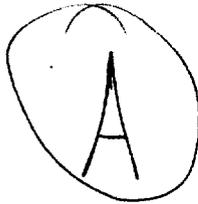


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Washington, D.C. 20460

ATTN: TSCA Section 8(e) Submission

- Ref: 1. [ ]
- 2. [ ], Twenty-eight day Oral Toxicity Study in the Rat (draft report), received July 28, 1993.
- 3. [ ], Acute Oral Toxicity to the Rat, report date 15 June 1992

03 AUG 17 AM 11:50  
OTS CBIC

Dear Sirs:

In compliance with the reporting requirements of TSCA Section 8(e) Substantial Risk Information, we are submitting the enclosed Draft Study Report (Ref. 2 & 3) for your review.

The draft report indicates the results of a twenty-eight day rat oral toxicity study performed on the following material manufactured by [ ] for which we filed a Premanufacture Notification [ ]:

Specific chemical name: [ ]  
Chemical Abstract Service number: [ ]  
Generic Name: BENZENEALKANENTRILE, 4-ALKYL-ALPHA, ALPHA-DIALKYL

The signs observed in the twenty-eight day study may indicate potential neurotoxic effects at high dose levels. Also observed were liver effects at high dose levels; however the significance of these effects are not known.

RECEIVED  
10-6-93

The potential neurotoxic signs observed in the twenty-eight day study are similar to the signs observed in the acute oral toxicity study. However, the signs in the acute oral study were transient. As a result, the acute oral toxicity study was not considered reportable under TSCA 8(e) at the time of receipt. Supported by the new information obtained from the twenty-eight day study, the results of the acute study suggest potential neurotoxicity, and we are submitting this study as required under TSCA Section 8(e).

We have changed the Material Safety Data Sheet to reflect the results of these studies. We have included additional statements related to the potential neurotoxicity and organ specific effects of the subject material. We will continue to advise the use of splash goggles or face shield when eye contact might occur, use of chemical resistant gloves, and use of a NIOSH approved respirator when inhalation of high concentrations may occur. The ventilation would meet ACGIH criteria.

As indicated in the PMN, the subject material will be used for fragrances to be used in cosmetic and household products. The maximum anticipated percent in a fragrance formulation was reported to be [ ]. The fragrance formulation will be further diluted in the final product. For example, a typical level of 0.5% of a fragrance formulation is used in these soap and detergents. Therefore, the final concentration of the subject material in a consumer product is anticipated to be very low. Because the potential neurotoxic effects were observed predominately at high dose level, we would not consider the levels to be used in the production of fragrance formulations and final products to represent a health risk to workers and consumers, respectively.

We have included sanitized and unsanitized versions of the Study Reports and request that you maintain this communication as "CONFIDENTIAL BUSINESS INFORMATION".

If you have any questions or comments, please contact me at [ ].

[ ]

CONFIDENTIAL

NOTE

This report is considered by the Study Director to be the 'final draft' and has been submitted to the HRC Quality Assurance Department for Audit.

The sponsor is requested to review this document and communicate any comments to the Study Director as soon as possible. When these comments have been received and on completion of the QA audit, the FINAL REPORT containing Study Director and QA Statements will be issued.

PLEASE NOTE

In compliance with GLP any changes to the final report after the date of issue will be in the form of a separate amendment to the report.

Date: 27 July 1993

V.1.

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JUL 28 1993

TWENTY-EIGHT DAY  
ORAL TOXICITY STUDY IN THE RAT

Addressee:

Testing facility:

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

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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.

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.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

---

Stuart M. Denton, B.Sc.,  
Study Director.  
Huntingdon Research Centre Ltd.

---

Date

7

## QUALITY ASSURANCE STATEMENT

This report has been audited by the Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

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

---

Huntingdon Research Centre Ltd.

(9)

# QUALITY ASSURANCE STATEMENT

## DATES OF STUDY INSPECTIONS

Inspections were made by the Quality Assurance Department of the various phases of the study described in this report. The dates on which the inspections were made and the dates on which the 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		
Pre-experimental Period		
Experimental Period		

---

Huntingdon Research Centre Ltd.

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(9)

## RESPONSIBLE PERSONNEL

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Stuart M. Denton, B.Sc.,  
Study Director,  
Department of Industrial Toxicology.

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Study Supervisor,  
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Head, Department of Analytical Chemistry & Pharmacy.

I. Suzanne Dawe, M.Sc., C.Chem., M.R.S.C.,  
Head of Formulation Analysis,  
Department of Analytical Chemistry & Pharmacy.

## SUMMARY

This study was performed to assess the systemic toxicity of \_\_\_\_\_ to the rat. The method followed was that outlined in Annex V, Part B, Method B7 in the EEC Directive 84/449/EEC and OECD Guideline for Testing of Chemicals No. 407 "Repeated dose oral toxicity - Rodent: 28-day or 14-day study".

\_\_\_\_\_ a clear colourless liquid, intended for use as a fragrance enhancer, was administered by oral gavage, once daily, to groups of five male and five female rats for a period of not less than twenty-eight consecutive days, at fixed dosage levels of 15, 150 and 500 mg/kg/day. The \_\_\_\_\_ substance was prepared as suspensions in corn oil at concentrations of 0.3, 3.0 or 10% w/v.

A further group of rats (five males and five females) was used as a concurrent control receiving the vehicle (corn oil) alone.

Bodyweights, food consumption and clinical observations were recorded during the study. Blood samples for clinical investigations were taken on Day 27 and all animals were killed and examined macroscopically (males on Day 29 and females on Day 30). Histological examination of specified tissues was then initiated.

The following comments relating to real or possible treatment effects are made in summary:

**Clinical signs.** Hunched posture and abnormal gait were observed on most occasions throughout the study for rats receiving 500 mg/kg/day. Abnormal gait was seen during the last few days of the study in rats receiving 150 mg/kg/day. This sign was also evident in one male and one female accompanied by hunched posture on Day 2 at 150 mg/kg/day.

**Haematology.** Longer thrombotest times were recorded for male and female rats treated at 500 mg/kg/day.

**Biochemistry.** At the 500 mg/kg/day level, increased values were observed for glucose, urea nitrogen, chloride (in males), glutamic-pyruvic transaminase and phosphorus (in females) and decreased values for cholesterol.

**Organ weights.** Increased adjusted liver weights were seen in males and females and lower adjusted spleen and absolute adrenal weights in males only were recorded for rats treated at 500 mg/kg/day.

Increased adjusted liver weights were also recorded in male rats dosed at 150 mg/kg/day.

**Macroscopic pathology.** Liver enlargement was observed in all male rats and 4/5 female rats treated at 500 mg/kg/day.

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**Microscopic pathology.** Centrilobular or generalised minimal enlargement of hepatocytes was seen in 5/5 males and 4/5 females receiving the high dosage level and 2/5 males receiving the intermediate dosage level. This was considered to be related to treatment. No similar changes were seen in males at the low dosage level or in controls, nor in any females from either control or other treated groups.

**Other findings.** There were no notable changes seen in the remaining parameters measured in this study namely bodyweight and food and water consumption.

### **Conclusion**

Based on the results of this study it was considered that administration of \_\_\_\_\_ at 150 mg/kg/day represents a no-observed toxic effect level (NOTEL) in the rat and that in accordance with EEC Council Directive 79/831/EEC, Annex VI, Part 11 (D), as described in Commission Directive 91/325/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, , intended for use as a fragrance enhancer, when repeatedly administered orally for a period of 28 consecutive days.

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

EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B7. Sub-acute toxicity (oral).

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

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 test substance was assessed for its toxicity to rats when administered orally, once daily for a minimum of twenty-eight consecutive days. Dosage levels of 15, 150 and 500 mg/kg/day were selected on the basis of available toxicity data (rat acute oral  $LD_{50} > 2$  g/kg, HRC Report no. 131/AC) and a 7-day preliminary oral toxicity study in rats (HRC Schedule no. Appendix 9) conducted at HRC. The results of the preliminary study indicated that the limit dose of 1000 mg/kg/day would not be tolerated by the rat, so the high level dosage was selected as 500 mg/kg/day.

The study protocol was approved by the Study Director and HRC Management on 26 January 1993 and by the Sponsor on 1 February 1993.

Rats were received from the supplier on 27 January 1993 and dosing commenced on 4 February 1993. Rats were killed following a four-week treatment period (males on 4 March and females on 5 March 1993).

The experimental phase of the study was conducted between 11 January 1993 and 14 July 1993.

## TEST SUBSTANCE

Identity:

Chemical name:

Lot number:

5364

Expiry:

10 January 1993

Purity:

> 98%

Appearance:

Clear colourless liquid

Storage conditions:

Room temperature in the dark

Date received:

3 January 1992

## EXPERIMENTAL PROCEDURE

### ANIMAL MANAGEMENT

A total of 22 male and 22 female healthy CD rats of Sprague-Dawley origin (CrI:CD® BR VAF PLUS™) was ordered from Charles River (U.K.) Ltd., Margate, Kent, England.

The rats were  $28 \pm 1$  days old, in a weight range of 65 to 91 g on arrival (27 January 1993). An eight-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 five 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 6).

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 7).

The rats were housed in Building R17, Room 7. Animal room temperature was controlled in the range 19 to 22°C and relative humidity was controlled in the range of 29 to 57% RH, the slightly low humidity value (29% RH) was recorded during the treatment period on Days 8, 9 and 12, however, the low humidity values were not considered to have affected the integrity of the study. 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.

Two days before the start of treatment each animal was weighed and forty rats were randomly allocated to four groups, each consisting of five males and five 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 five rats) constituting each group were distributed in batteries in such a manner that possible environmental influences arising from their spatial distribution were 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

The test substance was prepared initially in corn oil (high dose level). A series of formulations were prepared by further direct dilution of the high dose level.

Formulations were prepared freshly each day.

The chemical stability and homogeneity of test substance formulations in corn oil were assessed prior to the start of treatment by HRC Department of Analytical Chemistry and were found to be satisfactory (Appendix 8).

Concentration analyses of formulations prepared for administration on Day 1 were also performed by HRC Department of Analytical Chemistry. Results of these analyses were found to be satisfactory and are presented in Appendix 8.

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 the 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 this laboratory (HRC Schedule no. . Other levels were selected on the basis of the key dosages relative to EEC labelling requirements.

Groups of ten rats were dosed as follows:

Group	Cage label/ colour code	Treatment	Dosages (mg/kg/day)	No. of rats		Rat numbers	
				♂	♀	♂	♀
1	White	Control - corn oil	-	5	5	1-5	21-25
2	Yellow		15	5	5	6-10	26-30
3	Green		150	5	5	11-15	31-35
4	Red		500	5	5	16-20	36-40

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 a similar dose volume.

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

Animals were treated once daily for at least twenty-eight consecutive days.

Prior to dosing, the test substance formulations were mixed by inversion ( $\times 20$ ).

## **OBSERVATIONS**

### **Clinical signs**

All animals were observed 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. On Saturdays and Sundays 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.

Gravimetric measurement of water consumption was initiated during Week 3 due to a suspected treatment-related effect on consumption seen early in the study.

