	347	351	351	348
	102	297	296	297	303	306	313	322	318	323	320	324	323	320	324	323
	103	288	294	290	293	305	304	313	314	320	325	324	321	324	331	337
	104	403	405	409	414	435	447	446	449	464	465	474	463	480	490	461
	105	322	335	330	326	344	351	351	352	353	359	360	366	371	369	370

APPENDIX 5

(Bodyweights - continued)

Group 4♀: High dose

Cage number	Animal number	Week														
		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
22	106	176	185	198	209	203	210	220	226	226	230	235	236	238	240	248
	107	195	219	245	259	268	277	278	292	295	300	306	316	307	324	324
	108	178	211	225	243	238	256	260	265	268	275	284	289	292	296	288
	109	189	208	230	255	263	260	273	276	285	285	289	294	298	290	299
	110	203	230	251	270	269	274	282	285	294	290	287	291	292	289	297
23	111	190	210	221	231	235	235	233	243	253	257	254	258	262	262	265
	112	165	190	200	211	214	223	232	238	240	241	246	246	255	258	256
	113	205	230	246	260	270	273	273	279	285	292	289	293	306	297	299
	114	178	186	201	211	217	216	220	226	229	231	234	240	243	243	248
	115	190	221	239	251	260	246	254	254	255	261	251	258	267	256	266
24	116	188	211	226	246	241	257	263	258	265	274	279	276	275	264	268
	117	188	201	215	231	239	239	246	257	259	262	263	272	270	263	271
	118	167	188	204	208	218	221	229	224	233	243	237	237	238	233	240
	119	194	215	223	234	244	255	266	267	272	279	284	281	283	287	288
	120	212	234	246	257	274	289	296	299	308	310	322	322	326	321	331

APPENDIX 5

(Bodyweights - continued)

Group 4♀: High dose

Cage number	Animal number	Week														
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
22	106	250	245													
	107	331	324													
	108	303	297													
	109	304	303													
	110	304	301													
23	111	261	267													
	112	262	263													
	113	298	302													
	114	253	252													
	115	266	257													
24	116	286	258	289	308	317	329	337	342	345	359	360	370	377	382	379
	117	278	268	277	278	284	281	287	288	297	300	300	300	301	301	299
	118	239	237	237	253	258	263	272	281	285	287	290	299	304	310	305
	119	285	288	296	300	313	313	315	311	316	320	319	320	324	330	326
	120	340	349	351	353	376	388	396	395	404	413	411	415	423	428	418

APPENDIX 6

Food consumption - cage mean values (g/rat/week)

Week	Group 1♂ Air control			Group 2♂ Low dose			Group 3♂ Inter dose			Group 4♂ High dose		
	Cage											
	1	2	3	4	5	6	7	8	9	10	11	12
-3	230	234	229	239	239	235	241	233	223	227	236	235
-2	230	238	233	243	242	240	239	225	224	227	243	241
-1	231	225	234	238	241	241	238	220	221	226	238	237
1	224	224	233	235	239	236	240	207	229	201	206	194
2	227	227	230	229	232	228	241	214	227	178	187	191
3	235	238	239	237	244	237	242	226	235	174	186	188
4	243	239	236	241	247	243	245	224	236	175	181	184
5	243	249	242	244	251	243	248	223	236	181	191	180
6	238	239	231	235	242	243	242	203	233	178	184	182
7	240	241	236	237	244	239	244	212	230	175	174	168
8	238	240	234	233	248	243	247	213	225	178	163	175
9	235	236	226	232	243	245	243	209	229	177	174	178
10	235	240	216	233	246	241	242	213	229	181	188	179
11	233	237	223	236	234	240	240	213	233	191	194	190
12	234	232	217	230	236	239	227	209	224	188	187	184
13	217	213	209	214	213	220	213	196	211	163	173	171
14			225			241			247			236
15			226			235			249			243
16			225			229			245			246
17			228			231			250			238
18			230			238			250			232
19			228			238			250			239
20			230			234			241			230
21			238			238			254			235
22			232			236			253			234
23			237			246			251			240
24			228			241			250			238
25			231			243			252			241
26			209			228			235			223

APPENDIX 6

(Food consumption - continued)

Week	Group 1♀ Air control			Group 2♀ Low dose			Group 3♀ Inter dose			Group 4♀ High dose		
	Cage											
	13	14	15	16	17	18	19	20	21	22	23	24
-3	152	167	161	155	163	165	156	153	169	158	153	156
-2	155	171	166	160	169	166	157	156	174	167	157	160
-1	160	181	173	169	172	173	163	159	180	167	157	171
1	158	175	179	167	169	171	164	158	176	156	148	159
2	162	180	182	169	177	177	169	159	180	145	137	154
3	167	177	184	170	177	175	170	163	179	137	130	151
4	164	178	179	171	176	183	165	159	176	137	132	142
5	166	179	177	164	175	181	167	161	180	147	134	153
6	165	175	175	165	168	175	169	158	178	137	133	146
7	167	176	177	170	180	181	167	161	171	140	128	142
8	168	172	168	166	173	177	164	156	166	131	125	136
9	171	169	168	167	173	177	160	161	167	133	131	136
10	165	168	172	166	174	178	167	158	168	135	131	130
11	161	167	169	165	175	174	157	157	169	145	140	130
12	166	169	172	170	172	183	160	156	169	145	136	140
13	144	144	142	146	146	159	136	127	147	134	127	121
14			169			181			176			168
15			172			173			181			190
16			169			173			184			187
17			170			173			183			186
18			177			175			178			183
19			172			177			184			181
20			172			173			179			181
21			178			181			186			186
22			172			181			187			179
23			167			178			177			179
24			162			182			186			189
25			163			174			181			185
26			125			129			132			131

APPENDIX 7

Ophthalmoscopy findings

Date of examination: 02.11.95
Week 13

Group	Animal no.	Eye	Comments
1♂ (Air control)	1 - 15		No abnormalities detected
1♀ (Air control)	61		No abnormalities detected
	62	R	Large discreet opacity, pole of posterior capsule
	63 - 75		No abnormalities detected
2♂ (Low dose)	16 - 30		No abnormalities detected
2♀ (Low dose)	76		No abnormalities detected
	77	L	Pinpoint posterior capsular opacity with hyaloid remnant
	78 - 90		No abnormalities detected
3♂ (Inter dose)	31		No abnormalities detected
	32	B	Slight lack of corneal lustre
	33 - 35		No abnormalities detected
	36	B	Posterior capsular irregular opacities
	37		No abnormalities detected
	38	L	Slight lack of corneal lustre
		R	Pinpoint corneal opacity in nasal quadrant
	39		No abnormalities detected
	40	B	Slight lack of corneal lustre
	41 - 45		No abnormalities detected
3♀ (Inter dose)	91 - 95		No abnormalities detected
	96	L	Persistent pupillary membranes
	97		No abnormalities detected
	98	L	Slight lack of corneal lustre
	99 - 103		No abnormalities detected
	104	B	Lack of corneal lustre
	105	B	Lack of corneal lustre

L Left eye
R Right eye
B Both eyes

APPENDIX 7

(Ophthalmoscopy findings - continued)

Date of examination: 02.11.95
Week 13

Group No.	Animal No.	Eye	Comments
4♂	46	B	Small diffuse corneal opacity in lower half
(High dose)	47	B	Pinpoint corneal opacity below centre
	48	R	Pinpoint corneal opacity below centre
	49	B	Pinpoint corneal opacity near centre
	50		No abnormalities detected
	51	R	Pinpoint corneal opacity below centre
		L	Small diffuse corneal opacity below centre
	52 - 56		No abnormalities detected
	57	R	Pinpoint corneal opacity below centre
	58		No abnormalities detected
	59	B	Slight lack of corneal lustre
	60		No abnormalities detected
4♀	106		No abnormalities detected
(High dose)	107	R	Pinpoint corneal opacity near centre
	108	L	Pinpoint corneal opacity near centre
	109	B	Diffuse corneal opacity (more obvious in Left eye)
	110		No abnormalities detected
	111	L	Lack of corneal lustre
		R	Pinpoint corneal opacity near centre
	112	L	Faint pinpoint corneal opacity
	113	B	Diffuse opacity in centre of cornea
	114		No abnormalities detected
	115	R	Pinpoint central corneal opacity plus small irregular posterior capsular opacity
		L	Pinpoint central corneal opacity plus pinpoint posterior capsular opacity
	116	R	Slight nasal quadrant keratitis
	117	L	Pinpoint corneal opacity
	118	B	Central band of corneal opacity/dryness
	119 - 120		No abnormalities detected

L Left eye
R Right eye
B Both eyes

APPENDIX 7

(Ophthalmoscopy findings - continued)

Date of examination: 01.02.96
Week 26

Group No.	Animal No.	Eye	Comments
1♂ (Air control)	11 - 15		No abnormalities detected
1♀ (Air control)	71 - 75		No abnormalities detected
2♂ (Low dose)	26 - 30		No abnormalities detected
2♀ (Low dose)	86 - 90		No abnormalities detected
3♂ (Inter dose)	41 - 45		No abnormalities detected
3♀ (Inter dose)	101		No abnormalities detected
	102	R	Retinal disruption around disc
	103 - 105		No abnormalities detected
4♂ (High dose)	56 - 60		No abnormalities detected
4♀ (High dose)	116 - 120		No abnormalities detected

R Right eye

APPENDIX 8

Haematology - individual values

Week 13 (6 November 1995)

Group	Animal no.	PCV	Hb	RBC	MCHC	MCV	MCH	Retic	WBC Total	N	L	E	B	M
		%	g/dl	10^{12}/l	g/dl	fl	pg	%	10^9/l	10^9/l	10^9/l	10^9/l	10^9/l	10^9/l
1♂ Air control	1	48.4	16.8	9.34	34.7	51.8	18.0	1.2	11.82	1.44	9.65	0.18	0.04	0.22
	2	48.9	16.9	9.10	34.6	53.7	18.6	1.3	16.20	2.03	13.21	0.21	0.04	0.38
	3	44.9	15.6	8.66	34.8	51.8	18.0	2.7	16.25	3.98	11.45	0.18	0.05	0.24
	4	43.6	15.8	8.30	36.2	52.6	19.0	2.8	18.61	4.47	13.07	0.17	0.06	0.36
	5	47.0	16.4	8.92	34.9	52.6	18.4	2.3	13.39	2.99	9.75	0.26	0.02	0.17
	6	47.6	16.8	9.38	35.3	50.8	17.9	1.8	12.49	1.85	9.65	0.26	0.03	0.31
	7	45.3	16.1	8.84	35.5	51.2	18.2	1.3	9.59	1.46	7.55	0.20	0.02	0.18
	8	44.9	15.9	8.61	35.4	52.1	18.5	2.3	16.54	1.74	14.00	0.20	0.04	0.23
	9	46.0	15.7	9.46	34.2	48.6	16.6	2.6	14.55	3.74	9.49	0.20	0.03	0.51
	10	45.3	16.1	8.72	35.6	51.9	18.5	2.6	13.42	3.17	9.62	0.10	0.03	0.30
	Mean	46.2	16.2	8.93	35.1	51.7	18.2	2.1	14.29	2.69	10.74	0.20	0.04	0.29
	sd	1.72	0.49	0.381	0.59	1.35	0.64	0.63	2.672	1.124	2.082	0.046	0.013	0.105
2♂ Low dose	16	45.8	15.7	8.44	34.3	54.3	18.6	2.0	16.15	2.94	12.18	0.07	0.06	0.42
	17	48.7	17.1	9.30	35.2	52.4	18.4	1.9	15.07	1.87	12.45	0.10	0.04	0.25
	18	45.3	15.7	8.30	34.7	54.6	18.9	1.0	18.16	4.15	13.22	0.17	0.06	0.23
	19	47.0	16.1	8.74	34.2	53.7	18.4	1.4	13.82	4.47	8.39	0.11	0.04	0.38
	20	47.1	15.9	9.24	33.8	51.0	17.2	2.0	13.89	2.15	10.67	0.22	0.05	0.32
	21	45.8	16.2	8.88	35.4	51.6	18.3	2.0	12.76	2.52	9.67	0.11	0.03	0.18
	22	45.4	16.1	8.40	35.5	54.0	19.2	2.9	10.10	3.17	6.24	0.30	0.01	0.21
	23	43.1	15.2	8.55	35.1	50.5	17.7	3.1	13.61	2.97	9.46	0.22	0.02	0.57
	24	47.2	16.7	9.14	35.4	51.6	18.2	2.4	15.15	2.85	11.39	0.18	0.04	0.31
	25	45.8	16.0	8.85	34.9	51.7	18.0	2.2	26.30	5.85	18.39	0.31	0.09	0.60
	Mean	46.1	16.1	8.78	34.9	52.5	18.3	2.1	15.50	3.29	11.21	0.18	0.04	0.35
	sd	1.50	0.53	0.361	0.58	1.48	0.57	0.62	4.347	1.203	3.274	0.084	0.023	0.146

