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LAW OFFICES

**KELLER AND HECKMAN**

1001 G STREET, N.W.  
SUITE 500 WEST  
WASHINGTON, D.C. 20001  
TELEPHONE (202) 434-4100  
TELEX 49 95551 "KELMAN"  
TELECOPIER (202) 434-4646

BOULEVARD LOUIS SCHMIDT 87  
B-1040 BRUSSELS  
TELEPHONE 32(2) 732 52 80  
TELECOPIER 32(2) 732 53 92

JOSEPH E. KELLER (1907-1994)  
JEROME H. HECKMAN  
WILLIAM H. BORGHESEANI, JR.  
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SCIENTIFIC STAFF  
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CHARLES V. BREDER, PH. D.  
ROBERT A. MATHEWS, PH. D., D.A.B.T.  
JOHN P. MODDERMAN, PH. D.  
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February 17, 1995

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Office of Pollution Prevention and Toxics  
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Washington, D.C. 20460

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Re: Supplemental Data for TSCA § 8(e) Submission

Dear Sir or Madam:

At the request of our client, Velsicol Chemical Corporation, we are delivering the following final toxicity study report in conformance with section 8(e) of Toxic Substances Control Act (TSCA) (15 U.S.C. § 2607(e)):

*Isodecyl Benzoate; Twenty-Eight Day Oral Toxicity Study in the Rat With Functional Observational Battery*

Velsicol's initial TSCA § 8(e) filing, which was submitted on November 10, 1994, consisted of the interim report for this study, which was conducted under a Section 5(e) consent order for PMN P-90-549 (CASRN 131298-44-7). This supplemental submission provides the Agency with the final study report.

In this study, exposure of rats to isodecyl benzoate at 1000 mg/kg/day for 28-days resulted in behavioral changes, including altered appearance, palpebral closure in the arena, tremor, increased hindlimb grip strength, and increased hindlimb splay. Females appeared to be more affected than males. In addition, enlarged livers and higher liver and kidney weights were observed in certain test animals at this dosage level. If you have any

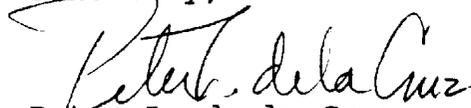
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February 17, 1995  
Page 2

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questions concerning this submission, please contact me at 202-434-4141 or Mr. David J. Mason at Velsicol at 708-635-3422.

Sincerely,

  
Peter L. de la Cruz

Enclosure

cc: Ken Moss  
OPPT/CCD/NCB

Phillip K. Cobb, Esq.  
David J. Mason

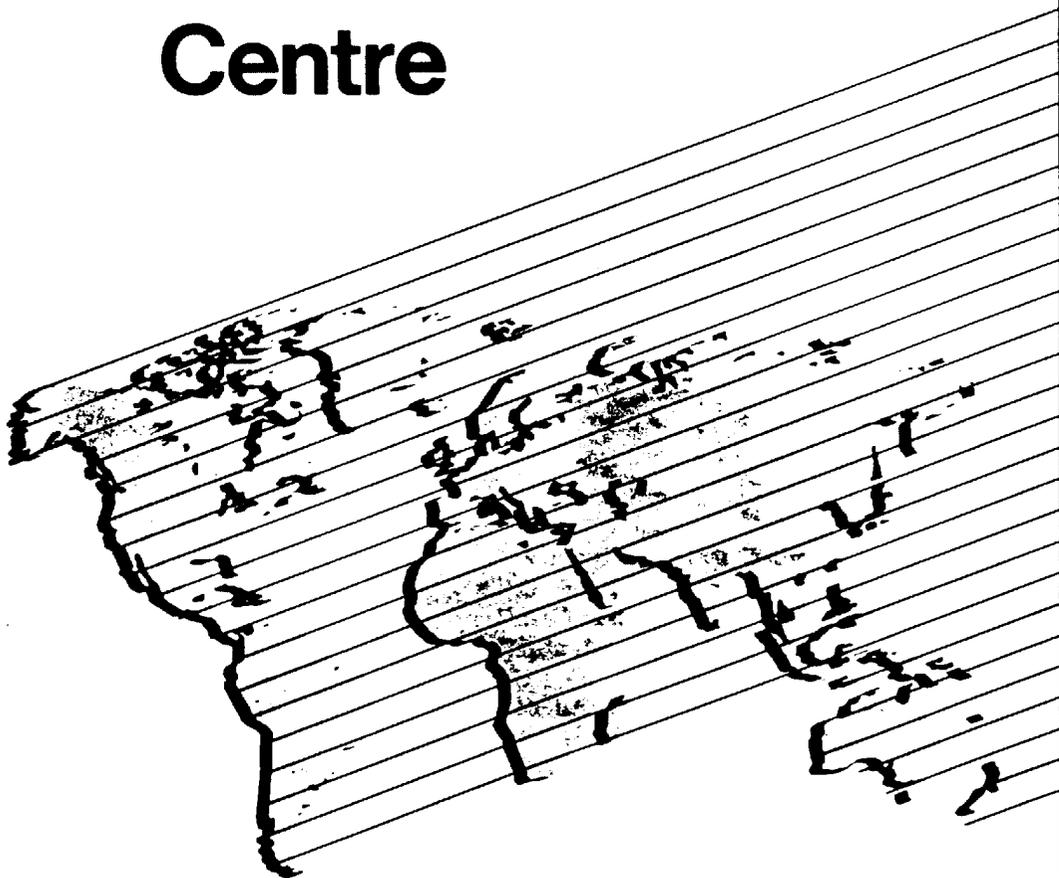
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# **HRC** Report

**ISODECYL BENZOATE**

**TWENTY-EIGHT DAY ORAL TOXICITY  
STUDY IN THE RAT WITH FUNCTIONAL  
OBSERVATIONAL BATTERY**

**Huntingdon  
Research  
Centre**



**CONFIDENTIAL**

**VCL 206/942848**

**ISODECYL BENZOATE**  
**TWENTY-EIGHT DAY ORAL TOXICITY STUDY IN THE RAT WITH**  
**FUNCTIONAL OBSERVATIONAL BATTERY**

**Sponsor**

Velsicol Chemical Corporation,  
10400 West Higgins Road,  
Rosemont,  
IL 60018-3713,  
U.S.A.

**Testing facility**

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

Report issued 9 February 1995

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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.

*Sarah A. Allan*

Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Huntingdon Research Centre Ltd.

*9 February 95*

Date

*David J. Mason Feb 16, 1995*

DAVID J. MASON, M.S.  
Director, SHEA,  
Velsicol Chemical Corporation

### 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	-	1 August 1994
Pre-experimental Period	19 - 24 August 1994	25 August 1994
Experimental Period	1 - 9 September 1994	9 September 1994
	21 - 23 September 1994	23 September 1994

Date of reporting audit findings to the  
Study Director and HRC Management

9 February 1995

  
Jacqueline Cahill,  
Audit Team Supervisor,  
Department of Quality Assurance,  
Huntingdon Research Centre Ltd.

9/2/95  
Date

**RESPONSIBLE PERSONNEL**

**STUDY MANAGEMENT**

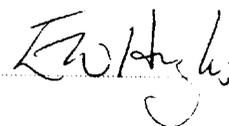
Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director.



Andrew D. Hawkins, B.Sc. (Hons.),  
Study Supervisor.



Elizabeth W. Hughes, B.A., M.Sc.,  
Behavioural Scientist.



**PATHOLOGY**

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



**ANALYTICAL CHEMISTRY**

Alan Anderson, B.Sc. (Hons.), C.Chem., F.R.S.C.,  
Head, Department of Analytical Chemistry & Pharmacy.



## SUMMARY

This study was performed to assess the systemic toxicity of Isodecyl benzoate to the rat. The method followed was that outlined in Annex to Directive 92/69/EEC (OJ No. L:383A, 29.12.92), Part B, Method B.7. Sub-acute toxicity (oral) and OECD Guidelines for Testing of Chemicals No. 407, "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-day study".

Isodecyl benzoate, a colourless liquid intended for use as a plasticizer, was administered by oral gavage, once daily to groups of eight male and eight female rats for 29 consecutive days, at fixed dosage levels of 15, 150 and 1000 mg/kg/day. The test material was prepared as emulsions in corn oil at concentrations of 0.3, 3.0 and 20.0% w/v, and was administered to rats at a dosage volume of 5 ml/kg/day. A further group of rats (eight males and eight females) was held as a control receiving corn oil alone at an equivalent dose volume (5 ml/kg/day).

Bodyweights, food consumption and clinical observations were recorded during the study. A functional observational battery was performed for all animals during the week prior to commencement of treatment and subsequently during Week 2 and 4. Blood samples for clinical investigations were taken from five male and five female rats from each group on Day 28. Five male and five female animals from each group were killed and examined macroscopically on Day 30, remaining animals were killed and discarded. Histopathological examination of specified tissues from animals that underwent a *post mortem* examination was then initiated.

The following comments are made in summary:

### Clinical signs

An appearance indicative of a slight lack of grooming was noted for high dosage group animals during Week 4.

### Functional observational battery

Behavioural changes were noted for rats from the high dosage group, with females affected more than males, and included altered appearance, palpebral closure in the arena, tremor, increased hindlimb grip strength and increased hindlimb splay.

### Biochemistry

Lower than control glucose levels were recorded for rats of both sexes receiving 1000 mg/kg/day

### Organ weights

Higher liver weights (males and females) and higher kidney weights (males only) were recorded for high dosage group animals in comparison with controls.

### Macroscopic pathology

An enlarged liver was recorded for four male and two female rats from the high dosage group.

### **Microscopic pathology**

Centrilobular hepatocyte enlargement was noted for male and female rats from the high dosage group. A dosage-related incidence of eosinophilic intracytoplasmic droplets in proximal convoluted tubules was noted in the kidneys of male rats treated at 1000 or 150 mg/kg/day. This finding is specific to male rats and is not predictive for a similar effect in man.

### **Other findings**

There were no differences from control in other parameters measured, namely bodyweights, food or water consumption, haematology and biochemistry, that were considered to be related to treatment.

### **Conclusion**

Behavioural changes, suggestive of an effect on the nervous system, were recorded for animals treated at 1000 mg/kg/day. These findings were considered sufficient to reject the high dosage level as a no-observed-adverse-effect-level (NOAEL).

The only treatment-related effect seen at the intermediate dosage level of 150 mg/kg/day was in the kidney and was not considered predictive for a similar effect in man. It was therefore concluded that 150 mg/kg/day represents the no-observed-adverse-effect-level (NOAEL) in the rat for Isodecyl benzoate when administered to the rat for 28 days. The low dosage of 15 mg/kg/day represents the no-observed-effect-level (NOEL).

According to the EEC Council Directive 92/69/EEC Annex VI, Part II (D) as described in Commission Directive 93/21/EEC, labelling with the R48 risk phrase is not required.

## INTRODUCTION

The study was designed to assess the systemic toxicity to the rat of the test substance Isodecyl benzoate when repeatedly administered orally for a period of at least twenty-eight consecutive days.

The procedure used is described in this report. The procedure complied with that described in:

EEC Methods for the determination of toxicity, Annex to Directive 92/69/EEC (OJ No. L383A, 29.12.92), Part B, Method B.7. Sub-acute toxicity (oral).

OECD Guideline for Testing of Chemicals No. 407, "Repeated Dose Oral Toxicity - Rodent: 28-days or 14-day study". Adopted: 12 May 1981.

EPA Health Effects Testing Guidelines, Subpart G - Neurotoxicity § 798.6050, Functional observational battery September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart E provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

The albino rat was chosen as the test species as it has been shown to be a suitable model for this type of study and is the species recommended in the test guidelines.

The rats were dosed orally by gavage as the test substance may be ingested accidentally.

The high dosage level of 1000 mg/kg/day was selected on the basis of available toxicity information, notably acute oral toxicity data (oral LD<sub>50</sub> rat >5.0 g/kg; Hazleton Laboratories America Inc, Project Identification HLA 70504073) and a preliminary study performed at WIL Research, USA (Project No. WIL-15217A).

The low and intermediate dosage levels were selected as being key dosages with respect to EEC Labelling requirements.

The protocol was approved by the Study Director and HRC Management on 18 July 1994 and by the Sponsor on 15 September 1994.

The experimental phase of the study was conducted between 25 August 1994 and 16 January 1995.

## TEST SUBSTANCE

Identity:	Isodecyl benzoate
Chemical class:	Benzoate ester
Intended use:	Plasticizer
Appearance:	Colourless liquid
Storage conditions:	Room temperature
Batch number:	C5-8
Expiry date:	15 May 1996
Purity:	95 - 99%
Date received:	13 June 1994

## EXPERIMENTAL PROCEDURE

### ANIMAL MANAGEMENT

A total of 37 male and 37 female healthy CD rats of Sprague-Dawley origin (CrI: CD® BR VAF PLUS™) was received from Charles River (UK) Ltd, Margate, Kent, England on 10 August 1994.

The rats were  $36 \pm 1$  days old, in a weight range of  $\pm 10\%$  of the mean weight per sex on arrival. A fifteen-day acclimatisation period was allowed between delivery of the animals and start of treatment.

All rats were initially caged, as far as possible, in groups of four according to sex in metal cages with wire mesh floors.

A standard pelleted laboratory rodent diet (Special Diet Services Rat and Mouse Maintenance Diet) and drinking water were provided *ad libitum*, except as noted under **CLINICAL PATHOLOGY**.

The batches of diet used for the study had been analysed for nutrients, possible contaminants and micro-organisms (Appendix 7).

Results of the routine physical and chemical examination of drinking water at source, as conducted, usually weekly by the supplier, are made available to Huntingdon Research Centre Ltd as quarterly summaries (Appendix 8).

Animal room temperature was controlled in the range 16.5°C to 21°C and relative humidity was controlled in the range 31 to 69% RH. These parameters were continuously monitored using a Kent Clearspan M105 7-day chart recorder. Air exchange was maintained at approximately 19 air changes per hour and lighting was controlled to provide 12 hours artificial light (0700 - 1900 hours) in each 24-hour period.

The health status of all animals was monitored, by daily observation throughout the acclimatisation period, to ensure that the rats selected for final assignment to the study were satisfactory.

One week prior to the start of treatment each animal was weighed and sixty-four rats were randomly allocated to four groups, each consisting of eight males and eight females. This allocation was carried out using a computer program, so that the weight distribution within each group was similar and the initial group mean bodyweights were approximately equalised.

Each rat was identified within each cage by ear-punch and individually by tail mark (tattoo).

Following the commencement of treatment spare animals were removed from the study. No further investigations were performed on these animals.

The cages (each containing four rats) constituting each group were distributed in batteries in such a manner that possible environmental influences arising from their spatial distribution was equilibrated, as far as possible, for all treatments (Figure 1).

Each cage was identified by a coloured label according to group. Each label displayed the study schedule number, cage number, sex, individual animal numbers and the initials of the Study Director and Home Office licensees.

### TEST SUBSTANCE PREPARATION

A 20% w/v formulation of the test substance was prepared by stirring the appropriate weight of material in with the vehicle (corn oil) and subsequently homogenising. Further formulations (3.0 and 0.3% w/v) were prepared in a similar manner.

Formulations were prepared freshly on each day of use.

The chemical stability and homogeneity of test substance formulations were assessed prior to the start of treatment. Concentration analyses of formulations prepared for administration on Day 1 were subsequently performed. Results of all analyses are presented in the **FORMULATION ANALYSIS REPORT**.

The absorption of the test substance was not determined in this study.

Data concerning the analytical purity and homogeneity of the test substance and its stability under specified conditions of storage are the responsibility of the Sponsor.

### TREATMENT PROCEDURE

The high dosage was selected on the basis of available toxicity data and a preliminary oral toxicity investigation performed at WIL Research, USA. The intermediate and low dosage levels were selected on the basis of the key dosages relative to EEC labelling requirements.

Groups of sixteen rats (eight males and eight females) were dosed as follows:

Group	Cage label/ colour code	Treatment	Dosages (mg/kg/day)	No. of rats		Rat numbers	
				♂	♀	♂	♀
1	White	Control, corn oil	-	8	8	1-8	33-40
2	Yellow	Isodecyl benzoate	15	8	8	9-16	41-48
3	Green	Isodecyl benzoate	150	8	8	17-24	49-56
4	Red	Isodecyl benzoate	1000	8	8	25-32	57-64

The test substance was administered by oral gavage to rats of Groups 2 to 4 inclusive using a syringe and rubber catheter at a dose volume of 5 ml/kg/day.

Control animals received the vehicle (corn oil) at the same dose volume.

Each animal received a constant dosage based on its most recently recorded bodyweight.

Animals were treated once daily for twenty-nine consecutive days. Treatment commenced on 25 August 1994 and the last day of dosing was 22 September 1994.

Prior to dosing, the test substance formulations for Groups 2 to 4 were mixed by inversion ( $\times 20$ ), subsequent mixing using a magnetic stirrer was also undertaken for a period of at least 10 minutes before dosing commenced.

## **OBSERVATIONS**

### **Clinical signs**

All animals were observed at least twice daily for signs of ill health, behavioural changes or toxicosis. Any observed changes were recorded.

All animals were checked early in each working day and again in the late afternoon to look for dead or moribund animals. This would permit a *post mortem* examination to be undertaken during the working part of that day. On Saturdays, Sundays and public holidays a similar procedure was followed except that the final check was carried out at approximately mid-day.

### **Bodyweight**

All rats were weighed prior to dosing on Day 1 (Week 0) and subsequently at weekly intervals throughout the study.

### **Food consumption**

The quantity of food consumed in each cage was measured at weekly intervals throughout the study.

### **Water consumption**

Daily monitoring by visual appraisal was maintained throughout the dosing period.

### **Functional observational battery**

A functional observational battery was performed for all animals on one occasion during the acclimatisation period, once during Week 2 and once during Week 4. Not all rats were tested in one day, but the time of testing was balanced across all groups. Observations performed in Week 2 and 4 were performed prior to dosing. Detailed results are presented in the **FUNCTIONAL OBSERVATIONAL BATTERY REPORT**.

The observational battery has previously been validated using positive control compounds<sup>1</sup>.

<sup>1</sup> NEWTON, D.F. *et al* (1992) "A Neurotoxicity Screen in rats following treatment with Acrylamide, Carbaryl or p,p'-DDT" Toxicology Letters Supplement 1992, p 184

## CLINICAL PATHOLOGY

### Removal of blood samples

Food was withdrawn overnight prior to collection of samples. Blood was withdrawn under light ether anaesthesia from the orbital sinus of five male and five female rats (lowest animal numbers) from each group prior to termination (Day 28, Week 4).

The collected blood samples were divided as follows:

EDTA anticoagulant tubes	.... for haematological investigations
Citrate anticoagulant tubes	.... for coagulation test
Heparin anticoagulant microtainer tubes*	.... for biochemical tests

\* Microtainer, brand plasma separator tube, Becton Dickinson, Rutherford, New Jersey, USA

All the tubes were then mechanically agitated for at least five minutes and the microtainer tubes were subsequently centrifuged for a minimum period of two minutes (3000 'g').

The estimations performed are listed below, together with an abbreviated title (for use in Tables and Appendices), the methods and the units of measurement applicable.

### Haematology

The following parameters were analysed with an Ortho ELT-1500 analyser, using standard Ortho methodology:

	Units
Packed cell volume (PCV)	%
Haemoglobin (Hb)	g/dl
Red blood cell count (RBC)	$\times 10^6/\text{mm}^3$
Absolute indices:	
Mean corpuscular haemoglobin concentration (MCHC)	
Calculated: $\text{Hb (g/dl)} \times 100 \div \text{PCV (\%)} $	%
Mean corpuscular volume (MCV)	f
Calculated: $\text{PCV (\%)} \times 10 \div \text{RBC } (\times 10^6/\text{mm}^3)$	
Platelet count (Plts)	$\times 10^3/\text{mm}^3$
Total white blood cell count (WBC)	$\times 10^3/\text{mm}^3$

The following estimations were measured using the appropriate methodology:

Thrombotest (TT) - Method of Owren, P.A. (Lancet, 1959, ii, 754)	s
--	---

## Units

Differential white blood cell count (Diff) - namely:

Neutrophils	(N)	
Lymphocytes	(L)	
Eosinophils	(E)	
Basophils	(B)	$\times 10^3/\text{mm}^3$
Monocytes	(M)	

The percentage distribution of each cell type was determined by standard microscopy of a blood smear stained with modified Wright's stain counting 100 cells. Percentage values were then converted to absolute values by computer inevitably involving a "rounding off" in a proportion of the results. Hence, the measured total WBC may differ slightly from the calculated total for the differential count.

Additionally, blood film slides were examined for morphological abnormalities. If abnormal cells (see below) were observed when examining the stained slides, their presence has been recorded and included in the haematology appendix (Appendix 3).

- P Polychromasia
- H Hypochromasia
- A Anisocytosis
- R Rouleaux formation
- S Separate film report (generated for additional abnormalities)

NAD No abnormality detected

- 1 Slight
- 2 Moderate
- 3 Marked
- 4 Gross

### Biochemistry

The following parameters were analysed with a Hitachi 737 Clinical Chemistry Analyser:

	Units
Glucose - using BCL Test Kit (hexokinase mediated)	mg/dl
Total protein	g/dl
Albumin (Alb)	g/dl
Globulin (Glob)	g/dl
Calculated: Total protein (g/dl) minus Alb (g/dl)	
Albumin/Globulin ratio (A/G)	
Calculated from Total protein and Albumin concentrations	
Urea nitrogen (Urea Nitr)	mg/dl

	<b>Units</b>
Creatinine	mg/dl
Alkaline phosphatase (AP) - reaction temperature 30°C	mU/ml
Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase (ALT)' - using BCL Test Kit, reaction temperature 30°C	mU/ml
Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase (AST)' - using BCL Test Kit, reaction temperature 30°C	mU/ml
Total bilirubin (Bilirubin)	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Chloride (Cl)	mEq/l
Inorganic phosphorus (P)	mEq/l
Cholesterol (Chol)	mg/dl

## TERMINAL STUDIES

### Termination

After 29 days of treatment (Day 30) five male and five female rats per group (lowest animal numbers) were killed by carbon dioxide asphyxiation and a complete autopsy undertaken. The order of sacrifice was determined using a pre-set cage sequence. Specified organs were weighed and relevant tissue samples were fixed for microscopic examination. Remaining animals were killed by cervical dislocation and discarded. No further investigations were performed on these animals.

### Organ weight

The following organs from each of those animals undergoing *post mortem* examination were dissected free of fat and weighed:

adrenals	liver	seminal vesicles
brain	ovaries	spleen
epididymides	prostate	testes
kidneys		

Testes and epididymides were weighed individually and identified as left and right.

### Macroscopic pathology

The macroscopic appearance of the tissues of all rats *post mortem* was recorded and samples of the following tissues were preserved. Eyes were preserved in Davidson's fixative. Testes/epididymides were fixed in Bouin's solution and then transferred to 70% alcohol. All other tissues were preserved in 10% buffered formalin.

adrenals*	liver*	skin
aorta	lungs*	spleen*
brain (medullary, cerebellar and cortical sections)	lymph nodes (cervical and mesenteric)	sternum (for bone and marrow sections)
caecum	mammary glands	stomach
colon	oesophagus	testes including epididymis* (Bouin's as fixative)
duodenum	ovaries*	thymus (where present)
eyes (Davidson's fluid as fixative)	pancreas	tongue
femur (with joint)	pharynx	thyroid (with parathyroid)
head	pituitary	urinary bladder
heart*	prostate	trachea
ileum	rectum	vagina
jejunum	salivary gland	uterus (with cervix)
kidneys*	sciatic nerve	any macroscopically abnormal tissue*
larynx	seminal vesicles	
	skeletal muscle	

\* Tissues required for histopathological examination for rats from Groups 1 and 4

### Microscopic pathology

Fixed tissue samples required for microscopic examination were prepared by embedding in paraffin wax (m.p. 56°C); sections were cut at 4  $\mu\text{m}$  and stained with haematoxylin and eosin. A transverse section of each testis (left and right) and a full longitudinal section of each epididymis (left and right) were cut as near as possible to 2  $\mu\text{m}$  and stained with Periodic acid Schiff-haematoxylin. Microscopic examination of the testes was made with reference to the stages of the cycle of the seminiferous epithelium.

Microscopic examination of prepared slides (from tissues indicated under **Macroscopic pathology**) was carried out for five male and five female rats from Group 1 (Control group) and Group 4 (high dosage group) killed on Day 30 and the liver and kidneys from five male and five female rats from Group 2 (low dosage group) and Group 3 (intermediate dosage group).

Following approval from the Sponsor, microscopic examinations were also carried out on the brain (six sections) and sciatic nerve for all animals from the control and high dosage groups.

## STATISTICAL ANALYSES

All statistical analyses were carried out separately for males and females using the individual animal as the basic experimental unit.

The following sequence of statistical tests was used for bodyweight gains, 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 values different from the mode was analysed by Fisher's exact test (1) followed by Mantel's test for a trend in proportions (2). Otherwise:

Bartlett's test (3) was applied to test for heterogeneity of variance between treatments. If significant heterogeneity was found at the 1% level, 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 followed by Williams' test (5) for a dose related response.

If significant heterogeneity of variance was present and could not be removed by a logarithmic transformation, the Kruskal-Wallis analysis of ranks (4) was used. This analysis was followed by the non-parametric equivalent of Williams' test (Shirley's test (6)).

Covariate analysis of organ weight data (with final bodyweight as covariate) was also performed using adjusted weights for organs where a correlation between organ weight and bodyweight was established at the 10% level of significance (7).

Significant differences between control animals and those treated with the test substance have been expressed at the 5% (\*  $P < 0.05$ ) or 1% (\*\*  $P < 0.01$ ) level.

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2. MANTEL, N. (1963) *J. Amer. Statist. Ass.*, **58**, 690.
3. BARTLETT, M.S. (1937) *Proc. Roy. Soc.*, **A 160**, 268.
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5. WILLIAMS, D.A. (1971/2) *Biometrics*, **27**, 103 and **28**, 519.
6. SHIRLEY, E. (1977) *Biometrics*, **33**, 386.
7. ANGERVALL, L. and CARLSTROM, E. (1963) *J. Theoret. Biol.*, **4**, 254.

## **PROCEDURES**

The procedure used during the study were those documented in HRC Procedure Manuals.

## **ARCHIVES**

All specimens, raw data and study-related documents generated during the course of the study at HRC, together with a copy of the final report are lodged in the Huntingdon Research Centre Ltd. Archive.

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.

## RESULTS

### ANALYTICAL CHEMISTRY

The analytical results confirm that the doses were accurately formulated for Day 1 of the study. The results also confirm that specimen formulations were homogenous and stable for a period representing the time from preparation to completion of dosing.

### CLINICAL SIGNS

There were no mortalities.

An appearance indicative of a slight lack of grooming was noted during the last week of treatment for all males and females receiving 1000 mg/kg/day.

An increase in salivation following dosing, commonly associated with wet fur, was seen for all high dosage animals. These are common findings in rat orally-dosed studies and are considered to be related to the palatability of the test material and not toxicologically important.

There were no clinical findings for rats treated at 150 or 15 mg/kg/day or for controls.

### BODYWEIGHT GAINS (Figure 2, Table 1, Appendix 1)

There were no statistically significant differences in bodyweight gain between treated and control rats.

### FOOD CONSUMPTION (Figure 3, Table 2, Appendix 2)

There were no differences in food consumption that were considered to be related to treatment. However, variation was seen (*eg* 15% higher than control for high dosage group females) but considering there were only 2 cages/sex/group and there was no statistical significance an effect of treatment was not suspected.

### WATER CONSUMPTION

Visual assessment of water consumption throughout the study revealed no differences considered to be related to treatment.

## FUNCTIONAL OBSERVATIONAL BATTERY

Exposure to Isodecyl benzoate resulted in behavioural changes at 1000 mg/kg/day. Females appeared to be more affected than males. The behavioural changes were generally only observed during Week 4 and included altered appearance (facial staining and badly groomed appearance), palpebral closure in the arena, the observation of tremor, increased hindlimb grip strength, and increased hindlimb splay.

The observations of increased hindlimb splay and the presence of tremor suggest an effect on the nervous system. Some of the additional observations, changes in appearance and the suggestion of effects on activity/rearing, can be considered non-specific. However, in the presence of the changes in splay and tremor this again suggests some effect on the nervous system.

The relevance of the observation of increased hindlimb grip strength is unclear as the expectation would be for a decrease in grip strength.

At lower dosages, there was no evidence of an effect of treatment.

### Conclusion

Exposure to Isodecyl benzoate for 28 days resulted in behavioural changes which were confined to 1000 mg/kg/day with females showing more changes compared with males. There was no evidence of an effect of treatment at lower dosages.

## HAEMATOLOGY (Table 3, Appendix 3)

There were no differences from control that were considered to be related to treatment with Isodecyl benzoate.

Lymphocyte count, and consequently total white blood cell count, was statistically significantly lower than control for male rats treated at 1000 mg/kg/day. Statistically significantly lower total white blood cell count was also recorded for males receiving 150 mg/kg/day. Individual values overlapped between the groups and the majority of values were within the expected range for rats of this age and strain (males,  $wbc \times 10^3/mm^3$ : 5 percentile - 7.6, median - 11.4, 95 percentile - 16.3; lymphocytes  $\times 10^3/mm^3$ ; 5 percentile - 6.54, median - 9.57, 95 percentile - 13.80). Therefore these differences from control were considered to be attributable to chance.

Statistically significantly lower than control mean corpuscular volume (MCV) was recorded for females receiving 1000 mg/kg/day. There was considerable overlap between the groups and the majority of values were within the background range for rats of this age and strain. This minor difference from control was not considered to be related to treatment.

The occurrence of slight polychromasia and/or slight anisocytosis is not uncommon in young laboratory rats and at the incidence seen in this study was not considered to be related to treatment with the test material.

**BIOCHEMISTRY** (Table 4, Appendix 4)

Glucose levels were statistically significantly lower than control for males and females receiving 1000 mg/kg/day and for females treated at 150 or 15 mg/kg/day. All individual values for rats from the intermediate and low dosage groups were within the expected range for rats of this age and strain and in the absence of a dosage-relationship a treatment-related effect at 150 or 15 mg/kg/day was not suspected.

Statistically significantly higher than control albumin was recorded for males receiving 1000 or 150 mg/kg/day, the resulting increase in A/G ratio also attained statistical significance for these groups though total protein remained unaffected. For females receiving 1000 mg/kg/day statistically significantly higher than control total protein was recorded. These differences from control were minor and a treatment-related effect on protein was not suspected.

Creatinine and sodium levels for high dosage males were statistically significantly higher than for control. The difference from control was considered to be due, in part, to high values for one animal (no. 28). For most remaining animals, individual values were within the expected range for rats of this age and strain. These differences from control were considered to have arisen by chance.

For females receiving 1000 mg/kg/day glutamic-pyruvic transaminase (GPT) was statistically significantly lower than control. However, individual values overlapped between the groups and the majority were within the expected range for rats of this age and strain. This difference was considered to be attributable to natural variation.

**ORGAN WEIGHTS** (Table 5, Appendix 5)

For rats of both sexes treated at 1000 mg/kg/day higher than control liver weight (bodyweight-adjusted) was recorded.

Kidney weights (bodyweight-adjusted) were statistically significantly higher than control for males treated at 1000 mg/kg/day.

Statistically significantly lower than control ovary (bodyweight-adjusted) and higher than control spleen weights were recorded for high dosage females. However, individual values overlapped to a large extent and these were not considered to be treatment-related effects.

There were no other statistically significant differences between treated and control rats

**MACROSCOPIC PATHOLOGY** (Table 6, Appendix 6)

The macroscopic *post mortem* examination performed at termination revealed enlarged liver for four males and two females receiving 1000 mg/kg/day.

There were no other macroscopic changes observed that were considered to be related to treatment.

**MICROSCOPIC PATHOLOGY** (Table 7, Appendix 6)**Treatment-related findings****Liver**

Centrilobular hepatocyte enlargement in male and female rats receiving 1000 mg/kg/day.

Treatment-related findings	Control		15 mg/kg/day		150 mg/kg/day		1000 mg/kg/day	
	♂	♀	♂	♀	♂	♀	♂	♀
Centrilobular hepatocyte enlargement	0	0	0	0	0	0	4*	3
Total number of animals examined	5	5	5	5	5	5	5	5

\* p < 0.05 with Fisher's Exact Test

These changes probably account for the increased liver weights recorded in this group.

No corresponding changes were seen in rats receiving 15 mg/kg/day and 150 mg/kg/day.

**Kidney**

A dosage-related incidence of eosinophilic intracytoplasmic droplets in the proximal convoluted tubules in male rats receiving 150 mg/kg/day and 1000 mg/kg/day.

Treatment-related findings	Control		15 mg/kg/day		150 mg/kg/day		1000 mg/kg/day	
	♂	♀	♂	♀	♂	♀	♂	♀
Eosinophilic intracytoplasmic droplets in proximal convoluted tubules	Total	0	0	0	4*	0	5**	0
	Trace	0	0	0	0	2	0	0
	Minimal	0	0	0	0	2	0	0
	Moderate	0	0	0	0	0	0	5**
Total number of animals examined	5	5	5	5	5	5	5	5

\* p < 0.05, \*\* p < 0.01 with Fisher's Exact Test

This change accounts for the increased organ weights recorded.

No corresponding changes were seen in male rats receiving 15 mg/kg/day or any of the female rats.

**Incidental findings**

Examination of brain and sciatic nerve revealed only occasional degenerate axons in the sciatic nerve of both control rats and those receiving 1000 mg/kg/day. These were considered normal background lesions.

No changes were considered to be sufficient to explain the clinical neurological signs.

All other changes were considered to be of no toxicological importance.

**Conclusion**

Centrilobular hepatocyte enlargement in male and female rats receiving 1000 mg/kg/day.

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules in male rats receiving 150 or 1000 mg/kg/day.

No treatment-related changes were seen in male rats receiving 15 mg/kg/day or female rats receiving 15 or 150 mg/kg/day.

## DISCUSSION

Isodecyl benzoate, a plasticizer, was administered to rats by oral gavage for 29 days at dosages of 1000, 150 or 15 mg/kg/day.

Treatment-related effects were seen in the liver and kidneys of high dosage group rats and there were also behavioural changes noted for this group.

In the liver centrilobular hepatocyte enlargement with associated macroscopic enlargement and increased liver weights were recorded. This liver finding was considered to be adaptive in nature and probably related to metabolism of the test substance. However, there were no biochemical changes (eg raised hepatocellular enzymes) to support this view. The toxicological significance of the lower glucose levels for rats treated at 1000 mg/kg/day remains unclear.