## 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 all rats prior to termination (Week 4).

The collected blood samples were divided as follows:

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

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

All 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 counts (RBC)	$\times 10^9/\text{mm}^3$
Absolute indices:	
Mean corpuscular haemoglobin concentration (MCHC) Calculated: $\text{Hb (g/dl)} \times 100 \div \text{PCV (\%)} $	%
Mean corpuscular volume (MCV) Calculated: $\text{PCV (\%)} \times 10 \div \text{RBC } (\times 10^9/\text{mm}^3)$	fl
Platelet counts (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

Differential white blood cell count (Diff) - namely:

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

Additional blood film slides were prepared and examined for morphological abnormalities. Abnormal cells (see below) observed when examining the stained slides were recorded and included in the haematology appendix.

- 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) Calculated: Total protein (g/dl) minus Alb (g/dl)	g/dl
Albumin/Globulin ratio (A/G) Calculated from Total protein and Albumin concentrations	g/dl
Urea nitrogen (Urea Nitr)	mg/dl
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 a minimum of 28 days of treatment all animals were killed (males Day 29, females Day 30) by carbon dioxide asphyxiation and a complete autopsy undertaken. The order of sacrifice was determined using pre-set cage sequence. Specified organs were weighed and relevant tissue samples were fixed for microscopic examination.

### Organ weight

The following organs from each animal were dissected free of fat and weighed:

adrenals	liver
brain	ovaries
kidneys	spleen
testes (with epididymides)	

### Macroscopic pathology

The macroscopic appearance of the tissues of all rats was recorded and samples of the following 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)
duodenum	ovaries	thymus (where present)
eyes (Davidson's fluid as fixative)	pancreas	thyroid (with parathyroid)
femur (with joint)	pharynx	tongue
head	pituitary	trachea
heart*	prostate	urinary bladder
ileum	rectum	uterus
jejunum	salivary gland	vagina
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.

Microscopic examination of prepared slides (from tissues indicated under Macroscopic pathology) was carried out for all rats of Group 1 (control group) and Group 4 (high dosage group) killed on Day 29 or 30.

Following documented approval from the Sponsor, microscopic examinations were extended to include the livers of all male and female rats of the low and intermediate 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, organ weight and clinical pathology data:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 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 are expressed at the 5% (\*  $P < 0.05$ ) or 1% (\*\*  $P < 0.01$ ) level.

## REFERENCES

1. FISHER, R.A. (1932) *Statistical methods for research workers*, 4th ed., Oliver and Boyd.
2. MANTEL, N. (1963) *J. Amer. Statist. Ass.*, **58**, 690.
3. BARTLETT, M.S. (1937) *Proc. Roy. Soc., A*, **160**, 268.
4. KRUSKAL, W.H. and WALLIS, W.A. (1952/3) *J. Amer. Statist. Ass.*, **47**, 583 and **48**, 907.
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.

## GOOD LABORATORY PRACTICE

### Regulations

The study was conducted in compliance with the principles of Good Laboratory Practice as set forth in:

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.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

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

## **QUALITY ASSURANCE DEPARTMENT REVIEW**

The Department of Quality Assurance reviewed the study protocol prior to commencement of treatment and conducted inspections of the various phases of the study as required by the above Good Laboratory Practice principles. The dates on which the findings of these inspections were reported to the Study Director and to HRC Management are specified earlier in this report.

The final report was reviewed by HRC's Department of Quality Assurance comparing individual findings against raw data and comparing the statements and results presented in the report with individual data presented in the appendices of the report.

## **PROCEDURES**

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

## **ARCHIVES**

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

Such 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

### OBSERVATIONS

#### Clinical signs (Table 1, Appendix 5).

For rats receiving 500 mg/kg/day, hunched posture and abnormal gait were observed on most occasions throughout the study.

Abnormal gait was seen during the last few days of the study in rats receiving 150 mg/kg/day. This sign was also evident in one male and one female, accompanied by hunched posture on Day 2.

Additional signs were related to the mode of dosing or the nature of the dose formulation. Increased salivation frequently seen in all rats dosed at 500 and 150 mg/kg/day, was generally accompanied by wet fur. This sign is commonly observed in gavage studies and may be related to test substance palatability. Greasy fur, commonly seen during the latter half of the study in all rats dosed at 500 and 150 mg/kg/day, is usually observed when corn oil is used as a vehicle and may indicate the spreading of dose residue during grooming. Red/brown staining around the mouth, seen in all animals dosed at 500 mg/kg/day and the majority of animals at 150 mg/kg/day is related to the stress of the dosing procedure. Loose faeces were observed in the cage tray for all animals receiving 500 or 150 mg/kg/day on Days 28 and 29 and probably result from the anaesthesia administered during the blood sampling procedure. A swollen area around the right eye was noted for one male rat treated at 15 mg/kg/day from Day 27 to termination (Day 29); this observation was related to trauma caused during the blood sampling procedure on Day 27. No other signs were seen at this dosage level. None of these signs are considered to be toxicologically important.

No clinical observations were observed in the control group.

#### Bodyweights (Figure 2, Table 2, Appendix 1)

There were no apparent differences in actual bodyweights and no significant difference in overall bodyweight gains between control and treated rats of both sexes.

#### Food consumption (Figure 3, Table 3)

The food consumption fluctuated amongst the groups during the study. Notably a slight increase was observed during Weeks 3 and 4 for male rats receiving 500 and 150 mg/kg/day and for female rats receiving 500 mg/kg/day. However, in the absence of any effect on bodyweight, this change was considered to be coincidental.

## **Water consumption (Figure 4, Table 4)**

Gravimetric measurement during the third week of the study revealed increased consumption for males and females receiving 150 or 500 mg/kg/day in comparison with controls. This probably reflects the increased salivation seen in these animals and may arise from unpalatability of the test substance.

## **CLINICAL PATHOLOGY**

### **Haematology (Table 5, Appendix 2)**

Significantly longer thrombotest times ( $P < 0.05$  or  $0.01$ ) were recorded for male and female rats treated at 500 mg/kg/day in comparison with controls. The remaining statistical differences from controls were marginal, within the expected range and were therefore considered to be of no toxicological importance.

The occurrence of polychromasia and anisocytosis among young laboratory rats is not uncommon and at the incidence seen in this study (on one occasion for each condition) was not considered to be toxicologically significant.

### **Biochemistry (Table 6, Appendix 3)**

At the 500 mg/kg/day level, slight but statistically significant increases in urea nitrogen levels (males) and glutamic-pyruvic transaminase and phosphorus levels (females) and a decrease in cholesterol (males) were considered to be related to treatment.

The marginally lower alkaline phosphatase activities noted for females receiving 500 mg/kg/day were considered to be of no toxicological importance in the absence of a dosage relationship within the treated female groups.

Other statistically significant differences, namely glucose (males), chlorine (males), alkaline phosphatase (females) were marginal, within the expected range and therefore of no toxicological importance.

There were no statistically significant differences in biochemical parameters between controls and rats treated at 150 or 15 mg/kg/day.

## **TERMINAL PROCEDURES**

### **Organ weights (Table 7, Appendix 4)**

Statistically significantly higher ( $P < 0.01$ ) liver weights (adjusted to take bodyweight into consideration) in males and females and lower ( $P < 0.01$ ) adjusted spleen and ( $P < 0.05$ ) absolute adrenal weights in males only were observed for rats treated 500 mg/kg/day in comparison with controls.

Statistically significantly higher ( $P < 0.01$ ) adjusted liver weights were recorded in male rats dosed at 150 mg/kg/day in comparison with controls.

Covariate analysis revealed no other statistically significant differences between the organ weights of treated and control rats.

**Macroscopic pathology (Table 8, Appendix 5)**

The macroscopic examination performed at the terminal kill revealed the following change:

**Liver** - Enlargement was observed in all male rats and 4/5 female rats treated with 500 mg/kg/day compared with 1/10 control rats.

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

**Microscopic pathology (Table 9, Appendix 5)**

**Treatment-related findings** - Centrilobular or generalised minimal enlargement of hepatocytes was seen in 5/5 males and 4/5 females receiving the high dosage level and 2/5 males receiving the intermediate dosage level. This was considered to be related to treatment. No similar changes were seen in males at the low dosage level or in controls, nor in any females from either control or other treated groups.

**Incidental findings** - The other histological changes seen were within the normally observed range of changes for animals of this species and age and were not considered to be of any toxicological significance.

A minimally increased cellularity of the splenic white pulp of 2/5 male animals at the high dosage level. This spleen change is within the normal spectrum of splenic histological diversity and is not considered to be related to treatment. No changes were seen that might account for the weight decrease recorded for the spleen in male rats of the highest dosage group.

No changes were seen that might account for the weight decrease recorded for adrenal glands in male rats of the highest dosage group.

## DISCUSSION

In this rat subacute study with . . . , there was evidence of a treatment-related effect on the liver. Among rats dosed at 500 mg/kg/day, increased liver weights, macroscopically enlarged livers and centrilobular or generalised hepatocyte enlargement were noted. This is considered to be a toxic effect on the liver and is seen further in disturbances in biochemical parameters namely cholesterol level (males) and glutamic-pyruvic transaminase level (females). The low incidence of the microscopic liver change and, in the absence of any disturbances in biochemical parameters, the effect seen in the intermediate dosage group (150 mg/kg/day), namely increased liver weight, was considered to be a reduced continuation of the treatment-related effect seen for the high dosage animals and was considered to be an adaptive response.

The clinical signs observed in rats receiving 500 mg/kg/day (hunched posture and abnormal gait) were indicative of discomfort and poor health. The high dosage of 500 mg/kg/day was rejected as the dosage level at which no-toxic effects were observed.

As the only treatment-related effects seen among rats treated at 150 mg/kg/day was adaptive, this dosage level was chosen as the no-observed toxic effect level (NOTEL) for . . . in the rat.

No treatment-related effects were seen among rats treated at 15 mg/kg/day.

## CONCLUSION

Based on the results of this study it was considered that administration of \_\_\_\_\_ at 150 mg/kg/day represents a no-observed toxic effect level (NOTEL) in the rat and that in accordance with EEC Council Directive 79/831/EEC, Annex VI, Part 11(D), as described in Commission Directive 91/325/EEC, labelling with the R48 risk phrase is not required.