sd Standard deviation

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	LUC 10^9/l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10^9/l	TT s
1♂ Air control	1	0.28	-	+	-	-	-	-	-	-	-	1042	23
	2	0.32	-	-	-	-	-	-	-	-	-	927	26
	3	0.35	-	+	-	+	-	-	-	-	-	1116	27
	4	0.49	-	+	-	-	-	+	-	-	-	1177	25
	5	0.20	-	-	-	-	-	-	-	-	-	975	27
	6	0.40	-	++	-	+++	-	+	-	-	-	1209	25
	7	0.17	-	+	-	+	-	-	-	-	-	1042	23
	8	0.33	-	+	-	+++	-	+	-	-	-	1023	25
	9	0.58	+	++	-	+	-	-	-	-	-	1036	23
	10	0.21	-	++	-	+++	-	+	-	-	-	1033	23
	Mean	0.33										1058	25
	sd	0.130										86.4	1.5
2♂ Low dose	16	0.49	-	+	-	+	-	-	-	-	-	907	28
	17	0.36	-	+	-	+++	-	+	-	-	-	1277	28
	18	0.33	-	-	-	-	-	-	-	-	-	976	23
	19	0.43	-	+	-	-	-	-	-	-	-	1054	24
	20	0.48	-	++	-	+	-	-	-	-	-	1129	25
	21	0.27	-	+	-	-	-	-	-	-	-	986	26
	22	0.17	-	-	-	-	-	-	-	-	-	1180	24
	23	0.36	-	++	-	+	-	+	+	-	-	1122	25
	24	0.38	-	+	-	+	-	+	-	-	-	1168	26
	25	1.04	-	+	-	+	-	+	-	-	-	1104	26
	Mean	0.43										1090	25
	sd	0.234										110.8	1.5

sd Standard deviation

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	PCV %	Hb g/dl	RBC 10^{12}/l	MCHC g/dl	MCV fl	MCH pg	Retic %	WBC Total 10^9/l	N 10^9/l	L 10^9/l	E 10^9/l	B 10^9/l	M 10^9/l
3♂ Inter dose	31	45.2	15.9	8.79	35.1	51.4	18.1	2.7	13.92	1.93	10.92	0.23	0.04	0.39
	32	48.7	16.8	9.84	34.5	49.5	17.1	2.6	20.34	4.07	15.16	0.17	0.08	0.35
	33	45.5	16.0	8.57	35.2	53.1	18.7	2.1	17.80	4.94	11.60	0.15	0.05	0.50
	34	43.6	14.9	8.43	34.3	51.7	17.7	4.6	16.36	3.41	11.95	0.41	0.04	0.27
	35	46.3	16.2	8.91	35.1	52.0	18.2	3.3	10.05	3.49	5.92	0.15	0.02	0.25
	36	45.2	15.5	9.32	34.4	48.5	16.7	3.2	13.76	2.60	10.11	0.17	0.04	0.44
	37	45.0	15.9	8.36	35.4	53.9	19.1	3.0	13.33	1.66	10.75	0.25	0.04	0.30
	38	47.6	16.6	9.10	34.8	52.3	18.2	4.6	13.45	1.99	10.86	0.12	0.03	0.19
	39	46.5	16.5	9.07	35.6	51.2	18.2	2.8	13.72	1.93	10.92	0.17	0.04	0.35
	40	44.8	16.0	8.75	35.7	51.2	18.3	3.1	11.28	1.82	8.93	0.13	0.02	0.16
	Mean	45.8	16.0	8.91	35.0	51.5	18.0	3.2	14.40	2.78	10.71	0.20	0.04	0.32
	sd	1.48	0.55	0.445	0.50	1.58	0.71	0.81	3.028	1.131	2.327	0.086	0.017	0.107
4♂ High dose	46	46.3	16.4	8.52	35.5	54.3	19.3	2.5	12.35	4.29	7.07	0.45	0.02	0.28
	47	49.5	17.7	10.01	35.6	49.5	17.6	1.3	8.18	1.94	5.89	0.16	0.01	0.06
	48	47.6	16.8	9.12	35.3	52.1	18.4	1.8	17.63	1.75	15.07	0.20	0.07	0.16
	49	48.2	16.8	9.08	34.8	53.1	18.5	3.0	20.60	4.38	15.19	0.28	0.06	0.28
	50	47.3	16.0	9.24	33.9	51.1	17.3	1.9	17.98	2.08	15.21	0.12	0.07	0.15
	51	51.1	17.8	9.96	34.9	51.3	17.9	1.2	14.24	1.34	11.64	0.27	0.04	0.45
	52	51.9	18.1	10.26	34.8	50.6	17.6	1.9	13.88	3.11	9.63	0.36	0.04	0.38
	53	48.8	16.9	10.06	34.7	48.5	16.8	1.5	12.51	2.48	9.20	0.07	0.02	0.43
	54	46.4	16.5	8.71	35.5	53.4	19.0	1.1	18.55	3.20	13.74	0.33	0.06	0.47
	55	57.6	20.5	11.18	35.6	51.6	18.4	1.7	11.33	2.47	8.19	0.14	0.04	0.19
	Mean	49.5	17.4	9.61	35.1	51.6	18.1	1.8	14.73	2.70	11.08	0.24	0.04	0.29
	sd	3.41	1.29	0.818	0.54	1.78	0.78	0.59	3.859	1.032	3.563	0.120	0.022	0.143

sd Standard deviation

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	LUC 10^9/l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10^9/l	TT s
3♂ Inter dose	31	0.40	-	+	-	-	-	-	-	-	-	1096	25
	32	0.52	-	++	-	-	-	-	-	-	-	1281	26
	33	0.56	-	+	-	+	-	-	-	-	-	993	25
	34	0.28	-	+	-	-	-	-	-	-	-	1648	23
	35	0.22	-	+	-	+	-	-	-	-	-	998	27
	36	0.40	-	++	-	+	-	-	-	-	-	978	25
	37	0.34	-	+	-	-	-	-	-	-	-	1229	24
	38	0.26	-	+	-	-	-	-	-	-	-	1040	27
	39	0.31	-	+	-	+	-	-	-	-	-	954	27
	40	0.21	-	+	-	-	-	-	-	-	-	1069	26
	Mean	0.35										1129	26
	sd	0.120										211.9	1.2
4♂ High dose	46	0.23	-	+	-	-	-	-	-	-	-	1072	25
	47	0.12	-	++	-	-	-	-	-	-	-	1025	24
	48	0.38	-	+	-	+	-	-	-	-	-	1191	26
	49	0.40	-	+	-	-	-	-	-	-	-	1154	25
	50	0.35	-	+	-	-	-	-	-	-	-	838	21
	51	0.50	-	+	-	-	-	-	-	-	-	863	26
	52	0.37	-	+	-	-	-	-	+	-	-	935	27
	53	0.31	-	++	-	-	-	-	-	-	-	1174	25
	54	0.75	-	+	-	-	-	-	-	-	-	850	29
	55	0.30	-	+	-	-	-	-	-	-	-	854	30
	Mean	0.37										996	26
	sd	0.168										145.0	2.4

sd Standard deviation

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	PCV %	Hb g/dl	RBC 10^{12}/l	MCHC g/dl	MCV fl	MCH pg	Retic %	WBC Total 10^9/l	N 10^9/l	L 10^9/l	E 10^9/l	B 10^9/l	M 10^9/l
1♀ Air control	61	42.0	15.3	7.70	36.4	54.5	19.8	1.2	9.17	1.94	6.79	0.04	0.02	0.15
	62	46.2	16.1	8.46	34.9	54.7	19.1	1.3	14.02	2.36	10.98	0.19	0.03	0.20
	63	45.7	15.5	8.43	33.9	54.2	18.3	1.8	11.97	2.24	8.98	0.21	0.02	0.18
	64	44.5	15.5	8.24	34.8	54.0	18.8	1.4	6.79	0.61	5.69	0.08	0.01	0.19
	65	45.1	15.5	8.19	34.4	55.1	18.9	2.0	11.55	2.33	8.06	0.36	0.02	0.39
	66	43.7	15.4	8.25	35.2	52.9	18.7	1.5	7.28	1.45	5.43	0.11	0.01	0.14
	67	46.3	16.4	8.51	35.4	54.4	19.3	1.5	6.45	1.38	4.74	0.06	0.01	0.15
	68	45.4	15.9	8.33	34.9	54.6	19.1	1.9	8.33	1.33	6.47	0.08	0.03	0.21
	69	43.5	15.4	7.78	35.5	55.8	19.8	1.7	7.91	1.03	6.47	0.12	0.01	0.15
	70	42.1	14.9	7.88	35.3	53.4	18.9	2.0	6.27	1.07	4.87	0.10	0.01	0.12
	Mean	44.5	15.6	8.18	35.1	54.4	19.1	1.6	8.97	1.57	6.85	0.14	0.02	0.19
	sd	1.58	0.43	0.291	0.67	0.82	0.47	0.29	2.666	0.611	1.974	0.095	0.008	0.077
2♀ Low dose	76	45.8	16.6	8.85	36.2	51.8	18.7	0.7	8.34	1.11	6.74	0.12	0.01	0.16
	77	45.3	15.6	8.22	34.4	55.0	19.0	1.3	7.64	1.24	5.71	0.11	0.02	0.36
	78	45.2	16.0	8.42	35.5	53.7	19.0	2.3	8.83	1.64	6.53	0.17	0.02	0.21
	79	46.8	15.9	8.09	34.0	57.9	19.7	1.5	8.60	1.34	6.68	0.15	0.01	0.17
	80	45.5	16.2	8.47	35.6	53.7	19.1	2.1	7.64	1.57	5.43	0.31	0.01	0.19
	81	46.6	16.3	8.96	35.1	52.0	18.2	0.7	8.90	3.11	5.23	0.18	0.02	0.20
	82	45.1	15.9	8.44	35.2	53.4	18.8	1.6	9.79	1.88	6.95	0.12	0.02	0.40
	83	43.6	15.5	7.88	35.5	55.4	19.7	1.5	6.23	0.77	5.05	0.15	0.01	0.15
	84	44.3	15.5	8.27	35.0	53.5	18.7	2.2	9.74	1.95	7.21	0.16	0.02	0.18
	85	44.9	15.5	8.60	34.6	52.2	18.1	2.0	9.00	1.21	7.17	0.23	0.01	0.19
	Mean	45.3	15.9	8.42	35.1	53.9	18.9	1.6	8.47	1.58	6.27	0.17	0.02	0.22
	sd	0.96	0.38	0.329	0.65	1.85	0.53	0.58	1.074	0.646	0.830	0.060	0.005	0.086

sd Standard deviation

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	LUC 10^9/l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10^9/l	TT s
1♀ Air control	61	0.22	-	-	-	-	-	-	-	-	-	1016	21
	62	0.26	-	-	-	-	-	-	-	-	-	693	ctd
	63	0.34	-	-	-	-	-	-	-	-	-	1122	ctd
	64	0.21	-	-	-	-	-	-	-	-	-	954	21
	65	0.40	-	-	-	-	-	-	-	-	-	981	21
	66	0.14	-	+	-	-	-	-	-	-	-	1077	19
	67	0.10	-	-	-	-	-	-	-	-	-	1096	20
	68	0.20	-	-	-	-	-	-	-	-	-	1066	21
	69	0.14	-	-	-	-	-	-	-	-	-	1040	23
	70	0.11	-	-	-	-	-	-	-	-	-	1135	20
	Mean	0.21										1018	21
	sd	0.099										128.2	1.0
2♀ Low dose	76	0.19	-	+	-	-	-	-	-	-	-	979	19
	77	0.21	-	-	-	-	-	-	-	-	-	978	20
	78	0.26	-	+	-	-	-	-	-	-	-	1043	21
	79	0.25	-	-	-	-	-	-	-	-	-	1038	ctd
	80	0.14	-	-	-	-	-	-	-	-	-	1092	22
	81	0.16	-	+	-	-	-	-	-	-	-	979	22
	82	0.42	-	-	-	-	-	-	-	-	-	1085	20
	83	0.10	-	-	-	-	-	-	-	-	-	1234	23
	84	0.21	-	-	-	-	-	-	-	-	-	945	ctd
	85	0.19	-	-	-	-	-	-	-	-	-	944	20
	Mean	0.21										1032	21
	sd	0.087										89.0	1.2