The microscopic finding of increased incidence and degree of eosinophilic inclusions in proximal tubular epithelium was observed in the kidneys of high dosage group and to a lesser extent, intermediate dosage group male rats, and is characteristic of light hydrocarbon nephropathy syndrome<sup>1</sup>. This finding, whilst treatment-related, is not considered predictive for a similar effect in man.

Behavioural changes, suggestive of an effect on the nervous system, were recorded. However, there were no histopathological changes to support these findings and other than evidence of poor grooming in Week 4, the animals remained clinically normal throughout the treatment period. The toxicological importance of these findings is unclear, but sufficient to reject the high dosage of 1000 mg/kg/day as a no-observed-adverse-effect-level (NOAEL).

The only treatment-related finding at the intermediate dosage level of 150 mg/kg/day was the microscopic finding of eosinophilic inclusions in kidney tubules. Therefore, this intermediate dosage can be considered to represent the NOAEL in the rat for Isodecyl benzoate when administered orally to the rat for 28 days. As there were no effects at this dosage of 150 mg/kg/day that were considered to represent the potential of the test substance to cause serious damage to health, the risk phrase R48 is not required for Isodecyl benzoate.

There were no treatment-related effects seen at the low dosage of 15 mg/kg/day and this dosage therefore represents the no-observed-effect-level (NOEL) in the rat.

<sup>1</sup> ALDEN, C.L. (1986) A review of unique male rat hydrocarbon nephropathy. *Tox. Path.* vol 14, no. 1, page 109 - 111.

## CONCLUSION

Behavioural changes, suggestive of an effect on the nervous system, were recorded for animals treated at 1000 mg/kg/day. These findings were considered sufficient to reject the high dosage level as a no-observed-adverse-effect level (NOAEL).

The only treatment-related effect seen at the intermediate dosage level of 150 mg/kg/day was in the kidney and was not considered predictive for a similar effect in man. It was therefore concluded that 150 mg/kg/day represents the no-observed-adverse-effect-level (NOAEL) in the rat for Isodecyl benzoate when administered to the rat for 28 days. The low dosage of 15 mg/kg/day represents the no-observed-effect-level (NOEL).

According to the EEC Council Directive 92/69/EEC Annex VI, Part II (D) as described in Commission Directive 93/21/EEC, labelling with the R48 risk phrase is not required.

FIGURE 1

## Group arrangement in batteries

Group	Cage label/ colour code	Treatment	Dosages (mg/kg/day)	No. of rats		Rat numbers	
				♂	♀	♂	♀
1	White	Control, corn oil	-	8	8	1-8	33-40
2	Yellow	Isodecyl benzoate	15	8	8	9-16	41-48
3	Green	Isodecyl benzoate	150	8	8	17-24	49-56
4	Red	Isodecyl benzoate	1000	8	8	25-32	57-64

♂	
1	2
3	4

♀	
1	2
3	4

**FIGURE 2**  
**Bodyweights - group mean values**

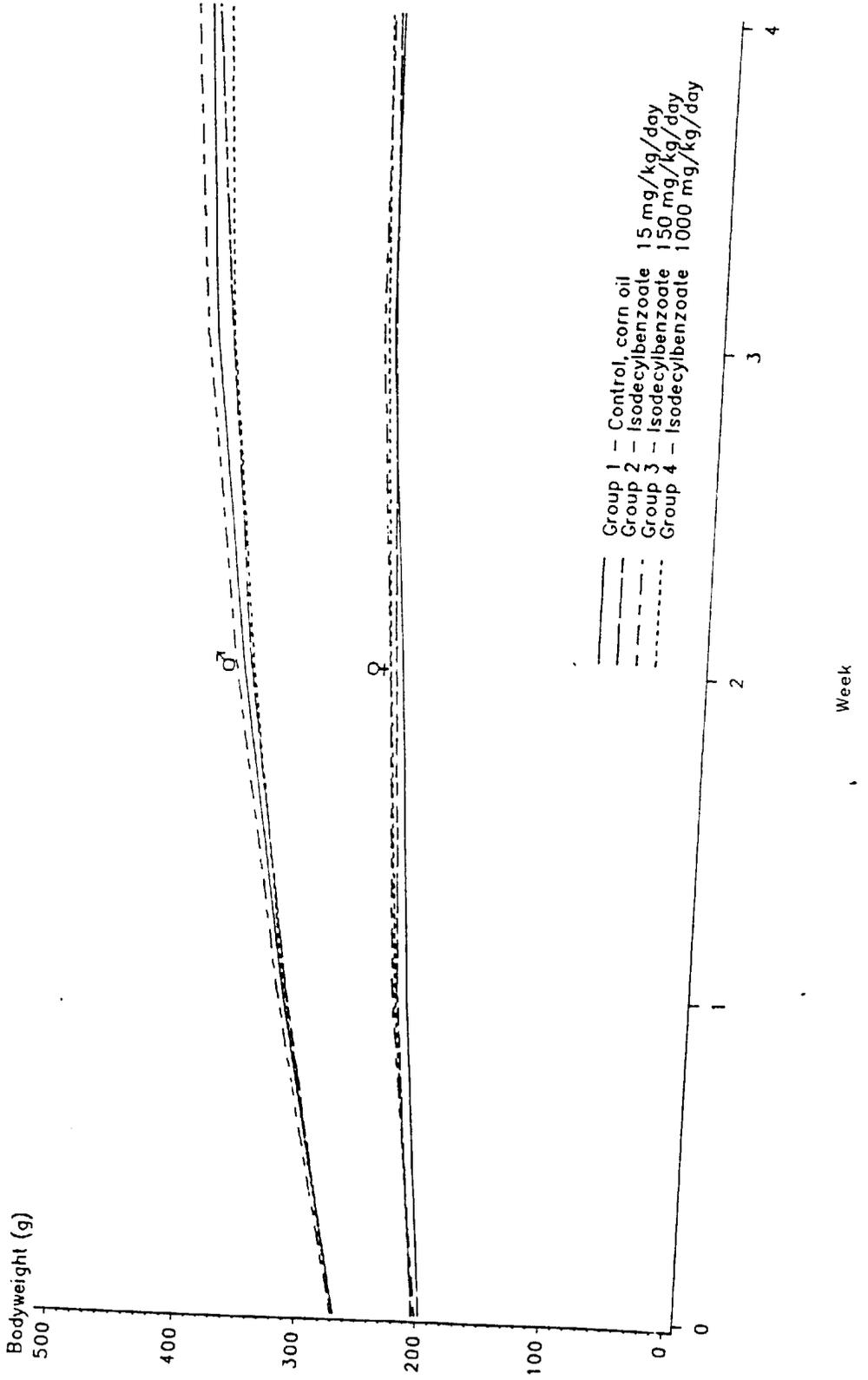
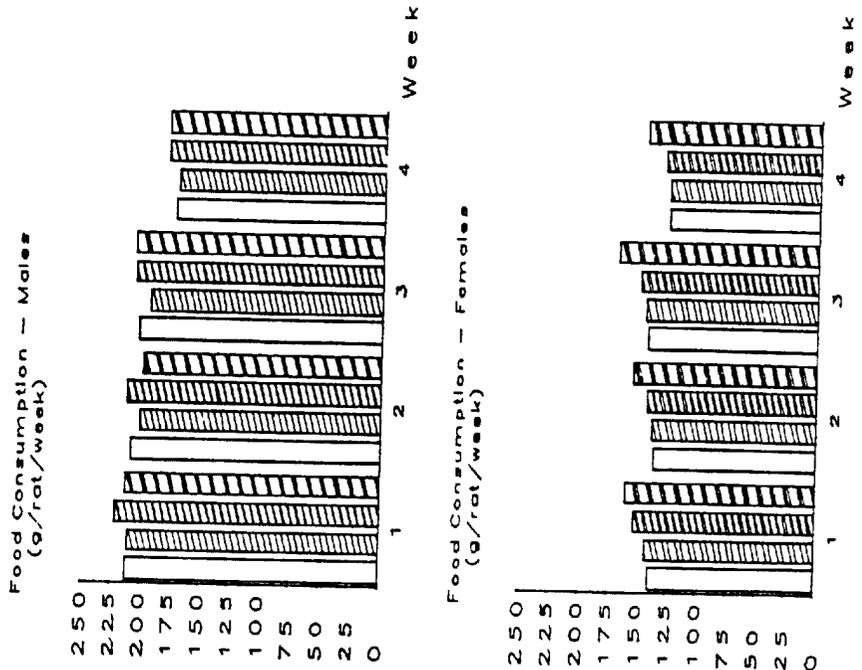


FIGURE 3

Food consumption - group mean values



- Group 1 - Control, corn oil
- Group 2 - Isodecyl benzoate 15 mg/kg/day
- Group 3 - Isodecyl benzoate 150 mg/kg/day
- Group 4 - Isodecyl benzoate 1000 mg/kg/day

TABLE 1

Bodyweights - group mean values (g)

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 15	3♂ 150	4♂ 1000	1♀ Control	2♀ 15	3♀ 150	4♀ 1000
0	268	269	271	269	198	204	201	203
1	324	320	328	323	221	228	233	230
2	369	363	375	360	238	243	248	248
3	404	392	411	390	259	257	267	263
4	422	416	433	407	266	268	273	275
Gain Wk 0 - 4	154	147	162	138	68	64	72	72
% of control	-	95	105	90	-	94	106	106

No statistical significance ( $P > 0.05$ ) for bodyweight gain

TABLE 2

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

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 15	3♂ 150	4♂ 1000	1♀ Control	2♀ 15	3♀ 150	4♀ 1000
1	212	210	222	214	140	144	154	161
2	209	202	213	200	138	139	144	156
3	204	195	206	207	144	145	150	169
4	174	172	181	180	127	128	132	147
Cumulative consumption	799	779	822	801	549	556	580	633
% of control	-	97	103	100	-	101	106	115

No statistical significance ( $P > 0.05$ ) for cumulative consumption

TABLE 3

## Haematology - group mean values

Week 4

Group/ dosage mg/kg/day	PCV %	Hb g/dl	RBC $\times 10^6/mm^3$	MCHC %	MCV fl	WBC + Diff $\times 10^3/mm^3$					Plts $\times 10^3/mm^3$	TT s	
						Total	N	L	E	B			M
1♂ Control	56	15.3	6.9	27.4	81	13.8	LT 2.91	10.45	0.12	NA 0.00	0.28	868	21
2♂ 15	56	15.4	7.2	27.3	79	11.8	2.04	9.52	0.06	0.00	0.18	672	22
3♂ 150	57	15.2	7.2	26.9	79	* 9.2	1.31	7.79	0.07	0.00	0.05	1014	21
4♂ 1000	56	15.4	7.0	27.5	81	** 8.0	1.41	** 6.47	0.02	0.00	0.07	762	22
1♀ Control	56	15.1	6.9	26.8	82	6.8	1.17	5.61	0.02	NA 0.00	0.02	919	20
2♀ 15	58	15.5	7.1	26.9	81	8.3	1.07	7.20	0.00	0.00	0.06	865	19
3♀ 150	56	14.9	6.9	26.6	81	6.4	1.37	4.89	0.14	0.00	0.01	922	19
4♀ 1000	55	14.7	6.8	26.9	80	* 7.0	1.58	5.35	0.02	0.00	0.05	887	19

\* P &lt; 0.05

\*\* P &lt; 0.01

LT Data log-transformed

NA No analysis necessary; all values the same

**TABLE 4**  
**Biochemistry - group mean values**

Group/ dosage mg/kg/day	Glu- cose mg/dl	Protein g/dl		A/G	Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl	
		Total	Alb														Glob
1♂ Control	121	6.6	2.7	3.9	0.69	10	0.5	383	25	56	0.1	144	3.6	5.7	4.9	98	65
2♂ 15	135	6.5	2.7	3.8	0.72	10	0.4	348	29	51	0.1	144	3.9	5.6	4.7	99	67
3♂ 150	112	6.6	2.9	3.7	0.77	10	0.5	342	28	53	0.1	146	3.6	5.6	4.8	99	69
4♂ 1000	** 89	6.6	3.0	3.6	0.82	10	0.6	374	25	52	0.1	147	3.7	5.5	5.0	100	61
1♀ Control	126	6.7	3.0	3.7	0.81	12	0.5	236	27	49	0.1	145	3.6	5.7	4.0	101	73
2♀ 15	* 112	6.7	3.0	3.7	0.83	13	0.5	173	25	53	0.1	145	3.5	5.7	4.3	101	74
3♀ 150	* 118	6.9	3.0	3.9	0.78	12	0.5	218	23	51	0.1	144	3.4	5.6	4.0	100	69
4♀ 1000	** 102	7.2	3.1	4.1	0.78	14	0.6	232	21	52	0.1	144	3.4	5.7	4.3	100	87

\* P &lt; 0.05

\*\* P &lt; 0.01

F Frequency analysis

TABLE 5

## Organ weights - group mean values

Week 5

Group/ dosage mg/kg/day	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes		Seminal Vesicle g	Epididymides		Prost- ate g
							L g	R g		Left g	Right g	
Unadjusted means												
1♂ Control	430	2.18	22.3	0.93	3.43	60.3	1.781	1.809	1.14	0.525	0.495	0.81
2♂ 15	410	2.09	20.4	0.87	3.25	58.5	1.660	1.666	1.08	0.496	0.497	0.86
3♂ 150	431	2.07	20.0	0.84	3.49	57.5	1.790	1.761	1.08	0.489	0.487	0.75
4♂ 1000	418	2.13	27.8	0.84	3.76	64.2	1.761	1.758	0.88	0.536	0.496	0.84
Adjusted means												
1♂		-	21.8	0.91	3.38	-	1.772	1.801	-	-	0.493	-
2♂		-	21.2	0.92	3.34	-	1.676	1.679	-	-	0.502	-
3♂		-	19.4 **	0.81	3.43 *	-	1.778	1.752	-	-	0.484	-
4♂		-	28.1	0.86	3.79	-	1.766	1.763	-	-	0.498	-

\* P &lt; 0.05

\*\* P &lt; 0.01

**TABLE 5**  
**(Organ weights - continued)**

Week 5

Group/ dosage mg/kg/day	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
Unadjusted means							
1♀ Control	266	1.93	11.9	0.61	2.38	74.4	114.6
2♀ 15	263	1.96	11.6	0.67	2.21	74.9	109.2
3♀ 150	271	1.92	12.3	0.66	2.43	81.0	113.6
4♀ 1000	274	1.95	18.7	0.73	2.65	80.1	96.8
Adjusted means							
1♀		-	12.0	-	-	-	116.2
2♀		-	11.8	-	-	-	112.4
3♀		-	12.2	-	-	-	112.1
4♀		-	18.5	-	-	-	93.5

\* P &lt; 0.05

\*\* P &lt; 0.01

**TABLE 6**  
**Macroscopic pathology incidence summary**

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Removal reason: Terminal	1	2	3	4	1	2	3	4
Animals on study Animals completed	8 5							
<b>Lymph Nodes - Cervical</b> Enlarged	2	3	3	2	0	0	2	2
<b>Liver</b> Median cleft, pale subcapsular area/s Enlarged	0 0	0 0	0 0	1 4	0 0	0 0	0 0	0 2
<b>Spleen</b> Clear fluid-filled cyst/s Capsule thickened	0 0	0 0	0 0	0 0	1 1	0 0	0 0	0 1
<b>Stomach Antrum Mucosa</b> White nodule/s	1	0	0	0	1	0	2	1
<b>Kidneys</b> Increased pelvic dilatation	1	0	0	0	0	0	0	0
<b>Ovaries</b> Clear fluid-filled cyst	0	0	0	0	0	0	0	1
<b>Uterus</b> Fluid distension Thickened	0 0	0 0	0 0	0 0	0 0	1 0	1 1	0 0
<b>Cervix</b> Swollen	0	0	0	0	0	0	1	0

**TABLE 7**  
**Microscopic pathology incidence summary**

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
<b>Animals on study</b>	8	8	8	8	8	8	8	8
<b>Animals completed</b>	5	5	5	5	5	5	5	5
<b>Lungs</b>	5	0	0	5	5	0	0	5
Examined	2	0	0	0	2	0	0	4
No abnormalities detected	3	0	0	5	2	0	0	1
Vascular congestion	3	0	0	3	3	0	0	1
Intra-alveolar recent haemorrhage (Total)	3	0	0	3	3	0	0	1
Minimal								
<b>Heart</b>	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected								
<b>Spleen</b>	5	0	0	5	5	0	0	5
Examined	0	0	0	0	0	0	0	0
No abnormalities detected	0	0	0	0	4	0	0	1
Capsular inflammation (Total)	0	0	0	0	1	0	0	1
Minimal	0	0	0	0	1	0	0	1
Capsular thickening (Total)	0	0	0	0	1	0	0	1
Minimal								
<b>Liver</b>	5	5	5	5	5	5	5	5
Examined	0	0	0	0	0	0	0	0
No abnormalities detected	0	0	0	0	0	0	0	0
Centrilobular hepatocyte enlargement (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Moderate								
<b>Kidneys</b>	5	5	5	5	5	5	5	5
Examined	2	5	1	0	5	5	3	2
No abnormalities detected	0	0	0	0	0	0	0	0
Pyelonephritis (Total)	0	0	0	0	1	0	0	0
Minimal					1			

TABLE 7  
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	8	8	8	8	8	8	8	8
Animals completed	5	5	5	5	5	5	5	5
<b>Kidneys</b>	(Continued)							
Cortical scarring (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Basophilic cortical tubules (Total)	0	0	1	0	0	0	1	3
Minimal	0	0	1	0	0	0	1	2
Moderate	0	0	0	0	0	0	0	1
Cortical cyst	1	0	0	0	0	0	1	0
Medullary tubule mineralisation (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Tubule mineralisation at the corticomedullary junction (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	1
Moderate	2	0	0	0	0	0	0	0
Pelvic dilation (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Eosinophilic intracytoplasmic droplets in proximal convoluted tubules (Total)	0	0	4	5	0	0	0	0
Trace	0	0	2	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
<b>Epididymides</b>	5	5	5	5	5	5	5	5
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0
<b>Testes</b>	5	5	5	5	5	5	5	5
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0
Tubules lined only by Sertoli cells (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
<b>Ovaries</b>	0	0	0	0	0	0	0	0
Examined	0	0	0	0	0	0	0	0
No abnormalities detected	0	0	0	0	0	0	0	0
Group 1	0	0	0	0	0	0	0	0
Group 2	0	0	0	0	0	0	0	0
Group 3	0	0	0	0	0	0	0	0
Group 4	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0

**TABLE 7**  
**(Microscopic pathology incidence summary - continued)**

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	8	8	8	8	8	8	8	8
Animals completed	5	5	5	5	5	5	5	5
<b>Ovaries</b>	<b>(Continued)</b>							
Follicular cyst	0	0	0	0	0	0	0	1
Luteal cyst(s)	0	0	0	0	0	0	0	0
<b>Adrenals</b>								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
<b>Sciatic Nerve</b>								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	4	0	0	2	3	0	0	4
Degenerate fibres (Total)	1	0	0	3	2	0	0	1
Trace	1	0	0	3	2	0	0	1
<b>Brain</b>								
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	5	0	0	5	5	0	0	5
<b>Lymph Nodes - Cervical</b>								
Examined	2	0	0	2	0	0	0	2
Lymphoid proliferation (Total)	2	0	0	2	0	0	0	2
Moderate	2	0	0	2	0	0	0	2
<b>Stomach</b>								
Examined	1	0	0	0	1	0	0	1
Focus of ectopic non-glandular epithelium within the glandular mucosa	1	0	0	0	1	0	0	1

## APPENDIX 1

## Bodyweights - individual values (g)

Group 1♂  
Control

Cage number	Animal number	Week				
		0	1	2	3	4
1	1	274	349	409	469	499
	2	265	303	335	366	376
	3	263	324	362	380	393
	4	278	339	396	444	460
2	5	273	332	383	426	444
	6	261	303	339	361	380
	7	260	306	347	371	389
	8	272	334	379	412	435

Group 2♂  
15 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
3	9	248	282	319	339	367
	10	275	331	373	408	426
	11	275	330	387	420	454
	12	272	330	375	420	446
4	13	260	314	351	375	382
	14	275	320	354	370	400
	15	264	318	362	393	418
	16	282	337	381	408	436

Group 3♂  
150 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
5	17	269	322	369	399	417
	18	290	352	403	451	472
	19	269	327	372	404	422
	20	260	304	344	379	402
6	21	279	345	402	445	473
	22	262	319	358	387	407
	23	256	325	382	422	453
	24	281	329	370	402	417

Group 4♂  
1000 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
7	25	269	322	358	379	381
	26	261	306	333	347	357
	27	277	346	391	430	450
	28	269	315	352	378	398
8	29	283	357	414	463	490
	30	271	316	353	379	399
	31	260	311	347	381	390
	32	264	307	335	366	389

## APPENDIX 1

## (Bodyweights - continued)

Group 1 ♀  
Control

Cage number	Animal number	Week				
		0	1	2	3	4
9	33	192	219	228	254	259
	34	201	218	246	269	273
	35	207	232	248	264	269
	36	204	231	238	262	268
10	37	197	220	241	260	269
	38	197	216	238	255	257
	39	188	209	227	248	259
	40	195	220	238	256	270

Group 2 ♀  
15 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
11	41	199	224	241	249	266
	42	204	229	248	257	273
	43	209	238	256	269	277
	44	202	227	233	251	253
12	45	206	229	240	260	276
	46	209	237	254	268	284
	47	183	202	218	225	226
	48	217	240	256	277	289

Group 3 ♀  
150 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
13	49	202	223	242	262	273
	50	220	248	267	289	302
	51	200	232	251	259	262
	52	195	215	225	246	251
14	53	196	230	251	269	268
	54	207	250	264	290	294
	55	186	230	232	245	247
	56	203	232	253	277	287

Group 4 ♀  
1000 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
15	57	212	244	266	281	292
	58	192	213	236	248	248
	59	204	226	237	261	271
	60	204	230	240	263	273
16	61	212	241	249	274	292
	62	201	234	258	269	276
	63	191	222	238	245	265
	64	208	231	257	266	280

## APPENDIX 2

## Food consumption - cage mean values (g/rat/week)

Group 1♂  
Control

Week	Cage	
	1	2
1	211	213
2	213	205
3	212	197
4	177	170

Group 2♂  
15 mg/kg/day

Week	Cage	
	3	4
1	207	214
2	203	201
3	200	189
4	173	171

Group 3♂  
150 mg/kg/day

Week	Cage	
	5	6
1	215	230
2	205	221
3	193	220
4	174	187

Group 4♂  
1000 mg/kg/day

Week	Cage	
	7	8
1	214	213
2	193	206
3	197	217
4	169	191

Group 1♀  
Control

Week	Cage	
	9	10
1	142	138
2	135	140
3	141	146
4	126	129

Group 2♀  
15 mg/kg/day

Week	Cage	
	11	12
1	138	149
2	133	146
3	142	149
4	121	134

Group 3♀  
150 mg/kg/day

Week	Cage	
	13	14
1	148	159
2	139	148
3	145	156
4	129	134

Group 4♀  
1000 mg/kg/day

Week	Cage	
	15	16
1	154	167
2	148	164
3	162	175
4	136	158

## APPENDIX 3

## Haematology - individual values

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	PCV %	Hb g/dl	RBC $\times 10^6/mm^3$	MCHC %	MCV fl	WBC + Diff $\times 10^3/mm^3$					Pits $\times 10^3/mm^3$	TT s	
							Total	N	L	E	B			M
1♂ Control	1P <sub>1</sub> A <sub>1</sub>	51	14.6	6.1	28.6	84	14.8	2.66	11.40	0.30	0.00	0.44	975	21
	2	57	15.1	7.2	26.5	79	8.3	1.58	6.47	0.08	0.00	0.17	937	22
	3A <sub>1</sub>	59	16.0	7.7	27.1	77	11.8	1.65	9.68	0.24	0.00	0.24	735	20
	4P <sub>1</sub> A <sub>1</sub>	54	15.2	6.6	28.1	82	20.0	7.00	12.60	0.00	0.00	0.40	847	21
	5	58	15.6	7.0	26.9	83	13.9	1.67	12.09	0.00	0.00	0.14	844	23
	Mean SD	56 3.3	15.3 0.53	6.9 0.61	27.4 0.88	81 2.9	13.8 4.29	2.91 2.328	10.45 2.482	0.12 0.139	0.00 0.000	0.28 0.135	868 93.4	21 1.1
2♂ 15	9	57	15.2	7.3	26.7	78	12.1	1.33	10.65	0.12	0.00	0.00	668	23
	10	57	15.4	7.2	27.0	79	9.1	2.00	7.01	0.00	0.00	0.09	685	25
	11	55	15.2	6.7	27.6	82	17.2	3.96	12.56	0.00	0.00	0.69	741	23
	12	56	15.4	7.3	27.5	77	12.3	2.09	10.09	0.00	0.00	0.12	566	20
	13	57	15.9	7.4	27.9	77	8.3	0.83	7.30	0.17	0.00	0.00	702	21
	Mean SD	56 0.9	15.4 0.29	7.2 0.28	27.3 0.48	79 2.1	11.8 3.50	2.04 1.189	9.52 2.349	0.06 0.081	0.00 0.000	0.18 0.290	672 65.3	22 1.9
3♂ 150	17A <sub>1</sub>	56	15.7	6.8	28.0	82	8.2	1.80	6.31	0.08	0.00	0.00	956	20
	18	ctd	ctd	ctd	nr	nr	ctd	ctd	ctd	ctd	ctd	ctd	ctd	21
	19P <sub>1</sub> A <sub>1</sub>	56	15.0	7.2	26.8	78	10.1	1.31	8.59	0.10	0.00	0.10	829	21
	20	58	15.5	7.3	26.7	79	10.9	1.20	9.70	0.00	0.00	0.00	909	21
	21	56	14.6	7.3	26.1	77	7.6	0.91	6.54	0.08	0.00	0.08	1360	23
	Mean SD	57 1.0	15.2 0.50	7.2 0.24	26.9 0.80	79 2.2	9.2 1.56	1.31 0.371	7.79 1.637	0.07 0.044	0.00 0.000	0.05 0.053	1014 236.9	21 1.3
4♂ 1000	25P <sub>1</sub>	56	15.6	7.0	27.9	80	6.6	1.39	5.15	0.00	0.00	0.07	844	23
	26	57	14.9	7.4	26.1	77	6.4	1.28	4.93	0.00	0.00	0.19	710	23
	27	58	16.2	7.0	27.9	83	9.4	2.07	7.24	0.00	0.00	0.09	730	23
	28	56	15.4	7.1	27.5	79	8.2	1.56	6.56	0.08	0.00	0.00	768	20
	29P <sub>1</sub> A <sub>1</sub>	53	15.0	6.3	28.3	84	9.2	0.74	8.46	0.00	0.00	0.00	760	20
	Mean SD	56 1.9	15.4 0.52	7.0 0.40	27.5 0.85	81 2.9	8.0 1.41	1.41 0.481	6.47 1.473	0.02 0.036	0.00 0.000	0.07 0.078	762 51.2	22 1.6

SD Standard deviation  
ctd Clotted sample  
nr Not recorded  
P<sub>1</sub> Slight polychromasia  
A<sub>1</sub> Slight anisocytosis

## APPENDIX 3

## (Haematology - continued)

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	PCV %	Hb g/dl	RBC $\times 10^6/mm^3$	MCHC %	MCV fl	WBC + Diff $\times 10^3/mm^3$					Plts $\times 10^3/mm^3$	TT s	
							Total	N	L	E	B			M
1♀ Control	33P <sub>1</sub>	55	15.2	6.8	27.6	81	6.8	0.54	6.26	0.00	0.00	0.00	819	21
	34P <sub>1</sub> A <sub>1</sub>	54	14.4	6.7	26.7	81	6.8	1.09	5.71	0.00	0.00	0.00	1002	19
	35	59	15.2	7.1	25.8	83	8.3	1.83	6.39	0.08	0.00	0.00	891	21
	36	56	15.4	6.7	27.5	84	6.3	0.88	5.42	0.00	0.00	0.00	1082	20
	37P <sub>1</sub> A <sub>1</sub>	58	15.4	7.0	26.6	83	5.9	1.53	4.25	0.00	0.00	0.12	801	18
	Mean SD	56 2.1	15.1 0.41	6.9 0.18	26.8 0.74	82 1.3	6.8 0.91	1.17 0.513	5.61 0.855	0.02 0.036	0.00 0.000	0.02 0.054	919 120.6	20 1.3
2♀ 15	41	59	15.6	7.1	26.4	83	10.8	1.08	9.50	0.00	0.00	0.22	940	19
	42	59	16.5	7.2	28.0	82	8.6	1.38	7.22	0.00	0.00	0.00	817	20
	43	57	15.2	6.9	26.7	83	5.8	0.81	4.99	0.00	0.00	0.00	778	19
	44	59	15.4	7.4	26.1	80	8.5	1.11	7.40	0.00	0.00	0.00	792	19
	45	54	14.8	6.8	27.4	79	7.9	0.95	6.87	0.00	0.00	0.08	1000	18
	Mean SD	58 2.2	15.5 0.63	7.1 0.24	26.9 0.77	81 1.8	8.3 1.79	1.07 0.212	7.20 1.606	0.00 0.000	0.00 0.000	0.06 0.096	865 98.8	19 0.7
3♀ 150	49A <sub>1</sub>	54	14.4	6.6	26.7	82	7.7	2.77	4.70	0.23	0.00	0.00	837	19
	50	58	15.4	7.1	26.6	82	7.3	1.24	5.91	0.15	0.00	0.00	929	20
	51	57	15.0	7.0	26.3	81	6.6	1.25	5.28	0.07	0.00	0.00	799	19
	52	56	15.0	6.9	26.8	81	4.5	1.22	3.02	0.23	0.00	0.05	977	19
	53	55	14.5	6.8	26.4	81	5.9	0.35	5.55	0.00	0.00	0.00	1070	ctd
	Mean SD	56 1.6	14.9 0.41	6.9 0.19	26.6 0.21	81 0.5	6.4 1.26	1.37 0.874	4.89 1.136	0.14 0.101	0.00 0.000	0.01 0.022	922 108.8	19 0.5
4♀ 1000	57	55	15.0	7.0	27.3	79	5.5	0.88	4.51	0.00	0.00	0.11	1212	20
	58	56	14.8	6.9	26.4	81	9.3	3.35	5.95	0.00	0.00	0.00	760	ctd
	59A <sub>1</sub>	51	13.7	6.3	26.9	81	3.7	0.67	3.00	0.00	0.00	0.04	953	17
	60A <sub>1</sub>	56	15.2	7.1	27.1	79	10.2	1.63	8.36	0.10	0.00	0.10	714	19
	61	55	14.8	6.7	26.9	82	6.3	1.39	4.91	0.00	0.00	0.00	798	19
	Mean SD	55 2.1	14.7 0.58	6.8 0.32	26.9 0.33	80 1.3	7.0 2.70	1.58 1.059	5.35 1.990	0.02 0.045	0.00 0.000	0.05 0.053	887 202.5	19 1.2

SD Standard deviation  
ctd Clotted sample  
P<sub>1</sub> Slight polychromasia  
A<sub>1</sub> Slight anisocytosis

APPENDIX 4

Biochemistry - individual values

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	Glu- cose mg/dl	Protein g/dl		A/G	Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	Bili- rubin mg/dl	Na mEq/ l	K mEq/ mEq/dl	Ca mEq/ mEq/dl	P mEq/ mEq/dl	Cl mEq/ mEq/dl	Chol mg/dl	
			Total	Alb														Glob
1 <sup>δ</sup> Control	1	121	7.0	2.6	4.4	0.59	11	0.4	300	25	50	0.1	143	3.8	5.6	5.1	97	57
	2	113	6.4	2.6	3.8	0.68	11	0.5	555	27	58	0.1	144	3.5	5.6	4.7	99	65
	3	132	6.8	2.9	3.9	0.74	9	0.5	315	23	65	0.1	145	3.2	5.8	5.0	98	66
	4	113	6.6	2.6	4.0	0.65	10	0.5	325	25	56	0.1	144	3.5	5.7	4.7	99	66
	5	128	6.2	2.7	3.5	0.77	11	0.5	418	24	50	0.1	144	3.8	5.6	5.2	99	69
	Mean	121	6.6	2.7	3.9	0.69	10	0.5	383	25	56	0.1	144	3.6	5.7	4.9	98	65
	SD	8.6	0.32	0.13	0.33	0.072	0.9	0.04	106.9	1.5	6.3	0.00	0.7	0.25	0.09	0.23	0.9	4.5
2 <sup>δ</sup> 15	9	134	6.1	2.7	3.4	0.79	10	0.4	290	30	59	0.2	144	3.6	5.5	4.7	99	69
	10	125	6.6	2.7	3.9	0.69	10	0.5	437	27	53	0.1	146	3.6	5.6	4.8	102	52
	11	131	6.5	2.7	3.8	0.71	8	0.5	353	29	47	0.1	143	3.8	5.5	4.7	98	58
	12	153	6.9	2.7	4.2	0.64	9	0.3	397	35	53	0.1	142	4.7	5.6	4.9	99	70
	13	133	6.6	2.9	3.7	0.78	12	0.4	264	22	44	0.1	143	3.8	5.7	4.6	99	87
	Mean	135	6.5	2.7	3.8	0.72	10	0.4	348	29	51	0.1	144	3.9	5.6	4.7	99	67
	SD	10.5	0.29	0.09	0.29	0.063	1.5	0.08	72.1	4.7	5.8	0.04	1.5	0.46	0.08	0.11	1.5	13.4
3 <sup>δ</sup> 150	17	103	6.8	2.9	3.9	0.74	12	0.5	432	38	55	0.1	144	3.5	5.7	4.9	98	79
	18	114	6.7	2.8	3.9	0.72	10	0.5	300	27	51	0.1	146	3.4	5.6	4.8	100	62
	19	117	6.6	2.9	3.7	0.78	10	0.5	370	23	55	0.1	146	3.5	5.5	4.6	100	85
	20	100	6.3	2.8	3.5	0.80	9	0.5	316	29	53	0.1	147	3.3	5.6	5.2	100	65
	21	124	6.5	2.9	3.6	0.81	9	0.5	292	25	50	0.1	146	4.2	5.6	4.7	99	53
	Mean	112	6.6	2.9	3.7	0.77	10	0.5	342	28	53	0.1	146	3.6	5.6	4.8	99	69
	SD	10.0	0.19	0.05	0.18	0.039	1.2	0.00	58.8	5.8	2.3	0.00	1.1	0.36	0.07	0.23	0.9	13.0
4 <sup>δ</sup> 1000	25	97	6.5	3.1	3.4	0.91	14	0.6	419	31	57	0.1	145	3.9	5.7	5.1	100	52
	26	80	6.9	3.2	3.7	0.86	10	0.6	368	23	49	0.1	146	3.3	5.3	5.1	99	46
	27	99	6.7	2.9	3.8	0.76	8	0.5	408	27	48	0.1	145	3.6	5.6	4.8	100	79
	28	84	6.4	2.8	3.6	0.78	10	0.7	300	22	52	0.1	150	3.7	5.5	5.2	102	57
	29	83	6.5	2.9	3.6	0.81	10	0.5	377	22	53	0.1	147	4.2	5.5	5.0	99	73
	Mean	89	6.6	3.0	3.6	0.82	10	0.6	374	25	52	0.1	147	3.7	5.5	5.0	100	61
	SD	8.7	0.20	0.16	0.15	0.061	2.2	0.08	46.6	3.9	3.6	0.00	2.1	0.34	0.15	0.15	1.2	14.0