FIGURE 1

Group and cage arrangement in batteries

Group	Cage label/ colour code	Treatment	Dosages (mg/kg/day)	No. of rats		Rat numbers	
				♂	♀	♂	♀
1	White	Control - corn oil	-	5	5	1-5	21-25
2	Yellow		15	5	5	6-10	26-30
3	Green		150	5	5	11-15	31-35
4	Red		500	5	5	16-20	36-40

♂	
1	2
3	4

♀	
1	2
3	4

FIGURE 2

Bodyweights - group mean values

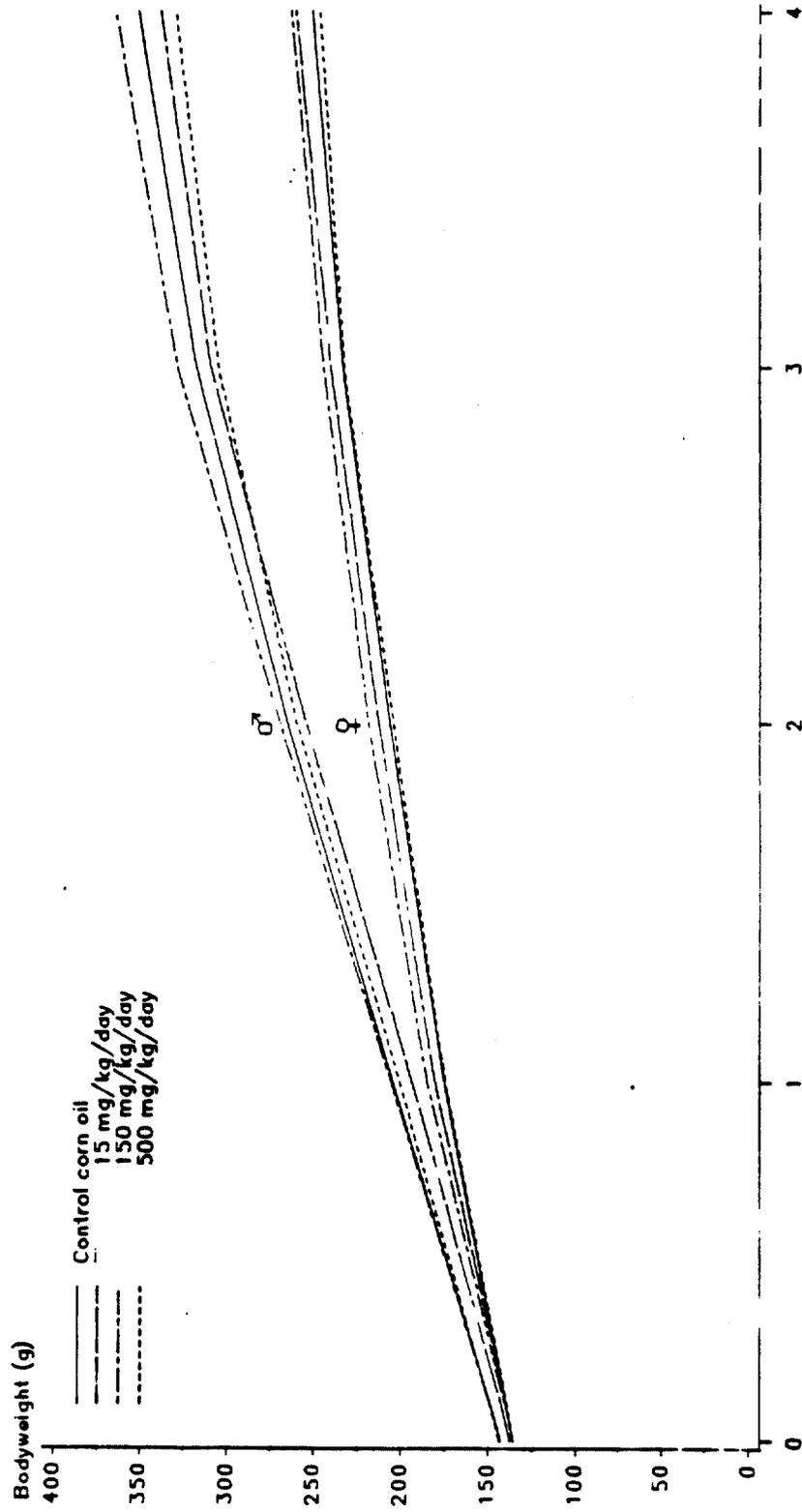


FIGURE 3

Food consumption - group mean values

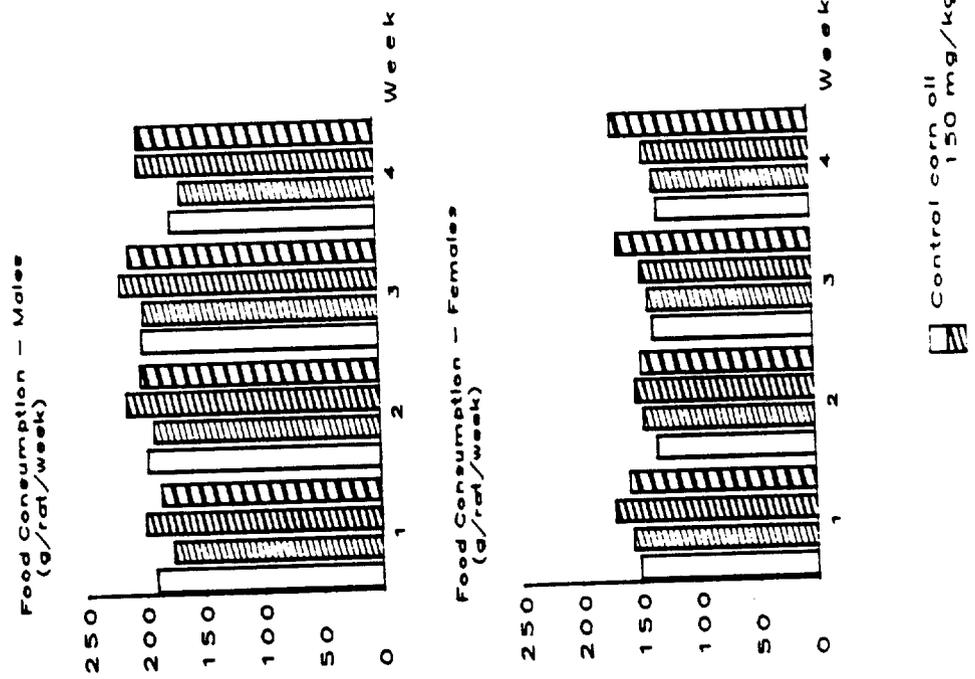


FIGURE 4

Water consumption - group mean values

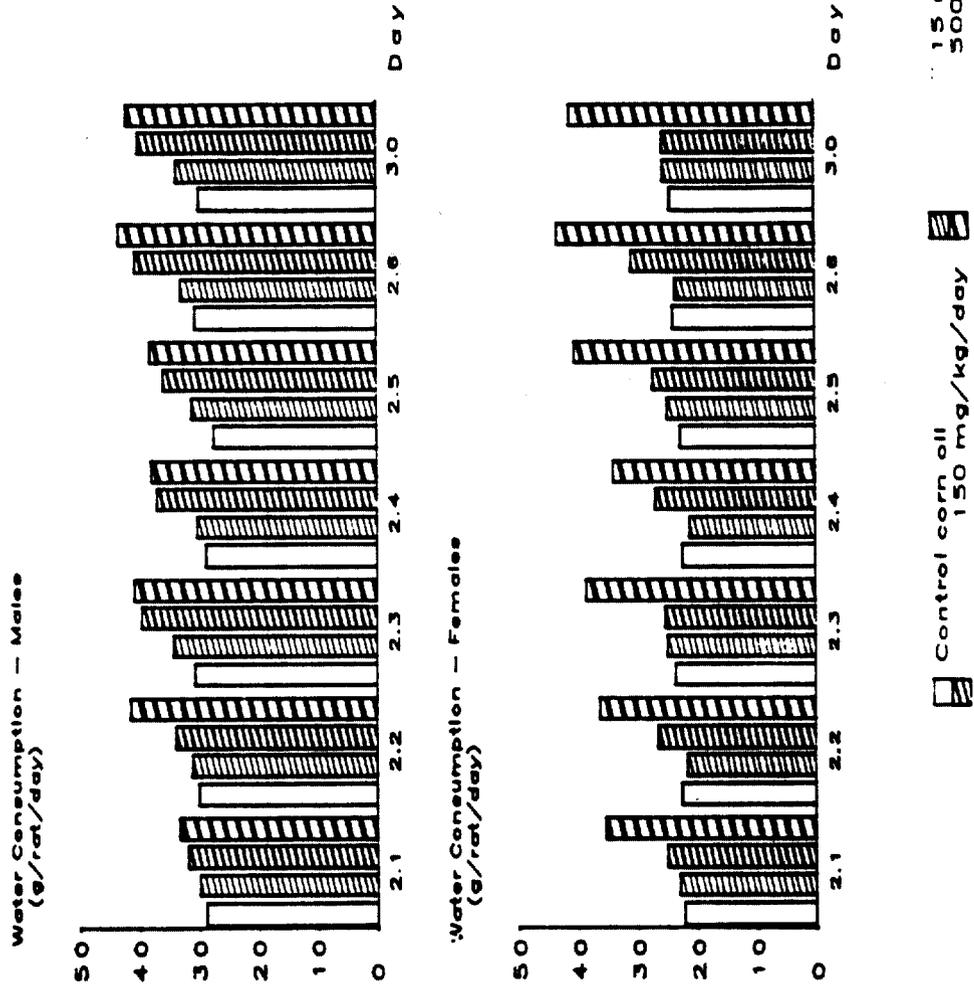


TABLE 1

Signs of reaction to treatment following oral administration of

Group/ dosage mg/kg/day	Clinical signs	Earliest appearance (Day)	Last appearance (Day)	Maximum consecutive duration (days)	Animal number				
					Maximum severity of observed signs				
					(1)	(2)	(3)	(4)	(5)
1♂ Corn oil	-	-	-	-	-	-	-	-	-
2♂ 15	-	-	-	-	(6)	(7)	(8)	(9)	(10)
					-	-	-	-	-
3♂ 150	Increased salivation	2	28	11	(11)	(12)	(13)	(14)	(15)
	Greasy fur	21	29	9	B	B	B	B	B
	Wet fur	23	28	6	A	A	A	A	A
	Hunched posture	2	2	1	A	A	A	A	A
	Abnormal gait - walking on toes	2	29	3	-	-	-	-	A
	Red/brown staining around mouth	4	9	1	+	+	+	+	+
	Loose faeces on cage tray	28	28	1	-	+	+	+	+
					20% loose faeces/ 80% normal faeces. Individuals not identified.				
4♂ 500	Increased salivation	2	28	27	(16)	(17)	(18)	(19)	(20)
	Greasy fur	20	29	10	B	B	B	B	B
	Wet fur	13	28	16	B	B	B	B	B
	Hunched posture	2	29	21	A	A	A	A	A
	Abnormal gait - walking on toes	2	29	21	+	+	+	+	+
	Red/brown staining around mouth	2	14	3	+	+	+	+	+
	Loose faeces on cage tray	28	29	2	40% loose faeces/ 60% normal faeces. Individuals not identified.				

A Slight response

B Moderate response

+/- Sign present or absent

TABLE 1

(Signs of reaction to treatment - continued)

Group/ dosage mg/kg/day	Clinical signs	Earliest appearance (Day)	Last appearance (Day)	Maximum consecutive duration (days)	Animal number				
					Maximum severity of observed signs				
1♀ Corn oil	-	-	-	-	(21)	(22)	(23)	(24)	(25)
					-	-	-	-	-
2♀ 15	-	-	-	-	(26)	(27)	(28)	(29)	(30)
					-	-	-	-	-
3♀ 150	Increased salivation	2	29	13	(31)	(32)	(33)	(34)	(35)
	Greasy fur	21	30	10	B	A	A	A	B
	Wet fur	23	28	6	A	A	A	A	A
	Hunched posture	2	2	1	-	-	-	-	A
	Abnormal gait - walking on toes	2	30	4	+	+	+	+	+
	Red/brown staining around mouth	2	9	1	-	+	+	+	+
	Diarrhoea on cage tray	28	28	1	20% diarrhoea/ 80% normal faeces. Individuals not identified.				
4♀ 500	Increased salivation	2	29	28	(36)	(37)	(38)	(39)	(40)
	Greasy fur	20	30	11	B	B	B	B	B
	Wet fur	13	29	17	B	B	B	B	B
	Hunched posture	2	30	22	A	A	A	A	A
	Abnormal gait - walking on toes	2	30	22	+	+	+	+	+
	Diarrhoea on cage tray	28	28	1	40% diarrhoea/ 60% normal faeces. Individuals not identified.				