sd Standard deviation
ctd Clotted Sample

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	PCV %	Hb g/dl	RBC 10^{12}/l	MCHC g/dl	MCV fl	MCH pg	Retic %	WBC Total 10^9/l	N 10^9/l	L 10^9/l	E 10^9/l	B 10^9/l	M 10^9/l
3♀ Inter dose	91	48.4	16.9	9.04	34.8	53.6	18.7	1.2	14.27	2.94	10.16	0.24	0.03	0.44
	92	44.2	15.3	8.28	34.6	53.4	18.5	2.8	7.38	1.08	5.80	0.16	0.02	0.18
	93	44.0	15.5	7.83	35.3	56.1	19.8	1.6	8.91	1.85	6.44	0.21	0.02	0.21
	94	43.5	15.2	7.67	34.9	56.7	19.8	1.3	9.05	1.47	7.00	0.10	0.02	0.24
	95	44.6	16.0	7.98	35.9	55.8	20.0	1.6	7.31	0.87	6.10	0.13	0.02	0.08
	96	45.6	16.2	9.14	35.4	49.9	17.7	1.1	8.49	0.84	7.11	0.30	0.01	0.09
	97	46.4	16.1	8.63	34.7	53.8	18.7	1.0	5.76	0.97	4.44	0.11	0.01	0.14
	98	45.1	16.0	8.24	35.5	54.7	19.4	1.9	9.19	1.76	6.87	0.15	0.02	0.20
	99	43.2	15.2	8.16	35.2	52.9	18.6	3.2	11.11	2.61	7.51	0.20	0.02	0.44
	100	45.0	16.0	8.36	35.6	53.9	19.2	1.9	6.45	0.74	5.24	0.21	0.01	0.10
	Mean	45.0	15.8	8.33	35.2	54.1	19.0	1.8	8.79	1.51	6.67	0.18	0.02	0.21
	sd	1.53	0.54	0.483	0.43	1.94	0.73	0.73	2.463	0.772	1.541	0.063	0.006	0.132
4♀ High dose	106	45.1	16.0	8.68	35.4	51.9	18.4	1.7	6.33	1.58	4.31	0.09	0.01	0.19
	107	46.9	16.7	8.85	35.5	53.0	18.8	1.6	7.87	1.74	5.53	0.14	0.01	0.23
	108	48.4	16.8	9.07	34.7	53.3	18.5	0.8	7.15	1.82	4.99	0.07	0.01	0.14
	109	46.3	16.4	8.59	35.6	53.9	19.2	0.9	9.56	2.60	6.65	0.08	0.02	0.11
	110	46.7	16.5	8.59	35.4	54.4	19.2	1.6	9.11	1.37	7.43	0.09	0.02	0.09
	111	45.8	15.9	8.49	34.8	53.9	18.8	2.7	5.51	1.03	4.31	0.05	0.00	0.06
	112	46.1	16.4	8.24	35.6	56.0	19.9	2.0	5.00	0.70	4.09	0.06	0.00	0.07
	113	49.9	17.6	8.81	35.3	56.6	20.0	2.2	8.21	1.52	6.27	0.16	0.02	0.10
	114	45.3	16.0	8.65	35.4	52.4	18.5	1.7	9.18	2.38	6.19	0.24	0.01	0.21
	115	46.9	16.6	8.31	35.4	56.4	20.0	1.4	6.24	1.18	4.68	0.14	0.01	0.12
	Mean	46.7	16.5	8.63	35.3	54.2	19.1	1.7	7.42	1.59	5.45	0.11	0.01	0.13
	sd	1.45	0.50	0.248	0.31	1.66	0.64	0.57	1.616	0.582	1.147	0.058	0.007	0.059

sd Standard deviation

APPENDIX 8

(Haematology - continued)

Week 13 (6 November 1995)

Group	Animal no.	LUC 10^9/l	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast	Plt 10^9/l	TT s
3♀ Inter dose	91	0.46	-	-	-	-	-	-	-	-	-	986	22
	92	0.14	-	-	-	-	-	-	-	-	-	1075	20
	93	0.18	-	-	-	-	-	-	-	-	-	1196	20
	94	0.23	-	-	-	-	-	-	-	-	-	887	19
	95	0.12	-	-	-	-	-	-	-	-	-	1303	21
	96	0.14	-	+	-	-	-	-	-	-	-	955	22
	97	0.10	-	-	-	-	-	-	-	-	-	1053	23
	98	0.19	-	-	-	-	-	-	-	-	-	608	21
	99	0.34	-	-	-	-	-	-	-	-	-	700	ctd
	100	0.14	-	-	-	-	-	-	-	-	-	1087	22
	Mean	0.20										985	21
	sd	0.113										211.5	1.1
4♀ High dose	106	0.15	-	+	-	-	-	-	-	-	-	1149	24
	107	0.23	-	-	-	-	-	-	-	-	-	869	22
	108	0.12	-	-	-	-	-	-	-	-	-	1006	23
	109	0.11	-	-	-	-	-	-	-	-	-	989	24
	110	0.11	-	-	-	-	-	-	-	-	-	1154	22
	111	0.07	-	+	-	-	-	-	-	-	-	1114	24
	112	0.08	-	-	-	-	-	-	-	-	-	1046	22
	113	0.13	-	-	-	-	-	-	-	-	-	1157	23
	114	0.14	-	+	-	-	-	-	-	-	-	368	24
	115	0.11	-	-	-	-	-	-	-	-	-	1204	20
	Mean	0.13										1006	23
	sd	0.044										245.8	1.3

sd Standard deviation
ctd Clotted Sample

APPENDIX 8

(Haematology - continued)

Week 26 (2 February 1996)

Group	Animal no.	PCV %	Hb g/dl	RBC 10^{12}/l	TT s
1♂ Air control	11	43.7	14.7	9.11	23
	12	42.7	14.7	8.81	25
	13	45.1	15.1	9.01	ctd
	14	43.0	14.3	8.67	22
	15	45.6	15.2	8.94	23
	Mean	44.0	14.8	8.91	23
	sd	1.28	0.36	0.172	1.2
2♂ Low dose	26	42.4	14.3	7.60	23
	27	42.8	14.6	8.05	ctd
	28	43.1	14.6	8.81	24
	29	44.0	14.6	8.80	22
	30	43.4	14.8	8.62	24
	Mean	43.1	14.6	8.38	23
	sd	0.61	0.18	0.533	0.8
3♂ Inter dose	41	45.2	15.2	8.38	23
	42	45.8	15.6	9.53	24
	43	42.7	14.1	7.70	20
	44	44.6	14.9	7.89	21
	45	50.3	16.8	9.31	23
	Mean	45.7	15.3	8.56	22
	sd	2.81	0.99	0.825	1.4
4♂ High dose	56	44.7	15.1	8.69	24
	57	44.8	15.2	8.58	25
	58	44.2	14.8	9.10	26
	59	46.1	16.0	8.96	26
	60	44.5	15.4	8.71	26
	Mean	44.9	15.3	8.81	25
	sd	0.73	0.45	0.214	0.7

sd Standard deviation
ctd Clotted Sample

APPENDIX 8

(Haematology - continued)

Week 26 (2 February 1996)

Group	Animal no.	PCV %	Hb g/dl	RBC 10^{12}/l	TT s
1♀ Air control	71	41.1	14.4	7.54	19
	72	43.9	15.0	7.76	22
	73	41.0	13.6	7.88	21
	74	44.6	15.4	8.05	20
	75	42.1	14.4	7.62	20
	Mean	42.5	14.6	7.77	20
	sd	1.64	0.68	0.204	1.2
2♀ Low dose	86	45.3	15.3	8.85	21
	87	42.8	14.4	7.81	24
	88	42.6	14.9	7.60	19
	89	44.0	15.3	8.17	21
	90	42.5	14.8	7.59	20
	Mean	43.4	14.9	8.00	21
	sd	1.20	0.38	0.528	1.7
3♀ Inter dose	101	41.9	14.2	7.94	ctd
	102	41.5	14.1	7.72	20
	103	45.1	15.7	8.13	19
	104	43.6	14.8	8.54	ctd
	105	40.3	13.7	7.36	18
	Mean	42.5	14.5	7.94	19
	sd	1.88	0.78	0.442	0.8
4♀ High dose	116	41.2	13.8	7.46	18
	117	42.4	14.7	7.87	19
	118	39.2	13.3	6.97	17
	119	43.7	15.0	7.83	22
	120	42.8	14.6	8.06	22
	Mean	41.9	14.3	7.64	20
	sd	1.74	0.70	0.432	2.1

sd Standard deviation
ctd Clotted Sample

APPENDIX 9

Biochemistry - individual values

Week 13 (6 November 1995)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
1♂ Air control	1	139	6.5	2.7	3.8	13	0.5	159	24	56	1	194	<0.1	144	4.2	5.3	3.8	103	61
	2	112	6.8	2.8	4.0	11	0.5	144	32	54	1	64	<0.1	144	3.7	5.4	3.3	103	64
	3	126	6.5	2.8	3.7	14	0.5	123	33	65	1	74	<0.1	144	3.7	5.3	3.6	103	51
	4	110	7.3	2.9	4.4	12	0.5	213	36	47	1	48	0.1	144	3.7	5.6	3.4	102	74
	5	99	6.9	2.8	4.1	12	0.5	125	28	52	2	56	<0.1	145	3.4	5.5	3.1	103	73
	6	137	6.7	2.8	3.9	13	0.5	149	32	63	<1	64	0.2	145	3.8	5.3	3.2	103	70
	7	126	6.8	2.9	3.9	11	0.5	198	29	57	1	54	0.1	144	3.8	5.4	3.4	102	107
	8	165	6.2	2.9	3.3	13	0.5	114	30	71	<1	61	0.1	146	3.4	5.1	3.4	105	65
	9	155	7.4	2.9	4.5	11	0.6	169	29	53	<1	51	<0.1	145	3.4	5.7	3.4	103	87
	10	115	6.8	2.8	4.0	13	0.5	117	29	54	<1	55	0.1	144	3.3	5.3	3.6	103	72
	Mean	128	6.8	2.8	4.0	12	0.5	151	30	57	<1	72	<0.1	145	3.6	5.4	3.4	103	72
	sd	20.8	0.36	0.07	0.34	1.1	0.03	34.1	3.3	7.1		43.5		0.7	0.27	0.17	0.20	0.8	15.4
2♂ Low dose	16	116	6.9	2.8	4.1	13	0.5	130	31	72	3	72	0.1	146	3.5	5.3	3.6	104	71
	17	128	6.8	2.9	3.9	15	0.5	163	30	59	<1	79	0.1	145	3.3	5.3	3.3	103	68
	18	118	6.4	2.8	3.6	13	0.5	128	33	65	1	83	0.1	145	3.7	5.3	3.4	105	78
	19	153	7.0	2.8	4.2	12	0.5	107	26	39	1	53	<0.1	147	3.3	5.6	3.4	102	90
	20	117	7.0	2.8	4.2	13	0.5	245	33	61	<1	83	0.1	146	3.2	5.4	3.5	103	84
	21	110	6.6	2.8	3.8	11	0.4	109	24	52	1	142	0.1	145	3.5	5.2	3.2	104	75
	22	125	7.1	2.9	4.2	12	0.5	132	33	53	1	89	0.1	144	3.5	5.4	3.4	103	81
	23	128	6.8	3.0	3.8	11	0.5	139	36	66	1	76	0.1	144	3.6	5.3	3.3	103	49
	24	115	6.6	2.8	3.8	13	0.5	136	24	49	1	75	0.1	145	3.5	5.3	3.2	103	83
	25	141	6.8	2.7	4.1	10	0.5	165	28	51	2	69	0.1	143	3.6	5.4	3.5	102	63
	Mean	125	6.8	2.8	4.0	12	0.5	145	30	57	<2	82	<0.1	145	3.5	5.4	3.4	103	74
	sd	13.2	0.22	0.08	0.22	1.4	0.03	39.8	4.2	9.7		23.2		1.2	0.16	0.11	0.13	0.9	12.0

sd Standard deviation

APPENDIX 9

(Biochemistry - continued)