SD Standard deviation

**APPENDIX 4**  
**(Biochemistry - continued)**

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	Glu- cose mg/dl	Protein g/dl		A/G	Urea Nitri- mine mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
			Total	Alb													
1♀ Control	33	118	6.5	2.8	3.7	0.76	13	0.5	205	21	40	144	3.5	5.7	4.4	100	49
	34	124	6.3	2.9	3.4	0.85	11	0.5	160	21	62	144	3.3	5.6	4.3	101	65
	35	124	7.1	3.2	3.9	0.82	9	0.5	258	31	50	145	4.0	5.8	3.9	102	77
	36	135	6.6	3.1	3.5	0.89	11	0.5	199	30	42	145	3.6	5.7	4.2	101	81
	37	129	7.1	3.0	4.1	0.73	14	0.5	358	32	52	145	3.7	5.6	3.4	102	92
	Mean	126	6.7	3.0	3.7	0.81	12	0.5	236	27	49	145	3.6	5.7	4.0	101	73
	SD	6.4	0.36	0.16	0.29	0.065	1.9	0.00	76.6	5.5	8.8	0.04	0.5	0.26	0.08	0.40	0.8
2♀ 15	41	106	6.9	3.2	3.7	0.86	15	0.5	246	31	61	146	3.3	5.7	4.4	101	75
	42	104	6.6	2.8	3.8	0.74	10	0.4	161	26	55	145	3.6	5.7	4.3	102	63
	43	116	7.0	3.3	3.7	0.89	13	0.4	124	26	54	145	3.6	5.6	4.0	102	79
	44	119	6.8	2.9	3.9	0.74	12	0.5	186	19	47	143	3.6	5.5	4.4	99	71
	45	114	6.3	3.0	3.3	0.91	13	0.5	149	25	49	144	3.4	5.8	4.3	99	80
	Mean	112	6.7	3.0	3.7	0.83	13	0.5	173	25	53	145	3.5	5.7	4.3	101	74
SD	6.5	0.28	0.21	0.23	0.082	1.8	0.05	46.4	4.3	5.5	0.04	1.1	0.14	0.11	0.16	1.5	6.9
3♀ 150	49	128	7.2	2.8	4.4	0.64	10	0.5	374	23	43	142	3.3	5.6	4.2	99	59
	50	122	6.5	2.9	3.6	0.81	13	0.5	202	19	50	142	3.8	5.6	4.2	100	76
	51	113	7.2	3.2	4.0	0.80	10	0.6	178	26	57	145	3.6	5.5	3.7	103	65
	52	105	7.1	3.2	3.9	0.82	12	0.5	184	23	52	144	3.2	5.7	3.6	99	68
	53	120	6.7	3.0	3.7	0.81	13	0.5	152	23	51	146	3.3	5.4	4.3	101	79
	Mean	118	6.9	3.0	3.9	0.78	12	0.5	218	23	51	144	3.4	5.6	4.0	100	69
SD	8.8	0.32	0.18	0.31	0.076	1.5	0.04	89.0	2.5	5.0	0.00	1.8	0.25	0.11	0.32	1.7	8.1
4♀ 1000	57	92	7.2	3.1	4.1	0.76	20	0.8	236	24	70	145	3.1	5.7	4.2	101	79
	58	110	7.6	3.1	4.5	0.69	11	0.6	281	22	53	144	3.4	5.8	4.3	100	98
	59	94	7.3	3.2	4.1	0.78	15	0.6	225	22	49	145	3.3	5.5	4.2	101	101
	60	104	7.3	3.1	4.2	0.74	13	0.5	179	18	42	143	3.4	5.8	4.1	100	85
	61	112	6.6	3.2	3.4	0.94	13	0.4	237	18	48	145	3.7	5.7	4.8	99	73
	Mean	102	7.2	3.1	4.1	0.78	14	0.6	232	21	52	144	3.4	5.7	4.3	100	87
SD	9.1	0.37	0.05	0.40	0.094	3.4	0.15	36.4	2.7	10.6	0.05	0.9	0.22	0.12	0.28	0.8	12.0

SD Standard deviation

## APPENDIX 5

## Organ weights - individual values

Week 5 (23 September 1994)

Group/ dosage mg/kg/day	Animal no.	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g	Prost- ate g
1♂ Control	1	484	2.15	24.1	1.23	3.94	53.3	1.889	1.880	1.03	0.569	0.535	0.80
	2	373	2.14	18.5	0.68	2.84	52.1	1.726	1.761	1.12	0.560	0.509	0.85
	3	387	2.05	20.5	0.84	2.94	53.7	1.705	1.691	1.15	0.511	0.463	0.79
	4	457	2.24	24.7	1.08	3.54	78.8	1.758	1.814	0.95	0.489	0.481	0.73
	5	447	2.31	23.8	0.84	3.90	63.7	1.829	1.897	1.43	0.494	0.489	0.91
	Mean SD	430 47.3	2.18 0.099	22.3 2.69	0.93 0.221	3.43 0.520	60.3 11.33	1.781 0.0763	1.809 0.0852	1.14 0.184	0.525 0.0375	0.495 0.0276	0.81 0.066
2♂ 15	9	359	1.98	15.6	0.75	2.83	55.2	1.506	1.516	0.89	0.468	0.461	0.80
	10	416	2.15	21.7	1.04	3.37	53.9	1.729	1.824	0.87	0.495	0.479	0.95
	11	451	2.13	24.1	1.13	3.58	60.8	1.703	1.665	1.79	0.556	0.543	1.05
	12	443	2.09	21.7	0.78	3.45	60.8	1.819	1.789	0.86	0.464	0.513	0.87
	13	380	2.11	18.7	0.67	3.02	61.9	1.542	1.535	0.97	0.496	0.491	0.62
	Mean SD	410 39.5	2.09 0.066	20.4 3.28	0.87 0.200	3.25 0.315	58.5 3.68	1.660 0.1318	1.666 0.1412	1.08 0.403	0.496 0.0368	0.497 0.0317	0.86 0.164
3♂ 150	17	412	2.09	20.0	0.76	3.50	65.2	1.866	1.849	1.10	0.496	0.491	0.59
	18	467	2.08	24.0	1.11	3.39	51.4	1.775	1.728	1.45	0.526	0.498	0.81
	19	416	2.14	19.8	0.74	3.48	52.5	1.814	1.739	0.88	0.437	0.459	0.72
	20	392	1.98	15.0	0.82	3.63	62.8	1.719	1.706	1.11	0.451	0.464	1.05
	21	470	2.05	21.1	0.78	3.46	55.6	1.775	1.782	0.89	0.534	0.523	0.61
	Mean SD	431 35.0	2.07 0.059	20.0 3.25	0.84 0.153	3.49 0.090	57.5 6.19	1.790 0.0544	1.761 0.0565	1.08 0.231	0.489 0.0436	0.487 0.0262	0.75 0.185
4♂ 1000	25	394	1.96	25.7	0.61	3.29	56.5	1.648	1.633	0.77	0.526	0.469	0.66
	26	358	2.23	26.0	0.72	3.59	64.5	1.729	1.782	1.19	0.553	0.487	0.75
	27	452	2.16	30.6	0.81	4.04	70.9	1.785	1.782	0.63	0.497	0.503	0.73
	28	397	2.14	24.7	0.88	3.51	65.9	1.820	1.831	0.80	0.565	0.513	0.92
	29	490	2.19	32.1	1.19	4.59	63.1	1.821	1.764	1.04	0.537	0.508	1.14
	Mean SD	418 52.6	2.13 0.106	27.8 3.31	0.84 0.218	3.76 0.444	64.2 5.20	1.761 0.0732	1.758 0.0744	0.88 0.226	0.536 0.0262	0.496 0.0180	0.84 0.192

SD Standard deviation

**APPENDIX 5**  
(Organ weights - continued)

Week 5 (23 September 1994)

Group/ dosage mg/kg/day	Animal no.	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Control	33	254	1.96	11.6	0.58	2.16	83.5	136.8
	34	270	1.92	11.8	0.66	2.31	86.0	105.4
	35	274	1.99	12.3	0.51	2.51	64.8	89.9
	36	266	1.84	12.3	0.67	2.79	70.6	127.5
	37	265	1.95	11.4	0.63	2.11	67.2	113.2
	Mean	266	1.93	11.9	0.61	2.38	74.4	114.6
	SD	7.4	0.055	0.40	0.067	0.281	9.69	18.41
2♀ 15	41	260	2.02	11.3	0.72	2.04	68.0	85.5
	42	262	2.02	12.4	0.65	2.50	87.9	108.7
	43	273	2.06	12.0	0.65	1.98	72.2	108.7
	44	252	1.90	10.2	0.63	2.08	75.8	106.4
	45	268	1.78	12.0	0.72	2.47	70.8	136.8
	Mean	263	1.96	11.6	0.67	2.21	74.9	109.2
	SD	8.0	0.117	0.88	0.044	0.250	7.77	18.25
3♀ 150	49	270	1.90	10.5	0.64	2.30	77.6	111.3
	50	293	1.98	11.6	0.70	2.69	77.0	136.6
	51	261	1.80	13.4	0.62	2.26	80.6	101.1
	52	248	1.90	12.9	0.62	2.35	88.5	99.2
	53	282	2.01	13.2	0.71	2.53	81.4	119.7
	Mean	271	1.92	12.3	0.66	2.43	81.0	113.6
	SD	17.7	0.082	1.22	0.046	0.181	4.59	15.29
4♀ 1000	57	284	1.92	21.6	0.61	3.02	87.2	100.5
	58	243	1.99	16.5	0.81	2.53	76.0	68.4
	59	277	1.91	17.8	0.66	2.77	76.4	112.4
	60	273	1.99	16.9	0.66	2.34	67.7	95.5
	61	290	1.93	20.9	0.89	2.58	93.0	107.3
	Mean	274	1.95	18.7	0.73	2.65	80.1	96.8
	SD	18.4	0.037	2.37	0.117	0.262	10.01	17.14

SD Standard deviation



**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 1 ♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Pelvic dilation: (Minimal , Unilateral)

**Lymph Nodes - Cervical**

Lymphoid proliferation: (Moderate)

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 2♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Cortical scarring: (Minimal , Focal , Unilateral)

Basophilic cortical tubules: (Minimal , Focal , Unilateral)

Cortical cyst

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 3♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Kidneys**

Increased pelvic dilatation: (Right , Minimal)

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Kidneys**

Pelvic dilation: (Minimal , Unilateral)

The following tissues were considered normal:

Heart; Spleen; Liver; Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 0 mg/kg/day  
Rat No/Sex: 4♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Lymph Nodes - Cervical**

Lymphoid proliferation: (Moderate)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 5♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**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**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Sciatic Nerve**

Degenerate fibres: (Trace)

**Stomach**

Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Epididymides; Testes; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 6♂

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 7♂

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 8♂

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

(Pathology - continued)

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 9♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 10♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 11♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 15 mg/kg/day  
Rat No/Sex: 12♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 13♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 14♂

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 15♂

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 16♂

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 17♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Trace)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 18♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Trace)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 19♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Basophilic cortical tubules: (Minimal , Focal , Unilateral)

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Minimal)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 20♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

**Compound:** Isodecyl benzoate

**Dosage Level:** 150 mg/kg/day

**Rat No/Sex:** 21 ♂ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Minimal)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 22♂

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 23♂

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 24♂

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 25♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing commonly seen during Week 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Liver**

Median cleft, pale subcapsular area/s: (One) 2mm  
Enlarged: 25.677g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

The following tissues were considered normal:

Heart; Spleen; Liver : (W.N.L.); Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 26♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

**Liver**  
Enlarged: 25.988g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**  
Vascular congestion

**Liver**  
Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**  
Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Lymph Nodes - Cervical**  
Lymphoid proliferation: (Moderate)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 26♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 27♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

**Liver**  
Enlarged: 30.568g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**  
Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Liver**  
Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**  
Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Testes**  
Tubules lined only by sertoli cells: (Minimal , Focal , Unilateral)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 27♂ - continued

**MICROSCOPIC FINDINGS - continued**

**Sciatic Nerve**

Degenerate fibres: (Trace)

**Lymph Nodes - Cervical**

Lymphoid proliferation: (Moderate)

The following tissues were considered normal:

Heart; Spleen; Epididymides; Adrenals; Brain

Pathologist: G.Lawson

## APPENDIX 6

(Pathology - continued)

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 28♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Heart; Spleen; Epididymides; Testes; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 29♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Liver**

Enlarged: 32.108g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Sciatic Nerve**

Degenerate fibres: (Trace)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 29♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Epididymides; Testes; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 30♂

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 31♂

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 32♂

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 33 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Stomach Antrum Mucosa**

White nodule, near to limiting ridge

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Stomach**

Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 34♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 35♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Pyelonephritis: (Minimal , Focal , Bilateral)

**Ovaries**

Luteal cyst(s)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 36♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Intra-alveolar recent haemorrhage: (Minimal , Focal)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 0 mg/kg/day  
Rat No/Sex: 37♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Spleen**

Clear fluid-filled cyst/s: (A few) Capsular 2mm  
Capsule thickened: Patchy

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Spleen**

Capsular inflammation: (Minimal , Focal)  
Capsular thickening: (Minimal , Focal)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Heart; Liver; Kidneys; Ovaries; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 38♀

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 39♀

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

**Compound:** Isodecyl benzoate

**Dosage Level:** 0 mg/kg/day

**Rat No/Sex:** 40♀

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 41 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 42♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 15 mg/kg/day  
Rat No/Sex: 43♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 44 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 15 mg/kg/day  
Rat No/Sex: 45♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 46♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 47♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 48♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 49♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Stomach Antrum Mucosa**

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 50♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

(Pathology - continued)

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 51 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 52♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Thickened: (Minimal)

**Cervix**

Swollen

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Basophilic cortical tubules: (Minimal , Focal , Unilateral)  
Cortical cyst: (Unilateral)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 53♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
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:

**Kidneys**  
Medullary tubule mineralisation: (Minimal , Focal , Unilateral)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 54♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 55♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 56♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 57♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight to moderate increase in salivation following dosing commonly seen from Day 6 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Liver**  
Enlarged: 21.648g

**Spleen**  
Capsule thickened: Patchy

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**  
Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Spleen**  
Capsular inflammation: (Minimal , Focal)  
Capsular thickening: (Minimal , Focal)

**Liver**  
Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**  
Basophilic cortical tubules: (Moderate , Focal , Bilateral)  
Tubule mineralisation at the corticomedullary junction: (Minimal , Focal)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 57♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 58♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing commonly seen during Week 2 to 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
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:

**Kidneys**  
Basophilic cortical tubules: (Minimal , Focal , Unilateral)

**Lymph Nodes - Cervical**  
Lymphoid proliferation: (Moderate)

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

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 58♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 59♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight to moderate increase in salivation following dosing commonly seen during Week 2 to 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Basophilic cortical tubules: (Minimal , Focal , Bilateral)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Lungs; Heart; Spleen; Ovaries; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 60♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing commonly seen during Week 2 to 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 61 ♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

**Liver**  
Enlarged: 20.905g

**Ovaries**  
Clear fluid-filled cyst: (Left) 2mm

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Liver**  
Centrilobular hepatocyte enlargement: (Moderate)

**Ovaries**  
Follicular cyst: (Unilateral)

**Lymph Nodes - Cervical**  
Lymphoid proliferation: (Moderate)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 61 ♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Lungs; Heart; Spleen; Kidneys; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 62♀

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 63♀

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 64♀

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

## APPENDIX 7

## Special Diet Services Rat and Mouse Maintenance Diet

## Composition and quality assurance aspects of diet

SDS Rat and Mouse No. 1 SQC 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.
<b>Contaminants</b>			<b>Maximum concentration</b>	
Fluoride			20	mg/kg
Nitrate (as NaNO <sub>3</sub> )			30	mg/kg
Nitrite (as NaNO <sub>2</sub> )			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 7

(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 per g diet

**APPENDIX 8****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.

VCL 206/942848

**FUNCTIONAL OBSERVATIONAL BATTERY  
REPORT**

Elizabeth W. Hughes,  
Behavioural Scientist.

This battery comprised 3 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed in a test arena and the third set comprised handling/specific testing of the animal.

- |  |   |
|--|---|
| <p>1. <b>Home cage observations</b></p> <p>Posture in the cage<br/>           Presence of convulsions, tremors, twitches<br/>           Presence of spontaneous vocalisations<br/>           Palpebral closure</p>   | <p>2. <b>Observations in the arena</b></p> <p>Occurrence of convulsions, tremors, twitches<br/>           Activity counts<br/>           Level of arousal<br/>           Rearing count<br/>           Grooming<br/>           Piloerection<br/>           Assessment of gait<br/>           Record presence of faecal boluses, urine</p>                          |
| <p>3. <b>Observations in the hand</b></p> <p>Occurrence of convulsions, tremors, twitches<br/>           Ease of removing rat from cage<br/>           Ease of handling rat<br/>           Salivation/lacrimation<br/>           Palpebral closure<br/>           Exophthalmus<br/>           Piloerection<br/>           Vocalisation on handling</p> | <p>4. <b>Manipulations</b></p> <p>Approach response<br/>           Touch response<br/>           Startle response<br/>           Righting reflex<br/>           Tail pinch response<br/>           Pupil response<br/>           Grip strength, fore and hindlimb<br/>           Landing foot splay<br/>           Body temperature<br/>           Bodyweight</p> |

At any point during the observations comments could be made about behaviour as free text.

The observational battery has previously been validated using positive control compounds<sup>1</sup>.

<sup>1</sup> NEWTON, D.F. *et al* (1992) "A Neurotoxicity Screen in rats following treatment with Acrylamide, Carbaryl or p,p'-DDT" Toxicology Letters Supplement 1992, p 184

## RESULTS OF THE FUNCTIONAL OBSERVATIONAL BATTERY

The data from the observational battery has been handled in the following manner. The observational endpoints such as ease of handling, activity, etc., have been tabulated for frequency of occurrence for each group. The following data was subjected to statistical analysis: rearing and activity counts, grip strength, hind limb splay, bodyweight and temperature.

### Observational endpoints

Among females, there were clear effects of treatment at 1000 mg/kg/day by Week 4 including:

The incidence of facial staining was increased and in conjunction with this the majority of animals at this dosage appeared "badly groomed".

One animal (no. 57) showed body tremor while being held in the hand.

In the arena, palpebral closure was observed in 4/8 animals. These same animals showed body tremor including animal no. 57 which was noted above as showing tremor while being held. Of the 4 animals with tremor, a total of two animals were classed as having moderate tremor including no. 57 which showed moderate continuous tremor.

Among males, there were only slight differences in the observational endpoints during the course of the study. Although during Week 4 there were few remarkable changes observed, in view of the changes observed in the females, the pattern of responses for individual animals was examined with the following result:

There was a low incidence of males at 1000 mg/kg/day showing slight body tremor during the recording of temperature. Two of these animals also showed slight body tremors in the arena with the third animal showing moderate continuous body tremor in the arena. All three animals showed palpebral closure in the arena. There was also an observation that in the righting response, some males were leaving one of their hindfeet tucked under the body. This was observed among all groups but the highest incidence was recorded at 1000 mg/kg/day.

### Grip strength

There were no statistically significant differences in forelimb grip strength.

There was a slight but statistically significant increase in the hindlimb grip strength of males at 1000 mg/kg/day during Week 2. There were no significant differences at Week 4. Among females at 1000 mg/kg/day, there was a statistically significant increase in hindlimb grip strength during Week 4.

### **Hindlimb splay**

Hindlimb splay was statistically significantly increased. Among males at 1000 mg/kg/day, a significant increase was confined to Week 2. Among females at 1000 mg/kg/day, however, hindlimb splay was significantly increased during Weeks 2 and 4.

### **Rearing counts**

Among males, the frequency of rearing in the arena was considered comparable among the groups. Among females, it was noted that counts among females at 1000 mg/kg/day were slightly lower than controls during Week 4. Differences failed to attain statistical significance.

### **Activity counts**

Among males, activity counts were variable among the groups. There was no indication of an effect of treatment. Among females, it was noted that counts among females at 1000 mg/kg/day were slightly lower than controls during Week 4. Differences failed to attain statistical significance.

### **Temperature**

There were no statistically significant differences in temperature among the groups.

### **Bodyweight**

Bodyweights were generally comparable among the groups.

### **Summary of effects**

Exposure to isodecyl benzoate was characterized by behavioural changes at 1000 mg/kg: These changes were generally not observed at Week 2 but were observed at Week 4.

The incidence of facial staining was increased among females at Week 4.  
Badly groomed appearance was observed among females at Week 4.  
Among females there was an association of tremor and palpebral closure in the arena.  
Among males there was an association of tremor in the arena, palpebral closure in the arena and tremor during body temperature.  
Hindlimb grip strength was increased among males at Week 2 and among females at Week 4.  
Hindlimb splay was increased among males during Week 2 and among females during Week 2 and 4.

There was also a suggestion at Week 4 of decreased activity and rearing counts for females treated at 1000 mg/kg/day.

### Discussion

Exposure to Isodecyl benzoate resulted in behavioural changes at 1000 mg/kg/day. Females appeared to be more affected than males. The behavioural changes were generally only observed during Week 4 and included altered appearance (facial staining and badly groomed appearance), palpebral closure in the arena, the observation of tremor, increased hindlimb grip strength, and increased hindlimb splay.

The observations of increased hindlimb splay and the presence of tremor suggest an effect on the nervous system.

In respect of the finding of tremor, in general the degree of the tremor was considered slight and was generally only observed when animals were in the arena. Controls also showed this response, however, there was a suggestion of a clustering of effects in animals at 1000 mg/kg/day: tremor in association with palpebral closure, and tremor during temperature (for males only). It was also noted that one male and one female at 1000 mg/kg/day showed moderate, continuous tremor while in the arena. This was not observed among controls.

Some of the additional observations, changes in appearance and the suggestion of effects on activity/rearing, can be considered non-specific. However, in the presence of the changes in splay and tremor this again suggests some effect on the nervous system.

The relevance of the observation of increased hindlimb grip strength was unclear as the expectation would be for a decrease in grip strength.

At lower dosages, there was no clear evidence of an effect of treatment.

### Conclusion

Exposure of Isodecyl benzoate resulted in behavioural changes which were confined to 1000 mg/kg/day with females showing more changes compared with males. There was no evidence of an effect of treatment at lower dosages.

## GROUP SUMMARY OF OBSERVATIONS - MALES

Group	Pre-dose				Week 2				Week 4			
	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
<b>OBSERVATIONS</b>												
<b>HOME CAGE</b>												
posture, sitting	7	8	8	7	6	8	7	7	7	7	8	8
<b>REMOVAL FROM CAGE</b>												
removing, easy	8	8	8	8	7	8	8	8	8	8	8	8
handling, easy	6	7	7	7	8	8	8	8	7	8	7	7
salivation	1	0	0	0	1	1	0	2	1	1	0	1
vocalising	0	0	0	0	1	1	0	0	1	1	0	1
<b>IN THE ARENA</b>												
grooming	1	4	2	5	4	3	4	2	1	1	2	3
tremors												
slight	1	1	2	2	2	2	3	4	2	4	3	3
moderate	0	0	0	0	0	0	0	0	0	1	0	1
arousal, alert	6	7	6	7	6	7	6	5	2	3	5	6
piloerection	0	0	0	0	0	0	0	0	0	0	0	0
defecation	4	5	3	2	2	2	1	1	3	3	2	2
urine present	2	3	4	3	3	3	2	1	4	3	6	4
<b>GAIT</b>												
walking on toes	0	0	0	1	0	3	1	1	2	1	1	2
swaying	0	0	0	0	0	1	0	0	0	0	1	0
limbs splayed	0	0	0	0	0	0	0	0	0	0	0	0
hunched	0	0	0	0	0	0	1	0	0	0	0	1
unable to assess	1	1	0	0	2	1	2	3	5	4	0	0
<b>MANIPULATIONS</b>												
approach, a reaction	6	6	5	7	7	7	7	7	4	4	8	5
touch, a reaction	5	4	6	6	6	5	7	3	3	1	6	5
startle (present)	8	8	8	8	8	8	8	8	6	7	6	7
righting, immediate	5	7	7	8	7	6	6	8	5	7	8	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	7	8	8	7	7	7	7	7	7
<b>ADDITIONAL COMMENTS</b>												
facial staining	3	5	2	3	1	2	0	0	1	1	0	0
body groomed	0	0	0	0	0	0	0	0	0	0	0	0
palpebral closure in arena	1	0	0	0	0	0	1	2	3	3	2	3
slight body tremors												
during temperature	0	1	0	0	0	3	2	1	0	1	1	3
position of feet during												
righting	0	0	0	0	0	0	0	0	1	1	2	3

Numbers reflect the number of animals either showing the response or with the indicated score

## GROUP SUMMARY OF OBSERVATIONS - MALES

Group	Pre-dose				Week 2				Week 4			
	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
<b>OBSERVATIONS</b>												
<b>HOME CAGE</b>												
posture, sitting	7	8	8	7	6	8	7	7	7	7	8	8
<b>REMOVAL FROM CAGE</b>												
removing, easy	8	8	8	8	7	8	8	8	8	8	8	8
handling, easy	6	7	7	7	8	8	8	8	7	8	7	7
salivation	1	0	0	0	1	1	0	2	1	1	0	1
vocalising	0	0	0	0	1	1	0	0	1	1	0	1
<b>IN THE ARENA</b>												
grooming	1	4	2	5	4	3	4	2	1	1	2	3
tremors												
slight	1	1	2	2	2	2	3	4	2	4	3	3
moderate	0	0	0	0	0	0	0	0	0	1	0	0
arousal, alert	6	7	6	7	6	7	6	5	2	3	5	0
piloerection	0	0	0	0	0	0	0	0	0	0	0	0
defecation	4	5	3	2	2	2	1	1	3	3	2	2
urine present	2	3	4	3	3	3	2	1	4	3	6	4
<b>GAIT</b>												
walking on toes	0	0	0	1	0	3	1	1	2	1	1	2
swaying	0	0	0	0	0	1	0	0	0	0	1	0
limbs splayed	0	0	0	0	0	0	0	0	0	0	0	0
hunched	0	0	0	0	0	0	1	0	0	0	0	1
unable to assess	1	1	0	0	2	1	2	3	5	4	0	0
<b>MANIPULATIONS</b>												
approach, a reaction	6	6	5	7	7	7	7	7	4	4	8	5
touch, a reaction	5	4	6	6	6	5	7	3	3	1	6	5
startle (present)	8	8	8	8	8	8	8	8	6	7	6	7
righting, immediate	5	7	7	8	7	6	6	8	5	7	8	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	7	8	8	7	7	7	7	7	7
<b>ADDITIONAL COMMENTS</b>												
facial staining	3	5	2	3	1	2	0	0	1	1	0	0
body groomed	0	0	0	0	0	0	0	0	0	0	0	0
palpebral closure in arena	1	0	0	0	0	0	1	2	3	3	2	3
slight body tremors												
during temperature	0	1	0	0	0	2	2	1	0	1	1	3
position of feet during												
righting	0	0	0	0	0	0	0	0	1	1	2	3

Numbers reflect the number of animals either showing the response or with the indicated score

## GROUP SUMMARY OF OBSERVATIONS - FEMALES

Group	Pre-dose				Week 2				Week 4			
	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
<b>OBSERVATIONS</b>												
<b>HOME CAGE</b>												
posture, sitting	7	5	6	7	8	5	8	8	8	6	6	8
<b>REMOVAL FROM CAGE</b>												
removing, easy	7	8	8	8	7	7	7	8	8	7	7	8
handling, easy	6	4	6	5	6	5	7	7	5	6	5	6
salivation	0	2	0	0	0	0	0	0	0	2	0	0
vocalising	2	3	1	2	1	4	2	2	2	2	2	1
tremors in the hand	0	0	0	0	0	0	0	0	0	0	0	1
<b>IN THE ARENA</b>												
grooming	5	2	1	6	3	4	4	3	2	2	4	5
tremors												
slight	1	0	0	1	2	1	1	1	0	1	0	2
moderate	0	0	0	0	0	0	0	0	0	0	0	2
arousal, alert	6	8	8	8	6	8	7	6	6	7	5	6
piloerection	0	0	0	0	0	0	0	1	0	0	0	0
defecation	0	0	0	1	0	0	0	0	0	0	0	0
urine present	1	1	0	1	1	1	0	0	0	0	1	0
<b>GAIT</b>												
walking on toes	3	3	4	2	4	5	6	6	4	5	6	5
swaying	0	0	0	0	0	0	0	0	0	1	0	0
limbs splayed	0	0	0	0	0	0	1	0	0	0	0	1
hunched	1	1	0	0	1	0	0	3	0	0	0	0
unable to assess	1	0	0	0	0	0	0	0	0	0	0	1
<b>MANIPULATIONS</b>												
approach, a reaction	7	6	6	7	8	7	8	7	8	6	8	6
touch, a reaction	7	4	6	5	6	6	5	6	5	5	5	3
startle (present)	8	8	8	8	8	8	8	8	6	6	6	7
righting, immediate	5	7	7	8	5	8	7	6	6	6	7	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	8	7	8	8	8	8	7	7	8
<b>ADDITIONAL COMMENTS</b>												
facial staining	1	2	2	3	2	2	1	3	0	2	1	4
badly groomed	0	0	0	0	0	0	0	0	0	0	0	6
palpebral closure in arena	1	0	0	0	0	0	0	1	0	0	0	4
slight body tremors												
during temperature	1	0	0	0	2	1	0	1	0	1	0	1
position of feet during												
righting	0	0	0	0	0	0	0	0	0	0	0	0

Numbers reflect the number of animals either showing the response or with the indicated score

**GROUP MEAN FORELIMB GRIP STRENGTH**

Predose

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	0.64	0.64
2	0.58	0.68
3	0.62	0.64
4	0.69	0.69

Week 2

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	0.95	0.87
2	0.90	0.94
3	0.94	0.86
4	1.00	0.90

Week 4

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	1.05	1.00
2	1.11	1.00
3	1.15	0.97
4	1.16	1.02

No statistical significance ( $P > 0.05$ )

**GROUP MEAN HINDLIMB GRIP STRENGTH**

Predose

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.60	0.67
2	0.55	0.68
3	0.56	0.70
4	0.64	0.74

Week 2

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.74	0.71
2	0.73	0.80
3	0.79	0.81
4	0.87+	0.85

Week 4

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.94	0.84
2	0.84	0.89
3	0.98	0.88
4	1.06	1.03+

+ P &lt; 0.05

## GROUP MEAN SPLAY VALUES

Predose

Group	Mean splay values (cm)	
	Males	Females
1	9.4	7.8
2	10.2	9.0
3	8.3	8.8
4	9.3	9.0

Week 2

Group	Mean splay values (cm)	
	Males	Females
1	9.0	8.0
2	10.7	9.7
3	10.8	8.8
4	11.8+	10.6++

Week 4

Group	Mean splay values (cm)	
	Males	Females
1	9.4	8.4
2	10.3	9.3
3	11.0	9.1
4	10.4	11.1++

+ P &lt; 0.05

++ P &lt; 0.01

## GROUP MEAN REARING COUNTS

Predose

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

Week 2

Group	Mean rearing counts	
	Males	Females
1	8	13
2	10	17
3	11	16
4	8	12

Week 4

Group	Mean rearing counts	
	Males	Females
1	5	13
2	5	15
3	8	14
4	8	8

No statistical significance ( $P > 0.05$ )

### GROUP MEAN ACTIVITY COUNTS

Pre-dose

Group	Mean activity counts	
	Males	Females
1	11	13
2	14	15
3	13	16
4	10	13

Week 2

Group	Mean activity counts	
	Males	Females
1	7	13
2	11	17
3	11	16
4	7	13

Week 4

Group	Mean activity counts	
	Males	Females
1	6	14
2	5	17
3	10	16
4	6	8

No statistical significance ( $P > 0.05$ )

**GROUP MEAN TEMPERATURE**

## Predose

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.2
2	37.9	38.3
3	37.9	38.4
4	37.9	38.1

## Week 2

Group	Mean temperature (°C)	
	Males	Females
1	37.6	38.0
2	37.8	38.5
3	37.7	38.0
4	37.9	38.2

## Week 4

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.5
2	38.2	38.4
3	37.8	38.3
4	37.9	37.9

No statistical significance ( $P > 0.05$ )

### GROUP MEAN BODYWEIGHTS

#### Predose

Group	Mean bodyweights (g)	
	Males	Females
1	241	188
2	239	192
3	241	194
4	240	191

#### Week 2

Group	Mean bodyweights (g)	
	Males	Females
1	346	235
2	344	240
3	352	244
4	342	244

#### Week 4

Group	Mean bodyweights (g)	
	Males	Females
1	417	267
2	405	267
3	429	271
4	402	271

No statistical significance ( $P > 0.05$ )

## KEY TO FUNCTIONAL OBSERVATIONAL BATTERY

### Posture

- S      Sitting/standing in cage
- R      Rearing in cage
- Cl     Climbing up cage

### Ease of removal from cage

- 2      Easy (little resistance)
- 3      Slightly awkward

### Ease of handling

- 2      Easy (little resistance)
- 3      Slightly awkward

### Arousal

- 2,3,4,5    Increasing levels of arousal with 4-being alert

### Gait

- T      Walking on toes
- A      Swaying/lurching gait
- H      Hindlimbs splayed
- HU     Hunched

The numbers associated with gait indicate the degree of effect

- 1,2,3    Increasing degree of effect

- U      Unable to assess gait - insufficient movement to assess gait
- N      Normal gait

### Approach

- 1      No reaction
- 2      Sniffs
- 3      Approaches and sniffs
- 4      Freezes
- O      Other

### Touch

- 1      No reaction
- 2      Turns
- 3      Walks away
- 4      Freezes
- O      Other

**Startle**

- |   |                   |
|---|-------------------|
| 1 | No reaction       |
| 2 | Ear twitch        |
| 3 | Noticeable flinch |
| 4 | Noticeable jump   |