A Slight response  
 B Moderate response  
 +/- Sign present or absent

**TABLE 2**

**Bodyweights - group mean values (g)**

Week	Group and dosage (mg/kg/day)							
	1♂ Corn oil	2♂ 15	3♂ 150	4♂ 500	1♀ Corn oil	2♀ 15	3♀ 150	4♀ 500
0	142	137	142	143	135	135	135	135
1	205	194	206	201	175	181	185	175
2	265	255	270	260	206	213	219	205
3	319	310	329	306	234	241	245	233
4	352	340	366	331	252	262	264	248
<b>Gain Weeks 0-4</b>	210	203	223	188	117	127	129	113

Blank Non-significant

**TABLE 3**

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

Week	Group and dosage (mg/kg/day)							
	1♂ Corn oil	2♂	3♂	4♂	1♀ Corn oil	2♀	3♀	4♀
1	190	176	200	186	151	156	171	159
2	197	191	214	202	134	145	152	147
3	200	198	218	210	137	140	146	166
4	174	165	201	201	131	134	142	168
Group mean cumulative value	761	730	833	799	553	575	611	640
% of control value	-	96	109	105	-	104	110	116

Statistical analysis not performed as only 1 cage/sex/group

TABLE 4

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

Week 3	Group and dosage (mg/kg/day)							
	1♂ Corn oil	2♂ 15	3♂ 150	4♂ 500	1♀ Corn oil	2♀ 15	3♀ 150	4♀ 500
Day 15	28.8	29.8	31.8	33.2	22.2	23.0	25.0	35.4
Day 16	30.0	31.0	33.8	41.4	22.6	21.6	26.6	36.4
Day 17	30.6	34.2	39.6	40.8	23.6	25.0	25.4	38.6
Day 18	28.8	30.2	37.0	38.0	22.4	21.2	27.0	34.0
Day 19	27.4	31.2	36.0	38.2	22.8	25.0	27.4	40.6
Day 20	30.6	33.0	40.8	43.6	24.0	23.6	31.0	43.4
Day 21	30.0	33.8	40.4	42.2	24.4	25.6	25.8	41.2
Group mean cumulative value	206.2	223.2	259.4	277.4	162.0	162.0	188.2	269.6
% of control value	-	108	126	135	-	102	116	166

Statistical analysis not performed as only 1 cage/sex/group

**TABLE 5**

**Haematology - group mean values**

Week 4 (2 March 1993)

Group/ dosage mg/kg/day	PCV %	Hb g/dl	RBC $\times 10^9/mm^3$	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♂ Corn oil	53	15.6	6.8	29.3	78	13.6	2.52	K 10.82	0.00	NA 0.00	0.28	1032	21
2♂ 15	52	15.5	6.8	29.6	77	13.4	3.09	10.03	0.10	0.00	0.18	1125	22
3♂ 150	53	15.8	6.8	29.7	78	16.5	2.89	13.52	0.00	0.00	0.13	1088	22
4♂ 500	52	15.7	6.9	** 30.2	76	14.4	1.74	12.58	0.09	0.00	** 0.00	1055	** 27
1♀ Corn oil	52	15.0	6.7	29.1	77	9.7	0.77	8.90	0.05	0.00	NA 0.00	K 1143	20
2♀ 15	52	15.2	6.7	29.2	77	10.7	1.60	9.09	0.03	0.00	0.02	1022	21
3♀ 150	52	15.1	6.8	29.2	76	10.4	0.92	9.47	0.02	0.00	0.01	1069	21
4♀ 500	52	15.4	6.8	29.5	77	10.2	1.29	8.79	0.04	0.00	** 0.10	971	* 23

Blank Non-significant

\* P<0.05

\*\* P<0.01

K Kruskal-Wallis analysis

NA No analysis necessary, all data values the same

TABLE 6

Biochemistry - group mean values

Week 4 (2 March 1993)

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/ mEq/	Ca mEq/ mEq/	P mEq/ mEq/	Cl mEq/ mEq/	Chol mg/dl	
		Total	Alb														Glob
1♂ Corn oil	104	6.2	2.9	3.3	0.88	10	0.5	496	28	57	0.1	142	3.8	5.2	5.0	95	74
2♂ 15	110	6.3	2.9	3.4	0.87	10	0.5	525	31	57	0.1	142	3.9	5.2	5.2	96	76
3♂ 150	112	6.2	2.9	3.3	0.88	14	0.5	511	33	58	0.1	143	4.1	5.2	5.5	96	70
4♂ 500	** 130	6.5	2.9	3.6	0.82	** 20	0.5	513	31	67	0.2	141	4.1	5.2	5.1	** 98	** 39
1♀ Corn oil	116	6.3	3.1	3.2	0.96	12	0.5	367	18	46	0.1	142	3.8	5.4	4.2	98	77
2♀ 15	107	6.1	3.0	3.1	0.97	12	0.5	252	19	48	0.1	142	3.9	5.4	4.2	97	75
3♀ 150	119	6.4	3.1	3.3	0.94	13	0.5	326	21	51	0.1	142	3.8	5.3	4.2	99	70
4♀ 500	134	6.4	3.1	3.3	0.94	15	0.6	* 264	25	51	0.2	143	3.7	5.4	4.8	* 98	62

Blank Non-significant

\* P<0.05

\*\* P<0.01

K Kruskal-Wallis analysis

F Frequency analysis

**TABLE 7**

**Organ weights - group mean values**

Week 5 (4 March 1993)

Group/ dosage mg/kg/day	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Corn oil	347	A 1.95 (1.94)	A 18.5 (18.0)	A 0.91 (0.89)	A 2.98 (2.94)	54.0	3.91
2♂ 15	335	1.94 (1.95)	19.0 (19.6)	0.75 (0.77)	2.92 (2.96)	48.6	3.96
3♂ 150	359	1.93 (1.91)	19.6 (18.2)	0.81 (0.77)	3.31 (3.21)	50.8	3.71
4♂ 500	326	1.90 (1.92)	25.9 ** (27.2)	0.62 ** (0.62)	3.13 (3.22)	46.9*	3.95

Blank Non-significant

\* P<0.05

\*\* P<0.01

A Values, adjusted for final bodyweight, given in parentheses

**TABLE 7**  
**(Organ weights - continued)**

Week 5 (5 March 1993)

Group/ dosage mg/kg/day	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Corn oil	250	A 1.81 (1.81)	A 12.4 (12.6)	A 0.61 (0.62)	A 2.27 (2.29)	67.2	92.5
2♀ 15	258	1.87 (1.86)	13.8 (13.4)	0.66 (0.64)	2.26 (2.23)	64.5	96.8
3♀ 150	262	1.88 (1.86)	15.5 ** (14.8)	0.62 (0.60)	2.16 (2.18)	66.8	102.5
4♀ 500	243	1.86 (1.88)	18.5 ** (19.3)	0.56 (0.59)	2.36 (2.42)	76.4	99.1

Blank Non-significant

\*\* P<0.01

A Values, adjusted for final bodyweight given in parentheses

TABLE 8

Macroscopic pathology incidence summary

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study Animals completed	5 5							
Fur Stained	1	0	0	0	0	0	0	0
Skin Scab/s	0	2	0	0	0	0	0	0
Eyes Damaged Congested	0 0	1 0	0 0	0 1	0 0	0 0	0 0	0 0
Incisors Pale	0	0	1	0	0	0	1	1
Lymph Nodes - Cervical Enlarged	5	5	3	5	1	3	1	2
Thymus Enlarged	0	0	0	0	0	0	1	0
Lungs Petechiae Not collapsed Congested	2 1 0	2 0 0	0 0 0	1 0 0	0 0 2	1 0 0	2 0 0	2 0 0
Heart 1 - subcapular area/s - median cleft Enlarged Pale subcapular area/s	1 1 0	0 1 0	0 1 1	0 5 1	0 0 0	1 0 0	0 1 0	1 4 0

**TABLE 8**  
**(Macroscopic 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 Animals completed	5 5							
Spleen Capsule thickened area/s Torsioned	0 0	0 1	1 0	2 0	2 0	1 0	0 0	1 0
Stomach White nodule/s, near to limiting ridge	0	3	0	0	0	0	0	0
Adrenals Enlarged	0	0	0	0	0	0	0	1
Kidneys Increased pelvic dilatation	0	0	1	0	0	0	0	0
Uterus Fluid distension	0	0	0	0	1	2	2	3

**TABLE 9**  
**Microscopic pathology incidence summary**

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
<b>Animals on study</b>	5	5	5	5	5	5	5	5
<b>Animals completed</b>	5	5	5	5	5	5	5	5
<b>Heart</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5
<b>Spleen</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5
<b>Extramedullary haemopoiesis (Total)</b>								
Minimal	3	3	3	3	3	3	3	3
Increased cellularity of the white pulp (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Capacular thickening (Total)	0	0	0	0	0	0	0	0
Trace	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
<b>Liver</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5
<b>Parenchymal inflammatory cells (Total)</b>								
Trace	1	1	1	1	1	1	1	1
<b>Centrilobular hepatocyte enlargement (Total)</b>								
Minimal	0	0	0	0	0	0	0	0
Congested sinusae	1	1	1	1	1	1	1	1
<b>Generalised hepatocyte enlargement (Total)</b>								
Minimal	0	0	0	0	0	0	0	0
<b>Kidney</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5

TABLE 9  
(Microscopic pathology incidence summary - continued)

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	5	5	5	5	5	5	5	5
Animals completed	5	5	5	5	5	5	5	5
(Continued)								
<b>Kidneys</b>								
Mineral foci at the corticomedullary junction (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Eosinophilic droplets in cortical tubular epithelias (Total)	1	0	0	2	0	0	0	0
Minimal	1	0	0	2	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>Lungs</b>								
Examined	2	0	0	1	2	0	0	2
No abnormalities detected	1	0	0	0	1	0	0	1
Congestion	1	0	0	1	1	0	0	1
<b>Lymph Nodes - Cervical</b>								
Examined	5	0	0	5	1	0	0	2
No abnormalities detected	0	0	0	2	0	0	0	0
Plasmacytosis	2	0	0	2	0	0	0	1
Lymphoid proliferation	0	0	0	1	0	0	0	0
Prominent germinal centres	4	0	0	0	1	0	0	1
Prominent distended venules containing small lymphocytes	2	0	0	2	0	0	0	1
<b>Uterus</b>								
Examined	0	0	0	0	1	0	0	3
Luminal dilatation (Total)	0	0	0	0	1	0	0	3
Minimal	0	0	0	0	1	0	0	3
<b>Eyes</b>								
Examined	0	0	0	1	0	0	0	0
No abnormalities detected	0	0	0	1	0	0	0	0

## APPENDIX I

### Bodyweights - individual values (g)

**Group 1♂**  
Corn oil

Cage number	Animal number	Week				
		0	1	2	3	4
1	1	149	208	270	323	356
	2	128	181	236	282	313
	3	139	200	254	294	320
	4	155	220	281	338	374
	5	139	215	286	356	399

**Group 2♂**  
15 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
2	6	134	191	243	296	327
	7	133	200	264	318	351
	8	123	173	242	299	314
	9	131	179	234	284	315
	10	162	225	291	353	391

**Group 3♂**  
150 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
3	11	141	209	278	348	385
	12	130	187	245	303	337
	13	149	218	284	347	397
	14	137	192	248	292	318
	15	154	223	293	356	391