Week 13 (6 November 1995)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
3♂ Inter dose	31	113	6.7	2.8	3.9	12	0.4	200	28	46	1	89	0.1	145	3.3	5.5	3.3	103	78
	32	170	6.9	2.8	4.1	13	0.5	148	36	70	1	87	0.1	145	2.8	5.4	3.0	104	85
	33	170	6.6	2.8	3.8	14	0.5	103	27	45	<1	64	<0.1	144	3.3	5.2	3.1	103	78
	34	119	6.7	2.8	3.9	11	0.5	97	27	55	1	57	0.1	143	3.8	5.3	3.2	102	64
	35	126	6.8	2.6	4.2	14	0.5	172	36	63	2	57	0.1	144	3.5	5.2	3.5	103	85
	36	134	6.6	2.8	3.8	13	0.5	140	30	57	1	48	0.1	145	3.5	5.3	3.1	105	68
	37	144	6.7	2.7	4.0	11	0.5	111	39	87	3	74	0.1	142	3.8	5.3	3.0	100	90
	38	120	6.8	2.9	3.9	15	0.5	94	28	61	<1	163	0.1	144	3.6	5.3	3.2	103	91
	39	131	7.1	2.9	4.2	16	0.6	164	27	54	2	62	0.1	144	3.5	5.3	2.9	102	52
	40	147	7.0	2.9	4.1	14	0.5	138	27	57	<1	108	0.1	142	3.9	5.4	3.3	101	68
	Mean	137	6.8	2.8	4.0	13	0.5	137	31	60	<2	81	<0.1	144	3.5	5.3	3.2	103	76
	sd	20.2	0.17	0.09	0.15	1.6	0.05	35.5	4.6	12.2		34.1		1.1	0.32	0.09	0.18	1.4	12.6
4♂ High dose	46	107	6.7	2.8	3.9	14	0.5	186	47	73	1	47	0.1	144	3.3	5.2	3.8	102	68
	47	116	6.8	2.7	4.1	12	0.5	202	53	83	1	50	0.1	145	3.8	5.2	3.4	106	56
	48	130	6.6	2.6	4.0	15	0.7	106	30	63	3	74	0.1	144	3.5	5.2	3.5	103	46
	49	142	7.0	2.9	4.1	14	0.5	120	26	53	<1	71	0.1	145	3.4	5.4	3.1	103	87
	50	129	6.6	2.4	4.2	12	0.6	116	29	64	1	92	<0.1	145	3.8	5.2	3.6	105	51
	51	100	6.4	2.7	3.7	16	0.6	126	35	86	<1	65	0.1	146	3.5	5.1	3.0	104	64
	52	105	6.9	2.9	4.0	15	0.6	205	45	78	1	236	0.1	145	3.4	5.3	3.5	105	50
	53	110	6.6	2.8	3.8	17	0.5	200	33	75	1	70	0.1	146	3.5	5.2	3.7	106	46
	54	124	6.6	2.7	3.9	15	0.6	158	39	72	1	57	0.1	145	3.4	5.2	3.2	103	47
	55	103	7.0	2.6	4.4	20	0.6	126	30	57	2	118	0.1	143	3.9	5.3	3.3	103	55
	Mean	117	6.7	2.7	4.0	15	0.6	155	37	70	<2	88	<0.1	145	3.6	5.2	3.4	104	57
	sd	14.0	0.20	0.15	0.20	2.4	0.07	40.2	9.0	10.9		56.0		0.9	0.21	0.08	0.26	1.4	12.9

sd Standard deviation

APPENDIX 9

(Biochemistry - continued)

Week 13 (6 November 1995)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
1♀ Air control	61	111	7.4	3.3	4.1	13	0.6	79	26	47	<1	30	0.1	144	3.2	5.5	2.6	105	79
	62	105	6.9	2.8	4.1	17	0.6	85	39	77	3	194	0.1	143	3.6	5.0	3.0	104	97
	63	130	7.2	3.4	3.8	15	0.6	72	27	57	1	120	0.1	143	3.3	5.7	3.0	105	100
	64	122	7.1	3.6	3.5	21	0.8	54	24	50	2	78	0.1	142	3.6	5.6	2.8	104	88
	65	118	7.2	3.4	3.8	16	0.6	89	39	61	<1	68	0.1	145	3.2	5.4	2.7	105	56
	66	129	7.2	3.5	3.7	18	0.5	67	21	50	1	118	0.1	143	3.4	5.5	2.5	103	73
	67	120	7.4	3.4	4.0	15	0.6	62	30	50	1	48	0.2	145	2.8	5.6	3.0	104	85
	68	107	7.7	3.7	4.0	14	0.5	59	22	51	1	71	0.2	142	3.0	5.8	3.0	101	74
	69	107	7.2	3.1	4.1	17	0.7	68	24	48	1	51	0.1	142	3.4	5.3	2.7	103	61
	70	103	7.1	3.3	3.8	18	0.6	85	30	64	<1	72	0.1	143	3.7	5.5	3.0	105	79
	Mean	115	7.2	3.4	3.9	16	0.6	72	28	56	<2	85	0.1	143	3.3	5.5	2.8	104	79
	sd	10.0	0.22	0.25	0.20	2.3	0.09	12.1	6.4	9.5		47.7	0.04	1.1	0.28	0.22	0.19	1.3	14.1
2♀ Low dose	76	117	7.0	3.5	3.5	26	0.6	131	44	73	2	84	0.2	143	4.0	5.6	3.1	104	69
	77	110	7.5	3.5	4.0	16	0.6	79	29	52	<1	47	0.2	144	3.5	5.7	3.0	101	107
	78	107	7.0	3.2	3.8	17	0.6	68	40	71	3	78	0.1	143	3.8	5.5	2.7	106	63
	79	123	7.2	3.4	3.8	21	0.6	62	27	62	1	109	0.2	144	4.4	5.7	3.6	106	97
	80	145	6.8	3.3	3.5	17	0.7	85	28	47	1	44	0.1	144	2.5	5.1	2.1	104	88
	81	112	7.1	3.2	3.9	17	0.7	65	28	74	<1	67	0.1	145	3.8	5.4	2.3	108	73
	82	107	7.9	3.7	4.2	18	0.7	76	43	61	1	95	0.2	144	3.0	5.7	3.1	102	74
	83	123	6.7	3.0	3.7	16	0.6	106	24	52	1	61	<0.1	143	3.7	5.5	2.7	106	72
	84	131	7.5	3.4	4.1	23	0.7	112	25	69	1	149	0.2	142	3.7	5.5	2.0	104	77
	85	144	6.7	3.3	3.4	17	0.6	70	27	46	1	60	0.1	143	3.3	5.3	2.2	104	81
	Mean	122	7.1	3.4	3.8	19	0.6	85	32	61	<2	79	<0.2	144	3.6	5.5	2.7	105	80
	sd	14.2	0.39	0.20	0.27	3.4	0.05	23.2	7.7	10.9		31.9		0.8	0.53	0.19	0.52	2.1	13.5

sd Standard deviation

APPENDIX 9

(Biochemistry - continued)

Week 13 (6 November 1995)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
3♀	91	112	7.0	3.3	3.7	15	0.6	139	70	105	<1	101	0.1	143	3.9	5.3	2.9	104	66
Inter dose	92	118	7.1	3.4	3.7	14	0.6	59	30	52	1	61	0.1	144	3.5	5.4	2.4	104	87
	93	124	7.8	3.5	4.3	19	0.7	70	66	106	<1	55	0.2	143	3.4	5.7	2.2	106	96
	94	108	7.0	3.4	3.6	17	0.6	52	32	61	1	42	0.2	143	3.4	5.3	3.1	104	61
	95	123	7.3	3.2	4.1	22	0.6	71	54	90	<1	66	0.2	144	3.3	5.5	2.8	106	103
	96	121	7.0	3.3	3.7	23	0.7	96	23	53	2	50	0.1	146	3.2	5.4	2.4	105	85
	97	124	7.4	3.3	4.1	16	0.6	58	25	48	<1	59	0.1	143	3.7	5.4	2.1	105	96
	98	143	6.8	3.0	3.8	15	0.7	87	20	61	2	251	<0.1	142	3.3	4.9	2.2	103	86
	99	127	7.8	3.0	4.8	18	0.7	72	23	46	<1	114	0.1	141	3.5	5.5	2.3	102	107
	100	132	7.3	3.5	3.8	21	0.7	80	35	52	1	50	0.2	143	3.3	5.6	2.5	103	80
	Mean	123	7.3	3.3	4.0	18	0.7	78	38	67	<1	85	<0.2	143	3.5	5.4	2.5	104	87
	sd	9.9	0.34	0.18	0.37	3.2	0.05	25.2	18.6	23.6		62.8		1.3	0.21	0.22	0.33	1.3	14.9
4♀	106	106	6.9	2.9	4.0	21	0.7	83	33	65	<1	45	0.1	144	3.2	5.3	2.9	103	62
High dose	107	122	7.3	3.2	4.1	25	0.7	72	27	59	1	73	0.1	143	3.7	5.3	2.9	104	70
	108	128	7.1	3.5	3.6	22	0.8	114	34	64	2	34	0.1	145	3.4	5.2	2.9	106	68
	109	110	7.5	3.2	4.3	25	0.7	84	40	74	<1	34	0.1	143	3.2	5.4	2.7	104	67
	110	138	7.0	3.1	3.9	25	0.7	102	46	54	1	53	0.2	143	3.3	5.5	3.1	104	70
	111	91	6.9	3.0	3.9	16	0.7	116	24	62	<1	27	0.1	147	3.5	5.0	2.0	111	73
	112	105	7.0	3.2	3.8	23	0.6	176	35	66	1	49	0.1	146	3.4	5.4	2.5	106	81
	113	124	6.8	3.1	3.7	19	0.7	82	29	59	<1	54	0.1	146	3.4	5.2	2.5	106	58
	114	108	7.0	3.0	4.0	20	0.7	84	51	98	2	52	<0.1	144	3.3	5.3	2.6	106	58
	115	119	7.3	3.5	3.8	27	0.8	71	40	69	<1	99	0.1	144	3.3	5.5	2.4	106	94
	Mean	115	7.1	3.2	3.9	22	0.7	98	36	67	<1	52	<0.2	145	3.4	5.3	2.7	106	70
	sd	13.6	0.22	0.20	0.20	3.4	0.06	31.5	8.5	12.2		21.0		1.4	0.15	0.15	0.32	2.2	10.9

sd Standard deviation

APPENDIX 9

(Biochemistry - continued)

Week 26 (2 February 1996)

Group	Animal no.	Urea Nitr mg/dl	Creat-inine mg/dl	GPT mU/ml	GOT mU/ml	Chol mg/dl
1♂ Air control	11	11	0.5	31	52	87
	12	15	0.5	26	53	77
	13	12	0.5	24	62	125
	14	12	0.6	33	56	91
	15	12	0.6	23	61	100
	Mean	12	0.5	27	57	96
	sd	1.5	0.05	4.4	4.5	18.2
2♂ Low dose	26	10	0.6	29	58	101
	27	14	0.7	35	78	95
	28	10	0.7	31	52	132
	29	13	0.6	46	70	140
	30	10	0.6	31	81	64
	Mean	11	0.6	34	68	106
	sd	1.9	0.05	6.8	12.5	30.6
3♂ Inter dose	41	10	0.5	32	46	95
	42	11	0.5	24	64	69
	43	13	0.7	41	99	171
	44	12	0.6	27	62	105
	45	14	0.6	38	85	78
	Mean	12	0.6	32	71	104
	sd	1.6	0.08	7.2	20.8	40.2
4♂ High dose	56	14	0.5	28	49	61
	57	14	0.6	28	52	70
	58	13	0.6	50	80	50
	59	12	0.6	23	60	95
	60	14	0.5	36	75	68
	Mean	13	0.6	33	63	69
	sd	0.9	0.05	10.6	13.8	16.6

sd Standard deviation

APPENDIX 9

(Biochemistry - continued)

Week 26 (2 February 1996)