**Tail pinch**

- |   |  |
|---|--|
| 2 | Turns<br>2 turns immediately<br>3 violent turn |
| 3 | Walks away<br>1 and turns                      |
| 5 | Jumps forward                                  |
| 0 | Other  |

**Righting reflex**

- |   |                    |
|---|--------------------|
| 1 | Immediate reaction |
| 2 | Reaction slow      |

**Tremors**

- |   |                    |
|---|--------------------|
| N | No tremor observed |
| B | Body               |
| H | Head               |
| T | Tail               |

The numbers associated with tremors indicate the degree of effect  
1,2,3    Increasing degree of effect

**Salivation, vocalising, grooming, piloerection**

- |   |               |
|---|---------------|
| N | Not observed  |
| Y | Sign observed |

The associated numbers indicate the degree of response  
1,2,3    Increasing degree of effect

**Pupil reflex**

- |     |                                    |
|-----|------------------------------------|
| B   | Reflex observed both pupils        |
| L,R | Left or right pupil responded only |
| N   | No reflex both pupils              |

**Urine**

- |   |                   |
|---|-------------------|
| S | Small amount      |
| M | Moderate amount   |
| L | Large amount      |
| N | No urine observed |

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 1 males							
		1	2	3	4	5	6	7	8
IN THE CAGE									
Posture		S	S	R	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	2	2	2	3
Salivation		N	N	N	N	N	Y1	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		B1	N	N	N	N	N	N	N
Grooming		N	N	Y	N	N	N	N	N
Activity count		3	8	16	8	1	7	19	23
Arousal		3	4	4	4	3	4	4	4
Rearing count		3	7	19	8	1	6	19	21
Bolus count		2	0	0	0	2	5	0	1
Urine present		M	M	N	N	N	N	N	N
Gait		N	N	N	N	U	N	N	N
MANIPULATIONS									
Approach		0	1	1	2	3	3	3	3
Touch		1	1	4	4	4	2	1	2
Startle		4	3	3	4	3	3	3	2
Righting reflex		2	1	1	1	1	2	2	1
Tail pinch		3	3	3	3	2	2	3	3
turns						2	2		
vocalises		Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.4	37.7	37.7	38.0	37.4	38.0	37.9	37.9
Bodyweight (g)		236	237	235	250	240	232	241	256
GRIP STRENGTH (kg) #									
Forelimb		0.80	0.90	0.57	0.54	0.51	0.55	0.63	0.62
Hindlimb		0.67	0.61	0.63	0.48	0.55	0.57	0.57	0.77
FOOTSPRAY (cm) #									
		8.1	10.1	8.9	11.0	10.9	8.1	6.6	11.5

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 1 Approach response: head sway  
During manipulations: soft faeces
- 3 Slight brown nasal staining
- 5 In the arena: sat in corner
- 6 Slight brown nasal staining
- 8 In the arena: partially closed eyes on occasions  
Slight brown nasal staining

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 2 males							
		9	10	11	12	13	14	15	16
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	N	N	N	Y	N	Y	Y
Activity count		18	20	1	20	11	17	11	15
Arousal		4	4	3	4	4	4	4	4
Rearing count		23	13	0	16	13	11	15	10
Bolus count		2	3	4	3	0	0	0	1
Urine present		M	S	S	N	N	N	N	N
Gait		N	N	U	N	N	N	N	N
MANIPULATIONS									
Approach		3	3	1	3	3	2	3	1
Touch		4	3	4	2	1	1	1	1
Startle		4	3	3	3	3	2	3	3
Righting reflex		1	1	1	1	2	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises			Y2	Y2	Y2		Y2	Y1	
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.2	38.1	38.1	37.6	37.6	38.1	38.4	37.2
Bodyweight (g)		222	239	244	243	233	240	231	259
GRIP STRENGTH (kg)#									
Forelimb		0.50	0.60	0.52	0.67	0.47	0.61	0.57	0.75
Hindlimb		0.50	0.69	0.66	0.58	0.38	0.56	0.49	0.58
FOOTSPRAY (cm) #		12.3	7.7	10.3	8.6	10.8	10.8	10.7	10.7

# Values represent the mean of two trials

## Additional comments

## Animal no.

9 During manipulations: soft faeces  
 11 Slight brown nasal staining  
 In the arena: sat in corner  
 During temperature: slight body tremors  
 12 Slight brown nasal staining  
 13 In the arena: hind feet slipping  
 14 Slight brown nasal staining  
 15 Slight brown nasal staining  
 16 Slight brown nasal staining  
 Pupil reflex: slow response

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 3 males							
		17	18	19	20	21	22	23	24
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	3
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	B1	B1	N	N	N	N
Grooming		N	N	N	Y	N	Y	N	N
Activity count		16	17	10	18	5	15	18	6
Arousal		4	4	4	4	4	5	4	3
Rearing count		18	18	10	15	9	10	16	3
Bolus count		2	4	5	0	0	0	0	0
Urine present		M	N	M	N	N	S	N	M
Gait		N	N	N	N	N	N	N	N
MANIPULATIONS									
Approach		3	2	3	1	3	1	3	1
Touch		2	4	5	1	4	3	2	1
Startle		4	3	3	3	3	4	3	3
Righting reflex		1	1	1	2	1	1	1	1
Tail pinch		3	3	5	5	3	3	3	3
turns									
vocalises		Y1	Y1	Y1	Y2	Y1		Y1	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.5	37.6	38.1	38.1	37.6	37.7	38.0	37.5
Bodyweight (g)		240	254	236	229	244	234	234	255
GRIP STRENGTH (kg) #									
Forelimb		0.59	0.76	0.80	0.45	0.37	0.69	0.45	0.90
Hindlimb		0.45	0.66	0.61	0.57	0.41	0.74	0.48	0.53
FOOTSPLAY (cm) #		8.9	8.5	9.2	6.8	5.5	9.9	6.1	11.4

# Values represent the mean of two trials

## Additional comments

Animal no.

18 During manipulations: soft faeces

22 Slight brown nasal staining, brown staining on head

24 Slight brown nasal staining

In the arena: soft faeces

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 4 males							
		25	26	27	28	29	30	31	32
IN THE CAGE									
Posture		S	S	S	S	S	S	S	R
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	3
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	B1	N	B1	N	N
Grooming		N	N	Y	Y	Y	N	Y	Y
Activity count		19	11	11	5	9	6	12	10
Arousal		4	4	4	3	4	4	4	4
Rearing count		19	15	14	5	10	7	14	11
Bolus count		0	4	0	3	0	0	0	0
Urine present		N	S	N	N	N	S	S	S
Gait		N	N	T1	N	N	N	N	N
MANIPULATIONS									
Approach		1	2	3	3	2	3	3	3
Touch		4	1	4	4	2	4	1	4
Startle		2	4	3	2	4	3	3	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises			Y1	Y2		Y1	Y1	Y1	Y1
Pupil reflex		B	B	B	B	N	B	B	B
Temperature (°C)		38.4	38.1	37.8	38.1	37.7	37.5	37.7	38.2
Bodyweight (g)		244	231	243	239	247	237	239	241
GRIP STRENGTH (kg) #									
Forelimb		0.81	0.65	0.66	0.73	0.57	0.87	0.56	0.65
Hindlimb		0.69	0.80	0.64	0.73	0.49	0.73	0.54	0.54
FOOTSPRAY (cm) #									
		11.7	10.1	7.4	9.1	8.8	8.1	6.7	12.9

# Values represent the mean of two trials

## Additional comments

Animal no.

- 27 During manipulations: soft faeces  
 28 Slight brown nasal staining, hair loss on neck  
 29 Slight brown nasal staining  
 Pupil reflex: both pupils constricted  
 30 Moderate brown nasal staining  
 Pupil reflex: left pupil appears misshapened and misplaced (reflex observed)  
 31 Hairloss on chin  
 32 During manipulations: soft faeces

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 1 females							
		33	34	35	36	37	38	39	40
IN THE CAGE									
Posture		S	CL	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	3	2	2
Handling		2	3	2	2	3	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	Y1	Y1	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	B1	N
Grooming		Y	Y	Y	N	Y	Y	N	N
Activity count		13	18	15	23	10	17	3	2
Arousal		5	4	4	4	4	4	4	3
Rearing count		3	18	18	19	6	22	5	1
Bolus count		0	0	0	0	0	0	0	0
Urine present		S	N	N	N	N	N	N	N
Gait		N	T1HU1	N	T1	N	T1	N	U
MANIPULATIONS									
Approach		2	3	3	2	1	2	2	3
Touch		4	2	4	2	1	4	3	4
Startle		4	4	3	3	3	4	3	3
Righting reflex		2	1	2	2	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y1		Y2	Y1	Y2	Y2		Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.7	38.0	38.2	38.3	38.0	38.3	37.9	38.5
Bodyweight (g)		179	193	191	192	185	188	180	193
GRIP STRENGTH (kg)#									
Forelimb		0.55	0.74	0.56	0.59	0.65	0.53	0.66	0.82
Hindlimb		0.64	0.85	0.69	0.60	0.71	0.71	0.64	0.55
FOOTSPRAY (cm) #									
		6.2	9.3	9.2	7.2	6.9	7.4	6.9	9.7

# Values represent the mean of two trials

## Additional comments

## Animal no.

34 Slight hairloss head and neck  
 37 In the arena: partially closed eyes on occasions  
 38 Slight kink near tip of tail  
 During manipulations: vocal and awkward to handle  
 40 Slight red staining around right eye, slight nasal staining  
 In the arena: limited walking  
 During temperature: slight body tremors

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 2 females							
		41	42	43	44	45	46	47	48
IN THE CAGE									
Posture		S	S	S	S	S	R	CL	R
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		3	3	2	2	2	3	2	3
Salivation		N	Y1	N	N	N	N	N	N
Vocalising		N	N	Y1	N	N	Y1	Y1	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		N	N	Y	N	Y	N	N	N
Activity count		17	10	20	14	14	17	13	13
Arousal		4	4	4	4	4	4	4	4
Rearing count		16	12	16	11	12	18	16	12
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	S	N	N
Gait		N	N	T1	N	N	T1	N	T1HUI
MANIPULATIONS									
Approach		3	2	1	1	3	2	0	2
Touch		4	1	1	1	1	3	0	2
Startle		3	3	4	3	3	3	3	3
Righting reflex		1	1	1	1	1	1	2	1
Tail pinch		3	3	3	3	3	3	0	3
turns									
vocalises		Y1	Y1	Y2	Y1	Y2	Y1		Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.2	38.4	38.2	38.3	38.1	37.8	39.1	38.1
Bodyweight (g)		185	189	199	187	193	205	176	205
GRIP STRENGTH (kg)#									
Forelimb		0.62	0.72	0.76	0.68	0.56	0.75	0.69	0.68
Hindlimb		0.72	0.73	0.80	0.75	0.55	0.69	0.70	0.49
FOOTSPLAY (cm) #		7.7	10.5	10.1	9.3	7.6	7.3	8.2	11.4

# Values represent the mean of two trials

## Additional comments

## Animal no.

45 Slight brown nasal staining  
 47 Tail pinch: stepped backwards then walked away  
 48 Slight brown nasal staining, hair loss on neck

VCL/206 Predose

OBSERVATIONS	Animal no	Group 3 females							
		49	50	51	52	53	54	55	56
IN THE CAGE									
Posture		S	R	S	S	S	R	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	3	2	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	Y1	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		N	N	N	N	Y	N	N	N
Activity count		19	13	14	14	22	13	15	18
Arousal		4	4	4	4	4	4	4	4
Rearing count		16	14	15	15	16	15	14	10
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		N	T1	T1	T1	N	N	N	T1
MANIPULATIONS									
Approach		2	3	3	1	2	1	2	3
Touch		1	4	4	3	1	3	4	3
Startle		3	3	4	3	3	3	3	2
Righting reflex		1	2	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y1	Y1	Y2	Y2	Y1		Y2	Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.1	38.3	38.3	38.7	37.6	38.9	38.7	38.3
Bodyweight (g)		192	206	193	182	191	199	187	199
GRIP STRENGTH (kg) #									
Forelimb		0.73	0.53	0.64	0.57	0.43	0.74	0.75	0.71
Hindlimb		0.55	0.59	0.66	0.82	0.71	0.72	0.81	0.73
FOOTSPLAY (cm) #									
		11.5	8.5	9.8	6.4	7.3	7.4	11.5	7.9

# Values represent the mean of two trials

## Additional comments

Animal no.

51 Slight brown nasal staining, slight hair loss on neck

53 Slight brown nasal staining

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 4 females							
		57	58	59	60	61	62	63	64
IN THE CAGE									
Posture		S	S	S	S	S	R	S	R
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	2	3	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	Y1	Y2	N	N	N	N
IN THE ARENA									
Tremors		N	B1	N	N	N	N	N	N
Grooming		N	N	Y	Y	Y	Y	Y	Y
Activity count		18	9	13	22	15	11	4	14
Arousal		4	4	4	4	4	4	4	4
Rearing count		13	8	13	24	17	10	7	14
Bolus count		0	0	0	1	0	0	0	0
Urine present		N	N	N	S	N	N	N	N
Gait		N	T1	T1	N	N	N	N	N
MANIPULATIONS									
Approach		2	5	1	3	3	2	3	2
Touch		1	4	4	4	1	1	4	3
Startle		3	3	4	3	3	4	3	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises			Y2	Y1	Y2	Y1	Y2	Y2	
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.4	38.3	38.5	37.2	38.0	38.6	37.1	38.3
Bodyweight (g)		199	182	193	191	197	187	183	197
GRIP STRENGTH (kg) #									
Forelimb		0.69	0.70	0.72	0.73	0.67	0.72	0.74	0.61
Hindlimb		0.65	0.83	0.83	0.75	0.71	0.78	0.68	0.68
FOOTSPLAY (cm) #		11.1	9.2	11.3	7.3	8.9	7.6	6.8	9.8

# Values represent the mean of two trials

## Additional comments

## Animal no.

57 Slight brown nasal staining

58 Slight brown nasal staining

63 Slight brown staining on nose

During manipulations: awkward to handle

## VCL/206 Week 2

OBSERVATIONS	Animal no	Group 1 males							
		1	2	3	4	5	6	7	8
IN THE CAGE									
Posture		R	S	S	S	S	S	R	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	3
Handling		2	2	2	2	2	2	2	2
Salivation		N	N	N	N	N	N	N	Y1
Vocalising		N	N	N	N	Y2	N	N	N
IN THE ARENA									
Tremors		N	B1	N	B1	N	N	N	N
Grooming		Y	Y	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		6	9	12	5	3	9	2	9
Arousal		4	4	4	4	3	4	3	4
Rearing count		9	10	15	8	1	7	7	9
Bolus count		0	0	0	0	4	0	0	3
Urine present		N	M	N	N	S	N	N	S
Gait		N	N	N	N	U	N	U	N
MANIPULATIONS									
Approach		1	3	3	3	3	3	3	3
Touch		1	4	4	4	4	4	1	4
Startle		3	3	4	3	3	3	3	3
Righting reflex		2	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	2	3	3	3
turns						3			
vocalises		Y2	Y2	Y2	Y1	Y3	Y1	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.0	37.6	37.5	37.7	37.2	37.2	37.1	38.2
Bodyweight (g)		372	320	342	365	352	320	336	362
GRIP STRENGTH (kg) #									
Forelimb		1.07	1.25	0.56	1.00	0.84	0.84	1.12	0.92
Hindlimb		0.71	0.76	0.71	0.58	0.83	0.66	0.74	0.93
FOOTSPRAY (cm) #									
		8.6	10.1	7.3	10.7	8.3	9.8	7.3	10.4

# Values represent the mean of two trials

## Additional comments

Animal no.

2 During manipulations: slight salivation

5 In the arena: sat in corner

7 In the arena: sat in corner

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 2 males							
		9	10	11	12	13	14	15	16
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									S
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Salivation		N	Y1	N	N	N	N	N	N
Vocalising		N	N	Y1	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	H1B1T1	N	N	N	N	B1
Grooming		Y	Y	N	N	N	N	Y	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		21	19	1	13	4	16	5	6
Arousal		4	4	2	4	4	4	4	4
Rearing count		21	16	0	9	7	13	8	3
Bolus count		1	0	7	0	0	0	0	0
Urine present		M	S	M	N	N	N	N	N
Gait		T2	A1T1	U	T1	N	N	N	N
MANIPULATIONS									
Approach		5	3	1	3	3	3	3	3
Touch		4	4	4	4	1	3	1	1
Startle		3	4	3	3	2	3	2	3
Righting reflex		2	1	1	1	1	2	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y2	Y2	Y2	Y2	Y1	Y1	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		37.8	38.5	38.0	37.3	36.9	37.9	38.0	38.0
Bodyweight (g)		304	349	358	361	331	337	347	362
GRIP STRENGTH (kg)#									
Forelimb		0.50	1.08	0.84	0.94	0.72	0.97	1.11	1.02
Hindlimb		0.69	0.84	0.77	0.61	0.59	0.69	0.79	0.90
FOOTSPRAY (cm) #		10.5	8.4	10.8	9.9	11.3	13.8	12.7	8.6

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 9 In the arena: slight salivation  
 11 In the arena: sat in corner  
 12 During temperature: slight body tremors  
 13 During temperature: slight body tremors  
 14 Slight brown nasal staining  
 16 During temperature: slight body tremors

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 3 males							
		17	18	19	20	21	22	23	24
IN THE CAGE									
Posture		S	S	S	R	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	B1	N	B1H1	N	N	N	B1
Grooming		Y	Y	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		18	6	12	1	17	15	14	1
Arousal		4	4	4	2	4	4	4	2
Rearing count		19	5	15	2	15	14	18	1
Bolus count		0	0	0	0	0	0	0	1
Urine present		N	N	N	N	N	M	S	M
Gait		N	N	HU1	U	T2	N	N	U
MANIPULATIONS									
Approach		3	2	3	3	3	2	3	1
Touch		3	1	3	2	4	4	3	4
Startle		3	3	3	3	3	3	3	4
Righting reflex		1	1	1	2	2	1	1	1
Tail pinch		3	3	3	5	3	3	3	3
turns									
vocalises		Y2	Y1	Y2		Y1	Y2	Y1	Y1
Pupil reflex		B	N	B	B	B	B	B	B
Temperature (°C)		38.6	37.1	37.8	37.6	38.6	37.8	37.0	37.3
Bodyweight (g)		347	376	348	321	375	334	364	354
GRIP STRENGTH (kg) #									
Forelimb		1.10	1.09	1.08	0.60	0.59	0.92	0.90	1.29
Hindlimb		0.57	0.90	0.79	0.75	0.85	0.87	0.77	0.85
FOOTSPRAY (cm) #		11.6	10.3	10.8	7.1	10.1	13.5	9.2	13.6

#Values represent the mean of two trials

## Additional comments

Animal no.

- 17 In the arena: slight salivation upon removal from arena  
During manipulations: soft faeces
- 18 Pupil reflex: both pupils constricted
- 20 In the arena: sat in corner, continuous body and tremors  
During temperature: slight body tremors
- 22 Slight brown nasal staining  
During temperature: slight body tremors
- 24 Slight brown nasal staining  
In the arena: sat in corner, partial to half closed eyes on occasions

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 4 males						
		25	26	27	28	29	30	31
IN THE CAGE								
Posture	R	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Salivation	N	N	Y1	N	N	N	Y1	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	B1	B1	N	N	B1	B1
Grooming	N	Y	N	N	Y	N	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	13	16	6	1	8	7	3	1
Arousal	4	4	4	3	4	4	3	2
Rearing count	13	14	6	1	11	12	3	1
Bolus count	0	0	0	3	0	0	0	0
Urine present	N	N	N	N	N	N	S	N
Gait	T1	N	N	U	N	N	U	U
MANIPULATIONS								
Approach	3	2	3	3	3	2	3	3
Touch	4	4	1	0	1	1	1	1
Startle	3	3	3	3	3	3	3	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y1	Y1	Y2			Y2	Y2	Y2
Pupil reflex	B	B	B	B	B	B	B	N
Temperature (°C)	38.2	38.0	37.7	38.6	37.0	37.8	37.5	38.4
Bodyweight (g)	339	319	369	330	366	345	341	328
GRIP STRENGTH (kg) #								
Forelimb	1.06	0.98	0.98	1.15	0.67	1.14	0.92	1.10
Hindlimb	0.87	0.94	0.86	0.84	0.70	1.17	0.87	0.76
FOOTSPRAY (cm) #								
	11.7	13.4	10.3	8.7	14.0	13.7	8.2	14.3

# Values represent the mean of two trials

## Additional comments

Animal no.

- 28 In the arena: sat in corner  
Touch response: body tremors  
During temperature: slight body tremors
- 31 Slight hairloss on neck  
In the arena: sat in corner, partially closed eyes on occasions
- 32 In the arena: sat in corner, partial to half closed eyes on occasions  
Pupil reflex: both pupils constricted

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 1 females							
		33	34	35	36	37	38	39	40
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	3	2	2	2
Handling		2	2	3	2	2	2	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	Y1	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	B1	B1
Grooming		Y	N	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		10	21	18	14	1	17	10	12
Arousal		4	4	4	4	3	4	5	4
Rearing count		12	11	16	17	1	22	13	10
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	M	N	N	N
Gait		N	T1	T1	T1HU1	U	T1	N	N
MANIPULATIONS									
Approach		3	3	3	3	3	2	3	3
Touch		2	3	1	2	3	1	3	3
Startle		3	3	3	3	3	3	3	3
Righting reflex		2	1	1	2	1	1	2	1
Tail pinch		3	3	3	0	3	3	3	3
turns							1		
vocalises		Y1	Y1	Y2	Y2		Y2		Y2
Pupil reflex		B	B	B	B	R	B	B	B
Temperature (°C)		38.7	37.1	38.4	38.8	38.7	37.6	37.9	37.0
Bodyweight (g)		224	241	239	246	234	237	226	233
GRIP STRENGTH (kg)#									
Forelimb		0.78	0.91	1.02	0.83	0.85	0.76	0.81	1.02
Hindlimb		0.70	0.68	0.81	0.55	0.71	0.71	0.77	0.75
FOOTSPRAY (cm) #		6.1	7.8	9.7	8.0	9.9	8.2	5.6	8.8

# Values represent the mean of two trials

## Additional comments

## Animal no.

36 In the arena: abdomen appeared distended  
Tail pinch: paused then walked away

37 In the arena: sat in corner, appears to lean to right side when sitting  
During manipulations: soft faeces  
Pupil reflex: left pupil no response

38 Slight brown nasal staining  
During manipulations: awkward to handle and vocalising

39 In the arena: occasional slipping of forelimbs  
During temperature: slight body tremors

40 Moderate brown nasal staining, slight brown staining on head and lower lip  
In the arena: abdomen appeared slight distended  
During temperature: slight body tremors

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 2 females						
		41	42	43	44	45	46	47
IN THE CAGE								
Posture	R	CL	S	CL	S	S	S	S
IN THE HAND								
Removing	2	3	2	2	2	2	2	2
Handling	2	3	3	3	2	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	Y1	Y1	Y1	N	Y1	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	B1
Grooming	Y	Y	Y	N	Y	N	N	Y
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	27	16	17	12	20	12	19	9
Arousal	4	4	4	4	4	4	4	4
Rearing count	25	14	18	16	16	21	19	10
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	S	N	N	N	N	N
Gait	T2	N	T2	N	N	T2	T1	T1
MANIPULATIONS								
Approach	1	3	3	2	3	3	3	3
Touch	1	2	1	4	2	2	2	4
Startle	4	3	4	3	3	3	3	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	2	3
turns							2	
vocalises	Y1	Y1	Y2	Y2	Y2	Y2	Y2	Y3
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (°C)	39.0	39.4	38.5	38.3	38.4	37.8	39.0	37.8
Bodyweight (g)	229	237	254	230	247	252	213	254
GRIP STRENGTH (kg) #								
Forelimb	0.85	1.19	1.12	1.02	0.66	0.95	0.67	1.05
Hindlimb	1.02	0.85	1.09	0.93	0.53	0.83	0.48	0.72
FOOTSPRAY (cm) #								
	9.5	11.7	10.8	9.6	7.7	8.9	7.6	11.9

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 41 Slight brown staining on head
- 43 In the arena: slight salivation upon removal from arena
- 46 Slight brown staining on head
- In the arena: abdomen appeared slightly distended
- 48 During manipulations: awkward to handle and vocalising
- During temperature: slight body tremors

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 3 females							
		49	50	51	52	53	54	55	56
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	3	2	2	2
Handling		2	2	2	2	3	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	Y1	N	N	Y1	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	B1	N
Grooming		Y	Y	N	N	N	Y	N	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		12	14	13	19	27	14	13	15
Arousal		4	4	4	4	5	4	4	4
Rearing count		12	11	20	16	23	11	17	19
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		T1	T1	N	T2	T2	T2H1	N	T1
MANIPULATIONS									
Approach		3	3	3	3	0	3	3	3
Touch		1	3	4	2	1	1	4	2
Startle		3	3	4	3	4	3	3	3
Righting reflex		1	2	1	1	1	1	1	1
Tail pinch		3	3	3	5	5	3	3	3
turns				1					
vocalises		Y2	Y2	Y2	Y2	Y1	Y2	Y2	Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		37.8	37.3	39.2	38.2	39.2	37.9	37.1	37.6
Bodyweight (g)		239	264	235	225	248	260	228	252
GRIP STRENGTH (kg) #									
Forelimb		0.84	1.05	0.85	0.86	0.66	0.90	0.87	0.89
Hindlimb		0.60	1.01	0.66	1.08	0.70	0.95	0.77	0.74
FOOTSPLAY (cm) #		9.0	11.4	10.2	5.2	7.2	8.1	9.4	9.7

# Values represent the mean of two trials

## Additional comments

Animal no.

- 50 In the arena: slight salivation upon removal from arena
- 52 Slight hairloss on neck
- 53 Slight hairloss on neck  
Approach response: head sway
- 54 In the arena: abdomen appeared slightly distended
- 56 Slight brown nasal staining

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 4 females							
		57	58	59	60	61	62	63	64
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	3	2	2	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	Y2	Y1	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	N	N	N	Y	N	Y	N
Pilo-erection		N	N	Y	N	N	N	N	N
Activity count		17	5	21	18	19	10	8	6
Arousal		4	3	4	4	4	4	4	3
Rearing count		7	5	22	16	19	10	13	4
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		T1HU1	N	T3HU1	T2	T1HU2	T1	T1	N
MANIPULATIONS									
Approach		1	3	3	3	3	3	3	3
Touch		4	1	2	3	3	4	1	4
Startle		3	3	3	3	3	2	3	3
Righting reflex		1	1	2	1	1	1	2	1
Tail pinch		3	3	0	3	3	3	3	2
turns									2
vocalises		Y1	Y2	Y1	Y2	Y2	Y2	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		37.5	38.2	39.1	38.0	39.2	38.6	37.6	37.7
Bodyweight (g)		258	227	241	249	254	249	228	244
GRIP STRENGTH (kg) #									
Forelimb		0.85	0.84	0.91	1.04	0.93	0.83	0.94	0.88
Hindlimb		0.77	0.94	0.94	0.71	0.88	0.76	0.94	0.88
FOOTSPRAY (cm) #		10.3	9.8	10.4	10.8	10.0	10.4	12.8	10.5

# Values represent the mean of two trials

## Additional comments

## Animal no.

57 Slight hairloss on neck  
 59 Slight brown staining head and neck  
 In the arena: abdomen appeared distended  
 Tail pinch: paused then walked away  
 61 Slight brown nasal staining  
 62 Slight brown nasal staining  
 In the arena: abdomen appeared slightly distended  
 63 During temperature: slight body tremors  
 64 In the arena: abdomen appeared slightly distended,  
 parial to half closed eyes occasionally, continuous  
 body tremors

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 1 males							
		1	2	3	4	5	6	7	8
IN THE CAGE									
Posture		S	S	S	S	S	S	R	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	3	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	Y1	N	N
Vocalising		N	N	N	N	Y2	N	N	N
IN THE ARENA									
Tremors		N	B1	N	B1	N	N	N	N
Grooming		N	N	N	N	N	N	N	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		1	2	13	1	1	6	1	25
Arousal		2	3	4	2	2	3	3	4
Rearing count		0	4	13	0	0	5	3	11
Bolus count		4	2	0	0	4	0	0	0
Urine present		N	S	S	N	S	S	N	N
Gait		U	U	T1	U	U	N	U	T2
MANIPULATIONS									
Approach		3	0	2	5	1	1	1	1
Touch		1	0	1	0	1	1	1	4
Startle		4	3	3	2	3	3	3	3
Righting reflex		2	1	1	1	2	1	2	1
Tail pinch		3	3	3	3	2	3	3	3
turns						2		1	
vocalises			Y2	Y1	Y2	Y2	Y1	Y2	Y2
Pupil reflex		B	B	B	L	B	B	B	B
Temperature (°C)		38.3	38.0	37.6	38.1	36.9	37.9	37.7	37.8
Bodyweight (g)		491	378	389	460	433	371	383	427
GRIP STRENGTH (kg) #									
Forelimb		1.14	1.18	0.84	1.21	0.78	1.05	1.09	1.13
Hindlimb		0.87	0.92	0.64	1.07	1.06	0.96	0.91	1.14
FOOTSPRAY (cm) #		8.7	10.3	9.5	10.9	8.0	9.2	6.4	12.4

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 1 In the arena: sat along edge of arena, ventral surface touching floor
- 2 In the arena: body held close to floor, sitting/rearing head first into corner  
Touch response: body tremors  
Approach response: body tremors  
Righting: left hindlimb tucked under body
- 3 During manipulations: slight nasal exudate observed
- 4 In the arena: sat in corner, partial to half closed eyes  
Touch response: body tremors  
Pupil reflex: too awkward to handle to assess right pupil
- 5 In the arena: sat in corner, partially closed eyes on occasions
- 6 Slight brown nasal staining
- 7 In the arena: sat in corner, partially closed eyes on occasions

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 2 males							
		9	10	11	12	13	14	15	16
IN THE CAGE									
Posture		S	S	S	S	R	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	Y1	N	N	N	N
Vocalising		N	N	Y2	N	N	N	N	N
IN THE ARENA									
Tremors		N	B1	B1	N	N	B1	B1	B2
Grooming		N	N	N	N	N	N	Y	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		13	4	1	16	3	1	1	1
Arousal		4	3	2	4	4	3	3	2
Rearing count		7	3	0	15	10	1	2	0
Bolus count		3	4	6	0	0	0	0	0
Urine present		M	S	N	N	N	S	N	N
Gait		N	N	U	T2	N	U	U	U
MANIPULATIONS									
Approach		1	1	1	3	3	3	3	1
Touch		1	1	1	1	1	4	1	1
Startle		2	3	3	3	3	3	3	3
Righting reflex		1	1	1	1	1	1	1	2
Tail pinch		3	3	2	3	3	3	3	3
turns				2					
vocalises		Y2	Y2	Y2	Y2		Y2	Y1	Y2
Pupil reflex		B	B	B	B	B	R	B	B
Temperature (°C)		38.4	38.4	38.8	37.9	37.8	37.7	38.1	38.1
Bodyweight (g)		357	415	438	439	379	383	403	423
GRIP STRENGTH (kg) #									
Forelimb		0.99	1.29	0.96	1.37	1.11	1.13	1.19	0.89
Hindlimb		0.99	0.98	0.82	0.80	0.69	0.87	0.76	0.85
FOOTSPLAY (cm) #		11.9	11.2	8.9	8.5	11.2	11.0	10.0	9.6

# Values represent the mean of two trials

## Additional comments

Animal no.