**Group 4♂**  
500 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
4	16	148	196	251	297	320
	17	146	204	261	295	311
	18	134	196	265	330	369
	19	155	219	278	320	340
	20	132	190	245	287	314

## APPENDIX 1

### (Bodyweights - continued)

Group 1♀  
Corn oil

Cage number	Animal number	Week				
		0	1	2	3	4
5	21	136	187	225	254	277
	22	129	167	195	215	227
	23	135	181	215	245	268
	24	140	174	203	240	260
	25	137	167	194	217	229

Group 2♀  
15 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
6	26	134	179	215	240	259
	27	133	179	209	237	256
	28	138	185	225	256	278
	29	126	165	188	214	231
	30	143	196	228	259	284

Group 3♀  
150 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
7	31	133	184	217	246	272
	32	128	171	207	233	248
	33	140	186	215	239	257
	34	134	183	217	248	265
	35	140	202	238	261	279

Group 4♀  
500 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
8	36	134	168	193	219	232
	37	134	172	200	229	242
	38	135	179	208	236	245
	39	142	193	234	268	285
	40	130	163	188	215	235

## APPENDIX 2

### Haematology - individual values

Week 4 (2 March 1993)

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♂ Corn oil	1	52	15.2	6.6	29.2	79	11.4	1.82	9.35	0.00	0.00	0.23	904	21
	2	56	16.1	7.2	28.8	78	9.4	2.07	7.14	0.00	0.00	0.19	1008	21
	3	52	15.2	6.6	29.2	79	14.3	1.14	13.01	0.00	0.00	0.14	1091	21
	4	55	16.1	7.1	29.3	77	16.2	4.37	11.50	0.00	0.00	0.32	1116	20
	5	51	15.2	6.6	29.8	77	16.8	3.19	13.10	0.00	0.00	0.50	1039	20
	Mean SD	53 2.2	15.6 0.49	6.8 0.30	29.3 0.36	78 1.0	13.6 3.16	2.52 1.272	10.82 2.557	0.00 0.000	0.00 0.000	0.28 0.142	1032 83.0	21 0.5
2♂ 15	6	51	15.2	6.6	29.8	77	12.0	2.76	8.76	0.24	0.00	0.24	1077	22
	7	55	16.1	7.3	29.3	75	13.8	2.48	10.90	0.00	0.00	0.41	1023	21
	8A	50	14.6	6.4	29.2	78	12.0	3.00	8.64	0.12	0.00	0.24	1042	22
	9	52	15.6	6.7	30.0	78	13.0	2.86	10.01	0.13	0.00	0.00	1290	20
	10	53	15.8	6.9	29.8	77	16.2	4.37	11.83	0.00	0.00	0.00	1191	23
	Mean SD	52 1.9	15.5 0.58	6.8 0.34	29.6 0.35	77 1.2	13.4 1.74	3.09 0.738	10.03 1.373	0.10 0.101	0.00 0.000	0.18 0.177	1125 113.1	22 1.1
3♂ 150	11	51	15.4	6.4	30.2	80	18.4	6.62	11.78	0.00	0.00	0.00	1206	23
	12	55	16.4	7.1	29.8	77	14.4	1.44	12.82	0.00	0.00	0.14	1232	22
	13	55	16.4	6.9	29.8	80	20.8	4.16	16.43	0.00	0.00	0.21	1026	23
	14	53	15.2	6.9	28.7	77	12.6	1.26	11.21	0.00	0.00	0.13	1073	20
	15	52	15.6	6.8	30.0	76	16.5	0.99	15.35	0.00	0.00	0.17	903	21
	Mean SD	53 1.8	15.8 0.57	6.8 0.26	29.7 0.58	78 1.9	16.5 3.23	2.89 2.444	13.52 2.273	0.00 0.000	0.00 0.000	0.13 0.079	1088 135.0	22 1.3
4♂ 500	16	54	16.1	7.2	29.8	75	14.0	1.40	12.60	0.00	0.00	0.00	952	26
	17	53	15.8	6.9	29.8	77	14.0	1.54	12.32	0.14	0.00	0.00	910	33
	18P	49	15.2	6.4	31.0	77	14.3	1.29	12.87	0.14	0.00	0.00	1247	24
	19	52	15.8	6.9	30.4	75	15.0	2.25	12.60	0.15	0.00	0.00	1002	28
	20	53	15.8	6.9	29.8	77	14.7	2.21	12.50	0.00	0.00	0.00	1164	25
	Mean SD	52 1.9	15.7 0.33	6.9 0.29	30.2 0.54	76 1.1	14.4 0.44	1.74 0.458	12.58 0.199	0.09 0.079	0.00 0.000	0.00 0.000	1055 144.2	27 3.6

SD Standard deviation

A Anisocytosis

P Polychromasia

APPENDIX 2

(Haematology - continued)

Week 4 (2 March 1993)

Group/ dosage mg/kg/day	Animal no.	PCV %	Hb g/dl	RBC $\times 10^9/mm^3$	MCHC %	MCV fl	WBC + Diff $\times 10^3/mm^3$					Plts $\times 10^9/mm^3$	TT s	
							Total	N	L	E	B			M
1♀ Corn oil	21	51	14.6	6.7	28.6	76	8.1	0.73	7.37	0.00	0.00	0.00	1073	20
	22	55	15.4	7.2	28.0	76	6.8	0.75	5.92	0.14	0.00	0.00	1110	21
	23	50	14.8	6.4	29.6	78	10.5	0.95	9.56	0.00	0.00	0.00	1185	20
	24	53	15.4	6.8	29.1	78	13.4	0.94	12.33	0.13	0.00	0.00	1217	20
	25	50	15.0	6.3	30.0	79	9.8	0.49	9.31	0.00	0.00	0.00	1130	21
	Mean SD	52 2.2	15.0 0.36	6.7 0.36	29.1 0.79	77 1.3	9.7 2.52	0.77 0.188	8.90 2.428	0.05 0.074	0.00 0.000	0.00 0.000	1143 57.9	20 0.5
2♀ 15	26	53	15.6	6.8	29.4	78	8.2	1.80	6.31	0.00	0.00	0.08	1155	20
	27	51	15.2	6.6	29.8	77	12.1	2.42	9.68	0.00	0.00	0.00	817	25
	28	54	15.8	6.9	29.3	78	7.2	0.43	6.77	0.00	0.00	0.00	1083	19
	29	51	14.3	6.7	28.0	76	13.7	1.37	12.33	0.00	0.00	0.00	933	21
	30	51	15.0	6.5	29.4	78	12.5	2.00	10.38	0.13	0.00	0.00	1121	21
	Mean SD	52 1.4	15.2 0.58	6.7 0.16	29.2 0.69	77 0.9	10.7 2.86	1.60 0.757	9.09 2.531	0.03 0.058	0.00 0.000	0.02 0.036	1022 142.4	21 2.3
3♀ 150	31	49	14.6	6.4	29.8	77	13.6	1.63	11.97	0.00	0.00	0.00	1025	22
	32	53	15.2	7.0	28.7	76	8.2	0.74	7.38	0.08	0.00	0.00	1090	20
	33	51	15.2	6.5	29.8	78	11.1	0.78	10.32	0.00	0.00	0.00	823	21
	34	52	15.1	7.0	29.0	74	7.4	0.52	6.81	0.00	0.00	0.07	860	20
	35	53	15.2	6.9	28.7	77	11.8	0.94	10.86	0.00	0.00	0.00	1549	21
	Mean SD	52 1.7	15.1 0.26	6.8 0.29	29.2 0.56	76 1.5	10.4 2.58	0.92 0.423	9.47 2.255	0.02 0.036	0.00 0.000	0.01 0.031	1069 290.2	21 0.8
4♀ 500	36	51	14.9	6.5	29.2	78	10.8	0.97	9.72	0.11	0.00	0.00	907	22
	37	52	15.4	6.7	29.6	78	10.6	0.85	9.65	0.00	0.00	0.11	923	23
	38	55	16.1	7.2	29.3	76	10.1	1.21	8.59	0.10	0.00	0.20	1034	28
	39	49	15.0	6.2	30.6	79	10.1	1.72	8.28	0.00	0.00	0.10	1009	23
	40	54	15.5	7.2	28.7	75	9.5	1.71	7.70	0.00	0.00	0.10	982	21
	Mean SD	52 2.4	15.4 0.48	6.8 0.44	29.5 0.70	77 1.6	10.2 0.51	1.29 0.407	8.79 0.879	0.04 0.058	0.00 0.000	0.10 0.071	971 54.6	23 2.7

SD Standard deviation

APPENDIX 3

Biochemistry - individual values

Week 4 (2 March 1993)

Group/ dosage mg/kg/day	Animal no.	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	
1δ Corn oil	1	5.8	2.7	3.1	0.87	12	0.4	347	19	48	0.1	142	3.9	5.3	4.8	97	91	
	2	6.5	3.0	3.5	0.86	9	0.5	608	39	59	0.1	142	3.8	5.3	5.0	96	66	
	3	108	5.8	2.9	1.00	11	0.5	503	29	67	0.1	143	4.1	5.0	4.9	97	55	
	4	133	6.3	2.9	3.4	0.85	9	0.4	430	27	53	0.1	142	3.8	5.3	5.0	94	85
	5	93	6.4	2.9	3.5	0.83	11	0.5	591	27	57	0.1	143	3.4	5.3	5.4	93	75
	Mean SD	6.2 17.8	2.9 0.34	3.3 0.27	0.88 0.068	10 1.3	0.5 0.05	496 109.7	28 7.2	57 7.1	0.1 0.00	142 0.5	3.8 0.25	5.2 0.13	5.0 0.23	95 1.8	74 14.4	
2δ 15	6	6.4	3.0	3.4	0.88	12	0.5	453	28	51	0.1	141	3.8	5.3	5.1	95	84	
	7	123	6.5	2.9	3.6	0.81	8	0.5	511	37	56	0.1	144	3.8	5.4	4.9	97	85
	8	113	6.1	2.8	3.3	0.85	10	0.5	516	27	47	0.1	141	4.4	5.2	5.0	98	58
	9	98	6.2	2.9	3.3	0.88	12	0.4	528	33	64	0.1	142	3.8	5.0	5.2	95	81
	10	125	6.3	3.0	3.3	0.91	10	0.6	617	31	66	0.1	143	3.5	5.1	6.0	96	70
	Mean SD	6.3 14.4	2.9 0.16	3.4 0.13	0.87 0.038	10 1.7	0.5 0.07	525 59.0	31 4.0	57 8.2	0.1 0.00	142 1.3	3.9 0.33	5.2 0.16	5.2 0.44	96 1.3	76 11.5	
3δ 150	11	119	6.3	2.8	3.5	0.80	18	0.4	511	26	47	0.1	142	3.4	5.3	5.6	96	76
	12	129	6.5	3.0	3.5	0.86	12	0.5	606	32	66	0.2	142	4.0	5.2	4.9	96	63
	13	102	6.4	3.0	3.4	0.88	14	0.5	508	33	55	0.1	143	5.2	5.3	5.9	95	74
	14	97	6.0	2.8	3.2	0.88	8	0.4	470	32	62	0.1	142	3.7	5.1	5.1	95	95
	15	115	5.9	2.9	3.0	0.87	18	0.5	461	41	62	0.1	144	4.4	5.3	5.9	99	44
	Mean SD	6.2 13.0	2.9 0.26	3.3 0.22	0.88 0.061	14 4.2	0.5 0.05	511 57.5	33 5.4	58 7.5	0.1 0.04	143 0.9	4.1 0.70	5.2 0.09	5.5 0.46	96 1.6	70 18.7	
4δ 500	16	139	6.4	2.8	3.6	0.78	20	0.5	539	36	65	0.1	140	4.0	5.1	5.3	97	23
	17	130	6.0	3.0	3.0	1.00	26	0.6	431	29	62	0.1	141	3.7	5.1	5.4	99	33
	18	128	6.7	2.9	3.8	0.76	18	0.5	504	24	61	0.2	142	4.2	5.3	4.8	95	63
	19	129	6.8	3.0	3.8	0.79	19	0.6	499	31	67	0.1	141	4.5	5.1	5.1	98	34
	20	123	6.8	3.0	3.8	0.79	18	0.5	590	35	80	0.4	142	4.2	5.3	5.1	100	40
	Mean SD	6.5 5.8	2.9 0.34	3.6 0.35	0.82 0.099	20 3.3	0.5 0.05	513 58.3	31 4.9	67 7.6	0.2 0.13	141 0.8	4.1 0.29	5.2 0.11	5.1 0.23	98 1.9	39 14.9	