Group	Animal no.	Urea Nitr mg/dl	Creat-inine mg/dl	GPT mU/ml	GOT mU/ml	Chol mg/dl
1♀ Air control	71	17	0.7	41	63	90
	72	17	0.7	36	94	99
	73	11	0.6	29	57	114
	74	14	0.6	24	52	102
	75	19	0.7	31	59	84
	Mean	16	0.7	32	65	98
	sd	3.1	0.05	6.5	16.7	11.5
2♀ Low dose	86	12	0.6	39	67	67
	87	28	1.1	92	283	83
	88	15	0.7	60	90	96
	89	17	0.6	32	76	100
	90	19	0.7	104	200	86
	Mean	18	0.7	65	143	86
	sd	6.1	0.21	31.8	94.8	12.9
3♀ Inter dose	101	16	0.6	32	53	118
	102	18	0.6	24	45	83
	103	20	0.7	46	91	85
	104	12	0.7	105	227	114
	105	20	0.7	70	153	149
	Mean	17	0.7	55	114	110
	sd	3.3	0.05	32.8	76.3	27.2
4♀ High dose	116	12	0.6	177	342	114
	117	15	0.6	187	452	187
	118	24	0.6	58	124	91
	119	19	0.7	28	75	83
	120	15	0.7	60	80	82
	Mean	17	0.6	102	215	111
	sd	4.6	0.05	74.2	172.0	44.2

sd Standard deviation

APPENDIX 10

Organ weights - individual values

Week 14

Group	Animal no.	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Prostate g	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g
1♂ Air control	1	538	2.06	1.62	15.4	0.86	2.95	55.5	1.066	1.729	1.704	2.15	0.691	0.671
	2	654	2.15	1.96	19.0	0.89	3.20	58.0	0.948	1.804	1.891	1.88	0.651	0.695
	3	597	2.11	1.81	19.0	0.90	3.07	53.7	1.156	1.667	1.770	1.69	0.716	0.815
	4	602	2.16	1.70	19.8	1.04	2.95	56.8	1.180	1.590	1.553	2.03	0.577	0.643
	5	648	2.17	1.85	19.5	0.96	3.39	61.9	0.554	1.810	1.823	1.69	0.639	0.726
	6	541	2.24	1.71	20.6	0.83	3.13	54.3	1.191	1.910	1.942	1.27	0.710	0.657
	7	527	2.13	1.61	18.3	0.82	2.79	54.7	1.014	1.615	1.707	1.74	0.657	0.709
	8	605	1.99	1.37	17.3	0.87	2.69	53.9	1.155	1.540	1.615	1.39	0.529	0.620
	9	739	2.18	1.84	23.5	1.29	3.40	61.8	1.187	1.970	2.069	2.09	0.770	0.761
	10	605	2.12	2.08	20.4	0.86	3.40	55.1	1.067	2.127	2.149	2.10	0.764	0.718
	Mean	605	2.13	1.75	19.3	0.93	3.10	56.6	1.052	1.776	1.822	1.80	0.670	0.702
	sd	64.0	0.068	0.200	2.16	0.143	0.253	3.08	0.1932	0.1859	0.1921	0.304	0.0767	0.0581
2♂ Low dose	16	616	2.06	1.71	22.0	0.99	3.40	54.1	1.251	1.748	1.711	1.53	0.778	0.736
	17	617	1.98	1.57	18.8	0.77	3.23	55.2	1.161	1.824	1.877	1.52	0.732	0.665
	18	494	2.11	1.64	14.6	1.03	2.95	49.8	0.808	1.741	1.718	1.64	0.615	0.605
	19	678	2.15	1.84	25.4	1.31	3.39	58.3	0.984	1.990	1.870	1.59	0.708	0.698
	20	639	2.17	1.82	19.8	0.96	2.82	40.9	1.023	1.791	1.784	2.17	0.679	0.661
	21	597	2.19	1.85	19.4	0.89	3.83	55.1	1.007	1.758	1.722	1.86	0.763	0.738
	22	547	2.11	2.01	19.7	1.43	3.11	50.5	1.175	1.814	1.813	2.13	0.803	0.752
	23	625	2.11	1.69	20.7	1.19	3.15	54.0	1.071	1.706	1.791	2.12	0.781	0.878
	24	567	1.91	1.57	17.7	1.05	2.97	47.5	0.807	1.699	1.666	1.75	0.578	0.628
	25	669	2.23	1.85	19.7	1.04	3.50	55.4	1.829	2.028	1.982	2.24	0.674	0.673
	Mean	605	2.10	1.75	19.8	1.07	3.23	52.1	1.112	1.810	1.793	1.86	0.711	0.703
	sd	56.2	0.098	0.142	2.78	0.195	0.300	5.06	0.2910	0.1130	0.0959	0.286	0.0748	0.0780

sd Standard deviation

HST 411/961219

APPENDIX 10

(Organ weights - continued)

Week 14

Group	Animal no.	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Prostate g	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g
3♂ Inter dose	31	629	2.11	2.06	19.4	0.96	3.45	59.5	0.961	1.945	1.941	2.39	0.807	0.758
	32	568	2.19	1.61	18.4	0.93	3.28	61.3	0.997	1.802	1.736	1.47	0.734	0.721
	33	582	2.00	1.52	18.5	0.84	2.91	44.2	0.976	1.772	1.718	1.96	0.671	0.642
	34	597	2.08	1.89	19.6	1.09	3.41	63.5	1.030	2.064	2.104	1.87	0.677	0.701
	35	564	2.08	1.77	16.0	0.78	2.96	48.2	0.772	1.749	1.782	2.10	0.611	0.678
	36	504	1.90	1.46	15.6	0.67	2.75	48.3	1.000	1.705	1.728	2.29	0.634	0.701
	37	555	2.02	1.80	17.8	0.86	3.24	63.1	0.855	1.634	1.604	1.50	0.642	0.657
	38	525	2.04	1.74	15.5	0.83	2.78	54.0	0.723	1.698	1.694	1.36	0.704	0.693
	39	624	2.08	1.78	17.7	0.80	3.51	65.4	1.215	1.963	1.922	1.60	0.700	0.777
	40	517	2.07	1.89	17.2	0.80	2.91	59.2	1.216	1.697	1.786	1.74	0.683	0.713
	Mean	566	2.06	1.75	17.6	0.85	3.12	56.7	0.975	1.803	1.802	1.83	0.686	0.704
	sd	42.8	0.077	0.182	1.50	0.115	0.289	7.49	0.1630	0.1407	0.1466	0.355	0.0558	0.0415
4♂ High dose	46	444	2.06	1.75	15.4	0.83	2.43	54.3	0.906	1.882	1.840	2.11	0.688	0.720
	47	396	1.73	1.59	10.8	0.49	2.41	44.6	1.009	1.573	1.633	1.05	0.587	0.586
	48	517	1.96	2.10	17.2	1.10	2.89	53.4	1.145	1.719	1.782	1.66	0.621	0.661
	49	564	2.14	1.99	18.0	1.14	3.13	68.1	1.072	2.075	2.149	2.30	0.796	0.840
	50	476	2.04	2.06	13.9	0.76	2.74	42.0	0.676	1.096	1.680	1.50	0.308	0.670
	51	487	1.95	1.95	13.8	0.58	2.50	61.8	1.163	1.851	1.794	1.38	0.619	0.690
	52	455	2.12	2.13	12.5	0.74	2.55	49.1	1.036	1.866	1.850	1.72	0.695	0.658
	53	481	2.06	2.37	14.7	0.90	2.77	51.6	0.853	1.740	1.740	1.70	0.636	0.683
	54	521	2.17	2.28	17.1	0.88	3.23	51.2	0.888	2.003	2.073	1.88	0.759	0.696
	55	498	1.99	2.63	15.9	0.66	3.00	53.6	1.020	2.069	2.120	1.17	0.753	0.758
	Mean	484	2.02	2.08	14.9	0.81	2.76	53.0	0.977	1.787	1.866	1.65	0.646	0.696
	sd	46.3	0.128	0.298	2.25	0.210	0.293	7.60	0.1480	0.2903	0.1842	0.392	0.1375	0.0673

sd Standard deviation

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APPENDIX 10

(Organ weights - continued)

Week 14

Group	Animal no.	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Air control	61	332	2.00	1.30	11.1	0.63	1.91	65.5	101.3
	62	296	1.92	1.39	9.4	0.68	1.68	74.5	86.5
	63	346	1.93	1.21	10.2	0.53	1.70	47.6	78.1
	64	344	1.94	1.28	11.1	0.60	1.75	60.7	80.8
	65	320	1.93	1.17	9.9	0.63	1.83	55.0	125.2
	66	298	1.97	1.17	9.4	0.41	1.84	60.2	73.6
	67	352	2.04	1.49	12.2	0.61	2.03	76.3	86.8
	68	315	1.92	1.23	12.4	0.54	2.16	67.9	85.5
	69	326	1.93	1.32	10.8	0.66	1.98	79.1	117.5
	70	312	1.95	1.44	9.8	0.64	1.77	80.0	78.0
	Mean	324	1.95	1.30	10.6	0.59	1.86	66.7	91.3
	sd	19.3	0.041	0.111	1.09	0.080	0.154	10.89	17.60
2♀ Low dose	76	307	2.00	1.23	10.4	0.60	1.88	57.9	79.6
	77	310	1.88	1.39	10.7	0.39	1.64	69.6	76.2
	78	358	2.07	1.35	13.4	0.56	2.49	74.2	105.3
	79	287	1.96	1.24	10.4	0.79	1.83	54.8	58.4
	80	348	2.08	1.34	10.5	0.47	1.79	73.0	102.7
	81	305	1.87	1.36	9.4	0.48	1.72	65.4	103.2
	82	332	1.97	1.16	10.7	0.49	1.65	59.1	109.7
	83	360	1.92	1.45	11.7	0.65	2.13	63.0	126.3
	84	304	1.96	1.32	10.5	0.52	1.91	65.3	82.7
	85	402	2.00	1.52	12.1	0.56	2.25	70.1	103.5
	Mean	331	1.97	1.34	11.0	0.55	1.93	65.2	94.8
	sd	35.3	0.071	0.108	1.13	0.112	0.278	6.57	19.95

sd Standard deviation

APPENDIX 10

(Organ weights - continued)

Week 14

Group	Animal no.	Body wt	Brain	Lungs	Liver	Spleen	Kidneys	Adrenals	Ovaries
		g	g	g	g	g	g	mg	mg
3♀ Inter dose	91	317	1.84	1.21	9.8	0.60	1.77	63.5	104.0
	92	312	1.85	1.19	10.1	0.44	1.65	45.0	74.0
	93	313	1.89	1.23	11.5	0.60	2.12	59.2	72.7
	94	340	1.93	1.36	12.0	0.61	2.01	64.2	127.3
	95	334	1.90	1.10	10.4	0.58	1.99	66.2	77.1
	96	334	1.94	1.32	10.6	0.63	1.80	61.9	103.8
	97	317	1.98	1.63	9.7	0.75	1.71	62.4	93.5
	98	307	2.00	1.14	10.6	0.62	1.89	59.2	89.9
	99	339	2.04	1.42	11.6	0.83	1.80	62.4	88.8
	100	319	1.95	1.24	10.2	0.47	1.44	61.3	77.4
	Mean	323	1.93	1.28	10.7	0.61	1.82	60.5	90.9
	sd	12.2	0.063	0.155	0.80	0.115	0.198	5.86	17.21
4♀ High dose	106	245	1.80	1.20	7.6	0.52	1.52	53.5	75.2
	107	330	1.89	1.56	10.7	0.58	2.02	65.3	78.1
	108	294	1.93	1.87	9.9	0.49	1.81	81.1	89.5
	109	304	2.00	1.48	9.2	0.54	1.73	65.6	83.8
	110	298	2.06	1.78	10.3	0.65	1.88	53.9	70.8
	111	270	1.96	1.41	8.2	0.55	1.67	50.1	70.5
	112	259	2.03	1.13	8.6	0.57	1.61	57.8	94.9
	113	301	1.88	1.79	9.2	0.52	1.73	59.8	99.2
	114	247	1.96	1.32	7.8	0.58	1.43	65.2	81.9
	115	264	1.81	1.41	9.4	0.50	1.79	73.7	40.2
	Mean	281	1.93	1.49	9.1	0.55	1.72	62.6	78.4
	sd	28.1	0.088	0.255	1.05	0.046	0.172	9.63	16.53

sd Standard deviation

APPENDIX 10

(Organ weights - continued)