- 11 Slight brown nasal staining  
In the arena: sat in corner
- 12 Slight hairloss on neck  
In the arena: occasional clicking of teeth
- 14 In the arena: sat in corner, partial to half closed eyes on occasions  
During righting reflex: urination observed, right hindlimb tucked under body  
Pupil reflex: left pupil dilated
- 15 During temperature: slight body tremors  
In the arena: sat in corner, partially closed eyes on occasions  
Pupil reflex: left pupil, slow response
- 16 During manipulations: slight salivation  
In the arena: sat in corner, partial to half closed eyes on occasions  
Pupil reflex: slow response

VCL/206 Week 4

OBSERVATIONS	Group 3 males								
	Animal no	17	18	19	20	21	22	23	24
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	B1	B1	B1	N	N	N	N
Grooming		Y	N	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		18	2	5	5	17	18	15	1
Arousal		4	3	3	4	4	4	4	2
Rearing count		15	1	6	6	10	11	15	0
Bolus count		0	0	4	0	0	0	0	4
Urine present		S	N	S	S	N	M	S	S
Gait		N	U	N	N	T2A1	N	N	U
MANIPULATIONS									
Approach		3	2	3	3	3	3	3	5
Touch		3	1	3	3	3	1	2	3
Startle		2	3	3	3	3	3	2	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	2	3	5	2	3	3	3
turns			2			2			
vocalises		Y2	Y2	Y2	Y2	Y3	Y2	Y1	Y2
Pupil reflex		R	B	R	R	B	B	B	B
Temperature (°C)		38.5	38.4	37.8	37.6	37.9	37.0	38.1	37.1
Bodyweight (g)		415	467	418	395	476	400	448	411
GRIP STRENGTH (kg) #									
Forelimb		1.24	1.41	1.40	0.98	0.91	0.78	1.13	1.39
Hindlimb		0.71	1.04	1.12	1.04	1.02	1.06	0.96	0.91
FOOTSPRAY (cm) #		12.5	12.6	9.7	8.5	13.6	11.2	9.1	11.3

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 17 Pupil reflex: left pupil no response
- 18 Slight hairloss on neck  
In the arena: sat in corner  
During temperature: slight body tremors
- 19 In the arena: partial to half closed eyes  
Tail pinch: slight salivation  
Pupil reflex: left pupil dilated
- 20 In the arena: hindfeet appeared to slip on one occasion  
During manipulations: soft faeces  
Righting: right hindlimb tucked under body  
Pupil reflex: left pupil dilated
- 21 In the arena: tail appeared slightly elevated  
Righting: right hindlimb tucked under body
- 22 During manipulations: salivation observed at end
- 24 In the arena: lying in the arena for full 3 minutes,  
partial to half closed eyes  
Pupil reflex: slow response

VCL/206 Week 4

OBSERVATIONS	Group 4 males								
	Animal no	25	26	27	28	29	30	31	32
IN THE CAGE									
Posture	S	S	S	S	S	S	S	S	S
IN THE HAND									
Removing	2	2	2	2	2	2	2	2	2
Handling	2	2	2	3	2	2	2	2	2
Tremors	N	N	N	N	N	N	N	N	N
Salivation	N	N	Y1	N	N	N	N	N	N
Vocalising	N	N	N	Y2	N	N	N	N	N
IN THE ARENA									
Tremors	N	N	B1	B2	N	N	N	B1	B1
Grooming	N	N	N	N	Y	N	Y	Y	Y
Pilo-erection	N	N	N	N	N	N	N	N	N
Activity count	12	7	9	1	10	4	2	6	6
Arousal	4	4	4	2	4	3	4	4	4
Rearing count	16	5	13	1	6	5	4	12	12
Bolus count	0	1	0	4	0	0	0	0	0
Urine present	S	S	N	N	S	N	S	N	N
Gait	T1	N	T1HU1	U	N	N	N	N	N
MANIPULATIONS									
Approach	3	2	1	2	3	1	2	1	1
Touch	0	4	1	0	2	1	4	1	1
Startle	3	3	3	3	3	2	3	3	3
Righting reflex	1	1	1	1	1	2	1	1	1
Tail pinch	3	3	3	3	2	3	3	3	3
turns					2				
vocalises	Y1	Y2	Y2	Y2		Y3	Y1		
Pupil reflex	B	B	B	B	B	R	B	B	B
Temperature (°C)	39.1	38.6	38.3	38.0	36.8	37.7	36.8	37.7	37.7
Bodyweight (g)	388	354	443	391	482	392	392	374	374
GRIP STRENGTH (kg) #									
Forelimb	1.33	1.11	1.17	1.18	0.68	1.29	1.14	1.37	1.37
Hindlimb	1.20	1.23	1.10	1.22	0.92	1.10	0.85	0.85	0.85
FOOTSPRAY (cm) #									
	11.5	12.0	11.3	9.9	8.1	9.6	8.7	12.2	12.2

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 25 Touch response: walked backwards  
Righting: right hindlimb tucked under body
- 28 Slight hairloss on neck  
In the arena: sat in corner, continuous tremors, partial to half closed eyes on occasions  
Touch response: body tremors  
Approach response: body tremors  
During righting reflex: urination observed, left hindlimb tucked under body  
During temperature: slight body tremors
- 29 Moderate hairloss head and neck  
In the arena: occasional clicking of teeth
- 30 Pupil reflex: left pupil no response
- 31 In the arena: partial to half closed eyes  
Righting: left hindlimb tucked under body  
Pupil reflex: slow response  
During temperature: slight body tremors
- 32 In the arena: partial to half closed eyes on occasions  
Tail pinch: slight salivation  
During temperature: slight body tremors

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 1 females							
		33	34	35	36	37	38	39	40
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	3	2	3	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	Y1	Y1	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		Y	N	Y	N	N	N	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		7	20	19	12	1	25	19	10
Arousal		5	4	4	4	3	4	4	4
Rearing count		5	15	21	13	1	24	19	8
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		N	T1	T2	N	U	N	T1	T1
MANIPULATIONS									
Approach		2	3	0	3	3	3	3	3
Touch		4	3	1	3	1	0	0	1
Startle		4	3	3	2	3	3	3	3
Righting reflex		2	1	1	1	1	1	1	2
Tail pinch		3	3	3	3	5	2	3	3
turns							2		
vocalises		Y1		Y2	Y1	Y1	Y2	Y1	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.5	39.1	38.5	38.9	37.2	39.4	38.2	38.2
Bodyweight (g)		258	273	273	275	271	261	252	272
GRIP STRENGTH (kg) #									
Forelimb		0.94	1.14	1.10	0.89	1.08	0.90	0.77	1.17
Hindlimb		0.73	0.89	1.05	0.68	0.89	0.68	0.80	0.98
FOOTSPLAY (cm) #		7.6	11.1	8.3	8.3	7.7	7.6	7.1	9.5

# Values represent the mean of two trials

## Additional comments

Animal no.

- 34 Tail pinch: slow reaction  
 35 Slight hairloss on neck  
 Approach response: body tremors  
 37 In the arena: sat in corner  
 38 Touch response: walked backwards  
 39 Slight hairloss on neck  
 Touch response: walked backwards

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 2 females							
		41	42	43	44	45	46	47	48
IN THE CAGE									
Posture		S	S	S	CL	S	S	R	S
IN THE HAND									
Removing		3	2	2	2	2	2	2	2
Handling		3	2	2	3	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		Y1	N	Y1	N	N	N	N	N
Vocalising		Y1	N	Y1	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	N	N	N	Y	N	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		23	1	24	19	24	10	25	7
Arousal		4	2	4	4	4	4	4	4
Rearing count		19	0	27	19	18	12	17	4
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		T2A1	U	T2	T1	N	T1	T2	N
MANIPULATIONS									
Approach		3	1	3	1	3	3	5	2
Touch		4	1	4	1	2	3	4	1
Startle		4	3	3	3	3	3	2	3
Righting reflex		1	1	1	1	1	2	2	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y2	Y1	Y2	Y2	Y2	Y2	Y2	Y3
Pupil reflex		B	B	B	B	B	B	B	R
Temperature (°C)		38.5	39.0	38.6	39.0	37.7	38.2	39.2	37.2
Bodyweight (g)		266	260	280	255	267	282	232	294
GRIP STRENGTH (kg) #									
Forelimb		0.64	1.15	1.15	1.06	0.86	1.15	0.92	1.07
Hindlimb		0.98	1.06	1.04	1.03	0.80	0.70	0.66	0.89
FOOTSPRAY (cm) #		9.1	10.3	9.7	10.2	8.5	8.0	8.4	10.7

# Values represent the mean of two trials

## Additional comments

Animal no.

- 41 In the arena: occasional walking on toes observed (marked)
- 42 In the arena: sat in corner  
Tail pinch: slow reaction
- 44 During manipulations: awkward to handle and vocalising
- 46 Slight brown nasal staining
- 47 Slight brown nasal staining
- 48 Pupil reflex: left pupil dilated  
During temperature: slight body tremors

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 3 females							
		49	50	51	52	53	54	55	56
IN THE CAGE									
Posture		S	R	S	CL	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	3	2	2	2
Handling		2	2	3	3	3	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	Y2	Y1	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		Y	Y	Y	N	N	N	N	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		11	26	6	28	11	19	8	18
Arousal		4	4	3	4	3	4	3	4
Rearing count		10	20	8	26	4	20	6	16
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	S	N	N	N	N
Gait		T1	T1	N	T2	T1	T1	T1	N
MANIPULATIONS									
Approach		3	3	3	3	3	3	3	3
Touch		1	3	3	2	1	1	3	2
Startle		3	3	2	2	3	3	3	3
Righting reflex		1	2	1	1	1	1	1	1
Tail pinch		3	3	2	3	2	3	3	3
turns		1		2		3			
vocalises		Y2	Y2		Y1	Y2	Y1	Y2	Y1
Pupil reflex		L	B	B	B	B	B	B	B
Temperature (°C)		39.2	38.8	38.0	39.2	38.0	38.0	37.9	37.6
Bodyweight (g)		270	291	263	243	277	290	251	284
GRIP STRENGTH (kg)#									
Forelimb		0.90	1.12	1.09	0.92	0.84	0.81	0.98	1.12
Hindlimb		0.75	0.90	1.03	0.73	1.05	0.98	0.90	0.74
FOOTSPRAY (cm) #		9.5	10.8	10.5	4.6	10.9	9.4	9.5	8.0

# Values represent the mean of two trials

## Additional comments

Animal no.

49 Pupil reflex: right pupil constricted

52 During manipulations: awkward to handle

53 Slight hairloss on neck, brown nasal staining

During manipulations: awkward to handle and vocalising

56 Tail pinch: slow reaction

VCL/206 Week 4

OBSERVATIONS	Group 4 females								
	Animal no	57	58	59	60	61	62	63	64
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	3	2	3	2	2	2
Tremors		B1	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	Y1	N	N	N	N	N
IN THE ARENA									
Tremors		B2	B2	N	N	N	N	B1	B1
Grooming		Y	N	Y	Y	Y	N	N	Y
Pilo-erection		N	N	Y	N	N	N	N	N
Activity count		7	1	18	14	5	6	5	5
Arousal		4	2	4	4	5	4	4	4
Rearing count		4	1	13	17	14	5	2	5
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		N	U	T3H1	T2	T1	N	T1	T1
MANIPULATIONS									
Approach		1	2	3	3	3	2	3	1
Touch		1	1	2	4	2	1	1	1
Startle		4	3	3	3	3	3	3	-
Righting reflex		1	1	1	2	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y2	Y2	Y2	Y2	Y1	Y1	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.2	37.9	37.7	37.1	38.7	39.0	37.4	37.4
Bodyweight (g)		288	247	271	269	287	276	258	270
GRIP STRENGTH (kg) #									
Forelimb		1.03	0.90	0.95	1.16	0.98	0.97	1.20	1.00
Hindlimb		1.07	0.94	1.12	1.05	1.15	0.82	0.98	1.11
FOOTSPLAY (cm) #		11.0	10.9	13.7	11.4	8.3	9.1	12.8	11.6

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 57 Badly groomed appearance  
In the arena: partial to half closed eyes, continuous tremor
- 58 Badly groomed appearance, slight brown nasal staining, area anterior to urogenital region appears slightly swollen  
In the arena: sat head into corner, partial to half closed eyes  
Pupil reflex: response slow
- 59 Badly groomed appearance, slight brown nasal staining, slight brown staining neck and muzzle
- 60 Badly groomed appearance.  
In the arena: half closed eyes on occasions
- 61 Slight brown staining neck
- 62 Badly groomed appearance
- 63 During temperature: slightly body tremors
- 64 Badly groomed appearance, slight brown staining head and neck  
In the arena: half closed eyes on occasions  
Tail pinch: slow reaction

**ISODECYL BENZOATE  
FORMULATION ANALYSIS**

**Authors:**

I. Suzanne Dawe,  
Lianne Martin.

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## INTRODUCTION

This report contains details of the analytical method used and the results obtained for:

The determination of concentrations of Isodecyl benzoate in dose formulations prepared for Day 1 of the study.

The determination of the stability (homogeneity and resuspendability) of Isodecyl benzoate in corn oil formulations.

The validation of the method of analysis for the determination of Isodecyl benzoate in corn oil formulations.

The formulations for this study were prepared as solutions of Isodecyl benzoate in corn oil by Pharmacy personnel at Huntingdon Research Centre Ltd.

**EXPERIMENTAL PROCEDURE****ANALYTICAL PROCEDURE****Apparatus and instrumentation**

High performance liquid chromatograph (HPLC):	As detailed in the chromatographic section.
Balances:	Mettler AT261, fitted with a LC-P45 printer. Sartorius 1712 MP8, fitted with a data print 7381.
Autodiluter:	Hamilton Microlab 1000.
Densitometer:	Stanton Redcroft, PAAR DMA 46.
General laboratory glassware.	

**Reagents**

Test material:	Isodecyl benzoate.
Supplier:	Velsicol Chemical Corporation.
Batch no.:	C5-8.
Stated purity:	95 - 99%.
Tetrahydrofuran:	Rathburn Chemicals Limited, HPLC grade.
Methanol:	Fisons plc, HPLC Solvent.
Sodium dihydrogen orthophosphate:	FSA Laboratory Supplies, AnalaR®.
Water:	Elgastat UHP-4, deionised reverse osmosis.

**Sample extraction**

A representative sample (approximately 1 ml) of test formulation was accurately weighed and dissolved in a suitable volume of tetrahydrofuran. The extract was appropriately diluted, initially using tetrahydrofuran and finally using mobile phase, to provide a solution containing Isodecyl benzoate in the expected concentration range 2 - 4 µg/ml.

The final solution was filtered (Whatman® PURADISC™ 25PP, 0.45  $\mu\text{m}$ ) and the concentration of Isodecyl benzoate was quantified by high performance liquid chromatography using ultraviolet detection as detailed in the following section.

### Typical chromatographic conditions

#### High performance liquid chromatograph (HPLC):

Pump:	Waters Associates 510.
Autosampler:	Waters Associates 712.
Detector:	Spectra Physics Spectra 100.
Data Handling:	Perkin Elmer Nelson Access*Chrom.
Analytical column:	LiChrospher 100 RP18e, 5 $\mu\text{m}$ , 125 $\times$ 4 mm id, Merck Limited.
Guard column:	LiChrospher 100 RP18e, 5 $\mu\text{m}$ , 4 $\times$ 4 mm id, Merck Limited.
Column temperature:	Ambient (nominally 21°C $\pm$ 1°C).
Mobile phase:	Methanol/0.05M aqueous sodium dihydrogen orthophosphate (90/10 v/v).
Flow rate:	1.2 ml/minute.
Detector wavelength:	UV, 236 nm.
Injection volume:	40 $\mu\text{l}$ .
Sensitivity:	5 - 19 mV.
Retention volume:	7 ml.

### Calibration

A primary standard solution was prepared for each analytical occasion by dissolving an accurately weighed quantity (50 mg) of Isodecyl benzoate in methanol. Solutions for instrument calibration, containing Isodecyl benzoate in the concentration range 1 - 5  $\mu\text{g/ml}$ , were prepared by appropriate dilution of the primary standard using mobile phase.

Calibration solutions were injected onto the HPLC, at the beginning and end of each sample analysis sequence, using the conditions detailed in the previous section.

### Calculation

The peak response for Isodecyl benzoate in each calibration chromatogram was measured and calibration curves were constructed by linear regression of standard response versus standard concentration. The response of the peak observed at the characteristic retention volume for Isodecyl benzoate in sample and procedural recovery chromatograms was measured and the concentration of Isodecyl benzoate was determined using the following equation:

$$\text{Concentration, mg/ml} = \frac{Y - I}{S} \times \frac{V}{W} \times D \times 10^{-3} \times \frac{100}{R}$$

- Where
- Y = Peak response in test chromatogram
  - I = Intercept derived from linear regression of calibration data
  - S = Slope derived from linear regression of calibration data
  - V = Dilution volume of sample (ml)
  - W = Weight of sample (g)
  - D = Density (g/ml)
  - R = Cumulative mean procedural recovery value at analysis

### Limit of detection

The limit of detection, defined as the concentration of Isodecyl benzoate in control matrix producing a peak response equivalent to  $3 \times$  baseline noise, was determined as 0.075 mg/ml.

### VALIDATION OF THE METHOD OF ANALYSIS

Procedural recoveries were prepared by fortifying control samples (1 ml) with known amounts of Isodecyl benzoate, either as a solution in tetrahydrofuran (inclusion levels  $< 20$  mg/ml) or as solid test material (inclusion levels  $\geq 20$  mg/ml). The prepared procedural recoveries were analysed in accordance with the analytical procedure.

The analytical procedure was validated at the low and high inclusion levels by determining six procedural recoveries at concentrations of 1 mg/ml and 200 mg/ml.

Procedural recoveries were determined for each inclusion level and analysed concurrently with test formulations.

**DETERMINATION OF CONCENTRATIONS OF ISODECYL BENZOATE IN DOSE FORMULATIONS PREPARED FOR DAY 1 OF THE STUDY**

Representative samples (approximately 20 ml) of freshly prepared dose formulations were thoroughly mixed by vigorous shaking and duplicate sub-samples (1 ml) were analysed in accordance with the analytical procedure.

**DETERMINATION OF THE STABILITY OF ISODECYL BENZOATE IN CORN OIL FORMULATIONS**

Freshly prepared specimen formulations (approximately 100 ml), containing Isodecyl benzoate at nominal concentrations of 1 mg/ml and 200 mg/ml, were each thoroughly mixed by inversion and magnetically stirred. After magnetic stirring for 5 minutes (0 hour) and 4 hours, samples (approximately 1 ml) were removed for analysis from points at approximately one-quarter, one-half and three-quarters the depth (representing the top, middle and bottom) of the formulation.

The magnetic stirring was discontinued and the remainder of each formulation was stored in the dark at ambient temperature during the day and refrigerated overnight. At a time point representing 24 hours after preparation, each formulation was re-mixed and sampled for analysis as above.

At each occasion, the three samples from each formulation were analysed in accordance with the analytical procedure.

## RESULTS

The mean concentrations of Isodecyl benzoate in dose formulations prepared for Day 1 of the study and the deviation of mean results from nominal values are summarised in Table 1. Mean results were within 5% of nominal concentrations. Individual analytical results and associated procedural recovery data are detailed in Table 2.

The results in Table 3 indicate that, at nominal concentrations of 1 mg/ml and 200 mg/ml, Isodecyl benzoate is stable in corn oil formulations during storage (ambient temperature during the day and refrigeration overnight) for 4 and 24 hours.

Procedural recovery data obtained during method validation and the determination of stability are presented in Table 4. The data demonstrate the accuracy and precision of the analytical method: a mean procedural recovery value of  $90.1\% \pm 6.05\text{ CV}$  ( $n=7$ ) was obtained for 1 mg/ml and  $93.1\% \pm 5.41\text{ CV}$  ( $n=7$ ) for 200 mg/ml. Results for the analysis of test samples were corrected for the appropriate mean procedural recovery value at analysis.

A typical calibration standard graph confirming the linearity of detector response for Isodecyl benzoate over the concentration range 1 - 5  $\mu\text{g/ml}$  is presented in Figure 1. Typical analytical chromatograms are presented in Figures 2 and 3. In Figure 2, the absence of a peak at the characteristic retention volume for Isodecyl benzoate in the control sample chromatogram demonstrates the specificity of the HPLC assay.

## CONCLUSION

The analytical results confirm that the doses were accurately formulated for Day 1 of the toxicity study. The results also confirm that specimen formulations were homogeneous and stable for a period representing the time from preparation to completion of dosing.

TABLE 1

Summary: mean concentrations of Isodecyl benzoate in dose formulations

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
1	Control	0	ND	-
	2	3	2.90	-3.3
	3	30	30.4	+1.3
	4	200	210	+5.0

ND None detected ( <0.075 mg/ml)

RME Relative mean error, representing the deviation from nominal

**TABLE 2**  
**Concentrations of Isodecyl benzoate in dose formulations**  
**(individual values)**

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean <sup>1</sup>
1	Control	0	ND	ND	ND		
	2	3	3.09	2.72	2.90	100.6	
	3	30	31.6	29.1	30.4	99.2	92.9
	4	200	214	207	210	98.3	

ND None detected (<0.075 mg/ml)

<sup>1</sup> Represents the cumulative mean procedural recovery value and includes procedural recovery data from Table 4

Results were calculated using unrounded figures and corrected for the appropriate mean procedural recovery value given in this Table

TABLE 3

## Stability of Isodecyl benzoate in corn oil formulations

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)				CV (%)	RME (%)
		Top	Middle	Bottom	Mean		
1	0	0.961	1.01	0.946	0.971	3.24	-
	4	1.06	1.05	0.980	1.03	4.28	+6.1
	24	1.02	0.928	0.983	0.978	4.91	+0.7
200	0	179	204	199	194	6.82	-
	4	209	174	186	190	9.18	-2.1
	24	204	207	201	204	1.33	+5.2

CV Coefficient of variation

RME Relative mean error, representing the deviation from time zero

Results were calculated using unrounded figures and corrected for the appropriate mean procedural recovery value in Table 4

TABLE 4

**Procedural recovery data for Isodecyl benzoate  
in corn oil formulations**

Analytical phase	Nominal fortification (mg/ml)	
	1	200
Validation	97.3	94.4
	82.3	88.8
	94.4	86.7
	85.4	95.7
	91.8	99.5
	86.4	88.5
Stability	93.0	97.8
Mean	90.1	93.1
CV (%)	6.05	5.41
Range	82.3 - 97.3	86.7 - 99.5
n	7	7

CV Coefficient of variation

n Number of determinations

Results are expressed as percent recovery and calculated using the following equation:

$$\% \text{ Recovery} = \frac{\text{Analysed concentration (mg/ml)}}{\text{Fortified concentration (mg/ml)}} \times 100$$

FIGURE 1

Typical calibration standard graph  
(Day 1)

Standard Sample	Component Area	Component Mass	% Rel. St. Dev.
STD1	88612	41.408	
STD2	172571	82.816	
STD3	264217	124.22	
STD4	348253	165.63	
STD5	409297	207.04	

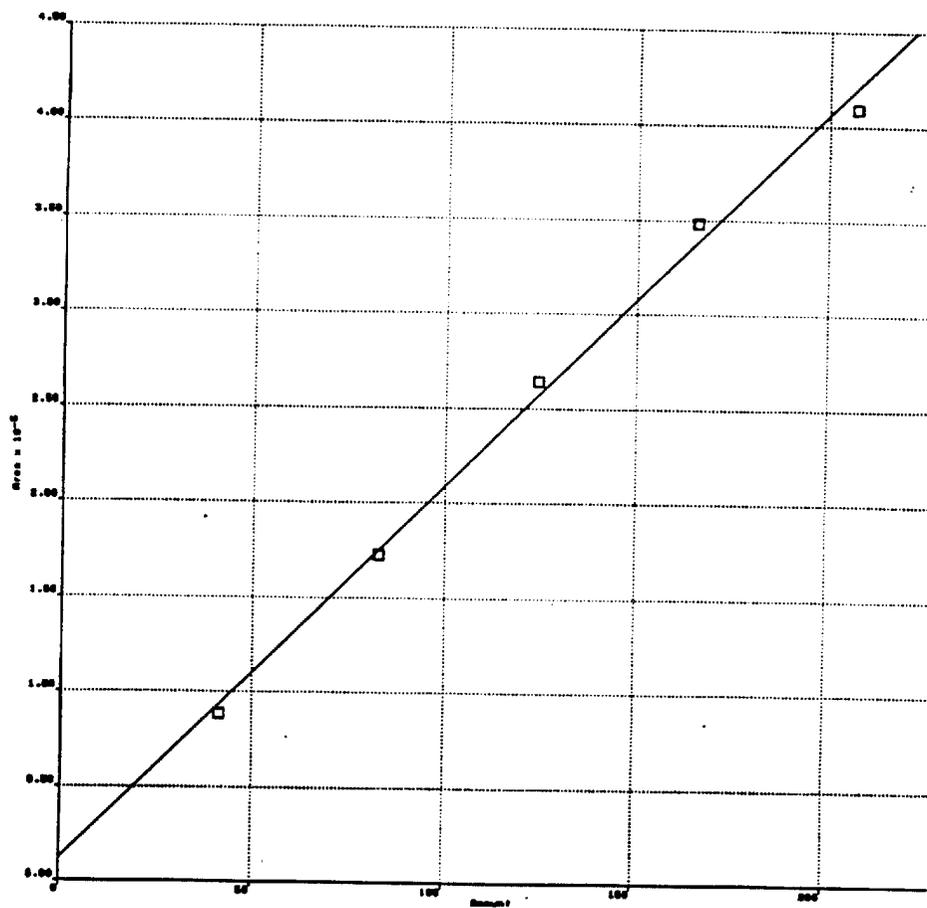
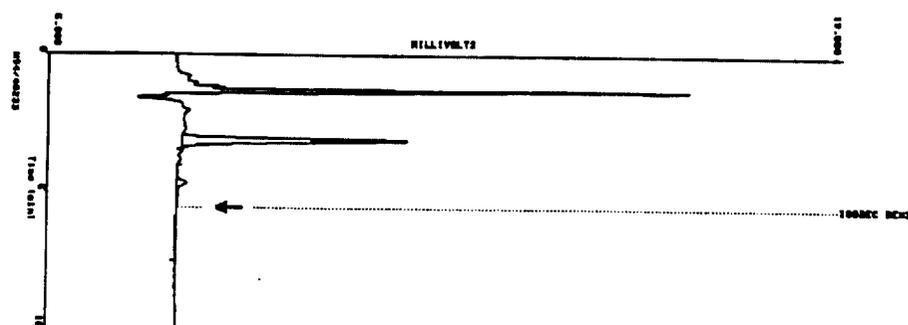


FIGURE 2

Typical sample chromatograms  
(Day 1)

Group 1, Control (1 g/500 ml)



Group 2, 3 mg/ml (1 g/1000 ml)

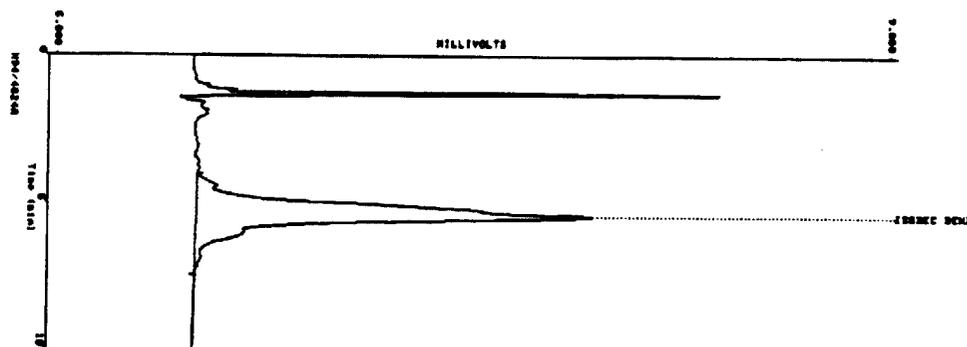
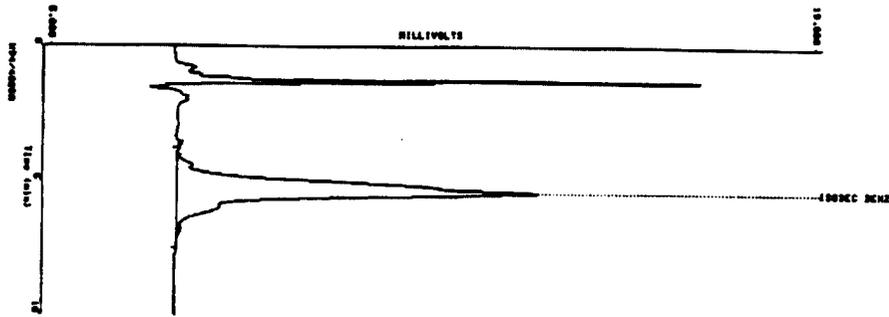


FIGURE 2

(continued)

Group 3, 30 mg/ml (1 g/10000 ml)



Group 4, 200 mg/ml (1 g/50000 ml)

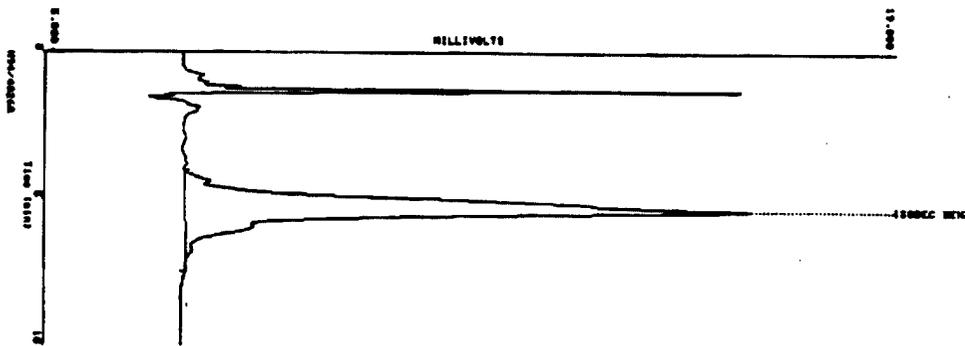
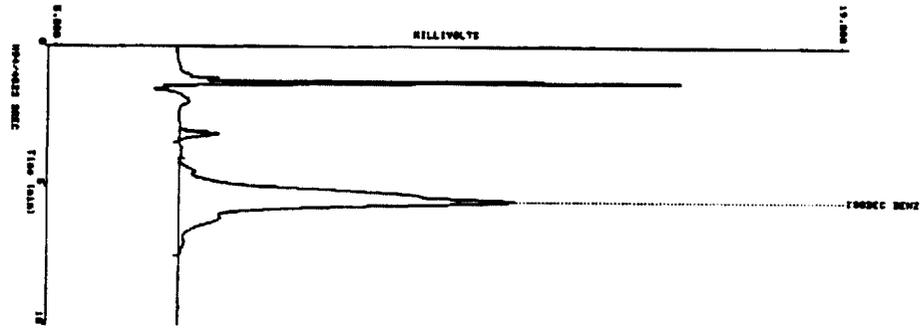


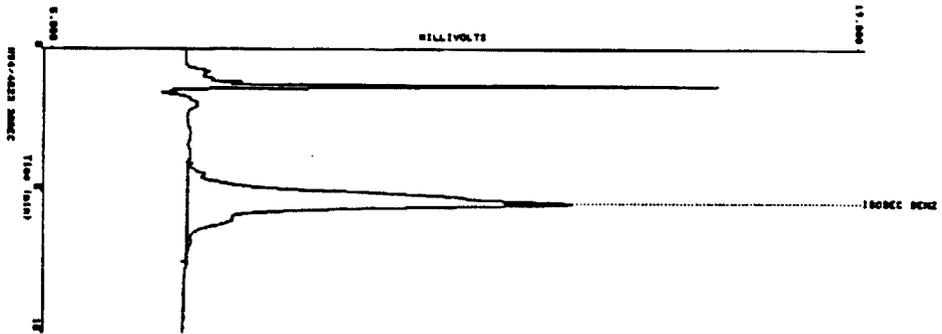
FIGURE 3

Typical procedural recovery chromatograms  
(Day 1)

3 mg/ml, 100.6%



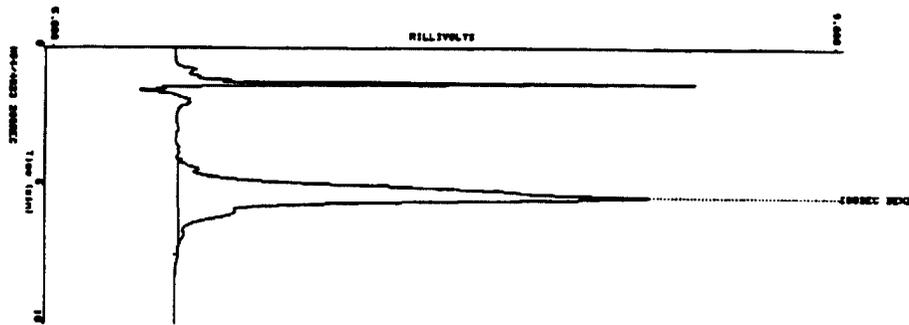
30 mg/ml, 99.2%



**FIGURE 3**

(continued)

200 mg/ml, 98.3%



# **HRC** Report

**ISODECYL BENZOATE**

**TWENTY-EIGHT DAY ORAL TOXICITY  
STUDY IN THE RAT WITH FUNCTIONAL  
OBSERVATIONAL BATTERY**

**Huntingdon  
Research  
Centre**

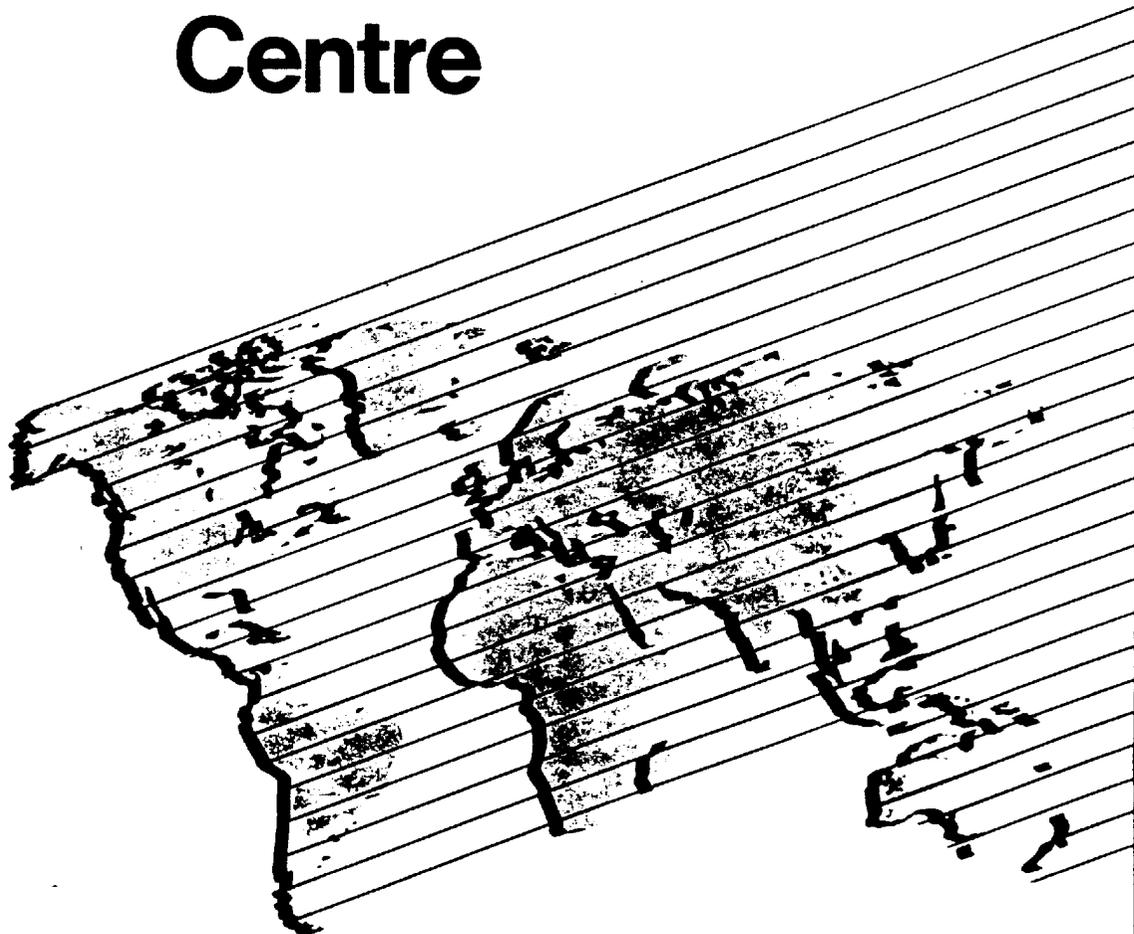
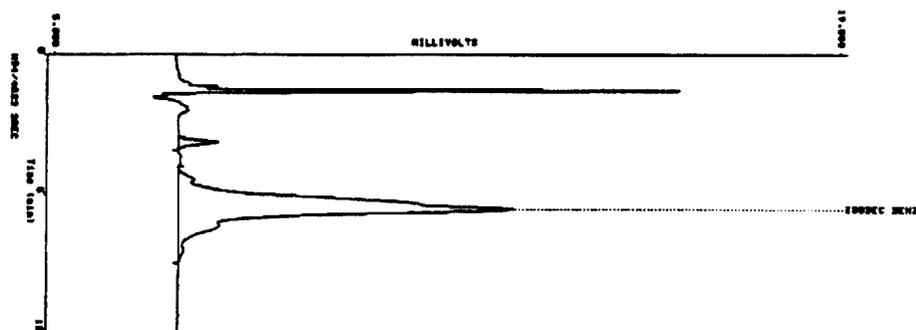