SD Standard deviation

APPENDIX 3

(Biochemistry - continued)

Week 4 (2 March 1993)

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/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
			Total	Alb													
19 Corn oil	21	127	6.3	3.0	3.3	0.91	9	0.4	377	18	45	0.1	143	3.9	5.4	4.1	98
	22	105	6.6	3.2	3.4	0.94	9	0.4	283	20	52	0.1	143	3.7	5.5	3.9	98
	23	112	6.0	2.9	3.1	0.94	13	0.4	464	18	46	0.2	141	4.2	5.3	4.1	99
	24	141	6.6	3.2	3.4	0.94	9	0.4	287	19	43	0.1	142	3.7	5.4	4.4	96
	25	93	5.8	3.0	2.8	1.07	19	0.7	424	13	46	0.1	143	3.5	5.2	4.3	100
	Mean	116	6.3	3.1	3.2	0.96	12	0.5	367	18	46	0.1	142	3.8	5.4	4.2	98
	SD	18.8	0.36	0.13	0.25	0.063	4.4	0.13	81.0	2.7	3.4	0.04	0.9	0.26	0.11	0.19	1.5
29 15	26	103	6.0	2.9	3.1	0.94	14	0.6	251	20	51	0.1	144	3.8	5.5	4.3	96
	27	99	5.8	2.9	2.9	1.00	13	0.5	220	20	53	0.1	141	4.2	5.3	4.3	99
	28	118	6.6	3.1	3.5	0.89	11	0.4	226	20	51	0.1	140	4.1	5.5	4.2	94
	29	101	6.1	3.2	2.9	1.10	12	0.6	238	12	37	0.1	140	3.3	5.3	4.3	97
	30	113	6.1	2.9	3.2	0.91	11	0.5	327	24	50	0.1	143	3.9	5.3	4.1	100
	Mean	107	6.1	3.0	3.1	0.97	12	0.5	252	19	48	0.1	142	3.9	5.4	4.2	97
	SD	8.3	0.29	0.14	0.25	0.085	1.3	0.08	43.4	4.4	6.5	0.00	1.8	0.35	0.11	0.09	2.4
39 150	31	115	6.5	3.3	3.2	1.03	15	0.6	306	22	55	0.1	140	4.0	5.3	4.4	98
	32	112	6.5	3.1	3.4	0.91	11	0.3	381	21	41	0.1	143	4.0	5.2	4.1	100
	33	121	6.3	3.0	3.3	0.91	14	0.5	328	16	49	0.1	143	3.6	5.3	4.4	99
	34	138	6.3	3.1	3.2	0.97	15	0.5	262	21	54	0.1	141	3.7	5.3	4.1	97
	35	109	6.5	3.0	3.5	0.86	10	0.6	353	23	54	0.1	141	3.9	5.4	4.2	99
	Mean	119	6.4	3.1	3.3	0.94	13	0.5	326	21	51	0.1	142	3.8	5.3	4.2	99
	SD	11.5	0.11	0.12	0.13	0.065	2.3	0.12	45.4	2.7	5.9	0.00	1.3	0.18	0.07	0.15	1.1
49 500	36	113	6.9	3.2	3.7	0.86	12	0.5	217	25	51	0.2	143	3.8	5.4	4.4	97
	37	127	6.1	3.2	2.9	1.10	14	0.5	236	21	45	0.1	142	3.3	5.3	4.3	100
	38	145	6.2	3.1	3.1	1.00	16	0.6	323	28	63	0.2	144	3.7	5.4	6.0	97
	39	159	6.5	3.0	3.5	0.86	15	0.6	317	20	45	0.1	141	4.5	5.5	5.0	96
	40	125	6.2	2.9	3.3	0.88	19	0.6	227	33	53	0.2	143	3.2	5.2	4.4	100
	Mean	134	6.4	3.1	3.3	0.94	15	0.6	264	25	51	0.2	143	3.7	5.4	4.8	98
	SD	18.1	0.33	0.13	0.32	0.107	2.6	0.05	51.6	5.3	7.4	0.05	1.1	0.51	0.11	0.72	1.9

SD Standard deviation

## APPENDIX 4

### Organ weights - individual values

Week 5 (4 March 1993)

Group/ dosage mg/kg/day	Animal no.	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Corn oil	1	353	1.82	15.9	0.88	2.83	49.8	3.91
	2	311	1.94	16.8	0.67	2.88	48.1	3.82
	3	313	1.90	16.6	0.70	2.83	54.2	4.06
	4	368	1.95	19.9	1.03	3.27	60.7	3.87
	5	392	2.12	23.3	1.25	3.07	57.2	3.90
	Mean SD	347 35.1	1.95 0.109	18.5 3.09	0.91 0.242	2.98 0.193	54.0 5.19	3.91 0.091
2♂ 15	6	324	1.99	17.0	0.85	2.67	45.0	3.72
	7	347	1.99	19.3	0.73	2.72	44.3	3.93
	8	309	1.86	16.0	0.78	2.53	55.1	4.19
	9	311	1.92	18.6	0.73	3.25	46.3	3.98
	10	383	1.96	24.2	0.68	3.43	52.3	3.98
	Mean SD	335 30.8	1.94 0.057	19.0 3.16	0.75 0.064	2.92 0.395	48.6 4.81	3.96 0.167
3♂ 150	11	379	1.84	20.6	0.87	3.18	50.5	3.54
	12	328	1.88	18.8	0.85	2.87	45.5	3.85
	13	391	2.00	24.2	0.83	3.57	49.9	3.88
	14	315	1.95	15.5	0.65	3.26	54.6	3.30
	15	381	1.98	19.1	0.85	3.68	53.4	3.97
	Mean SD	359 34.5	1.93 0.068	19.6 3.13	0.81 0.089	3.31 0.320	50.8 3.54	3.71 0.281
4♂ 500	16	314	1.88	23.1	0.59	2.97	46.8	4.03
	17	303	1.90	25.6	0.62	2.96	49.1	3.90
	18	367	2.04	30.4	0.72	3.46	50.3	4.13
	19	340	1.89	27.1	0.57	3.28	44.4	3.92
	20	308	1.79	23.1	0.59	2.96	43.9	3.76
	Mean SD	326 26.6	1.90 0.089	25.9 3.07	0.62 0.062	3.13 0.232	46.9 2.81	3.95 0.141

SD Standard deviation

APPENDIX 4

(Organ weights - continued)

Week 5 (5 March 1993)

Group/ dosage mg/kg/day	Animal no.	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Corn oil	21	278	1.85	13.2	0.55	2.15	72.0	70.9
	22	223	1.80	9.7	0.46	2.15	75.0	87.0
	23	264	1.80	13.1	0.77	2.43	62.6	102.6
	24	256	1.79	13.7	0.75	2.55	65.6	110.6
	25	231	1.80	12.3	0.53	2.09	61.0	91.6
	Mean SD	250 23.1	1.81 0.024	12.4 1.57	0.61 0.139	2.27 0.204	67.2 6.04	92.5 15.23
2♀ 15	26	251	1.86	12.9	0.54	2.10	62.9	92.0
	27	250	1.77	12.2	0.80	2.27	69.7	109.3
	28	278	2.02	15.6	0.51	2.32	72.5	86.8
	29	229	1.73	12.2	0.60	2.17	57.7	76.7
	30	283	1.97	16.2	0.83	2.45	59.8	119.3
	Mean SD	258 22.4	1.87 0.125	13.8 1.94	0.66 0.149	2.26 0.136	64.5 6.36	96.8 17.24
3♀ 150	31	268	1.82	15.6	0.64	2.21	53.5	111.9
	32	250	1.81	14.3	0.48	1.86	69.8	106.2
	33	254	1.90	15.2	0.74	2.09	70.8	100.6
	34	263	2.03	15.1	0.48	2.28	69.4	93.5
	35	273	1.82	17.1	0.76	2.37	70.4	100.5
	Mean SD	262 9.5	1.88 0.092	15.5 1.04	0.62 0.138	2.16 0.196	66.8 7.44	102.5 6.90
4♀ 500	36	231	1.78	18.5	0.56	2.15	69.9	110.3
	37	240	1.86	17.1	0.53	2.52	61.7	79.8
	38	240	1.80	18.8	0.52	2.57	103.7	98.6
	39	283	1.92	22.6	0.72	2.49	71.4	102.7
	40	223	1.92	15.4	0.49	2.09	75.3	104.1
	Mean SD	243 23.3	1.86 0.065	18.5 2.66	0.56 0.091	2.36 0.229	76.4 16.05	99.1 11.58

SD Standard deviation

## APPENDIX 5

### Clinical and pathological data relating to rats killed at termination

Group:	1	2	3	4
Compound:	Control			
Level (mg/kg/day):	0	15	150	500

In this appendix the clinical, macroscopic and microscopic findings relating to each animal are listed on one page. These findings are presented by an automated data collation system.