Week 27

Group	Animal no.	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Prostate g	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g
1♂ Air control	11	690	2.07	1.72	18.0	0.98	3.01	76.3	0.868	2.166	2.005	2.05	0.462	0.752
	12	505	2.14	1.76	14.1	0.92	2.81	43.3	1.387	1.624	1.695	2.46	0.754	0.765
	13	745	2.16	1.88	25.3	1.05	4.06	51.1	1.655	2.176	2.195	3.23	0.836	0.830
	14	782	2.13	2.01	22.0	1.05	3.28	53.6	0.980	1.838	1.842	2.11	0.768	0.808
	15	731	2.15	1.86	21.2	0.89	3.37	48.9	0.704	1.957	2.008	2.61	0.669	0.758
	Mean	691	2.13	1.85	20.1	0.98	3.31	54.6	1.119	1.952	1.949	2.49	0.698	0.783
	sd	109.0	0.034	0.114	4.24	0.073	0.477	12.69	0.3917	0.2327	0.1891	0.476	0.1446	0.0344
2♂ Low dose	26	792	2.24	1.83	23.3	1.29	4.57	63.1	1.212	1.915	1.973	1.67	0.907	0.833
	27	577	2.10	1.64	15.1	0.78	2.88	48.1	0.695	1.741	1.696	2.08	0.731	0.720
	28	814	2.23	1.93	24.9	1.31	3.89	48.8	1.109	1.959	1.919	2.31	0.645	0.849
	29	704	2.10	1.94	21.2	1.05	4.00	60.7	1.151	1.842	1.893	2.54	0.778	0.764
	30	618	2.12	1.73	16.6	1.02	3.12	45.2	0.853	1.624	1.655	2.34	0.621	0.673
	Mean	701	2.16	1.81	20.2	1.09	3.69	53.2	1.004	1.816	1.827	2.19	0.736	0.768
	sd	103.9	0.071	0.132	4.24	0.219	0.687	8.12	0.2203	0.1355	0.1422	0.335	0.1146	0.0744
3♂ Inter dose	41	822	2.18	2.18	26.3	1.01	4.01	50.8	1.749	1.944	2.052	2.66	0.730	0.831
	42	807	2.10	1.85	19.9	0.99	3.26	62.4	1.112	2.188	2.060	1.67	0.716	0.716
	43	703	2.22	1.80	25.2	1.15	3.44	48.7	0.802	1.568	1.504	2.04	0.566	0.583
	44	695	2.21	1.89	19.4	1.35	3.63	44.0	1.311	1.776	1.810	2.15	0.794	0.773
	45	581	2.22	1.91	16.0	0.95	2.63	55.3	0.851	1.939	1.972	1.74	0.835	0.796
	Mean	722	2.19	1.92	21.4	1.09	3.39	52.2	1.165	1.883	1.880	2.05	0.728	0.740
	sd	97.9	0.050	0.147	4.30	0.165	0.509	6.98	0.3859	0.2294	0.2328	0.395	0.1027	0.0971
4♂ High dose	56	586	2.15	1.82	17.5	1.13	3.19	52.3	1.079	1.975	2.003	1.95	0.839	0.865
	57	560	2.10	2.11	14.6	0.76	2.54	61.8	1.115	1.809	1.781	2.00	0.660	0.737
	58	646	1.97	2.02	15.9	0.95	2.72	52.1	0.823	1.496	1.565	2.06	0.614	0.647
	59	760	2.35	2.18	21.5	0.92	3.85	57.9	0.949	2.113	2.118	2.47	0.760	0.826
	60	638	2.08	1.97	19.4	1.13	3.28	52.2	0.945	1.777	1.778	2.26	0.649	0.637
	Mean	638	2.13	2.02	17.8	0.98	3.11	55.3	0.982	1.834	1.849	2.15	0.704	0.742
	sd	77.0	0.140	0.136	2.74	0.155	0.514	4.41	0.1171	0.2323	0.2159	0.215	0.0928	0.1028

sd Standard deviation

APPENDIX 10

(Organ weights - continued)

Week 27

Group	Animal no.	Body wt g	Brain g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Air control	72	418	1.83	1.92	12.3	0.90	1.78	81.4	95.8
	73	379	1.93	1.28	11.7	0.53	2.45	63.3	81.0
	74	317	2.03	1.46	10.3	0.77	2.06	51.0	103.9
	75	347	1.86	1.12	9.4	0.50	1.68	54.5	66.8
	Mean	365	1.91	1.44	10.9	0.67	1.99	62.6	86.9
	sd	43.4	0.088	0.345	1.32	0.194	0.344	13.59	16.40
2♀ Low dose	86	402	2.00	1.28	10.4	0.68	1.89	78.7	82.7
	87	389	2.00	1.41	11.0	0.74	1.97	73.7	114.4
	88	355	1.96	1.45	11.0	0.56	2.06	58.2	93.3
	89	315	1.82	1.18	9.5	0.57	1.80	55.6	61.7
	90	398	2.00	1.35	10.2	0.68	1.81	71.3	102.5
	Mean	372	1.95	1.33	10.4	0.64	1.91	67.5	90.9
	sd	37.0	0.077	0.107	0.61	0.077	0.107	10.08	20.08
3♀ Inter dose	101	345	1.99	1.43	12.6	0.64	2.10	71.3	50.9
	102	319	1.82	1.20	9.5	0.53	1.61	60.6	84.0
	103	331	1.88	1.22	10.6	0.56	2.14	70.1	82.3
	104	454	2.12	1.45	12.3	0.55	2.46	88.2	92.3
	105	358	1.86	1.56	13.9	0.62	2.25	91.3	38.3
	Mean	361	1.94	1.37	11.8	0.58	2.11	76.3	69.6
	sd	54.0	0.121	0.155	1.74	0.044	0.312	13.01	23.52
4♀ High dose	116	382	1.84	1.36	11.2	0.67	1.81	49.8	87.9
	117	297	1.84	1.26	9.5	0.46	1.77	64.9	34.9
	118	302	1.85	1.33	10.2	0.62	1.60	64.2	57.7
	119	325	1.93	1.45	9.2	0.55	1.71	55.6	69.7
	120	417	1.97	1.46	11.3	0.47	1.88	52.4	97.0
	Mean	345	1.89	1.37	10.3	0.55	1.75	57.4	69.4
	sd	52.5	0.059	0.085	0.94	0.092	0.104	6.86	24.65

sd Standard deviation

APPENDIX 11

Individual pathological findings

In this appendix the macroscopic and microscopic findings relating to each animal are listed.

The microscopic pathology was carried out by two pathologists. 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: Richard L. Gregson, M.Phil., Ph.D., C. Biol., M.I.Biol.,
Senior Pathologist
Department of Pathology

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

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 1♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Marked)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 2♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 3♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 4♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Marked)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 5♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Lungs
Pale subpleural foci: (Right , A few , Punctate)

Stomach
A caseous cyst: 3mm

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Three) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Trace , Focus , One lobe)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Marked)

Kidneys
Dilatation of the renal pelvis: (Minimal , Unilateral)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa
Cystic structure at limiting ridge

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 5♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 6♂ (Terminal)

MACROSCOPIC FINDINGS

Skin Scabs
Right side of face: (A few) 1mm

Skin Alopecia
Right side of face

Lymph Nodes - Cervical
Enlarged: 8mm

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

Skin
Epidermal erosion: (Minimal , Foci)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 6♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 7♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7m

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 8♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Stomach
A caseous cyst: 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart
Myocardial fibrosis: (Minimal , Focus)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Stomach
Cystic structure at limiting ridge

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 9♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 10♂ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Sides of face: (Diffuse)

Lymph Nodes - Cervical
Enlarged: 8mm
Congested

Liver
Pale subcapsular area/s: (One) median lobe, 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Liver
Subpleural area of rarefied hepatocytes: (Minimal , One lobe)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 11♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Median cleft, pale subcapsular area: 1mm

Testes
White subtunical striae: (Left)
Oedematous: (Left)

Epididymides
Small: (Left)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Epididymides
Spermatozoa absent - unilateral

Testes
Unilateral intratubular oedema
Unilateral pressure atrophy

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 11♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Liver : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 12♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm
Congested: (Minimal)

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 13♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 14♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm
Congested

Kidneys
Irregular cortical scarring: (Left , Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Kidneys
Subcapsular nephritis: (Trace , Focus , Unilateral)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 15♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Marked)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 16♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 17♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 18♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm
Congested: (Minimal)

Lymph Nodes - Deep Cervical
Enlarged: 3mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Lymph Nodes - Deep Cervical : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 19♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 20♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Larynx
Arytenoid - squamous epithelial hyperplasia: (Trace)
Arytenoid - subepithelial inflammation: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 21♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Marked)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 22♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm
Congested: (Minimal)

Spleen
Enlarged: (Minimal) 1.433g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Spleen
Red pulp congestion

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 23♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 24♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 25♂ (Terminal)

MACROSCOPIC FINDINGS

Skin Scabs
Left side of face: (A few) 1mm

Skin Alopecia
Sides of face

Lymph Nodes - Cervical
Enlarged: 11mm

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Marked)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

Skin
Epidermal erosion: (Minimal , Foci)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 26♂ (Recovery)

MACROSCOPIC FINDINGS

Skin Scabs
Sides of face: (A few , Punctate)

Skin Alopecia
Sides of face

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Skin
Scab: (Trace)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 27♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 28♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm
Congested: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 29♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 30♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Median cleft, pale subcapsular area: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Liver : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 31♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Arytenoid - squamous epithelial hyperplasia: (Minimal)
Arytenoid - subepithelial inflammation: (Minimal)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 32♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 3mm

Spleen
Clear fluid-filled cyst/s: (A few) capsular, 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Arytenoid - squamous epithelial hyperplasia: (Minimal)
Subepithelial inflammatory infiltration: (Minimal)
Arytenoid - subepithelial inflammation: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Lymph Nodes - Deep Cervical
Increased cellularity - generalised: (Moderate)

Spleen
Mesothelial capsular cyst(s)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 32♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 33♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm
Congested: (Minimal)

Stomach
A caseous cyst: 3mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Stomach
Cystic structure at limiting ridge

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 34♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - inflammation
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Arytenoid - squamous epithelial hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 35♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm
Congested: (Minimal)

Lungs
Pale subpleural foci: (A few , Punctate)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 36♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Moderate)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 37♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm
Congested: (Minimal)

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Two) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 38♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - hyperplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Trace)
Arytenoid - squamous epithelial hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 39♂ (Terminal)

MACROSCOPIC FINDINGS

Skin Scabs
Sides of face: (A few) 1mm

Skin Alopecia
Sides of face

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Trace)

Lymph Nodes - Cervical
Medullary plasmacytosis: (Moderate)

Skin
Scab
Epidermal hyperplasia: (Minimal)

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 40♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Stomach
A caseous cyst: 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Minimal)

Larynx
Arytenoid - squamous epithelial hyperplasia: (Minimal)

Lungs
Pneumonitis: (Moderate)
Bronchiolitis: (Moderate)
Prominent BALT: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Stomach
Cystic structure at limiting ridge

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 40♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea(including Bifurcation)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 41♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Congested: (Minimal) right, anterior lobe

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 42♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 43♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Lungs
Dark subpleural foci: (A few) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 44♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 45♂ (Recovery)

MACROSCOPIC FINDINGS

Extra-orbital Lachrymal Glands
Pale subcapsular foci

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Extra-orbital Lachrymal Glands
Vacuolation of groups of acinar cells: (Minimal , Areas)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 46♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Moderate)
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Ventrolateral epithelial hyperplasia: (Minimal)
Arytenoid - epithelial keratinisation: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

Trachea(including Bifurcation)
Tracheitis: (Minimal)
Epithelial hyperplasia: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)

Lungs
Inflammatory cell foci at terminal bronchioles: (Trace)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 46♂ - continued

MICROSCOPIC FINDINGS - continued

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Spleen
Reduced cellularity of the white pulp - generalised: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 47♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 4mm

Lungs
Not collapsed

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - granular eosinophilic inclusions: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)
Respiratory epithelium - squamous metaplasia: (Minimal , Focal)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Marked)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 47♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)

Lungs
Inflammatory cell foci at terminal bronchioles: (Trace)
Perivascular inflammatory cells: (Minimal , Focal)
Bronchiolar squamous cell metaplasia: (Minimal , Focal)

Thymus
Reduced cellularity of cortex: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Lymph Nodes - Deep Cervical
Medullary plasmacytosis: (Minimal)

Spleen
Reduced cellularity of the white pulp - PALS: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 48♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 4mm

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Moderate , Foci/areas)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Moderate)

Trachea(including Bifurcation)
Inflammatory cells in the lamina propria: (Moderate)
Epithelial hyperplasia at the carina: (Minimal)

Lungs
Perivascular inflammatory cells: (Minimal)
Prominent BALT: (Moderate)
Alveolar ducts - fibrosis: (Trace)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 48♂ - continued

MICROSCOPIC FINDINGS - continued

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Lymph Nodes - Deep Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Kidneys
Tubular basophilia: (Minimal , Area)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 49♂ (Terminal)