FIGURE 3

Typical procedural recovery chromatograms  
(Day 1)

3 mg/ml, 100.6%



30 mg/ml, 99.2%

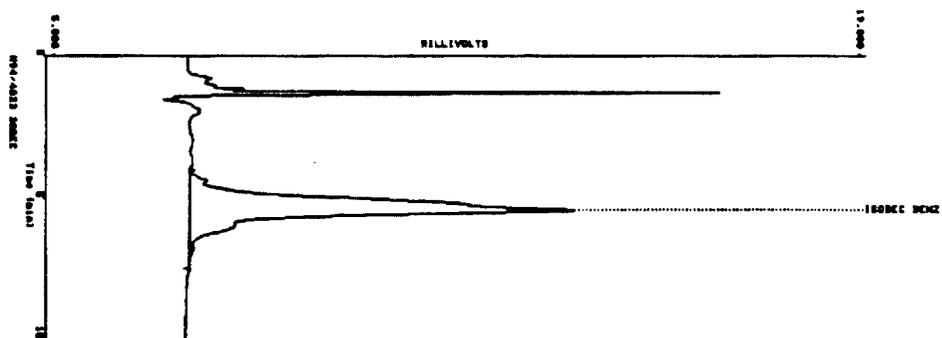
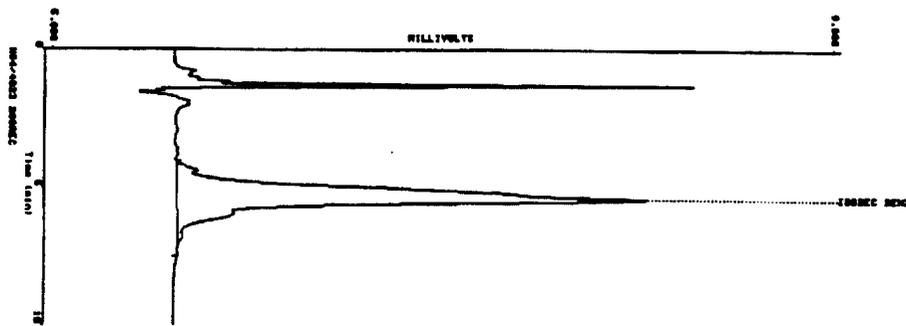


FIGURE 3

(continued)

200 mg/ml, 98.3%

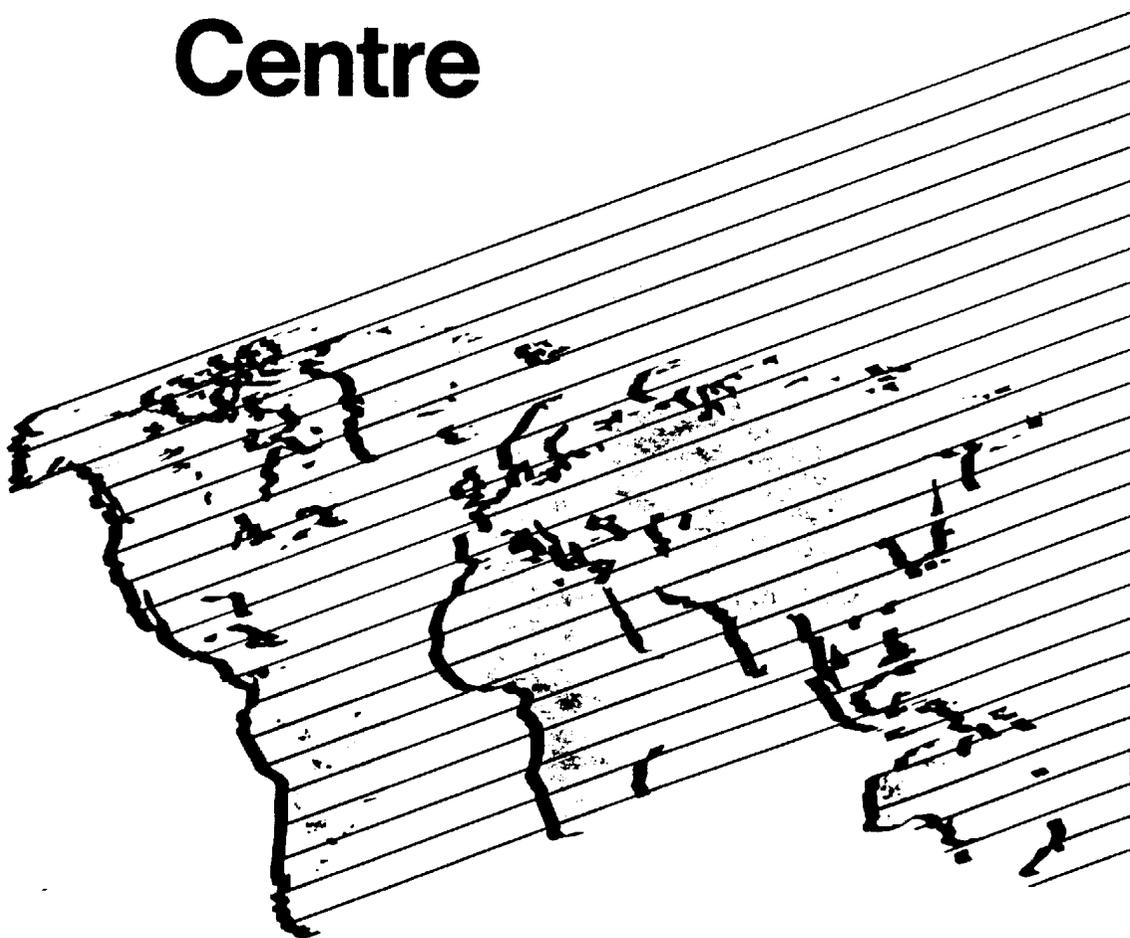


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STUDY IN THE RAT WITH FUNCTIONAL  
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Centre**



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**Huntingdon Research Centre Ltd**

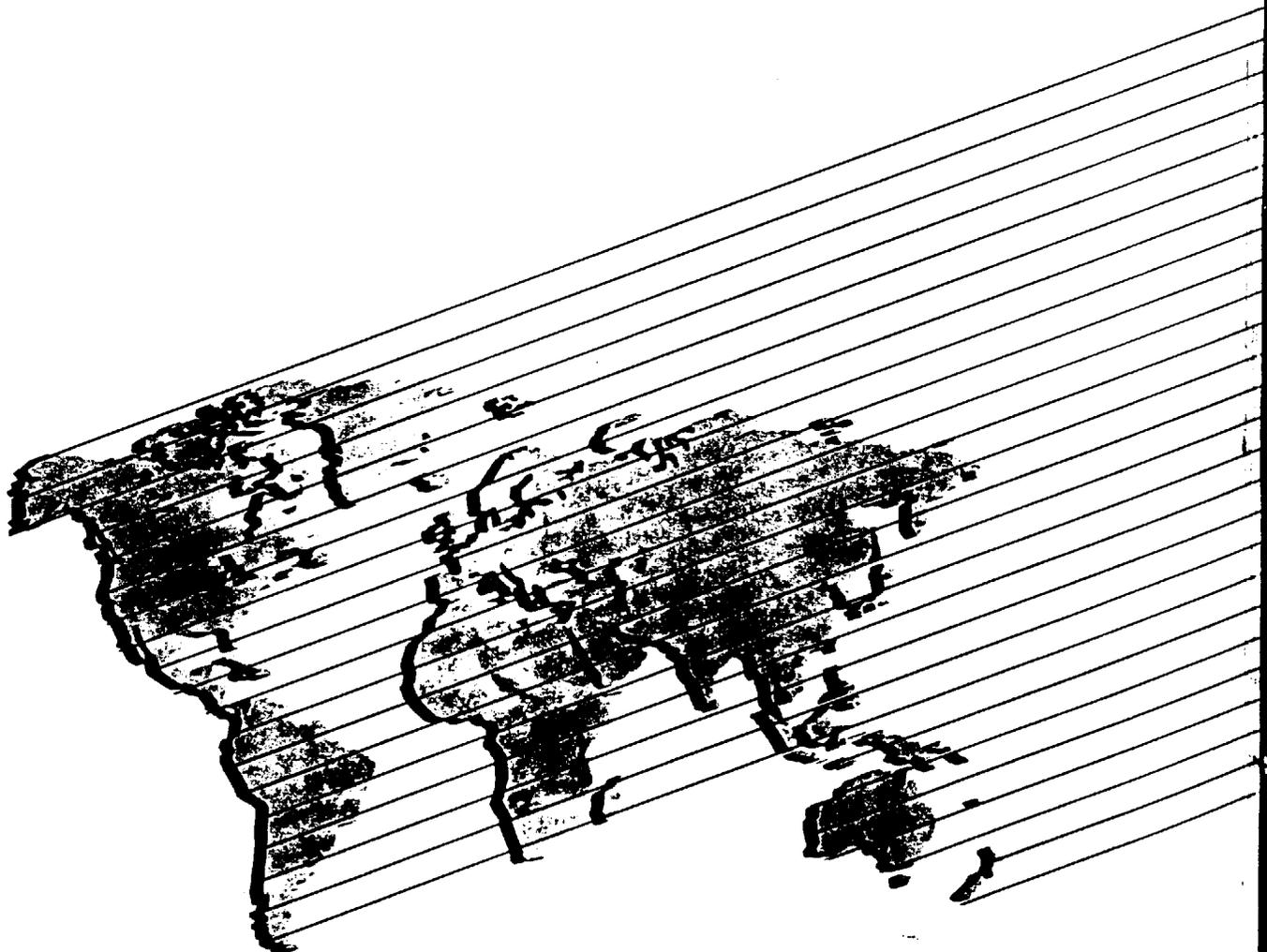
Huntingdon  
Cambridgeshire PE18 6ES  
England

***Telephone***

(01480) 890431

***Fax***

(01480) 890693



**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 26♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

**Liver**  
Enlarged: 25.988g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**  
Vascular congestion

**Liver**  
Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**  
Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Lymph Nodes - Cervical**  
Lymphoid proliferation: (Moderate)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 26♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Epididymides; Testes; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 27♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

**Liver**  
Enlarged: 30.568g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**  
Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Liver**  
Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**  
Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Testes**  
Tubules lined only by sertoli cells: (Minimal , Focal , Unilateral)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 27♂ - continued

**MICROSCOPIC FINDINGS - continued**

**Sciatic Nerve**

Degenerate fibres: (Trace)

**Lymph Nodes - Cervical**

Lymphoid proliferation: (Moderate)

The following tissues were considered normal:

Heart; Spleen; Epididymides; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 28♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Heart; Spleen; Epididymides; Testes; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 29♂ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Liver**

Enlarged: 32.108g

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Eosinophilic intracytoplasmic droplets in proximal convoluted tubules:  
(Moderate)

**Sciatic Nerve**

Degenerate fibres: (Trace)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 29♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Epididymides; Testes; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 30♂

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 31♂

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 32♂

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen during the latter half of the study and frequently associated with slightly wet fur during Week 4, and a slight lack of grooming seen on Day 30. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 0 mg/kg/day  
Rat No/Sex: 33 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Stomach Antrum Mucosa**  
White nodule, near to limiting ridge

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

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

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 34♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 0 mg/kg/day  
Rat No/Sex: 35♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Pyelonephritis: (Minimal , Focal , Bilateral)

**Ovaries**

Luteal cyst(s)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 36♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Intra-alveolar recent haemorrhage: (Minimal , Focal)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 37♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Spleen**

Clear fluid-filled cyst/s: (A few) Capsular 2mm  
Capsule thickened: Patchy

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion  
Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Spleen**

Capsular inflammation: (Minimal , Focal)  
Capsular thickening: (Minimal , Focal)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Heart; Liver; Kidneys; Ovaries; Adrenals; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 38♀

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 39♀

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 0 mg/kg/day

Rat No/Sex: 40♀

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 41 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 42♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 15 mg/kg/day  
Rat No/Sex: 43♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 44 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 15 mg/kg/day  
Rat No/Sex: 45 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 46♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 47♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 15 mg/kg/day

Rat No/Sex: 48♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 49♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Stomach Antrum Mucosa**

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 50♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 51 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver; Kidneys

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 52♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Thickened: (Minimal)

**Cervix**

Swollen

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Kidneys**

Basophilic cortical tubules: (Minimal , Focal , Unilateral)  
Cortical cyst: (Unilateral)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 53♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
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:

**Kidneys**  
Medullary tubule mineralisation: (Minimal , Focal , Unilateral)

The following tissues were considered normal:

Liver

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 54♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 150 mg/kg/day  
Rat No/Sex: 55♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 150 mg/kg/day

Rat No/Sex: 56♀

**CLINICAL FINDINGS**

No signs of ill health, behavioural change or reaction to treatment were noted.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 57♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight to moderate increase in salivation following dosing commonly seen from Day 6 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS****Liver**

Enlarged: 21.648g

**Spleen**

Capsule thickened: Patchy

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Lungs**

Vascular congestion

Intra-alveolar recent haemorrhage: (Minimal , Focal)

**Spleen**

Capsular inflammation: (Minimal , Focal)

Capsular thickening: (Minimal , Focal)

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Basophilic cortical tubules: (Moderate , Focal , Bilateral)

Tubule mineralisation at the corticomedullary junction: (Minimal , Focal)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 57♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 58♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing commonly seen during Week 2 to 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS****Lymph Nodes - Cervical**  
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:

**Kidneys**

Basophilic cortical tubules: (Minimal , Focal , Unilateral)

**Lymph Nodes - Cervical**

Lymphoid proliferation: (Moderate)

**Stomach**

Focus of ectopic non-glandular epithelium within the glandular mucosa

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 58♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

**Compound:** Isodecyl benzoate

**Dosage Level:** 1000 mg/kg/day

**Rat No/Sex:** 59♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight to moderate increase in salivation following dosing commonly seen during Week 2 to 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

**Kidneys**

Basophilic cortical tubules: (Minimal , Focal , Bilateral)

**Sciatic Nerve**

Degenerate fibres: (Trace)

The following tissues were considered normal:

Lungs; Heart; Spleen; Ovaries; Adrenals; Brain

**Pathologist:** G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 60♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing commonly seen during Week 2 to 4 and frequently associated with slightly wet fur, and a slight lack of grooming seen from Day 22. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

No abnormalities detected

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Lungs; Heart; Spleen; Liver; Kidneys; Ovaries; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6****(Pathology - continued)**

Compound: Isodecyl benzoate  
Dosage Level: 1000 mg/kg/day  
Rat No/Sex: 61 ♀ (Terminal)

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**  
Enlarged

**Liver**  
Enlarged: 20.905g

**Ovaries**  
Clear fluid-filled cyst: (Left) 2mm

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Liver**  
Centrilobular hepatocyte enlargement: (Moderate)

**Ovaries**  
Follicular cyst: (Unilateral)

**Lymph Nodes - Cervical**  
Lymphoid proliferation: (Moderate)

**APPENDIX 6**

**(Pathology - continued)**

Rat No/Sex: 61 ♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Lungs; Heart; Spleen; Kidneys; Adrenals; Sciatic Nerve; Brain

Pathologist: G.Lawson

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 62♀

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 63♀

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

**APPENDIX 6**

**(Pathology - continued)**

Compound: Isodecyl benzoate

Dosage Level: 1000 mg/kg/day

Rat No/Sex: 64♀

**CLINICAL FINDINGS**

Clinical signs comprised a slight increase in salivation following dosing seen intermittently during Week 2 to 4 and infrequently associated with slightly wet fur and a slight lack of grooming seen from Day 29. There were no other signs of ill health, behavioural change or reaction to treatment observed.

Sacrificed following completion of treatment period and no further investigations performed.

## APPENDIX 7

## Special Diet Services Rat and Mouse Maintenance Diet

## Composition and quality assurance aspects of diet

SDS Rat and Mouse No. 1 SQC 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 <sub>3</sub> )	30	mg/kg
Nitrite (as NaNO <sub>2</sub> )	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 7

(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 per g diet

**APPENDIX 8****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.

VCL 206/942848

**FUNCTIONAL OBSERVATIONAL BATTERY  
REPORT**

Elizabeth W. Hughes,  
Behavioural Scientist.

This battery comprised 3 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed in a test arena and the third set comprised handling/specific testing of the animal.

- |  |   |
|--|---|
| <p>1. <b>Home cage observations</b></p> <p>Posture in the cage<br/>           Presence of convulsions, tremors, twitches<br/>           Presence of spontaneous vocalisations<br/>           Palpebral closure</p>   | <p>2. <b>Observations in the arena</b></p> <p>Occurrence of convulsions, tremors, twitches<br/>           Activity counts<br/>           Level of arousal<br/>           Rearing count<br/>           Grooming<br/>           Piloerection<br/>           Assessment of gait<br/>           Record presence of faecal boluses, urine</p>                          |
| <p>3. <b>Observations in the hand</b></p> <p>Occurrence of convulsions, tremors, twitches<br/>           Ease of removing rat from cage<br/>           Ease of handling rat<br/>           Salivation/lacrimation<br/>           Palpebral closure<br/>           Exophthalmus<br/>           Piloerection<br/>           Vocalisation on handling</p> | <p>4. <b>Manipulations</b></p> <p>Approach response<br/>           Touch response<br/>           Startle response<br/>           Righting reflex<br/>           Tail pinch response<br/>           Pupil response<br/>           Grip strength, fore and hindlimb<br/>           Landing foot splay<br/>           Body temperature<br/>           Bodyweight</p> |

At any point during the observations comments could be made about behaviour as free text.

The observational battery has previously been validated using positive control compounds<sup>1</sup>.

<sup>1</sup> NEWTON, D.F. *et al* (1992) "A Neurotoxicity Screen in rats following treatment with Acrylamide, Carbaryl or p,p'-DDT" Toxicology Letters Supplement 1992, p 184

## RESULTS OF THE FUNCTIONAL OBSERVATIONAL BATTERY

The data from the observational battery has been handled in the following manner. The observational endpoints such as ease of handling, activity, etc., have been tabulated for frequency of occurrence for each group. The following data was subjected to statistical analysis: rearing and activity counts, grip strength, hind limb splay, bodyweight and temperature.

### Observational endpoints

Among females, there were clear effects of treatment at 1000 mg/kg/day by Week 4 including:

The incidence of facial staining was increased and in conjunction with this the majority of animals at this dosage appeared "badly groomed".

One animal (no. 57) showed body tremor while being held in the hand.

In the arena, palpebral closure was observed in 4/8 animals. These same animals showed body tremor including animal no. 57 which was noted above as showing tremor while being held. Of the 4 animals with tremor, a total of two animals were classed as having moderate tremor including no. 57 which showed moderate continuous tremor.

Among males, there were only slight differences in the observational endpoints during the course of the study. Although during Week 4 there were few remarkable changes observed, in view of the changes observed in the females, the pattern of responses for individual animals was examined with the following result:

There was a low incidence of males at 1000 mg/kg/day showing slight body tremor during the recording of temperature. Two of these animals also showed slight body tremors in the arena with the third animal showing moderate continuous body tremor in the arena. All three animals showed palpebral closure in the arena. There was also an observation that in the righting response, some males were leaving one of their hindfeet tucked under the body. This was observed among all groups but the highest incidence was recorded at 1000 mg/kg/day.

### Grip strength

There were no statistically significant differences in forelimb grip strength.

There was a slight but statistically significant increase in the hindlimb grip strength of males at 1000 mg/kg/day during Week 2. There were no significant differences at Week 4. Among females at 1000 mg/kg/day, there was a statistically significant increase in hindlimb grip strength during Week 4.

### **Hindlimb splay**

Hindlimb splay was statistically significantly increased. Among males at 1000 mg/kg/day, a significant increase was confined to Week 2. Among females at 1000 mg/kg/day, however, hindlimb splay was significantly increased during Weeks 2 and 4.

### **Rearing counts**

Among males, the frequency of rearing in the arena was considered comparable among the groups. Among females, it was noted that counts among females at 1000 mg/kg/day were slightly lower than controls during Week 4. Differences failed to attain statistical significance.

### **Activity counts**

Among males, activity counts were variable among the groups. There was no indication of an effect of treatment. Among females, it was noted that counts among females at 1000 mg/kg/day were slightly lower than controls during Week 4. Differences failed to attain statistical significance.

### **Temperature**

There were no statistically significant differences in temperature among the groups.

### **Bodyweight**

Bodyweights were generally comparable among the groups.

### **Summary of effects**

Exposure to isodecyl benzoate was characterized by behavioural changes at 1000 mg/kg: These changes were generally not observed at Week 2 but were observed at Week 4.

- The incidence of facial staining was increased among females at Week 4.
- Badly groomed appearance was observed among females at Week 4.
- Among females there was an association of tremor and palpebral closure in the arena.
- Among males there was an association of tremor in the arena, palpebral closure in the arena and tremor during body temperature.
- Hindlimb grip strength was increased among males at Week 2 and among females at Week 4.
- Hindlimb splay was increased among males during Week 2 and among females during Week 2 and 4.

There was also a suggestion at Week 4 of decreased activity and rearing counts for females treated at 1000 mg/kg/day.

### Discussion

Exposure to Isodecyl benzoate resulted in behavioural changes at 1000 mg/kg/day. Females appeared to be more affected than males. The behavioural changes were generally only observed during Week 4 and included altered appearance (facial staining and badly groomed appearance), palpebral closure in the arena, the observation of tremor, increased hindlimb grip strength, and increased hindlimb splay.

The observations of increased hindlimb splay and the presence of tremor suggest an effect on the nervous system.

In respect of the finding of tremor, in general the degree of the tremor was considered slight and was generally only observed when animals were in the arena. Controls also showed this response, however, there was a suggestion of a clustering of effects in animals at 1000 mg/kg/day: tremor in association with palpebral closure, and tremor during temperature (for males only). It was also noted that one male and one female at 1000 mg/kg/day showed moderate, continuous tremor while in the arena. This was not observed among controls.

Some of the additional observations, changes in appearance and the suggestion of effects on activity/rearing, can be considered non-specific. However, in the presence of the changes in splay and tremor this again suggests some effect on the nervous system.

The relevance of the observation of increased hindlimb grip strength was unclear as the expectation would be for a decrease in grip strength.

At lower dosages, there was no clear evidence of an effect of treatment.

### Conclusion

Exposure of Isodecyl benzoate resulted in behavioural changes which were confined to 1000 mg/kg/day with females showing more changes compared with males. There was no evidence of an effect of treatment at lower dosages.

## GROUP SUMMARY OF OBSERVATIONS - MALES

Group	Pre-dose				Week 2				Week 4			
	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
<b>OBSERVATIONS</b>												
<b>HOME CAGE</b>												
posture, sitting	7	8	8	7	6	8	7	7	7	7	8	8
<b>REMOVAL FROM CAGE</b>												
removing, easy	8	8	8	8	7	8	8	8	8	8	8	8
handling, easy	6	7	7	7	8	8	8	8	7	8	7	7
salivation	1	0	0	0	1	1	0	2	1	1	0	1
vocalising	0	0	0	0	1	1	0	0	1	1	0	1
<b>IN THE ARENA</b>												
grooming	1	4	2	5	4	3	4	2	1	1	2	3
tremors												
slight	1	1	2	2	2	2	3	4	2	4	3	3
moderate	0	0	0	0	0	0	0	0	0	1	0	1
arousal, alert	6	7	6	7	6	7	6	5	2	3	5	6
piloerection	0	0	0	0	0	0	0	0	0	0	0	0
defecation	4	5	3	2	2	2	1	1	3	3	2	2
urine present	2	3	4	3	3	3	2	1	4	3	6	4
<b>GAIT</b>												
walking on toes	0	0	0	1	0	3	1	1	2	1	1	2
swaying	0	0	0	0	0	1	0	0	0	0	1	0
limbs splayed	0	0	0	0	0	0	0	0	0	0	0	0
hunched	0	0	0	0	0	0	1	0	0	0	0	1
unable to assess	1	1	0	0	2	1	2	3	5	4	0	0
<b>MANIPULATIONS</b>												
approach, a reaction	6	6	5	7	7	7	7	7	4	4	8	5
touch, a reaction	5	4	6	6	6	5	7	3	3	1	6	5
startle (present)	8	8	8	8	8	8	8	8	6	7	6	7
righting, immediate	5	7	7	8	7	6	6	8	5	7	8	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	7	8	8	7	7	7	7	7	7
<b>ADDITIONAL COMMENTS</b>												
facial staining	3	5	2	3	1	2	0	0	1	1	0	0
body groomed	0	0	0	0	0	0	0	0	0	0	0	0
palpebral closure in arena	1	0	0	0	0	0	1	2	3	3	2	3
slight body tremors												
during temperature	0	1	0	0	0	3	2	1	0	1	1	3
position of feet during												
righting	0	0	0	0	0	0	0	0	1	1	2	3

Numbers reflect the number of animals either showing the response or with the indicated score

## GROUP SUMMARY OF OBSERVATIONS - MALES

Group	Pre-dose				Week 2				Week 4			
	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
<b>OBSERVATIONS</b>												
<b>HOME CAGE</b>												
posture, sitting	7	8	8	7	6	8	7	7	7	7	8	8
<b>REMOVAL FROM CAGE</b>												
removing, easy	8	8	8	8	7	8	8	8	8	8	8	8
handling, easy	6	7	7	7	8	8	8	8	7	8	7	7
salivation	1	0	0	0	1	1	0	2	1	1	0	1
vocalising	0	0	0	0	1	1	0	0	1	1	0	1
<b>IN THE ARENA</b>												
grooming	1	4	2	5	4	3	4	2	1	1	2	3
tremors												
slight	1	1	2	2	2	2	3	4	2	4	3	3
moderate	0	0	0	0	0	0	0	0	0	1	0	0
arousal, alert	6	7	6	7	6	7	6	5	2	3	5	0
piloerection	0	0	0	0	0	0	0	0	0	0	0	0
defecation	4	5	3	2	2	2	1	1	3	3	2	2
urine present	2	3	4	3	3	3	2	1	4	3	6	4
<b>GAIT</b>												
walking on toes	0	0	0	1	0	3	1	1	2	1	1	2
swaying	0	0	0	0	0	1	0	0	0	0	1	0
limbs splayed	0	0	0	0	0	0	0	0	0	0	0	0
hunched	0	0	0	0	0	0	1	0	0	0	0	1
unable to assess	1	1	0	0	2	1	2	3	5	4	0	0
<b>MANIPULATIONS</b>												
approach, a reaction	6	6	5	7	7	7	7	7	4	4	8	5
touch, a reaction	5	4	6	6	6	5	7	3	3	1	6	5
startle (present)	8	8	8	8	8	8	8	8	6	7	6	7
righting, immediate	5	7	7	8	7	6	6	8	5	7	8	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	7	8	8	7	7	7	7	7	7
<b>ADDITIONAL COMMENTS</b>												
facial staining	3	5	2	3	1	2	0	0	1	1	0	0
body groomed	0	0	0	0	0	0	0	0	0	0	0	0
palpebral closure in arena	1	0	0	0	0	0	1	2	3	3	2	3
slight body tremors												
during temperature	0	1	0	0	0	2	2	1	0	1	1	3
position of feet during												
righting	0	0	0	0	0	0	0	0	1	1	2	3

Numbers reflect the number of animals either showing the response or with the indicated score

## GROUP SUMMARY OF OBSERVATIONS - FEMALES

Group	Pre-dose				Week 2				Week 4			
	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
<b>OBSERVATIONS</b>												
<b>HOME CAGE</b>												
posture, sitting	7	5	6	7	8	5	8	8	8	6	6	8
<b>REMOVAL FROM CAGE</b>												
removing, easy	7	8	8	8	7	7	7	8	8	7	7	8
handling, easy	6	4	6	5	6	5	7	7	5	6	5	6
salivation	0	2	0	0	0	0	0	0	0	2	0	0
vocalising	2	3	1	2	1	4	2	2	2	2	2	1
tremors in the hand	0	0	0	0	0	0	0	0	0	0	0	1
<b>IN THE ARENA</b>												
grooming	5	2	1	6	3	4	4	3	2	2	4	5
tremors												
slight	1	0	0	1	2	1	1	1	0	1	0	2
moderate	0	0	0	0	0	0	0	0	0	0	0	2
arousal, alert	6	8	8	8	6	8	7	6	6	7	5	6
piloerection	0	0	0	0	0	0	0	1	0	0	0	0
defecation	0	0	0	1	0	0	0	0	0	0	0	0
urine present	1	1	0	1	1	1	0	0	0	0	1	0
<b>GAIT</b>												
walking on toes	3	3	4	2	4	5	6	6	4	5	6	5
swaying	0	0	0	0	0	0	0	0	0	1	0	0
limbs splayed	0	0	0	0	0	0	1	0	0	0	0	1
hunched	1	1	0	0	1	0	0	3	0	0	0	0
unable to assess	1	0	0	0	0	0	0	0	0	0	0	1
<b>MANIPULATIONS</b>												
approach, a reaction	7	6	6	7	8	7	8	7	8	6	8	6
touch, a reaction	7	4	6	5	6	6	5	6	5	5	5	3
startle (present)	8	8	8	8	8	8	8	8	6	6	6	7
righting, immediate	5	7	7	8	5	8	7	6	6	6	7	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	8	7	8	8	8	8	7	7	8
<b>ADDITIONAL COMMENTS</b>												
facial staining	1	2	2	3	2	2	1	3	0	2	1	4
badly groomed	0	0	0	0	0	0	0	0	0	0	0	6
palpebral closure in arena	1	0	0	0	0	0	0	1	0	0	0	4
slight body tremors												
during temperature	1	0	0	0	2	1	0	1	0	1	0	1
position of feet during												
righting	0	0	0	0	0	0	0	0	0	0	0	0

Numbers reflect the number of animals either showing the response or with the indicated score

**GROUP MEAN FORELIMB GRIP STRENGTH****Pre-dose**

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	0.64	0.64
2	0.58	0.68
3	0.62	0.64
4	0.69	0.69

**Week 2**

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	0.95	0.87
2	0.90	0.94
3	0.94	0.86
4	1.00	0.90

**Week 4**

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	1.05	1.00
2	1.11	1.00
3	1.15	0.97
4	1.16	1.02

No statistical significance ( $P > 0.05$ )

**GROUP MEAN HINDLIMB GRIP STRENGTH**

## Predose

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.60	0.67
2	0.55	0.68
3	0.56	0.70
4	0.64	0.74

## Week 2

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.74	0.71
2	0.73	0.80
3	0.79	0.81
4	0.87+	0.85

## Week 4

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.94	0.84
2	0.84	0.89
3	0.98	0.88
4	1.06	1.03+

+ P &lt; 0.05

## GROUP MEAN SPLAY VALUES

## Predose

Group	Mean splay values (cm)	
	Males	Females
1	9.4	7.8
2	10.2	9.0
3	8.3	8.8
4	9.3	9.0

## Week 2

Group	Mean splay values (cm)	
	Males	Females
1	9.0	8.0
2	10.7	9.7
3	10.8	8.8
4	11.8+	10.6++

## Week 4

Group	Mean splay values (cm)	
	Males	Females
1	9.4	8.4
2	10.3	9.3
3	11.0	9.1
4	10.4	11.1++

+ P &lt; 0.05

++ P &lt; 0.01

### GROUP MEAN REARING COUNTS

#### Predose

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

#### Week 2

Group	Mean rearing counts	
	Males	Females
1	8	13
2	10	17
3	11	16
4	8	12

#### Week 4

Group	Mean rearing counts	
	Males	Females
1	5	13
2	5	15
3	8	14
4	8	8

No statistical significance ( $P > 0.05$ )

## GROUP MEAN ACTIVITY COUNTS

Predose

Group	Mean activity counts	
	Males	Females
1	11	13
2	14	15
3	13	16
4	10	13

Week 2

Group	Mean activity counts	
	Males	Females
1	7	13
2	11	17
3	11	16
4	7	13

Week 4

Group	Mean activity counts	
	Males	Females
1	6	14
2	5	17
3	10	16
4	6	8

No statistical significance ( $P > 0.05$ )

**GROUP MEAN TEMPERATURE****Predose**

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.2
2	37.9	38.3
3	37.9	38.4
4	37.9	38.1

**Week 2**

Group	Mean temperature (°C)	
	Males	Females
1	37.6	38.0
2	37.8	38.5
3	37.7	38.0
4	37.9	38.2

**Week 4**

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.5
2	38.2	38.4
3	37.8	38.3
4	37.9	37.9

No statistical significance ( $P > 0.05$ )

### GROUP MEAN BODYWEIGHTS

#### Predose

Group	Mean bodyweights (g)	
	Males	Females
1	241	188
2	239	192
3	241	194
4	240	191

#### Week 2

Group	Mean bodyweights (g)	
	Males	Females
1	346	235
2	344	240
3	352	244
4	342	244

#### Week 4

Group	Mean bodyweights (g)	
	Males	Females
1	417	267
2	405	267
3	429	271
4	402	271

No statistical significance ( $P > 0.05$ )

## KEY TO FUNCTIONAL OBSERVATIONAL BATTERY

### Posture

- S      Sitting/standing in cage
- R      Rearing in cage
- Cl     Climbing up cage

### Ease of removal from cage

- 2      Easy (little resistance)
- 3      Slightly awkward

### Ease of handling

- 2      Easy (little resistance)
- 3      Slightly awkward

### Arousal

- 2,3,4,5    Increasing levels of arousal with 4-being alert

### Gait

- T      Walking on toes
- A      Swaying/lurching gait
- H      Hindlimbs splayed
- HU     Hunched

The numbers associated with gait indicate the degree of effect

- 1,2,3    Increasing degree of effect

- U      Unable to assess gait - insufficient movement to assess gait
- N      Normal gait

### Approach

- 1      No reaction
- 2      Sniffs
- 3      Approaches and sniffs
- 4      Freezes
- O      Other

### Touch

- 1      No reaction
- 2      Turns
- 3      Walks away
- 4      Freezes
- O      Other

Startle	
1	No reaction
2	Ear twitch
3	Noticeable flinch
4	Noticeable jump

Tail pinch	
2	Turns
	2 turns immediately
	3 violent turn
3	Walks away
	1 and turns
5	Jumps forward
0	Other

Righting reflex	
1	Immediate reaction
2	Reaction slow

Tremors	
N	No tremor observed
B	Body
H	Head
T	Tail

The numbers associated with tremors indicate the degree of effect  
1,2,3      Increasing degree of effect

Salivation, vocalising, grooming, piloerection	
N	Not observed
Y	Sign observed

The associated numbers indicate the degree of response  
1,2,3      Increasing degree of effect

Pupil reflex	
B	Reflex observed both pupils
L,R	Left or right pupil responded only
N	No reflex both pupils

Urine	
S	Small amount
M	Moderate amount
L	Large amount
N	No urine observed

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 1 males							
		1	2	3	4	5	6	7	8
IN THE CAGE									
Posture		S	S	R	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	2	2	2	3
Salivation		N	N	N	N	N	Y1	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		B1	N	N	N	N	N	N	N
Grooming		N	N	Y	N	N	N	N	N
Activity count		3	8	16	8	1	7	19	23
Arousal		3	4	4	4	3	4	4	4
Rearing count		3	7	19	8	1	6	19	21
Bolus count		2	0	0	0	2	5	0	1
Urine present		M	M	N	N	N	N	N	N
Gait		N	N	N	N	U	N	N	N
MANIPULATIONS									
Approach		0	1	1	2	3	3	3	3
Touch		1	1	4	4	4	2	1	2
Startle		4	3	3	4	3	3	3	2
Righting reflex		2	1	1	1	1	2	2	1
Tail pinch		3	3	3	3	2	2	3	3
turns						2	2		
vocalises		Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.4	37.7	37.7	38.0	37.4	38.0	37.9	37.9
Bodyweight (g)		236	237	235	250	240	232	241	256
GRIP STRENGTH (kg) #									
Forelimb		0.80	0.90	0.57	0.54	0.51	0.55	0.63	0.62
Hindlimb		0.67	0.61	0.63	0.48	0.55	0.57	0.57	0.77
FOOTSPRAY (cm) #		8.1	10.1	8.9	11.0	10.9	8.1	6.6	11.5

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 1 Approach response: head sway  
During manipulations: soft faeces
- 3 Slight brown nasal staining
- 5 In the arena: sat in corner
- 6 Slight brown nasal staining
- 8 In the arena: partially closed eyes on occasions  
Slight brown nasal staining

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 2 males							
		9	10	11	12	13	14	15	16
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	N	N	N	Y	N	Y	Y
Activity count		18	20	1	20	11	17	11	15
Arousal		4	4	3	4	4	4	4	4
Rearing count		23	13	0	16	13	11	15	10
Bolus count		2	3	4	3	0	0	0	1
Urine present		M	S	S	N	N	N	N	N
Gait		N	N	U	N	N	N	N	N
MANIPULATIONS									
Approach		3	3	1	3	3	2	3	1
Touch		4	3	4	2	1	1	1	1
Startle		4	3	3	3	3	2	3	3
Righting reflex		1	1	1	1	2	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises			Y2	Y2	Y2		Y2	Y1	
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.2	38.1	38.1	37.6	37.6	38.1	38.4	37.2
Bodyweight (g)		222	239	244	243	233	240	231	259
GRIP STRENGTH (kg) #									
Forelimb		0.50	0.60	0.52	0.67	0.47	0.61	0.57	0.75
Hindlimb		0.50	0.69	0.66	0.58	0.38	0.56	0.49	0.58
FOOTSPRAY (cm) #		12.3	7.7	10.3	8.6	10.8	10.8	10.7	10.7

# Values represent the mean of two trials

## Additional comments

## Animal no.

9 During manipulations: soft faeces  
 11 Slight brown nasal staining  
 In the arena: sat in corner  
 During temperature: slight body tremors  
 12 Slight brown nasal staining  
 13 In the arena: hind feet slipping  
 14 Slight brown nasal staining  
 15 Slight brown nasal staining  
 16 Slight brown nasal staining  
 Pupil reflex: slow response

VCL/206 Predose

OBSERVATIONS	Animal no	Group 3 males							
		17	18	19	20	21	22	23	24
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	3
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	B1	B1	N	N	N	N
Grooming		N	N	N	Y	N	Y	N	N
Activity count		16	17	10	18	5	15	18	6
Arousal		4	4	4	4	4	5	4	3
Rearing count		18	18	10	15	9	10	16	3
Bolus count		2	4	5	0	0	0	0	0
Urine present		M	N	M	N	N	S	N	M
Gait		N	N	N	N	N	N	N	N
MANIPULATIONS									
Approach		3	2	3	1	3	1	3	1
Touch		2	4	5	1	4	3	2	1
Startle		4	3	3	3	3	4	3	3
Righting reflex		1	1	1	2	1	1	1	1
Tail pinch		3	3	5	5	3	3	3	3
turns									
vocalises		Y1	Y1	Y1	Y2	Y1		Y1	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.5	37.6	38.1	38.1	37.6	37.7	38.0	37.5
Bodyweight (g)		240	254	236	229	244	234	234	255
GRIP STRENGTH (kg) #									
Forelimb		0.59	0.76	0.80	0.45	0.37	0.69	0.45	0.90
Hindlimb		0.45	0.66	0.61	0.57	0.41	0.74	0.48	0.53
FOOTSPRAY (cm) #									
		8.9	8.5	9.2	6.8	5.5	9.9	6.1	11.4

# Values represent the mean of two trials

## Additional comments

Animal no.