The following abbreviation is used:

W.N.L. - Within normal limits (macroscopic abnormality)



## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 1♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

#### Fur

Stained - periorbital region/s: (Right , Red , Brown , Minimal)

#### Lymph Nodes - Cervical

Enlarged: 8mm

#### Liver

Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Extramedullary haemopoiesis: (Minimal)

#### Kidneys

Eosinophilic droplets in corticl tubular epithelia: (Minimal)

#### Lymph Nodes - Cervical

Plasmacytosis

Prominent germinal centres

Prominent distended venules containing small lymphocytes

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 1♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Liver : (W.N.L.); Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 2♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 9mm

**Lungs**  
Petechiae: (A few)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Lymph Nodes - Cervical**  
Plasmacytosis

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals; Lungs : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:        "  
Dosage Level:    Corn oil  
Rat No/Sex:      3♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 7mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Extramedullary haemopoiesis: (Minimal)

**Lymph Nodes - Cervical**  
Prominent germinal centres

The following tissues were considered normal:

Heart; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 4♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 8mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Lymph Nodes - Cervical**  
Prominent germinal centres

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 5♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 10mm

**Lungs**

Petechiae: (A few)

Not collapsed

**Liver**

Enlarged: 23.292g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Extramedullary haemopoiesis: (Minimal)

**Liver**

Parenchymal inflammatory cells: (Trace , Foci)

Congested sinuses

**Lungs**

Congestion

**Lymph Nodes - Cervical**

Prominent germinal centres

Prominent distended venules containing small lymphocytes

**APPENDIX 5**

**(Pathology - continued)**

**Rat No/Sex: 5♂ - continued**

**MICROSCOPIC FINDINGS - continued**

**The following tissues were considered normal:**

**Heart; Kidneys; Adrenals**

**Pathologist: R.L.Gregson**

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 6♂ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 8mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound: ----  
Dosage Level: 15 mg/kg/day  
Rat No/Sex: 7♂ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

#### Skin Scabs

Infra-auricular region/s: (Right , One) 1mm

#### Lymph Nodes - Cervical

Enlarged: 9mm

#### Lungs

Petechiae: (A few)

#### Spleen

Torsioned: (Severe)

#### Stomach Antrum Mucosa

White nodule/s, near to limiting ridge: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 8♂ (Terminal)

### CLINICAL FINDINGS

The right eye was swollen following the blood sampling procedure on Day 27, 28 and 29. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Skin Scabs

Lip/s: (Right , Upper , One) 3mm

#### Eyes

Damaged: (Right)

#### Lymph Nodes - Cervical

Enlarged: 10mm

#### Stomach Antrum Mucosa

White nodule/s, near to limiting ridge: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

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

**Lymph Nodes - Cervical**

Enlarged: 8mm

**Stomach Antrum Mucosa**

White nodule/s, near to limiting ridge: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

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: 8mm

**Lungs**  
Petechiae: (A few)

**Liver**  
Enlarged: 24.193g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**  
Congested sinuses

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 11♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of the study and abnormal gait (walking on toes) on Day 27 to 29. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

Lymph Nodes - Cervical  
Enlarged: 10mm

Spleen  
Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

Liver  
Centrilobular hepatocyte enlargement: (Minimal)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 12♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of the study and abnormal gait (walking on toes) on Day 27 to 29 and red/brown staining around the mouth after dosing on Day 4. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Incisors

Pale: (Right , Lower)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 13♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of dosing, abnormal gait (walking on toes) on Day 27 to 29 and red/brown staining around the mouth after dosing on Day 4. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

Lymph Nodes - Cervical

Enlarged: 9mm

Liver

Enlarged: 24.174g

Pale subcapsular area/s: (One) median lobe, 2mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 14♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of dosing, abnormal gait (walking on toes) on Day 27 to 29 and red/brown staining around the mouth after dosing on Day 4, 7 and 9. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 15♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of dosing, abnormal gait (walking on toes) on Day 27 to 29 (and on Day 2 accompanied by hunched posture). Red/brown staining around the mouth was seen after dosing on Day 9 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 7mm

**Kidneys**

Increased pelvic dilatation: (Right , Minimal)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 16♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

#### Lymph Nodes - Cervical

Enlarged: 7mm

#### Liver

Enlarged: 23.098g

Pale subcapsular area/s: (A few , Punctate) left lobe

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Increased cellularity of the white pulp: (Minimal)

#### Liver

Congested sinuses

Generalised hepatocyte enlargement: (Minimal)

**APPENDIX 5**

**(Pathology - continued)**

**Rat No/Sex: 16♂ - continued**

**MICROSCOPIC FINDINGS - continued**

**The following tissues were considered normal:**

**Heart; Kidneys; Adrenals; Lymph Nodes - Cervical : (W.N.L.)**

**Pathologist: R.L.Gregson**

## APPENDIX 5

### (Pathology - continued)

Compound: -  
Dosage Level: 500 mg/kg/day  
Rat No/Sex: 17♂ (Terminal)

#### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

#### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 8mm

**Liver**  
Enlarged: 25.614g

**Spleen**  
Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

#### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Capsular thickening: (Trace)

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

**APPENDIX 5**

**(Pathology - continued)**

**Rat No/Sex: 17♂ - continued**

**MICROSCOPIC FINDINGS - continued**

**The following tissues were considered normal:**

**Heart; Kidneys; Adrenals; Lymph Nodes - Cervical : (W.N.L.)**

**Pathologist: R.L.Gregson**

## APPENDIX 5

### (Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 18♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

#### Lymph Nodes - Cervical

Enlarged: 8mm

#### Liver

Enlarged: 30.430g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Extramedullary haemopoiesis: (Minimal)  
Increased cellularity of the white pulp: (Minimal)

#### Liver

Centrilobular hepatocyte enlargement: (Minimal)  
Congested sinuses

#### Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Minimal)

#### Lymph Nodes - Cervical

Lymphoid proliferation

**APPENDIX 5**

**(Pathology - continued)**

**Rat No/Sex: 18♂ - continued**

**MICROSCOPIC FINDINGS - continued**

**The following tissues were considered normal:**

**Heart; Adrenals**

**Pathologist: R.L.Gregson**

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 19♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

#### Eyes

Congested: (Right , Minimal)

#### Lymph Nodes - Cervical

Enlarged: 9mm

#### Lungs

Petechiae: (A few)

#### Liver

Enlarged: 27.069g

#### Spleen

Capsule thickened area/s: (A few) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Capsular thickening: (Trace)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 19♂ - continued

**MICROSCOPIC FINDINGS - continued**

**Liver**

Congested sinuses  
Generalised hepatocyte enlargement: (Minimal)

**Lungs**

Congestion

**Lymph Nodes - Cervical**

Plasmacytosis  
Prominent distended venules containing small lymphocytes

The following tissues were considered normal:

Heart; Kidneys; Adrenals; Eyes : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 20♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 9mm

**Liver**  
Enlarged: 23.104g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

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

**Kidneys**  
Eosinophilic droplets in cortical tubular epithelia: (Minimal)

**Lymph Nodes - Cervical**  
Plasmacytosis  
Prominent distended venules containing small lymphocytes

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 20♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 21 ♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 22♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lungs**  
Congested

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**  
Parenchymal inflammatory cells: (Trace , Foci)

The following tissues were considered normal:

Heart; Spleen; Kidneys; Adrenals; Lungs : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 23♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 7mm

**Spleen**

Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Extramedullary haemopoiesis: (Minimal)

Capsular thickening: (Moderate , Area)

**Lymph Nodes - Cervical**

Prominent germinal centres

The following tissues were considered normal:

Heart; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 24♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

#### Uterus

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Uterus

Luminal dilatation: (Minimal)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 25♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

#### Lungs

Congested

#### Spleen

Capsule thickened area/s: (A few) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Capsular thickening: (Trace)

#### Liver

Congested sinuses

#### Lungs

Congestion

The following tissues were considered normal:

Heart; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 26♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 7mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 27♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

#### Lymph Nodes - Cervical

Enlarged: 9mm

#### Liver

Pale subcapsular area/s - median cleft: (One , Punctate)

#### Spleen

Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 28♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

#### Lungs

Petechiae: (A few)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 29♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

#### Uterus

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L. Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 30♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

Lymph Nodes - Cervical  
Enlarged: 10mm

Uterus  
Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 31 ♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Lungs

Petechiae: (A few)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 32♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, red/brown staining around the mouth on Day 4 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 33 ♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, red/brown staining around the mouth on Day 4 and 9 and loose faeces were seen on cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: (Minimal) 6mm

**Thymus**

Enlarged

**Lungs**

Petechiae: (A few)

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 34♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, red/brown staining around the mouth on Day 4 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Incisors

Pale: (Left , Lower)

#### Uterus

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 35 ♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30 (and on Day 2 accompanied by hunched posture) red/brown staining around the mouth on Day 2, 4 and 9 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Liver

Enlarged: (Minimal) 17.106g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Liver

Congested sinuses

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 36♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 3. Abnormal gait (walking on toes) and hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Lungs

Petechiae: (A few)

#### Liver

Enlarged: 18.507g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Extramedullary haemopoiesis: (Minimal)

#### Liver

Generalised hepatocyte enlargement: (Minimal)

The following tissues were considered normal:

Heart; Kidneys; Adrenals; Lungs : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 37♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and hunched posture was observed on the majority of occasions throughout the study and loose faeces on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Incisors

Pale: (Lower)

#### Liver

Enlarged: (Minimal) 17.057g

#### Uterus

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Liver

Generalised hepatocyte enlargement: (Minimal)

#### Uterus

Luminal dilatation: (Minimal)

**APPENDIX 5**

**(Pathology - continued)**

**Rat No/Sex: 37♀ - continued**

**MICROSCOPIC FINDINGS - continued**

**The following tissues were considered normal:**

**Heart; Spleen; Kidneys; Adrenals**

**Pathologist: R.L.Gregson**

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 38♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and slight hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 7mm

**Lungs**

Petechiae: (A few)

**Liver**

Enlarged: 18.823g

**Spleen**

Capsule thickened area/s: (A few) 1mm

**Adrenals**

Enlarged: 103.7mg

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Capsular thickening: (Trace)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 38♀ - continued

**MICROSCOPIC FINDINGS - continued**

**Liver**

Congested sinuses  
Generalised hepatocyte enlargement: (Minimal)

**Kidneys**

Mineral foci at the corticomedullary junction: (Minimal)

**Lungs**

Congestion

**Lymph Nodes - Cervical**

Plasmacytosis  
Prominent distended venules containing small lymphocytes

The following tissues were considered normal:

Heart; Adrenals : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 39♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and slight hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 8mm

**Liver**

Pale subcapsular area/s - median cleft: (One) 1mm

Enlarged: 22.577g

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Extramedullary haemopoiesis: (Minimal)

**Lymph Nodes - Cervical**

Prominent germinal centres

**Uterus**

Luminal dilatation: (Minimal)

**APPENDIX 5**

**(Pathology - continued)**

**Rat No/Sex: 39♀ - continued**

**MICROSCOPIC FINDINGS - continued**

**The following tissues were considered normal:**

**Heart; Liver : (W.N.L.); Kidneys; Adrenals**

**Pathologist: R.L.Gregson**

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 40♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and slight hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Uterus

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Liver

Generalised hepatocyte enlargement: (Minimal)

#### Uterus

Luminal dilatation: (Minimal)

The following tissues were considered normal:

Heart; Spleen; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 6

### Special Diet Services Rat and Mouse Maintenance Diet

#### Composition and quality assurance aspects of diet

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

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

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

#### Contaminants

#### Maximum concentration

Fluoride	20	mg/kg
Nitrate (as NaNO <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 6

(Aspects of diet - 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 7

### Quality assurance aspects of drinking water

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

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

These results include levels of:

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

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



**APPENDIX 8**

**The analysis of                    in corn oil**

**THE ANALYSIS OF  
IN CORN OIL FORMULATIONS**

**Authors:**

**Alan Anderson.  
I. Suzanne Dawe,  
S.Patel.**



**APPENDIX 8**  
**(Chemical analysis - continued)**

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## APPENDIX 8

### (Chemical analysis - continued)

#### INTRODUCTION

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

The determination of concentrations of                      n dose formulations prepared for Day 1 of of the study.

The determination of the chemical stability of                      in corn oil formulations.

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

The formulations for this study were prepared as solutions of                      in corn oil by Formulation personnel at Huntingdon Research Centre Limited.

## APPENDIX 8

(Chemical analysis - continued)

### EXPERIMENTAL PROCEDURE

#### ANALYTICAL PROCEDURE

##### Apparatus and instrumentation

High performance liquid chromatograph (HPLC):

Pump:

Autosampler:

Detector:

Integrator:

As detailed below or suitable alternative.

Spectra-Physics SP8770.

Waters Associates WISP model 710B.

Waters Associates model 481.