MACROSCOPIC FINDINGS

Tail
Pustule/s: (A few , Scabby) 1mm

Lymph Nodes - Cervical
Enlarged: 8mm

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 49♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Moderate)
Inflammatory cells in the lamina propria: (Moderate)
Eosinophilic epithelial inclusions: (Moderate)
Epithelial squamous metaplasia at the carina: (Moderate)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Spleen
Reduced cellularity of the white pulp - marginal zone: (Moderate)

Adrenals
Vacuolation of cells of the zona fasciculata: (Minimal)

Tail
Pustule

The following tissues were considered normal:

Pharynx; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 50♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 4mm

Lungs
Pale subpleural foci: (A few , Punctate)

Adipose Tissue
Minimal

Testes
Small: (Left)

Epididymides
Small: (Left)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)
Transitional epithelium - erosion: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 50♂ - continued

MICROSCOPIC FINDINGS - continued

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)
Eosinophilic epithelial inclusions: (Moderate)
Epithelial squamous metaplasia at the carina: (Minimal)

Lungs
Prominent BALT: (Minimal)
Alveolar ducts - fibrosis: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Lymph Nodes - Deep Cervical
Increased cellularity - generalised: (Moderate)

Epididymides
Spermatozoa absent - unilateral

Testes
Unilateral tubular atrophy: (Moderate)
Unilateral intratubular oedema: (Marked)

Eyes
Keratitis: (Minimal , Focal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 50♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 51♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Lungs
Not collapsed

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Minimal)
Subepithelial inflammatory infiltration: (Moderate)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)
Epithelial squamous metaplasia at the carina: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 51♂ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Prominent BALT: (Minimal)
Alveolar ducts - fibrosis: (Minimal)
Alveolar ducts - epithelial ciliated metaplasia: (Minimal)

Thymus
Reduced cellularity of cortex: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 52♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Lungs
Not collapsed
Consolidated: right, middle lobe, partially

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Moderate)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)
Epithelial squamous metaplasia at the carina: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 52♂ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Increased numbers of alveolar macrophages: (Minimal)
Prominent BALT: (Minimal)
Perivascular inflammatory cells: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Marked)

Spleen
Reduced cellularity of the white pulp - PALS: (Minimal)
Increased cellularity of the white pulp - marginal zone: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 53♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Not collapsed

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - erosion: (Moderate)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Moderate)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)
Epithelial squamous metaplasia at the carina: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 53♂ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Perivascular inflammatory cells: (Minimal)
Increased numbers of alveolar macrophages: (Minimal)

Heart
Myocardial fibrosis: (Moderate , Area)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Pharynx; Aorta; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 54♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Consolidated: right, middle lobe

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Ventrolateral epithelial hyperplasia: (Minimal)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 54♂ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Perivascular inflammatory cells: (Minimal)
Alveolar ducts - fibrosis: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Spleen
Reduced cellularity of the white pulp - marginal zone: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 55♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lungs
Not collapsed

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Ventrolateral epithelial erosion: (Minimal)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 55♂ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Epitheial squamous metaplasia: (Minimal)
Inflammatory cells in the lamina propria: (Moderate)
Epithelial hyperplasia at the carina: (Minimal)
Eosinophilic epithelial inclusions: (Minimal)
Epithelial squamous metaplasia at the carina: (Moderate)

Lungs
Perivascular inflammatory cells: (Minimal)
Alveolar ducts - fibrosis: (Minimal)
Increased numbers of alveolar macrophages: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Kidneys
Tubular dilatation: (Minimal , Area , Unilateral)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Prostate; Seminal Vesicles; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 56♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 57♂ (Recovery)

MACROSCOPIC FINDINGS

Tail
Tip missing

Lymph Nodes - Cervical
Enlarged: 6mm
Congested

Adipose Tissue
Minimal

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - inflammation: (Trace)
Transitional epithelium - hyperplasia: (Trace)

Larynx
Ventral epithelial squamous metaplasia: (Minimal , Focal)

Lymph Nodes - Cervical
Prominent distended venules containing small lymphocytes: (Moderate)
Medullary plasmacytosis: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 57♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 58♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - hyperplasia: (Minimal , Foci/areas)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 59♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Trace)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 60♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Lobe atelectatic: right, anterior

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)

Lungs
Perivascular inflammatory cells: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 61♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Uterus
Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 62♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Cranial region

Lymph Nodes - Cervical
Enlarged: 9mm

Bone
Sternum misshapen

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Marked)

Bone Marrow/sternum
Dislocation

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 63♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Scabs
Right side of face: (A few , Punctate)

Skin Alopecia
Sides of face
Cranial region: (Diffuse)
Right lumbar region

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Kidneys
Dystrophic mineralisation: (Minimal)

Skin
Epidermal erosion: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 63♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 64♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Cranial region: (Minimal , Diffuse)

Lymph Nodes - Cervical
Enlarged: 6mm

Lungs
Pale subpleural foci: (A few , Punctate)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 65♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Marked)
Medullary plasmacytosis: (Marked)

Kidneys
Dystrophic mineralisation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 66♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Dorsal thoracic region: (Diffuse)

Lymph Nodes - Cervical
Enlarged: 6mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus : (W.N.L.); Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 67♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Left scapular region
Right scapular region

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 68♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Left scapular region
Right scapular region

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 69♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Sides of face: (Minimal , Diffuse)
Right scapular region

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Kidneys
Dystrophic mineralisation: (Moderate)

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 70♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Sides of face
Left scapular region
Right scapular region

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Median cleft, pale subcapsular area: 1mm

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Spleen
Reduced cellularity of the white pulp - PALS: (Minimal)

Kidneys
Dystrophic mineralisation: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 70♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Pharynx; Larynx; Trachea(including Bifurcation); Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver : (W.N.L.); Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 71♀ (Intercurrent)

MACROSCOPIC FINDINGS

Fur
Stained - periorbital region/s: (Right , Red , Brown)

Skin Alopecia
Forelimbs

Eyes
Damaged: (Right)

Lymph Nodes - Cervical
Enlarged: (Minimal)
Congested

Skeletal Muscle
Haemorrhage - periorbital region: (Right)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Sinus erythrocytosis: (Minimal)

Skeletal Muscle
Periorbital haemorrhage

Eyes
Traumatic disruption: (Unilateral)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 71♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 72♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Not collapsed: right, middle lobe
Firm: right, middle lobe
Dark subpleural foci: (A few) 1mm

Forestomach
A crater-like depression, epithelial aspect: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Trace)
Aggregations of brown pigmented alveolar macrophages: (Moderate , Some lobes)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Stomach : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 73♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 74♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 5mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Air control

Rat No/Sex: 75♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm
Congested: (Minimal)

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 76♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Lymph Nodes - Cervical : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 77♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 78♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Kidneys
Pale cortical foci: (Right , Two) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Kidneys : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 79♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Scabs
Left side of face: (One) 1mm

Skin Alopecia
Sides of face
Forelimbs: (Minimal , Diffuse)

Lymph Nodes - Cervical
Enlarged: 7mm

Adrenals
Dark subcapsular foci: (Left , A few , Punctate)

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Uterus
Luminal dilatation: (Moderate)

Adrenals
Area(s) of cortical congestion: (Minimal)

Skin
Scab
Epidermal hyperplasia: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 79♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 80♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Cranial region: (Minimal , Diffuse)
Dorsal cervical region: (Minimal , Diffuse)

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 81♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Marked)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 82♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 83♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 84♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 85♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Two) 1mm

Uterus
Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Uterus
Luminal dilatation: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 86♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Adipose Tissue
Splenulus - omental: 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Medullary plasmacytosis: (Moderate)

Adipose Tissue
Splenulus

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 87♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 88♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 89♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Low

Rat No/Sex: 90♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 91♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Arytenoid - subepithelial inflammation: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Marked)

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 92♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Subepithelial inflammatory infiltration: (Trace)

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 93♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 94♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Trace)
Subepithelial inflammatory infiltration: (Minimal)

Trachea(including Bifurcation)
Epithelial squamous metaplasia at the carina: (Trace)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 95♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Trace)
Transitional epithelium - hyperplasia: (Moderate)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 96♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lungs
Pale subpleural foci: (A few , Punctate)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Trace , Area)
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Moderate)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 97♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 98♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Marked)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 99♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lungs
Pale subpleural foci: (A few , Punctate)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 100♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Adipose Tissue
Torsioned nodule/s: (One) right mesometrial, 4mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - granular eosinophilic inclusions: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Larynx
Subepithelial inflammatory infiltration: (Moderate)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

Adipose Tissue
Fat necrosis: (Area)

The following tissues were considered normal:

Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 101♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Ovaries
No corpora lutea visible

Uterus
Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Marked)

Ovaries
Absence of corpora lutea

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Uterus : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 102♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

. (Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 103♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Liver
Median cleft, pale subcapsular area: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs; Liver : (W.N.L.)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 104♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Adipose Tissue
Excessive

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: Intermediate

Rat No/Sex: 105♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Mammary Glands
Creamy fluid-filled cysts: (A few) 4mm

Ovaries
No corpora lutea visible

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Trace , Focus)

Lymph Nodes - Cervical
Medullary plasmacytosis: (Minimal)

Ovaries
Absence of corpora lutea

Mammary Glands
Secretory activity: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 106♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Left scapular region: (Diffuse)
Right scapular region; (Diffuse)

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Minimal)
Ventrolateral epithelial hyperplasia: (Minimal)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 106♀ - continued

MICROSCOPIC FINDINGS - continued

Kidneys
Dystrophic mineralisation: (Minimal)

The following tissues were considered normal:

Pharynx; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 107♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lymph Nodes - Deep Cervical
Enlarged: 3mm

Lungs
Not collapsed
Consolidated: right, middle lobe, partially

Uterus
Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Ventrolateral epithelial erosion: (Minimal)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 107♀ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Epitheial squamous metaplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)
Epithelial squamous metaplasia at the carina: (Minimal)

Lungs
Perivascular inflammatory cells: (Minimal)
Alveolar ducts - fibrosis
Increased numbers of alveolar macrophages: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Lymph Nodes - Deep Cervical
Increased cellularity - generalised: (Minimal)

Spleen
Reduced cellularity of the white pulp - PALS: (Minimal)
Increased cellularity of the white pulp - marginal zone: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus : (W.N.L.); Cervix; Oviduct; Ovaries; Thyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 108♀ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Left scapular region
Right scapular region

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Not collapsed
Pale subpleural foci: (Multiple) 1mm

Adipose Tissue
Minimal

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Moderate)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 108♀ - continued

MICROSCOPIC FINDINGS - continued

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Trace)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Moderate)
Epithelial squamous metaplasia at the carina: (Minimal)

Lungs
Alveolar ducts - fibrosis: (Minimal)
Increased numbers of alveolar macrophages: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 109♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Not collapsed

Adipose Tissue
Minimal

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Two) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - hyperplasia: (Moderate)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 109♀ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Moderate)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)

Lungs
Perivascular inflammatory cells: (Minimal)
Prominent BALT: (Minimal)
Alveolar ducts - fibrosis: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 110♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Lungs
Not collapsed
Pale subpleural foci: (A few , Punctate)
Dark subpleural focus: right, anterior lobe, 3mm

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Moderate)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Ventrolateral epithelial hyperplasia: (Minimal)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 110♀ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)

Lungs
Bronchiolar squamous cell metaplasia: (Minimal)
Perivascular inflammatory cells: (Moderate)
Alveolitis: (Moderate , Area , One lobe)
Bronchiolitis: (Minimal)
Alveolar ducts - fibrosis: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Moderate)

Lymph Nodes - Tracheobronchial
Increased cellularity - generalised: (Minimal)

Spleen
Reduced cellularity of the white pulp - PALS: (Minimal)
Increased cellularity of the white pulp - marginal zone: (Minimal)

Kidneys
Dystrophic mineralisation: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 111♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Not collapsed

Adipose Tissue
Minimal

Uterus
Fluid distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Respiratory epithelium - squamous metaplasia: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 111♀ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Alveolar ducts - fibrosis: (Minimal)
Increased numbers of alveolar macrophages: (Trace)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Spleen
Reduced cellularity of the white pulp - PALS: (Moderate)

Kidneys
Dystrophic mineralisation: (Minimal)

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Urinary Bladder; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 112♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Lungs
Not collapsed

Adipose Tissue
Minimal

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Moderate)
Respiratory epithelium - granular eosinophilic inclusions: (Moderate)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)
Transitional epithelium - erosion: (Minimal)
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Moderate)
Transitional epithelium - squamous metaplasia: (Moderate)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 112♀ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Perivascular inflammatory cells: (Trace)
Alveolar ducts - fibrosis: (Trace)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Minimal)

Kidneys
Dystrophic mineralisation: (Trace)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 113♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lungs
Not collapsed
Pale subpleural foci: (A few , Punctate)