18 During manipulations: soft faeces  
 22 Slight brown nasal staining, brown staining on head  
 24 Slight brown nasal staining  
 In the arena: soft faeces

VCL/206 Predose

OBSERVATIONS	Animal no	Group 4 males							
		25	26	27	28	29	30	31	32
IN THE CAGE									
Posture		S	S	S	S	S	S	S	R
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	3
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	B1	N	B1	N	N
Grooming		N	N	Y	Y	Y	N	Y	Y
Activity count		19	11	11	5	9	6	12	10
Arousal		4	4	4	3	4	4	4	4
Rearing count		19	15	14	5	10	7	14	11
Bolus count		0	4	0	3	0	0	0	0
Urine present		N	S	N	N	N	S	S	S
Gait		N	N	T1	N	N	N	N	N
MANIPULATIONS									
Approach		1	2	3	3	2	3	3	3
Touch		4	1	4	4	2	4	1	4
Startle		2	4	3	2	4	3	3	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises			Y1	Y2		Y1	Y1	Y1	Y1
Pupil reflex		B	B	B	B	N	B	B	B
Temperature (°C)		38.4	38.1	37.8	38.1	37.7	37.5	37.7	38.2
Bodyweight (g)		244	231	243	239	247	237	239	241
GRIP STRENGTH (kg) #									
Forelimb		0.81	0.65	0.66	0.73	0.57	0.87	0.56	0.65
Hindlimb		0.69	0.80	0.64	0.73	0.49	0.73	0.54	0.54
FOOTSPRAY (cm) #		11.7	10.1	7.4	9.1	8.8	8.1	6.7	12.9

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 27 During manipulations: soft faeces
- 28 Slight brown nasal staining, hair loss on neck
- 29 Slight brown nasal staining  
Pupil reflex: both pupils constricted
- 30 Moderate brown nasal staining  
Pupil reflex: left pupil appears misshapened and misplaced (reflex observed)
- 31 Hairloss on chin
- 32 During manipulations: soft faeces

VCL/206 Predose

OBSERVATIONS	Animal no	Group 1 females							
		33	34	35	36	37	38	39	40
IN THE CAGE									
Posture	S	CL	S	S	S	S	S	S	S
IN THE HAND									
Removing	2	2	2	2	2	3	2	2	2
Handling	2	3	2	2	3	2	2	2	2
Salivation	N	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	Y1	Y1	N	N	N
IN THE ARENA									
Tremors	N	N	N	N	N	N	B1	N	N
Grooming	Y	Y	Y	N	Y	Y	N	N	N
Activity count	13	18	15	23	10	17	3	2	2
Arousal	5	4	4	4	4	4	4	3	3
Rearing count	3	18	18	19	6	22	5	1	1
Bolus count	0	0	0	0	0	0	0	0	0
Urine present	S	N	N	N	N	N	N	N	N
Gait	N	T1HUI	N	T1	N	T1	N	N	U
MANIPULATIONS									
Approach	2	3	3	2	1	2	2	3	3
Touch	4	2	4	2	1	4	3	4	4
Startle	4	4	3	3	3	4	3	3	3
Righting reflex	2	1	2	2	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3	3
turns									
vocalises	Y1		Y2	Y1	Y2	Y2			Y1
Pupil reflex	B	B	B	B	B	B	B	B	B
Temperature (°C)	38.7	38.0	38.2	38.3	38.0	38.3	37.9	38.5	38.5
Bodyweight (g)	179	193	191	192	185	188	180	193	193
GRIP STRENGTH (kg) #									
Forelimb	0.55	0.74	0.56	0.59	0.65	0.53	0.66	0.82	0.82
Hindlimb	0.64	0.85	0.69	0.60	0.71	0.71	0.64	0.55	0.55
FOOTSPLAY (cm) #									
	6.2	9.3	9.2	7.2	6.9	7.4	6.9	9.7	9.7

# Values represent the mean of two trials

## Additional comments

Animal no.

- 34 Slight hairloss head and neck  
 37 In the arena: partially closed eyes on occasions  
 38 Slight kink near tip of tail  
 During manipulations: vocal and awkward to handle  
 40 Slight red staining around right eye, slight nasal staining  
 In the arena: limited walking  
 During temperature: slight body tremors

## VCL/206 Predose

OBSERVATIONS	Animal no	Group 2 females							
		41	42	43	44	45	46	47	48
IN THE CAGE									
Posture		S	S	S	S	S	R	CL	R
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		3	3	2	2	2	3	2	3
Salivation		N	Y1	N	N	N	N	N	N
Vocalising		N	N	Y1	N	N	Y1	Y1	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		N	N	Y	N	Y	N	N	N
Activity count		17	10	20	14	14	17	13	13
Arousal		4	4	4	4	4	4	4	4
Rearing count		16	12	16	11	12	18	16	12
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	S	N	N
Gait		N	N	T1	N	N	T1	N	T1HU1
MANIPULATIONS									
Approach		3	2	1	1	3	2	0	2
Touch		4	1	1	1	1	3	0	2
Startle		3	3	4	3	3	3	3	3
Righting reflex		1	1	1	1	1	1	2	1
Tail pinch		3	3	3	3	3	3	0	3
turns									
vocalises		Y1	Y1	Y2	Y1	Y2	Y1		Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.2	38.4	38.2	38.3	38.1	37.8	39.1	38.1
Bodyweight (g)		185	189	199	187	193	205	176	205
GRIP STRENGTH (kg) #									
Forelimb		0.62	0.72	0.76	0.68	0.56	0.75	0.69	0.68
Hindlimb		0.72	0.73	0.80	0.75	0.55	0.69	0.70	0.49
FOOTSPLAY (cm) #									
		7.7	10.5	10.1	9.3	7.6	7.3	8.2	11.4

# Values represent the mean of two trials

## Additional comments

Animal no.

45 Slight brown nasal staining

47 Tail pinch: stepped backwards then walked away

48 Slight brown nasal staining, hair loss on neck

VCL/206 Predose

OBSERVATIONS	Animal no	Group 3 females							
		49	50	51	52	53	54	55	56
IN THE CAGE									
Posture		S	R	S	S	S	R	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	3	2	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	Y1	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		N	N	N	N	Y	N	N	N
Activity count		19	13	14	14	22	13	15	18
Arousal		4	4	4	4	4	4	4	4
Rearing count		16	14	15	15	16	15	14	10
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		N	T1	T1	T1	N	N	N	T1
MANIPULATIONS									
Approach		2	3	3	1	2	1	2	3
Touch		1	4	4	3	1	3	4	3
Startle		3	3	4	3	3	3	3	2
Righting reflex		1	2	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y1	Y1	Y2	Y2	Y1		Y2	Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.1	38.3	38.3	38.7	37.6	38.9	38.7	38.3
Bodyweight (g)		192	206	193	182	191	199	187	199
GRIP STRENGTH (kg) #									
Forelimb		0.73	0.53	0.64	0.57	0.43	0.74	0.75	0.71
Hindlimb		0.55	0.59	0.66	0.82	0.71	0.72	0.81	0.73
FOOTSPRAY (cm) #		11.5	8.5	9.8	6.4	7.3	7.4	11.5	7.9

# Values represent the mean of two trials

## Additional comments

Animal no.

51 Slight brown nasal staining, slight hair loss on neck

53 Slight brown nasal staining

VCL/206 Predose

OBSERVATIONS	Animal no	Group 4 females							
		57	58	59	60	61	62	63	64
IN THE CAGE									
Posture		S	S	S	S	S	R	S	R
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	2	3	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	Y1	Y2	N	N	N	N
IN THE ARENA									
Tremors		N	B1	N	N	N	N	N	N
Grooming		N	N	Y	Y	Y	Y	Y	Y
Activity count		18	9	13	22	15	11	4	14
Arousal		4	4	4	4	4	4	4	4
Rearing count		13	8	13	24	17	10	7	14
Bolus count		0	0	0	1	0	0	0	0
Urine present		N	N	N	S	N	N	N	N
Gait		N	T1	T1	N	N	N	N	N
MANIPULATIONS									
Approach		2	5	1	3	3	2	3	2
Touch		1	4	4	4	1	1	4	3
Startle		3	3	4	3	3	4	3	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises			Y2	Y1	Y2	Y1	Y2	Y2	
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.4	38.3	38.5	37.2	38.0	38.6	37.1	38.3
Bodyweight (g)		199	182	193	191	197	187	183	197
GRIP STRENGTH (kg) #									
Forelimb		0.69	0.70	0.72	0.73	0.67	0.72	0.74	0.61
Hindlimb		0.65	0.83	0.83	0.75	0.71	0.78	0.68	0.68
FOOTSPRAY (cm) #									
		11.1	9.2	11.3	7.3	8.9	7.6	6.8	9.8

# Values represent the mean of two trials

## Additional comments

Animal no.

57 Slight brown nasal staining

58 Slight brown nasal staining

63 Slight brown staining on nose

During manipulations: awkward to handle

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 1 males							
		1	2	3	4	5	6	7	8
IN THE CAGE									
Posture		R	S	S	S	S	S	R	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	3
Handling		2	2	2	2	2	2	2	2
Salivation		N	N	N	N	N	N	N	Y1
Vocalising		N	N	N	N	Y2	N	N	N
IN THE ARENA									
Tremors		N	B1	N	B1	N	N	N	N
Grooming		Y	Y	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		6	9	12	5	3	9	2	9
Arousal		4	4	4	4	3	4	3	4
Rearing count		9	10	15	8	1	7	7	9
Bolus count		0	0	0	0	4	0	0	3
Urine present		N	M	N	N	S	N	N	S
Gait		N	N	N	N	U	N	U	N
MANIPULATIONS									
Approach		1	3	3	3	3	3	3	3
Touch		1	4	4	4	4	4	1	4
Startle		3	3	4	3	3	3	3	3
Righting reflex		2	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	2	3	3	3
turns						3			
vocalises		Y2	Y2	Y2	Y1	Y3	Y1	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.0	37.6	37.5	37.7	37.2	37.2	37.1	38.2
Bodyweight (g)		372	320	342	365	352	320	336	362
GRIP STRENGTH (kg) #									
Forelimb		1.07	1.25	0.56	1.00	0.84	0.84	1.12	0.92
Hindlimb		0.71	0.76	0.71	0.58	0.83	0.66	0.74	0.93
FOOTSPRAY (cm) #		8.6	10.1	7.3	10.7	8.3	9.8	7.3	10.4

# Values represent the mean of two trials

## Additional comments

Animal no.

2 During manipulations: slight salivation

5 In the arena: sat in corner

7 In the arena: sat in corner

VCL/206 Week 2

OBSERVATIONS	Group 2 males								
	Animal no	9	10	11	12	13	14	15	16
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Salivation		N	Y1	N	N	N	N	N	N
Vocalising		N	N	Y1	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	H1B1T1	N	N	N	N	B1
Grooming		Y	Y	N	N	N	N	Y	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		21	19	1	13	4	16	5	6
Arousal		4	4	2	4	4	4	4	4
Rearing count		21	16	0	9	7	13	8	3
Bolus count		1	0	7	0	0	0	0	0
Urine present		M	S	M	N	N	N	N	N
Gait		T2	A1T1	U	T1	N	N	N	N
MANIPULATIONS									
Approach		5	3	1	3	3	3	3	3
Touch		4	4	4	4	1	3	1	1
Startle		3	4	3	3	2	3	2	3
Righting reflex		2	1	1	1	1	2	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y2	Y2	Y2	Y2	Y1	Y1	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		37.8	38.5	38.0	37.3	36.9	37.9	38.0	38.0
Bodyweight (g)		304	349	358	361	331	337	347	362
GRIP STRENGTH (kg) #									
Forelimb		0.50	1.08	0.84	0.94	0.72	0.97	1.11	1.02
Hindlimb		0.69	0.84	0.77	0.61	0.59	0.69	0.79	0.90
FOOTSPRAY (cm) #									
		10.5	8.4	10.8	9.9	11.3	13.8	12.7	8.6

# Values represent the mean of two trials

## Additional comments

Animal no.

- 9 In the arena: slight salivation  
 11 In the arena: sat in corner  
 12 During temperature: slight body tremors  
 13 During temperature: slight body tremors  
 14 Slight brown nasal staining  
 16 During temperature: slight body tremors

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 3 males							
		17	18	19	20	21	22	23	24
IN THE CAGE									
Posture		S	S	S	R	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	B1	N	B1H1	N	N	N	B1
Grooming		Y	Y	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		18	6	12	1	17	15	14	1
Arousal		4	4	4	2	4	4	4	2
Rearing count		19	5	15	2	15	14	18	1
Bolus count		0	0	0	0	0	0	0	1
Urine present		N	N	N	N	N	M	S	M
Gait		N	N	HU1	U	T2	N	N	U
MANIPULATIONS									
Approach		3	2	3	3	3	2	3	1
Touch		3	1	3	2	4	4	3	4
Startle		3	3	3	3	3	3	3	4
Righting reflex		1	1	1	2	2	1	1	1
Tail pinch		3	3	3	5	3	3	3	3
turns									
vocalises		Y2	Y1	Y2		Y1	Y2	Y1	Y1
Pupil reflex		B	N	B	B	B	B	B	B
Temperature (°C)		38.6	37.1	37.8	37.6	38.6	37.8	37.0	37.3
Bodyweight (g)		347	376	348	321	375	334	364	354
GRIP STRENGTH (kg) #									
Forelimb		1.10	1.09	1.08	0.60	0.59	0.92	0.90	1.29
Hindlimb		0.57	0.90	0.79	0.75	0.85	0.87	0.77	0.85
FOOTSPRAY (cm) #		11.6	10.3	10.8	7.1	10.1	13.5	9.2	13.6

#Values represent the mean of two trials

## Additional comments

Animal no.

- 17 In the arena: slight salivation upon removal from arena  
During manipulations: soft faeces
- 18 Pupil reflex: both pupils constricted
- 20 In the arena: sat in corner, continuous body and tremors  
During temperature: slight body tremors
- 22 Slight brown nasal staining  
During temperature: slight body tremors
- 24 Slight brown nasal staining  
In the arena: sat in corner, partial to half closed eyes on occasions

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 4 males							
		25	26	27	28	29	30	31	32
IN THE CAGE									
Posture		R	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Salivation		N	N	Y1	N	N	N	Y1	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	B1	B1	N	N	B1	B1
Grooming		N	Y	N	N	Y	N	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		13	16	6	1	8	7	3	1
Arousal		4	4	4	3	4	4	3	2
Rearing count		13	14	6	1	11	12	3	1
Bolus count		0	0	0	3	0	0	0	0
Urine present		N	N	N	N	N	N	S	N
Gait		T1	N	N	U	N	N	U	U
MANIPULATIONS									
Approach		3	2	3	3	3	2	3	3
Touch		4	4	1	0	1	1	1	1
Startle		3	3	3	3	3	3	3	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y1	Y1	Y2			Y2	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	N
Temperature (°C)		38.2	38.0	37.7	38.6	37.0	37.8	37.5	38.4
Bodyweight (g)		339	319	369	330	366	345	341	328
GRIP STRENGTH (kg) #									
Forelimb		1.06	0.98	0.98	1.15	0.67	1.14	0.92	1.10
Hindlimb		0.87	0.94	0.86	0.84	0.70	1.17	0.87	0.76
FOOTSPRAY (cm) #		11.7	13.4	10.3	8.7	14.0	13.7	8.2	14.3

# Values represent the mean of two trials

## Additional comments

Animal no.

- 28 In the arena: sat in corner  
Touch response: body tremors  
During temperature: slight body tremors
- 31 Slight hairloss on neck  
In the arena: sat in corner, partially closed eyes on occasions
- 32 In the arena: sat in corner, partial to half closed eyes on occasions  
Pupil reflex: both pupils constricted

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 1 females							
		33	34	35	36	37	38	39	40
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	3	2	2	2
Handling		2	2	3	2	2	2	3	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	Y1	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	B1	B1
Grooming		Y	N	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		10	21	18	14	1	17	10	12
Arousal		4	4	4	4	3	4	5	4
Rearing count		12	11	16	17	1	22	13	10
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	M	N	N	N
Gait		N	T1	T1	T1HU1	U	T1	N	N
MANIPULATIONS									
Approach		3	3	3	3	3	2	3	3
Touch		2	3	1	2	3	1	3	3
Startle		3	3	3	3	3	3	3	3
Righting reflex		2	1	1	2	1	1	2	1
Tail pinch		3	3	3	0	3	3	3	3
turns							1		
vocalises		Y1	Y1	Y2	Y2		Y2		Y2
Pupil reflex		B	B	B	B	R	B	B	B
Temperature (°C)		38.7	37.1	38.4	38.8	38.7	37.6	37.9	37.0
Bodyweight (g)		224	241	239	246	234	237	226	233
GRIP STRENGTH (kg) #									
Forelimb		0.78	0.91	1.02	0.83	0.85	0.76	0.81	1.02
Hindlimb		0.70	0.68	0.81	0.55	0.71	0.71	0.77	0.75
FOOTSPRAY (cm) #									
		6.1	7.8	9.7	8.0	9.9	8.2	5.6	8.8

# Values represent the mean of two trials

## Additional comments

Animal no.

- 36 In the arena: abdomen appeared distended  
Tail pinch: paused then walked away
- 37 In the arena: sat in corner, appears to lean to right side when sitting  
During manipulations: soft faeces  
Pupil reflex: left pupil no response
- 38 Slight brown nasal staining  
During manipulations: awkward to handle and vocalising
- 39 In the arena: occasional slipping of forelimbs  
During temperature: slight body tremors
- 40 Moderate brown nasal staining, slight brown staining on head and lower lip  
In the arena: abdomen appeared slight distended  
During temperature: slight body tremors

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 2 females							
		41	42	43	44	45	46	47	48
IN THE CAGE									
Posture		R	CL	S	CL	S	S	S	S
IN THE HAND									
Removing		2	3	2	2	2	2	2	2
Handling		2	3	3	3	2	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	Y1	Y1	Y1	N	Y1	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	Y	Y	N	Y	N	N	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		27	16	17	12	20	12	19	9
Arousal		4	4	4	4	4	4	4	4
Rearing count		25	14	18	16	16	21	19	10
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	S	N	N	N	N	N
Gait		T2	N	T2	N	N	T2	T1	T1
MANIPULATIONS									
Approach		1	3	3	2	3	3	3	3
Touch		1	2	1	4	2	2	2	4
Startle		4	3	4	3	3	3	3	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	3	3	3	3	3	2	3
turns								2	
vocalises		Y1	Y1	Y2	Y2	Y2	Y2	Y2	Y3
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		39.0	39.4	38.5	38.3	38.4	37.8	39.0	37.8
Bodyweight (g)		229	237	254	230	247	252	213	254
GRIP STRENGTH (kg) #									
Forelimb		0.85	1.19	1.12	1.02	0.66	0.95	0.67	1.05
Hindlimb		1.02	0.85	1.09	0.93	0.53	0.83	0.48	0.72
FOOTSPRAY (cm) #									
		9.5	11.7	10.8	9.6	7.7	8.9	7.6	11.9

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 41 Slight brown staining on head
- 43 In the arena: slight salivation upon removal from arena
- 46 Slight brown staining on head
- 48 In the arena: abdomen appeared slightly distended  
During manipulations: awkward to handle and vocalising  
During temperature: slight body tremors

VCL/206 Week 2

OBSERVATIONS	Animal no	Group 3 females							
		49	50	51	52	53	54	55	56
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	3	2	2	2
Handling		2	2	2	2	3	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	Y1	N	N	Y1	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	B1	N
Grooming		Y	Y	N	N	N	Y	N	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		12	14	13	19	27	14	13	15
Arousal		4	4	4	4	5	4	4	4
Rearing count		12	11	20	16	23	11	17	19
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		T1	T1	N	T2	T2	T2H1	N	T1
MANIPULATIONS									
Approach		3	3	3	3	0	3	3	3
Touch		1	3	4	2	1	1	4	2
Startle		3	3	4	3	4	3	3	3
Righting reflex		1	2	1	1	1	1	1	1
Tail pinch		3	3	3	5	5	3	3	3
turns				1					
vocalises		Y2	Y2	Y2	Y2	Y1	Y2	Y2	Y1
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		37.8	37.3	39.2	38.2	39.2	37.9	37.1	37.6
Bodyweight (g)		239	264	235	225	248	260	228	252
GRIP STRENGTH (kg)#									
Forelimb		0.84	1.05	0.85	0.86	0.66	0.90	0.87	0.89
Hindlimb		0.60	1.01	0.66	1.08	0.70	0.95	0.77	0.74
FOOTSPRAY (cm) #		9.0	11.4	10.2	5.2	7.2	8.1	9.4	9.7

# Values represent the mean of two trials

## Additional comments

Animal no.

- 50 In the arena: slight salivation upon removal from arena
- 52 Slight hairloss on neck
- 53 Slight hairloss on neck  
Approach response: head sway
- 54 In the arena: abdomen appeared slightly distended
- 56 Slight brown nasal staining

VCL/206 Week 2

OBSERVATIONS	Group 4 females								
	Animal no	57	58	59	60	61	62	63	64
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	3	2	2	2	2	2
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	Y2	Y1	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	N	N	N	Y	N	Y	N
Pilo-erection		N	N	Y	N	N	N	N	N
Activity count		17	5	21	18	19	10	8	6
Arousal		4	3	4	4	4	4	4	3
Rearing count		7	5	22	16	19	10	13	4
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		T1HU1	N	T3HU1	T2	T1HU2	T1	T1	N
MANIPULATIONS									
Approach		1	3	3	3	3	3	3	3
Touch		4	1	2	3	3	4	1	4
Startle		3	3	3	3	3	2	3	3
Righting reflex		1	1	2	1	1	1	2	1
Tail pinch		3	3	0	3	3	3	3	2
turns									2
vocalises		Y1	Y2	Y1	Y2	Y2	Y2	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		37.5	38.2	39.1	38.0	39.2	38.6	37.6	37.7
Bodyweight (g)		258	227	241	249	254	249	228	244
GRIP STRENGTH (kg) #									
Forelimb		0.85	0.84	0.91	1.04	0.93	0.83	0.94	0.88
Hindlimb		0.77	0.94	0.94	0.71	0.88	0.76	0.94	0.88
FOOTSPRAY (cm) #									
		10.3	9.8	10.4	10.8	10.0	10.4	12.8	10.5

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 57 Slight hairloss on neck
- 59 Slight brown staining head and neck  
In the arena: abdomen appeared distended  
Tail pinch: paused then walked away
- 61 Slight brown nasal staining
- 62 Slight brown nasal staining  
In the arena: abdomen appeared slightly distended
- 63 During temperature: slight body tremors
- 64 In the arena: abdomen appeared slightly distended,  
parial to half closed eyes occasionally, continuous  
body tremors

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 1 males							
		1	2	3	4	5	6	7	8
IN THE CAGE									
Posture		S	S	S	S	S	S	R	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	3	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	Y1	N	N
IN THE ARENA									
Tremors		N	B1	N	B1	N	N	N	N
Grooming		N	N	N	N	N	N	N	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		1	2	13	1	1	6	1	25
Arousal		2	3	4	2	2	3	3	4
Rearing count		0	4	13	0	0	5	3	11
Bolus count		4	2	0	0	4	0	0	0
Urine present		N	S	S	N	S	S	N	N
Gait		U	U	T1	U	U	N	U	T2
MANIPULATIONS									
Approach		3	0	2	5	1	1	1	1
Touch		1	0	1	0	1	1	1	4
Startle		4	3	3	2	3	3	3	3
Righting reflex		2	1	1	1	2	1	2	1
Tail pinch		3	3	3	3	2	3	3	3
turns						2		1	
vocalises			Y2	Y1	Y2	Y2	Y1	Y2	Y2
Pupil reflex		B	B	B	L	B	B	B	B
Temperature (°C)		38.3	38.0	37.6	38.1	36.9	37.9	37.7	37.8
Bodyweight (g)		491	378	389	460	433	371	383	427
GRIP STRENGTH (kg)#									
Forelimb		1.14	1.18	0.84	1.21	0.78	1.05	1.09	1.13
Hindlimb		0.87	0.92	0.64	1.07	1.06	0.96	0.91	1.14
FOOTSPRAY (cm) #									
		8.7	10.3	9.5	10.9	8.0	9.2	6.4	12.4

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 1 In the arena: sat along edge of arena, ventral surface touching floor
- 2 In the arena: body held close to floor, sitting/rearing head first into corner  
Touch response: body tremors  
Approach response: body tremors  
Righting: left hindlimb tucked under body
- 3 During manipulations: slight nasal exudate observed
- 4 In the arena: sat in corner, partial to half closed eyes  
Touch response: body tremors  
Pupil reflex: too awkward to handle to assess right pupil
- 5 In the arena: sat in corner, partially closed eyes on occasions
- 6 Slight brown nasal staining
- 7 In the arena: sat in corner, partially closed eyes on occasions

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 2 males							
		9	10	11	12	13	14	15	16
IN THE CAGE									
Posture		S	S	S	S	R	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	2	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	Y1	N	N	N	N
Vocalising		N	N	Y2	N	N	N	N	N
IN THE ARENA									
Tremors		N	B1	B1	N	N	B1	B1	B2
Grooming		N	N	N	N	N	N	Y	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		13	4	1	16	3	1	1	1
Arousal		4	3	2	4	4	3	3	2
Rearing count		7	3	0	15	10	1	2	0
Bolus count		3	4	6	0	0	0	0	0
Urine present		M	S	N	N	N	S	N	N
Gait		N	N	U	T2	N	U	U	U
MANIPULATIONS									
Approach		1	1	1	3	3	3	3	1
Touch		1	1	1	1	1	4	1	1
Startle		2	3	3	3	3	3	3	3
Righting reflex		1	1	1	1	1	1	1	2
Tail pinch		3	3	2	3	3	3	3	3
turns				2					
vocalises		Y2	Y2	Y2	Y2		Y2	Y1	Y2
Pupil reflex		B	B	B	B	B	R	B	B
Temperature (°C)		38.4	38.4	38.8	37.9	37.8	37.7	38.1	38.1
Bodyweight (g)		357	415	438	439	379	383	403	423
GRIP STRENGTH (kg)#									
Forelimb		0.99	1.29	0.96	1.37	1.11	1.13	1.19	0.89
Hindlimb		0.99	0.98	0.82	0.80	0.69	0.87	0.76	0.85
FOOTSPRAY (cm) #		11.9	11.2	8.9	8.5	11.2	11.0	10.0	9.6

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 11 Slight brown nasal staining  
In the arena: sat in corner
- 12 Slight hairloss on neck  
In the arena: occasional clicking of teeth
- 14 In the arena: sat in corner, partial to half closed eyes on occasions  
During righting reflex: urination observed, right hindlimb tucked under body  
Pupil reflex: left pupil dilated  
During temperature: slight body tremors
- 15 In the arena: sat in corner, partially closed eyes on occasions  
Pupil reflex: left pupil, slow response  
During manipulations: slight salivation
- 16 In the arena: sat in corner, partial to half closed eyes on occasions  
Pupil reflex: slow response

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 3 males							
		17	18	19	20	21	22	23	24
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	N	N	N	N
IN THE ARENA									
Tremors		N	B1	B1	B1	N	N	N	N
Grooming		Y	N	Y	N	N	Y	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		18	2	5	5	17	18	15	1
Arousal		4	3	3	4	4	4	4	2
Rearing count		15	1	6	6	10	11	15	0
Bolus count		0	0	4	0	0	0	0	4
Urine present		S	N	S	S	N	M	S	S
Gait		N	U	N	N	T2A1	N	N	U
MANIPULATIONS									
Approach		3	2	3	3	3	3	3	5
Touch		3	1	3	3	3	1	2	3
Startle		2	3	3	3	3	3	2	3
Righting reflex		1	1	1	1	1	1	1	1
Tail pinch		3	2	3	5	2	3	3	3
turns			2			2			
vocalises		Y2	Y2	Y2	Y2	Y3	Y2	Y1	Y2
Pupil reflex		R	B	R	R	B	B	B	B
Temperature (°C)		38.5	38.4	37.8	37.6	37.9	37.0	38.1	37.1
Bodyweight (g)		415	467	418	395	476	400	448	411
GRIP STRENGTH (kg) #									
Forelimb		1.24	1.41	1.40	0.98	0.91	0.78	1.13	1.39
Hindlimb		0.71	1.04	1.12	1.04	1.02	1.06	0.96	0.91
FOOTSPRAY (cm) #		12.5	12.6	9.7	8.5	13.6	11.2	9.1	11.3

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 17 Pupil reflex: left pupil no response
- 18 Slight hairloss on neck  
In the arena: sat in corner  
During temperature: slight body tremors
- 19 In the arena: partial to half closed eyes  
Tail pinch: slight salivation  
Pupil reflex: left pupil dilated
- 20 In the arena: hindfeet appeared to slip on one occasion  
During manipulations: soft faeces  
Righting: right hindlimb tucked under body  
Pupil reflex: left pupil dilated
- 21 In the arena: tail appeared slightly elevated  
Righting: right hindlimb tucked under body
- 22 During manipulations: salivation observed at end
- 24 In the arena: lying in the arena for full 3 minutes,  
partial to half closed eyes  
Pupil reflex: slow response

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 4 males							
		25	26	27	28	29	30	31	32
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	2	3	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	Y1	N	N	N	N	N
Vocalising		N	N	N	Y2	N	N	N	N
IN THE ARENA									
Tremors		N	N	B1	B2	N	N	B1	B1
Grooming		N	N	N	N	Y	N	Y	Y
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		12	7	9	1	10	4	2	6
Arousal		4	4	4	2	4	3	4	4
Rearing count		16	5	13	1	6	5	4	12
Bolus count		0	1	0	4	0	0	0	0
Urine present		S	S	N	N	S	N	S	N
Gait		T1	N	T1HUI	U	N	N	N	N
MANIPULATIONS									
Approach		3	2	1	2	3	1	2	1
Touch		0	4	1	0	2	1	4	1
Startle		3	3	3	3	3	2	3	3
Righting reflex		1	1	1	1	1	2	1	1
Tail pinch		3	3	3	3	2	3	3	3
turns						2			
vocalises		Y1	Y2	Y2	Y2		Y3	Y1	
Pupil reflex		B	B	B	B	B	R	B	B
Temperature (°C)		39.1	38.6	38.3	38.0	36.8	37.7	36.8	37.7
Bodyweight (g)		388	354	443	391	482	392	392	374
GRIP STRENGTH (kg) #									
Forelimb		1.33	1.11	1.17	1.18	0.68	1.29	1.14	1.37
Hindlimb		1.20	1.23	1.10	1.22	0.92	1.10	0.85	0.85
FOOTSPRAY (cm) #									
		11.5	12.0	11.3	9.9	8.1	9.6	8.7	12.2

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 25 Touch response: walked backwards  
Righting: right hindlimb tucked under body
- 28 Slight hairloss on neck  
In the arena: sat in corner, continuous tremors, partial to half closed eyes on occasions  
Touch response: body tremors  
Approach response: body tremors  
During righting reflex: urination observed, left hindlimb tucked under body  
During temperature: slight body tremors
- 29 Moderate hairloss head and neck  
In the arena: occasional clicking of teeth
- 30 Pupil reflex: left pupil no response
- 31 In the arena: partial to half closed eyes  
Righting: left hindlimb tucked under body  
Pupil reflex: slow response
- 32 During temperature: slight body tremors  
In the arena: partial to half closed eyes on occasions  
Tail pinch: slight salivation  
During temperature: slight body tremors

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 1 females							
		33	34	35	36	37	38	39	40
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	3	2	2	3	2	3	2
Tremors		N	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	N	N	Y1	Y1	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	N
Grooming		Y	N	Y	N	N	N	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		7	20	19	12	1	25	19	10
Arousal		5	4	4	4	3	4	4	4
Rearing count		5	15	21	13	1	24	19	8
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		N	T1	T2	N	U	N	T1	T1
MANIPULATIONS									
Approach		2	3	0	3	3	3	3	3
Touch		4	3	1	3	1	0	0	1
Startle		4	3	3	2	3	3	3	3
Righting reflex		2	1	1	1	1	1	1	2
Tail pinch		3	3	3	3	5	2	3	3
turns							2		
vocalises		Y1		Y2	Y1	Y1	Y2	Y1	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.5	39.1	38.5	38.9	37.2	39.4	38.2	38.2
Bodyweight (g)		258	273	273	275	271	261	252	272
GRIP STRENGTH (kg)#									
Forelimb		0.94	1.14	1.10	0.89	1.08	0.90	0.77	1.17
Hindlimb		0.73	0.89	1.05	0.68	0.89	0.68	0.80	0.98
FOOTSPRAY (cm) #									
		7.6	11.1	8.3	8.3	7.7	7.6	7.1	9.5

# Values represent the mean of two trials

## Additional comments

## Animal no.

34 Tail pinch: slow reaction  
 35 Slight hairloss on neck  
 Approach response: body tremors  
 37 In the arena: sat in corner  
 38 Touch response: walked backwards  
 39 Slight hairloss on neck  
 Touch response: walked backwards

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 2 females							
		41	42	43	44	45	46	47	48
IN THE CAGE									
Posture		S	S	S	CL	S	S	R	S
IN THE HAND									
Removing		3	2	2	2	2	2	2	2
Handling		3	2	2	3	2	2	2	2
Tremors		N	N	N	N	N	N	N	N
Salivation		Y1	N	Y1	N	N	N	N	N
Vocalising		Y1	N	Y1	N	N	N	N	N
IN THE ARENA									
Tremors		N	N	N	N	N	N	N	B1
Grooming		Y	N	N	N	Y	N	N	N
Pilo-erection		N	N	N	N	N	N	N	N
Activity count		23	1	24	19	24	10	25	7
Arousal		4	2	4	4	4	4	4	4
Rearing count		19	0	27	19	18	12	17	4
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		T2A1	U	T2	T1	N	T1	T2	N
MANIPULATIONS									
Approach		3	1	3	1	3	3	5	2
Touch		4	1	4	1	2	3	4	1
Startle		4	3	3	3	3	3	2	3
Righting reflex		1	1	1	1	1	2	2	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y2	Y1	Y2	Y2	Y2	Y2	Y2	Y3
Pupil reflex		B	B	B	B	B	B	B	R
Temperature (°C)		38.5	39.0	38.6	39.0	37.7	38.2	39.2	37.2
Bodyweight (g)		266	260	280	255	267	282	232	294
GRIP STRENGTH (kg) #									
Forelimb		0.64	1.15	1.15	1.06	0.86	1.15	0.92	1.07
Hindlimb		0.98	1.06	1.04	1.03	0.80	0.70	0.66	0.89
FOOTSPRAY (cm) #									
		9.1	10.3	9.7	10.2	8.5	8.0	8.4	10.7

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 41 In the arena: occasional walking on toes observed (marked)
- 42 In the arena: sat in corner  
Tail pinch: slow reaction
- 44 During manipulations: awkward to handle and vocalising
- 46 Slight brown nasal staining
- 47 Slight brown nasal staining
- 48 Pupil reflex: left pupil dilated  
During temperature: slight body tremors

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 3 females							
		49	50	51	52	53	54	55	56
IN THE CAGE									
Posture	S	R	S	CL	S	S	S	S	S
IN THE HAND									
Removing	2	2	2	2	3	2	2	2	2
Handling	2	2	3	3	3	2	2	2	2
Tremors	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N
Vocalising	N	N	N	Y2	Y1	N	N	N	N
IN THE ARENA									
Tremors	N	N	N	N	N	N	N	N	N
Grooming	Y	Y	Y	N	N	N	N	N	Y
Pilo-erection	N	N	N	N	N	N	N	N	N
Activity count	11	26	6	28	11	19	8	18	18
Arousal	4	4	3	4	3	4	3	4	4
Rearing count	10	20	8	26	4	20	6	16	16
Bolus count	0	0	0	0	0	0	0	0	0
Urine present	N	N	N	S	N	N	N	N	N
Gait	T1	T1	N	T2	T1	T1	T1	T1	N
MANIPULATIONS									
Approach	3	3	3	3	3	3	3	3	3
Touch	1	3	3	2	1	1	3	2	2
Startle	3	3	2	2	3	3	3	3	3
Righting reflex	1	2	1	1	1	1	1	1	1
Tail pinch	3	3	2	3	2	3	3	3	3
turns	1		2		3				
vocalises	Y2	Y2		Y1	Y2	Y1	Y2	Y1	Y1
Pupil reflex	L	B	B	B	B	B	B	B	B
Temperature (°C)	39.2	38.8	38.0	39.2	38.0	38.0	37.9	37.6	37.6
Bodyweight (g)	270	291	263	243	277	290	251	284	284
GRIP STRENGTH (kg) #									
Forelimb	0.90	1.12	1.09	0.92	0.84	0.81	0.98	1.12	1.12
Hindlimb	0.75	0.90	1.03	0.73	1.05	0.98	0.90	0.74	0.74
FOOTSPLAY (cm) #									
	9.5	10.8	10.5	4.6	10.9	9.4	9.5	8.0	8.0

# Values represent the mean of two trials

## Additional comments

Animal no.

- 49 Pupil reflex: right pupil constricted  
52 During manipulations: awkward to handle  
53 Slight hairloss on neck, brown nasal staining  
During manipulations: awkward to handle and vocalising  
56 Tail pinch: slow reaction

VCL/206 Week 4

OBSERVATIONS	Animal no	Group 4 females							
		57	58	59	60	61	62	63	64
IN THE CAGE									
Posture		S	S	S	S	S	S	S	S
IN THE HAND									
Removing		2	2	2	2	2	2	2	2
Handling		2	2	3	2	3	2	2	2
Tremors		B1	N	N	N	N	N	N	N
Salivation		N	N	N	N	N	N	N	N
Vocalising		N	N	Y1	N	N	N	N	N
IN THE ARENA									
Tremors		B2	B2	N	N	N	N	B1	B1
Grooming		Y	N	Y	Y	Y	N	N	Y
Pilo-erection		N	N	Y	N	N	N	N	N
Activity count		7	1	18	14	5	6	5	5
Arousal		4	2	4	4	5	4	4	4
Rearing count		4	1	13	17	14	5	2	5
Bolus count		0	0	0	0	0	0	0	0
Urine present		N	N	N	N	N	N	N	N
Gait		N	U	T3H1	T2	T1	N	T1	T1
MANIPULATIONS									
Approach		1	2	3	3	3	2	3	1
Touch		1	1	2	4	2	1	1	1
Startle		4	3	3	3	3	3	3	3
Righting reflex		1	1	1	2	1	1	1	1
Tail pinch		3	3	3	3	3	3	3	3
turns									
vocalises		Y2	Y2	Y2	Y2	Y1	Y1	Y2	Y2
Pupil reflex		B	B	B	B	B	B	B	B
Temperature (°C)		38.2	37.9	37.7	37.1	38.7	39.0	37.4	37.4
Bodyweight (g)		288	247	271	269	287	276	258	270
GRIP STRENGTH (kg) #									
Forelimb		1.03	0.90	0.95	1.16	0.98	0.97	1.20	1.00
Hindlimb		1.07	0.94	1.12	1.05	1.15	0.82	0.98	1.11
FOOTSPRAY (cm) #									
		11.0	10.9	13.7	11.4	8.3	9.1	12.8	11.6

# Values represent the mean of two trials

## Additional comments

## Animal no.

- 57 Badly groomed appearance  
In the arena: partial to half closed eyes, continuous tremor
- 58 Badly groomed appearance, slight brown nasal staining, area anterior to urogenital region appears slightly swollen  
In the arena: sat head into corner, partial to half closed eyes  
Pupil reflex: response slow
- 59 Badly groomed appearance, slight brown nasal staining, slight brown staining neck and muzzle
- 60 Badly groomed appearance.  
In the arena: half closed eyes on occasions
- 61 Slight brown staining neck
- 62 Badly groomed appearance
- 63 During temperature: slightly body tremors
- 64 Badly groomed appearance, slight brown staining head and neck  
In the arena: half closed eyes on occasions  
Tail pinch: slow reaction

VCL 206/942848

**ISODECYL BENZOATE  
FORMULATION ANALYSIS**

**Authors:**

I. Suzanne Dawe,  
Lianne Martin.

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## INTRODUCTION

This report contains details of the analytical method used and the results obtained for:

The determination of concentrations of Isodecyl benzoate in dose formulations prepared for Day 1 of the study.

The determination of the stability (homogeneity and resuspendability) of Isodecyl benzoate in corn oil formulations.

The validation of the method of analysis for the determination of Isodecyl benzoate in corn oil formulations.

The formulations for this study were prepared as solutions of Isodecyl benzoate in corn oil by Pharmacy personnel at Huntingdon Research Centre Ltd.

## EXPERIMENTAL PROCEDURE

### ANALYTICAL PROCEDURE

#### Apparatus and instrumentation

High performance liquid chromatograph (HPLC):	As detailed in the chromatographic section.
Balances:	Mettler AT261, fitted with a LC-P45 printer. Sartorius 1712 MP8, fitted with a data print 7381.
Autodiluter:	Hamilton Microlab 1000.
Densitometer:	Stanton Redcroft, PAAR DMA 46.
General laboratory glassware.	

#### Reagents

Test material:	Isodecyl benzoate.
Supplier:	Velsicol Chemical Corporation.
Batch no.:	C5-8.
Stated purity:	95 - 99%.
Tetrahydrofuran:	Rathburn Chemicals Limited, HPLC grade.
Methanol:	Fisons plc, HPLC Solvent.
Sodium dihydrogen orthophosphate:	FSA Laboratory Supplies, AnalaR®.
Water:	Elgastat UHP-4, deionised reverse osmosis.

#### Sample extraction

A representative sample (approximately 1 ml) of test formulation was accurately weighed and dissolved in a suitable volume of tetrahydrofuran. The extract was appropriately diluted, initially using tetrahydrofuran and finally using mobile phase, to provide a solution containing Isodecyl benzoate in the expected concentration range 2 - 4  $\mu\text{g/ml}$ .

The final solution was filtered (Whatman® PURADISC™ 25PP, 0.45  $\mu\text{m}$ ) and the concentration of Isodecyl benzoate was quantified by high performance liquid chromatography using ultraviolet detection as detailed in the following section.

### Typical chromatographic conditions

#### High performance liquid chromatograph (HPLC):

Pump:	Waters Associates 510.
Autosampler:	Waters Associates 712.
Detector:	Spectra Physics Spectra 100.
Data Handling:	Perkin Elmer Nelson Access*Chrom.

Analytical column:	LiChrospher 100 RP18e, 5 $\mu\text{m}$ , 125 $\times$ 4 mm id, Merck Limited.
Guard column:	LiChrospher 100 RP18e, 5 $\mu\text{m}$ , 4 $\times$ 4 mm id, Merck Limited.
Column temperature:	Ambient (nominally 21°C $\pm$ 1°C).
Mobile phase:	Methanol/0.05M aqueous sodium dihydrogen orthophosphate (90/10 v/v).
Flow rate:	1.2 ml/minute.
Detector wavelength:	UV, 236 nm.
Injection volume:	40 $\mu\text{l}$ .
Sensitivity:	5 - 19 mV.
Retention volume:	7 ml.

### Calibration

A primary standard solution was prepared for each analytical occasion by dissolving an accurately weighed quantity (50 mg) of Isodecyl benzoate in methanol. Solutions for instrument calibration, containing Isodecyl benzoate in the concentration range 1 - 5  $\mu\text{g/ml}$ , were prepared by appropriate dilution of the primary standard using mobile phase.

Calibration solutions were injected onto the HPLC, at the beginning and end of each sample analysis sequence, using the conditions detailed in the previous section.

### Calculation

The peak response for Isodecyl benzoate in each calibration chromatogram was measured and calibration curves were constructed by linear regression of standard response versus standard concentration. The response of the peak observed at the characteristic retention volume for Isodecyl benzoate in sample and procedural recovery chromatograms was measured and the concentration of Isodecyl benzoate was determined using the following equation:

$$\text{Concentration, mg/ml} = \frac{Y - I}{S} \times \frac{V}{W} \times D \times 10^{-3} \times \frac{100}{R}$$

Where Y = Peak response in test chromatogram  
 I = Intercept derived from linear regression of calibration data  
 S = Slope derived from linear regression of calibration data  
 V = Dilution volume of sample (ml)  
 W = Weight of sample (g)  
 D = Density (g/ml)  
 R = Cumulative mean procedural recovery value at analysis

### Limit of detection

The limit of detection, defined as the concentration of Isodecyl benzoate in control matrix producing a peak response equivalent to  $3 \times$  baseline noise, was determined as 0.075 mg/ml.

### VALIDATION OF THE METHOD OF ANALYSIS

Procedural recoveries were prepared by fortifying control samples (1 ml) with known amounts of Isodecyl benzoate, either as a solution in tetrahydrofuran (inclusion levels  $< 20$  mg/ml) or as solid test material (inclusion levels  $\geq 20$  mg/ml). The prepared procedural recoveries were analysed in accordance with the analytical procedure.

The analytical procedure was validated at the low and high inclusion levels by determining six procedural recoveries at concentrations of 1 mg/ml and 200 mg/ml.

Procedural recoveries were determined for each inclusion level and analysed concurrently with test formulations.

**DETERMINATION OF CONCENTRATIONS OF ISODECYL BENZOATE IN DOSE FORMULATIONS PREPARED FOR DAY 1 OF THE STUDY**

Representative samples (approximately 20 ml) of freshly prepared dose formulations were thoroughly mixed by vigorous shaking and duplicate sub-samples (1 ml) were analysed in accordance with the analytical procedure.

**DETERMINATION OF THE STABILITY OF ISODECYL BENZOATE IN CORN OIL FORMULATIONS**

Freshly prepared specimen formulations (approximately 100 ml), containing Isodecyl benzoate at nominal concentrations of 1 mg/ml and 200 mg/ml, were each thoroughly mixed by inversion and magnetically stirred. After magnetic stirring for 5 minutes (0 hour) and 4 hours, samples (approximately 1 ml) were removed for analysis from points at approximately one-quarter, one-half and three-quarters the depth (representing the top, middle and bottom) of the formulation.

The magnetic stirring was discontinued and the remainder of each formulation was stored in the dark at ambient temperature during the day and refrigerated overnight. At a time point representing 24 hours after preparation, each formulation was re-mixed and sampled for analysis as above.

At each occasion, the three samples from each formulation were analysed in accordance with the analytical procedure.

## RESULTS

The mean concentrations of Isodecyl benzoate in dose formulations prepared for Day 1 of the study and the deviation of mean results from nominal values are summarised in Table 1. Mean results were within 5% of nominal concentrations. Individual analytical results and associated procedural recovery data are detailed in Table 2.

The results in Table 3 indicate that, at nominal concentrations of 1 mg/ml and 200 mg/ml, Isodecyl benzoate is stable in corn oil formulations during storage (ambient temperature during the day and refrigeration overnight) for 4 and 24 hours.

Procedural recovery data obtained during method validation and the determination of stability are presented in Table 4. The data demonstrate the accuracy and precision of the analytical method: a mean procedural recovery value of  $90.1\% \pm 6.05$  CV (n=7) was obtained for 1 mg/ml and  $93.1\% \pm 5.41$  CV (n=7) for 200 mg/ml. Results for the analysis of test samples were corrected for the appropriate mean procedural recovery value at analysis.

A typical calibration standard graph confirming the linearity of detector response for Isodecyl benzoate over the concentration range 1 - 5  $\mu$ g/ml is presented in Figure 1. Typical analytical chromatograms are presented in Figures 2 and 3. In Figure 2, the absence of a peak at the characteristic retention volume for Isodecyl benzoate in the control sample chromatogram demonstrates the specificity of the HPLC assay.

## CONCLUSION

The analytical results confirm that the doses were accurately formulated for Day 1 of the toxicity study. The results also confirm that specimen formulations were homogeneous and stable for a period representing the time from preparation to completion of dosing.

TABLE 1

Summary: mean concentrations of Isodecyl benzoate in dose formulations

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
1	Control	0	ND	-
	2	3	2.90	-3.3
	3	30	30.4	+1.3
	4	200	210	+5.0

ND None detected (<0.075 mg/ml)

RME Relative mean error, representing the deviation from nominal

TABLE 2

**Concentrations of Isodecyl benzoate in dose formulations  
(individual values)**

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean <sup>1</sup>
1	Control	0	ND	ND	ND		
	2	3	3.09	2.72	2.90	100.6	
	3	30	31.6	29.1	30.4	99.2	92.9
	4	200	214	207	210	98.3	

ND None detected (<0.075 mg/ml)

<sup>1</sup> Represents the cumulative mean procedural recovery value and includes procedural recovery data from Table 4

Results were calculated using unrounded figures and corrected for the appropriate mean procedural recovery value given in this Table

TABLE 3

## Stability of Isodecyl benzoate in corn oil formulations

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)				CV (%)	RME (%)
		Top	Middle	Bottom	Mean		
1	0	0.961	1.01	0.946	0.971	3.24	-
	4	1.06	1.05	0.980	1.03	4.28	+6.1
	24	1.02	0.928	0.983	0.978	4.91	+0.7
200	0	179	204	199	194	6.82	-
	4	209	174	186	190	9.18	-2.1
	24	204	207	201	204	1.33	+5.2

CV Coefficient of variation

RME Relative mean error, representing the deviation from time zero

Results were calculated using unrounded figures and corrected for the appropriate mean procedural recovery value in Table 4

TABLE 4

**Procedural recovery data for Isodecyl benzoate  
in corn oil formulations**

Analytical phase	Nominal fortification (mg/ml)	
	1	200
Validation	97.3	94.4
	82.3	88.8
	94.4	86.7
	85.4	95.7
	91.8	99.5
	86.4	88.5
Stability	93.0	97.8
Mean	90.1	93.1
CV (%)	6.05	5.41
Range	82.3 - 97.3	86.7 - 99.5
n	7	7

CV Coefficient of variation

n Number of determinations

Results are expressed as percent recovery and calculated using the following equation:

$$\% \text{ Recovery} = \frac{\text{Analysed concentration (mg/ml)}}{\text{Fortified concentration (mg/ml)}} \times 100$$

FIGURE 1

Typical calibration standard graph  
(Day 1)

Standard Sample	Component Area	Component Mass	Rel. St. Dev.
STD1	88612	41.408	
STD2	172571	82.816	
STD3	264217	124.22	
STD4	348253	165.63	
STD5	409297	207.04	

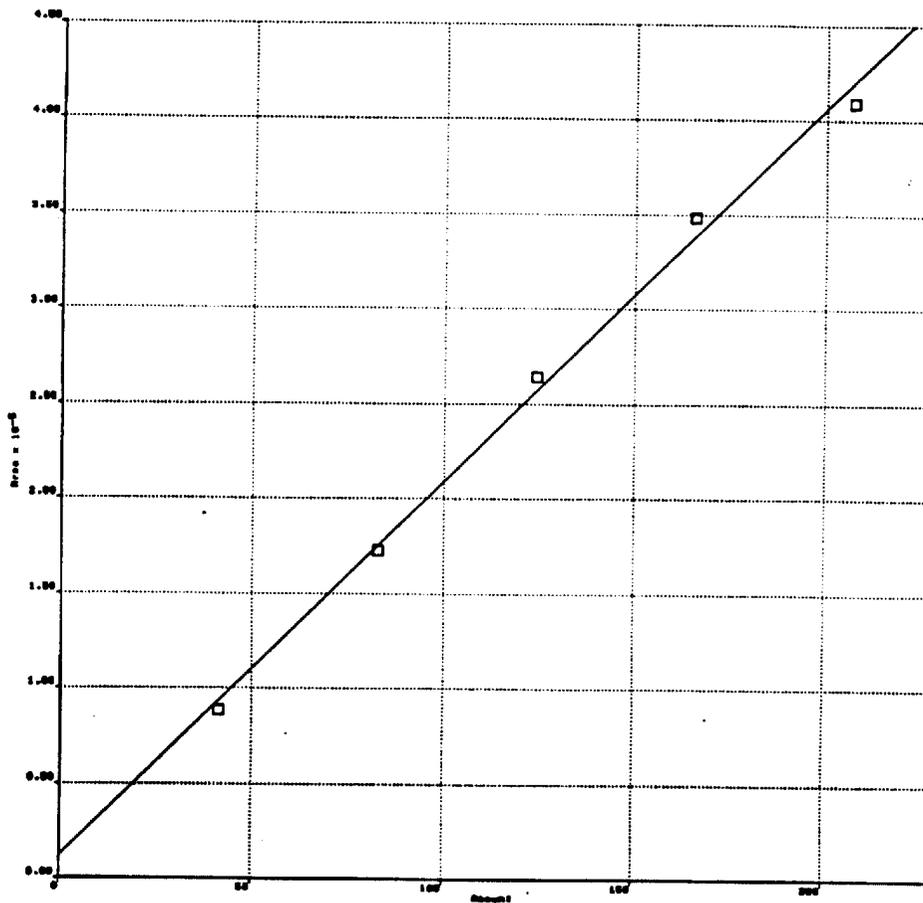
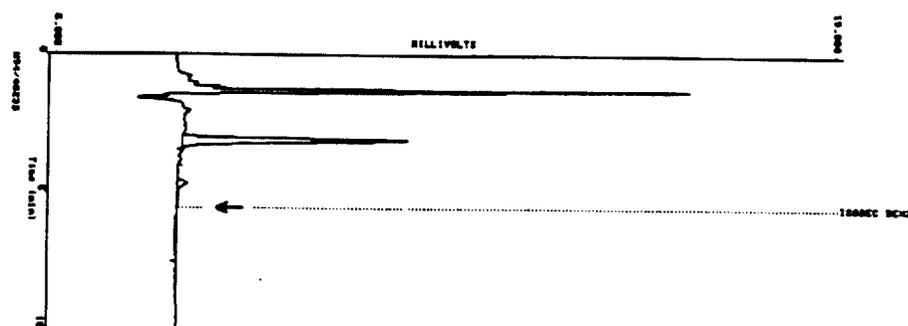


FIGURE 2

Typical sample chromatograms  
(Day 1)

Group 1, Control (1 g/500 ml)



Group 2, 3 mg/ml (1 g/1000 ml)

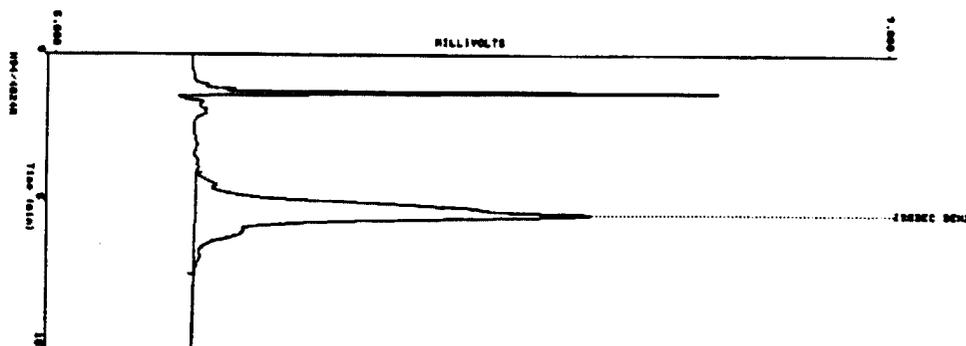
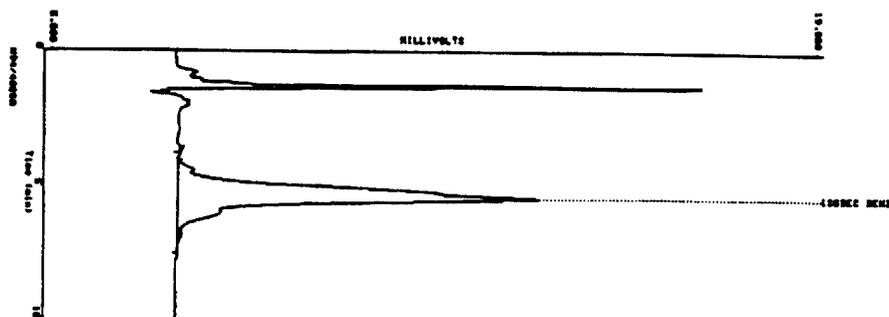


FIGURE 2

(continued)

Group 3, 30 mg/ml (1 g/10000 ml)



Group 4, 200 mg/ml (1 g/50000 ml)

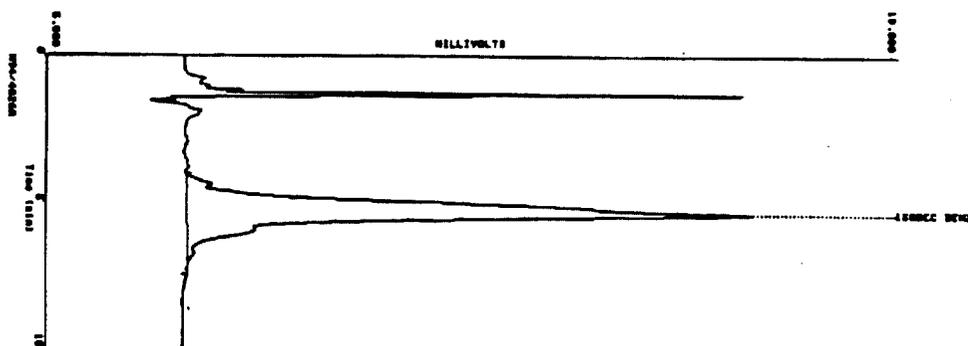
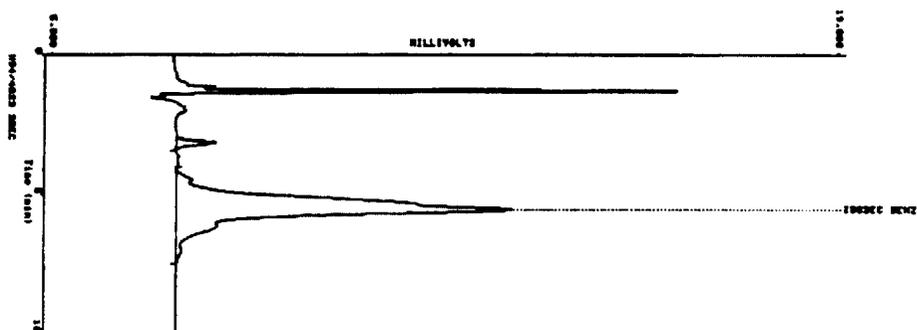


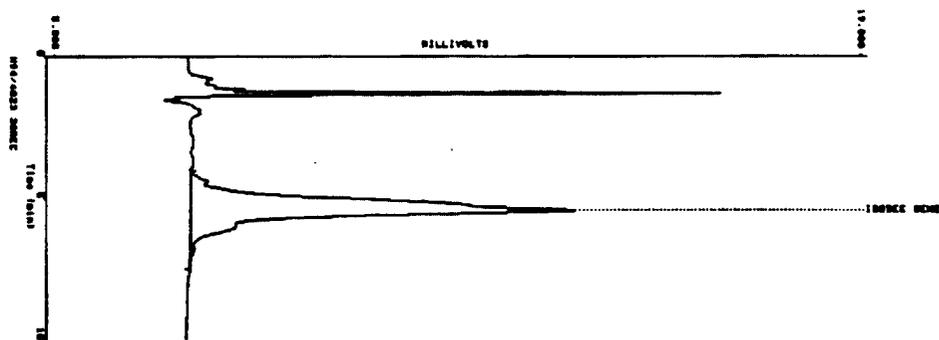
FIGURE 3

Typical procedural recovery chromatograms  
(Day 1)

3 mg/ml, 100.6%



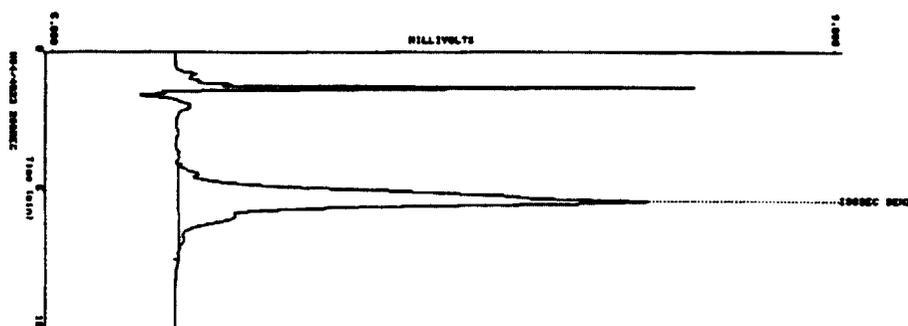
30 mg/ml, 99.2%



**FIGURE 3**

(continued)

200 mg/ml, 98.3%

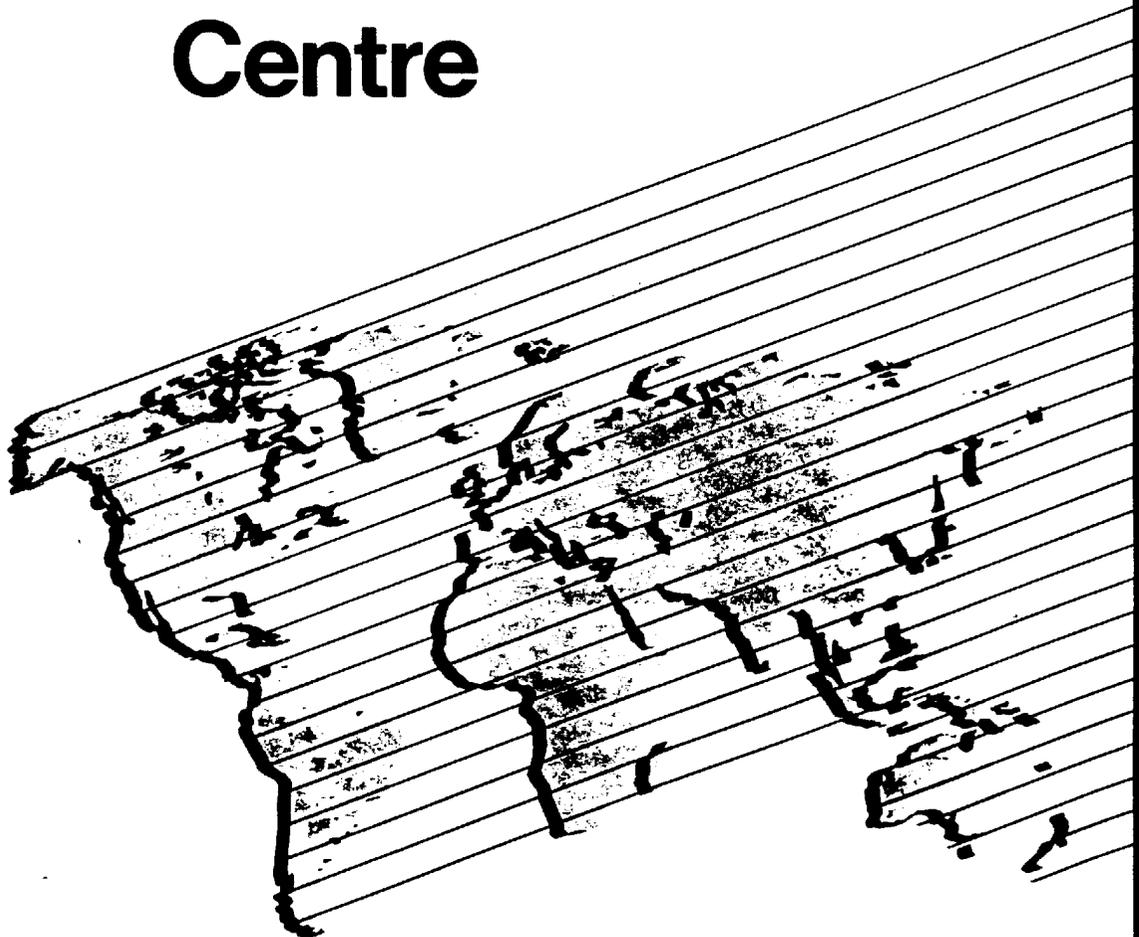


# **HRC** Report

**ISODECYL BENZOATE**

**TWENTY-EIGHT DAY ORAL TOXICITY  
STUDY IN THE RAT WITH FUNCTIONAL  
OBSERVATIONAL BATTERY**

**Huntingdon  
Research  
Centre**



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**Huntingdon Research Centre Ltd**

Huntingdon  
Cambridgeshire PE18 6ES  
England

***Telephone***

(01480) 890431

***Fax***

(01480) 890693