Lymph Nodes - Tracheobronchial
Enlarged: 4x2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - granular eosinophilic inclusions: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Moderate)
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Ventrolateral epithelial erosion: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Moderate)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 113♀ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial squamous metaplasia at the carina: (Minimal)

Lungs
Prominent BALT: (Minimal)
Perivascular inflammatory cells: (Minimal)
Alveolar ducts - fibrosis: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)

Lymph Nodes - Tracheobronchial
Increased cellularity - paracortex: (Moderate)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 114♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lungs
Not collapsed
Pale subpleural foci: (Multiple , Punctate)

Adipose Tissue
Minimal

Small Intestine
Contents minimal
Gaseous distension

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - inflammation: (Minimal)
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Moderate)
Transitional epithelium - granular eosinophilic inclusions: (Moderate)
Transitional epithelium - hyperplasia: (Minimal)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)
Olfactory epithelium - hyperplasia: (Minimal , Areas)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - squamous epithelial hyperplasia: (Moderate)
Subepithelial inflammatory infiltration: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 114♀ - continued

MICROSCOPIC FINDINGS - continued

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)
Epithelial hyperplasia at the carina: (Minimal)
Epithelial squamous metaplasia at the carina: (Minimal)

Lungs
Perivascular inflammatory cells: (Trace)
Alveolar ducts - fibrosis: (Trace)
Increased numbers of alveolar macrophages: (Minimal)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Moderate)
Medullary plasmacytosis: (Moderate)

Spleen
Increased cellularity of the white pulp - marginal zone: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Liver; Pancreas; Kidneys; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 115♀ (Terminal)

MACROSCOPIC FINDINGS

Lungs
Not collapsed
Pale subpleural foci: (A few , Punctate)

Adipose Tissue
Minimal

Kidneys
Irregular cortical scarring: (Right , Minimal)
Small: (Right)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - inflammation: (Trace)
Transitional epithelium - squamous metaplasia: (Minimal)
Exudative inflammatory cells (sinusitis): (Minimal)

Larynx
Ventrolateral epithelial squamous metaplasia: (Moderate)
Arytenoid - epithelial erosion/ulceration: (Minimal)
Arytenoid - squamous epithelial hyperplasia: (Moderate)

Trachea(including Bifurcation)
Epithelial hyperplasia: (Minimal)
Inflammatory cells in the lamina propria: (Minimal)

APPENDIX 11

(Pathology - continued)

Rat No/Sex: 115♀ - continued

MICROSCOPIC FINDINGS - continued

Lungs
Perivascular inflammatory cells: (Minimal)
Alveolar ducts - fibrosis: (Minimal)
Increased numbers of alveolar macrophages: (Minimal)

Kidneys
Cortical scarring: (Minimal)

The following tissues were considered normal:

Pharynx; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Urinary Bladder; Uterus; Cervix; Oviduct; Ovaries; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Skeletal Muscle; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Caecum; Colon; Rectum; Mammary Glands; Skin; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 116♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - inflammation: (Minimal , Area)

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)
Medullary plasmacytosis: (Minimal)

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 117♀ (Recovery)

MACROSCOPIC FINDINGS

Ovaries
No corpora lutea visible

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Respiratory epithelium - hyperplasia/prominent goblet cells: (Minimal)
Transitional epithelium - hyperplasia: (Minimal)

Ovaries
Absence of corpora lutea

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 118♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 6mm

Stomach Antrum Mucosa
White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Nasal Passages
Transitional epithelium - inflammation: (Minimal)
Transitional epithelium - hyperplasia: (Moderate , Area)

Lymph Nodes - Cervical
Medullary plasmacytosis: (Minimal)

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 119♀ (Recovery)

MACROSCOPIC FINDINGS

Fur
Coarse - sacral region

Lymph Nodes - Cervical
Enlarged: 6mm

Adipose Tissue
Torsioned nodule/s: (One) right mesometrial, 3mm

Spleen
Clear fluid-filled cyst/s: (Two) capsular, 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Medullary plasmacytosis: (Minimal)

Spleen
Mesothelial capsular cyst(s): (Moderate)

Adipose Tissue
Fat necrosis

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 11

(Pathology - continued)

Compound: Acetic anhydride

Dosage Level: High

Rat No/Sex: 120♀ (Recovery)

MACROSCOPIC FINDINGS

Uterus
Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Uterus
Luminal dilatation: (Minimal)

The following tissues were considered normal:

Nasal Passages; Larynx; Trachea(including Bifurcation); Lungs

Pathologist: R.L.Gregson

APPENDIX 12

Methods of sample collection and analysis for acetic anhydride

SAMPLE COLLECTION AND ANALYSIS

Throughout the study the methods of sample collection and sample analysis detailed in the study Inhalation Analytical Procedure (IAP) were followed (see Appendix A).

METHOD VALIDATION

All values quoted in this section relate to solution concentrations.

Specificity

The method was shown to resolve acetic anhydride from acetic acid. Using blank samples and solutions no interferences could be seen. As noted below, however, a peak corresponding to acetic anhydride was always observed following injections of acetic anhydride solutions.

Accuracy

The response to acetic anhydride was repeatably non-linear, however, using an appropriate calibration (logarithm of response against logarithm of concentration) the relative errors were shown to be less than 5% across the range of concentrations 4 to 85 μg/ml.

Storage stability

Solutions of acetic anhydride in acetone (4 to 85 μg/ml) stored in low density polypropylene vials at approximately 4°C were shown to be stable for 4 days.

Precision

The coefficient of variation for six injections was 0.5%, 1.8% and 2.0% for standards of concentration 106, 21 and 4 μg/ml respectively.

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

ACETIC ACID

Over the course the study a peak was observed to elute with a similar retention time to acetic acid. This peak was noted in both standards and samples. The response was variable and did not show a relationship to dose or concentration of acetic anhydride within the sample. This indicated that a degree of hydrolysis of acetic anhydride was taking place within the analysis system.

Attempts were made using both a capillary gas chromatograph and an infra-red spectrometer to confirm this conclusion.

The capillary gas chromatograph was used because of the inert nature of the analysis system. This was used in conjunction with standards made in dichloromethane, used to eliminate hydrolysis in solution. The peak eluting at a similar retention time to acetic acid was observed in both standards and samples. This confirmed the hypothesis that the peak was due to the hydrolysis of acetic anhydride during the course of the analysis and not the presence of acetic acid in the chamber.

Infra-red spectroscopy was used in an attempt to establish wether acetic acid was present in air sampled directly from the high dose chamber (without animals present). The spectrometer was unable to distinguish the responses due to acetic acid from those due to the acetic anhydride. Therefore no conclusion could be drawn.

Comparison of analysed chamber concentration to nominal chamber concentration showed fairly good agreement. This indicates little degradation of acetic anhydride was taking place within the chamber.

It is considered that the analysed chamber concentration for acetic anhydride is a true representation of the actual chamber concentrations and that no significant amount of acetic acid was present in the chamber.

CALCULATIONS

In order to minimise the cumulative errors which 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, any further calculation using these rounded numbers will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in the report.

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

APPENDIX A

Inhalation Analytical Procedure - HST/411

Analysis of acetic anhydride in air sample substrate

This method details the procedure to be used for the GLC assay of acetic anhydride in air samples at amounts in the range of 0.5 - 25 ppm.

Effective date: 8 September 1995

Reference Sponsor's methodology: No

Title:

Dated:

Amendments issued (see any separate attached pages for details)

Amendment number	Date of issue	Section affected

For the core method refer to the compund specific IAP :- Acetic Anhydride

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

APPENDIX A

Inhalation Analytical Procedure

for the analysis of

Acetic Anhydride

in air sample substrate

This contains the core method for the analysis of acetic anhydride. Study specific amendments will be detailed on the study IAP cover sheet.

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

APPENDIX A

1. **Compound details**

1.1	Name	Acetic anhydride.
1.2	Appearance	Liquid.
1.3	Storage	Room temperature.

2. **Reagents**

2.1	Acetic anhydride	Aldrich Chemical Company Ltd. Lot: 50218009 Purity: 99+ %
2.2	Acetone	Glass distilled, Rathburn Chemical Company Ltd.

3. **Apparatus**

3.1	Balance	Sartorius R200D, fitted with a data print YDP-01.
3.2	30 ml LDPE bottles	Nalgene Company.
3.3	1 ml gas-tight syringe	Hamilton Company.
3.4	1 μl plunger in needle syringe	Scientific Glass Engineering Ltd.
3.5	Dispensers	Bibby Pressmatic 2000 and Gilson Pipetteman.

4. **Preparation of chamber samples**

4.1 Using a syringe (50 ml or 100 ml) withdraw 100 ml of chamber air from the sampling port and discard to waste.

4.2 Using a syringe (1 ml) withdraw 1 ml of chamber air from the sampling port.

4.3 Inject the 1 ml air sample through the septum port of the GLC and start the run.

5. **Preparation of standards**

5.1 Weigh a known amount of acetic anhydride accurately (to 0.01 mg) into a LDPE bottle (30 ml).

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

APPENDIX A

5.2 Using a dispenser add acetone to the bottle. Seal the bottle and shake well to disolve the acetic anhydride.

5.3 Dilute the standard to prepare calibration standards stored in LDPE bottles (30 ml) within the range 4 to 85 μg/ml, by using a Gilson pipetteman with a polypropylene tip and a dispenser containing acetone.

5.4 Inject 1 μl of the standard through the septum port of the GLC and start the run.

5.5 See note 9.1 for storage details.

6. **Calibration**

6.1 Measure the peak area response of acetic anhydride of each 1 μl injection of calibration standard. Construct a log-log regression line to give the following equation:

$$\ln (A) = (S \times \ln (C)) + I$$

where S = Slope of calibration line
I = Intercept of calibration line
A = Acetic anhydride area response
C = Concentration of acetic anhydride (ppm) see note 9.2

7. **Typical chromatographic conditions**

7.1	Gas-Liquid Chromatography	As detailed below
	Chromatograph	Shimadzu GC-14A
	Integrator	Shimadzu C-R4A
7.1.1	Analytical column	DB-1701, 1 μm film, 15 m x 0.53 mm i.d.
7.1.2	Detector	Flame ionisation (range=1)
7.1.3	Temperature	Column = 35°C Injector = 100°C Detector = 200°C
7.1.4	Flow rates	Helium (carrier) 15 ml/min Hydrogen 30 ml/min (0.5 kg/cm^2) Air 300 ml/min (0.3 kg/cm^2)

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

APPENDIX A

7.1.5	Retention time for acetic anhydride	Approximately 3.0 minutes
7.1.6	Injection volume	Standards 1 µl Samples 1 ml
7.1.7	Analysis cycle	4 minutes (gas sample) 8 minutes (standard)

8. **Quality assurance measures**

8.1 **System suitability tests**

8.1.1	Column efficiency	acetic anhydride $N_{w1/2}$ = 9500
8.1.2	Asymmetry	acetic anhydride T = 1.1
8.1.3	Column capacity	acetic anhydride k' = 10

8.2 **Tests to assure the validity of reported results**

8.2.1 The top calibration standard will be compared against a second valid independantly prepared top calibration standard. The ratio of response factors will be acceptable if within the range 0.95 to 1.05.

8.2.2 The correlation coefficient of the calibration shall not be less than 0.999.

8.2.3 Quality check standards at 2 levels must be run on each day of analysis for the analysis to be regarded as valid.

8.2.4 The results of the quality check standards must lie within 5% of the nominal value except at the limit of quantification (LOQ), where 10% is acceptable. If the values differ from the nominal amount by more than the acceptable limit, the system will be investigated and recalibrated if necessary.

8.2.5 The population relative standard deviation of duplicate injections must be less than 5% except at the limit of quantification, where 10% is acceptable.

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

APPENDIX 12

(Methods of sample collection and analysis for acetic anhydride - continued)

APPENDIX A

9. **Notes**

9.1 **Storage of standard solutions**

4 days at 4°C (refrigerator)

9.2 **Conversion factor**

$$\text{Concentration in air (ppm)} = \frac{\text{solution conc. } (\mu g/ml) \times \text{liquid injection vol. } (\mu l) \times 24.45 \text{ l}}{\text{gas injection vol. (ml)} \times 102 \text{ g}}$$

where 24.45 l = molar volume of an ideal gas at 25°C and 760 mmHg

102 g = molecular weight of acetic anhydride

Prepared by M F Bimson
Project Coordinator

Approved by I S Gilkison
Head of Section, Aerosol Technology and Analysis