Spectra-Physics SP4270.

General laboratory glassware.

##### Reagents

Test material:

Supplier:

Batch no.:

Stated purity:

5364.

> 98%.

Acetonitrile:

Merck Ltd., Far UV HiPerSolv for HPLC

Tetrahydrofuran:

Rathburn Chemicals Ltd., HPLC grade

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 (50 or 100 ml) of tetrahydrofuran. The extract was appropriately diluted, initially using tetrahydrofuran and finally using mobile phase, to provide a solution containing the expected concentration range 2 - 4 µg/ml.

The final solution was filtered (Whatman PURADISC™ 25PP, 0.2µm) and the concentration of was quantified by high performance liquid chromatography using ultraviolet detection as detailed in the following section.

**APPENDIX 8****(Chemical analysis - continued)****Typical chromatographic conditions**

Analytical column:	Merck Limited, LiChrospher 100 RP-18e, 5 $\mu$ m, 125 mm x 4 mm ID.
Guard column:	Merck Limited, LiChrospher 100 RP-18e, 5 $\mu$ m, 4 mm x 4 mm ID.
Mobile phase:	Acetonitrile/water (1/1 v/v).
Flow rate:	1.0 ml/minute.
Detector wavelength:	UV, 215 nm.
Injection volume:	30 $\mu$ l.
Integrator attenuation:	16.
Retention volume:	Isomer I 9.5 ml. Isomer II 11.0 ml.

**Calibration**

A primary standard solution was prepared for each analytical occasion by dissolving an accurately weighed quantity (50 mg) of \_\_\_\_\_ in acetonitrile. Solutions for instrument calibration, containing \_\_\_\_\_ in the concentration range 1 - 5  $\mu$ 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.

## APPENDIX 8

### (Chemical analysis - continued)

#### Calculation

The peak response for each calibration chromatogram was measured with respect to each isomer 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 each isomer of in sample and procedural recovery chromatograms was measured and the concentration of was determined with respect to each isomer using the following equation:

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

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)

Results were corrected for the appropriate mean procedural recovery value at analysis.

#### Limit of detection

The limit of detection, defined as the concentration of 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

The analytical procedure was validated by fortifying a minimum of six samples (1 ml) of control vehicle with to concentrations of 1 mg/ml and 200 mg/ml, which were analysed in accordance with the analytical procedure. The test substance, , was added either as a solution in tetrahydrofuran (inclusion levels  $< 20$  mg/ml) or as neat test material (inclusion levels  $\geq 20$  mg/ml).

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

## APPENDIX 8

(Chemical analysis - continued)

### DETERMINATION OF CONCENTRATIONS OF . IN DOSE FORMULATIONS ANALYSED DURING 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 CHEMICAL STABILITY OF . IN CORN OIL FORMULATIONS

Representative samples (approximately 20 ml) of freshly-prepared specimen formulations, containing at nominal concentrations of 1 mg/ml and 200 mg/ml, were thoroughly mixed and duplicate sub-samples (approximately 1ml) were removed for analysis (0 hour).

The formulations were stored at ambient temperature in the dark. At time-points representing 4 hours and 24 hours storage, the formulations were mixed and sampled for analysis as above.

At each occasion, two sub-samples of each formulation were analysed in accordance with the analytical procedure.

## APPENDIX 8

### (Chemical analysis - continued)

#### RESULTS

The mean concentrations of \_\_\_\_\_ in dose formulations analysed on Day 1 of the study and the deviation of mean results from nominal values are summarised, with respect to both isomers, in Table 1. Mean results were within the range +2%/-6% of nominal concentrations. Individual analytical results and associated procedural recovery data are detailed in Table 2.

The results in Table 3 confirm that, at nominal concentrations of 1 mg/ml and 200 mg/ml, \_\_\_\_\_ is chemically stable in the corn oil formulation during storage in the dark at ambient temperature for 24 hours.

Procedural recovery data obtained during method validation and the determination of stability are presented in Table 4. The data confirm the precision and accuracy of the analytical method with respect to both isomers. Results for the analysis of the test sample are corrected for the appropriate mean procedural recovery value at analysis.

Typical calibration standard graphs are presented in Figure 1 which confirms the linearity of detector response for \_\_\_\_\_ over the concentration range 1 - 5 µg/ml. Typical analytical chromatograms are presented in Figures 2 and 3. In Figure 2, the absence of a peak at the characteristic retention volumes for each isomer of \_\_\_\_\_ in the control sample chromatogram demonstrates the specificity of the HPLC assay.

#### CONCLUSION

The analytical results confirm that the dose formulations were accurately prepared and were stable from the time of preparation to the completion of dosing.

**APPENDIX 8**

**(Chemical analysis - continued)**

**TABLE 1**

**Summary: mean concentrations of                      m dose formulations**

**1.1 With respect to isomer I**

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
1	Control	0	ND	-
	2	3	2.84	-5.3
	3	30	29.3	-2.3
	4	100	99.4	-0.6

**1.2 With respect to isomer II**

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
1	Control	0	ND	-
	2	3	2.93	-2.3
	3	30	30.0	0.0
	4	100	102	+2.0

**ND**    None detected (<0.075 mg/ml)

**RME**    Relative mean error, representing the deviation from nominal

**APPENDIX 8**

**(Chemical analysis - continued)**

**TABLE 2**

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

**1.1 With respect to isomer I**

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean
1	Control	0	ND	ND	ND		
	2	3	2.86	2.82	2.84	98.9	
	3	30	29.2	29.4	29.3	100.2	100.7
	4	100	99.2	99.5	99.4	100.5	

**2.2 With respect to isomer II**

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean
1	Control	0	ND	ND	ND		
	2	3	2.91	2.94	2.93	100.1	
	3	30	29.9	30.0	30.0	99.5	98.3
	4	100	104	101	102	99.8	

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

**APPENDIX 8**

**(Chemical analysis - continued)**

**TABLE 3**

**Chemical stability of 1 in corn oil formulations**

**3.1 With respect to isomer I**

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)			RME (%)
		Analysis 1	Analysis 2	Mean	
1	0	0.980	0.972	0.976	-
	4	0.962	0.986	0.974	-0.2
	24	1.01	0.995	1.00	+2.5
200	0	199	199	199	-
	4	198	199	199	0.0
	24	194	194	194	-2.5

**3.2 With respect to isomer II**

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)			RME (%)
		Analysis 1	Analysis 2	Mean	
1	0	0.990	0.991	0.990	-
	4	0.979	0.998	0.988	-0.2
	24	0.994	0.989	0.991	+0.1
200	0	202	203	203	-
	4	202	203	202	-0.5
	24	201	201	201	-1.0

ND None detected (<0.075 mg/ml)

RME Relative mean error, representing the deviation from time zero

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

## APPENDIX 8

(Chemical analysis - continued)

TABLE 4

Procedural recovery data for  
in corn oil formulations

(results are expressed as percent recovery)

4.1 With respect to isomer I

Analytical phase	Nominal level of fortification (mg/ml)	
	1	200
Validation	100.1	102.2
	100.6	101.8
	99.7	102.1
	100.0	102.0
	99.2	101.0
	100.0	101.6
Stability	100.0	102.0
	102.6	97.9
Mean	100.3	101.3
SD ( $\pm$ )	1.02	1.44
Range	99.2 - 102.6	97.9 - 102.2
n	8	8

SD Standard deviation  
n Number of determinations

**APPENDIX 8****(Chemical analysis - continued)****TABLE 4****(continued)**

(results are expressed as percent recovery)

**4.2 With respect to isomer II**

Analytical phase	Nominal level of fortification (mg/ml)	
	1	200
Validation	97.2	98.4
	97.5	97.6
	96.8	98.2
	96.9	98.1
	97.1	98.0
	97.5	97.7
Stability	98.4	101.0
	98.9	98.9
Mean	97.5	98.5
SD ( $\pm$ )	0.74	1.09
Range	96.8 - 98.9	97.6 - 101.0
n	8	8

SD Standard deviation  
n Number of determinations

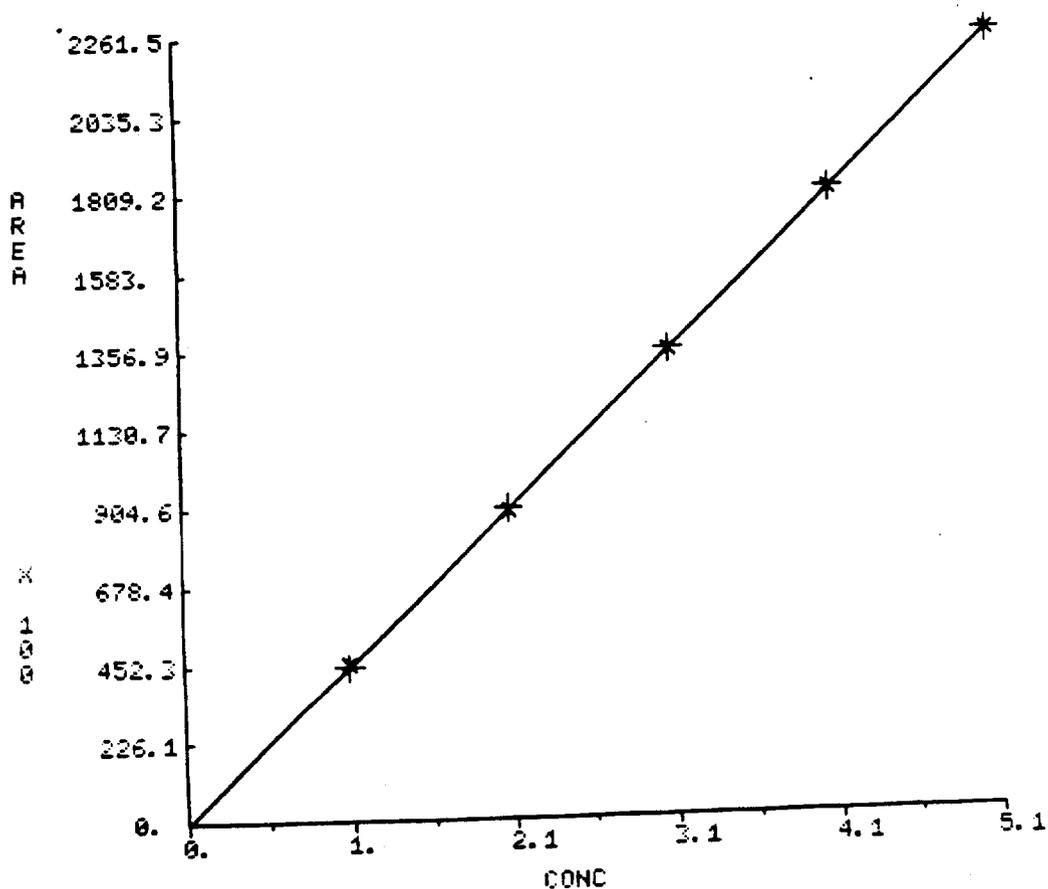
APPENDIX 8

(Chemical analysis - continued)

FIGURE 1

Typical calibration standard graph  
(Day 1)

With respect to isomer I



COEFFICIENTS OF LEAST SQUARES FIT TO A LINEAR EQUATION  
KA= 0.                    KB= 44065.805            KC=-528.29951  
CORRELATION COEFFICIENT OF X-Y PAIRS = 0.9999668  
COEFFICIENT OF DETERMINATION = 0.9999336

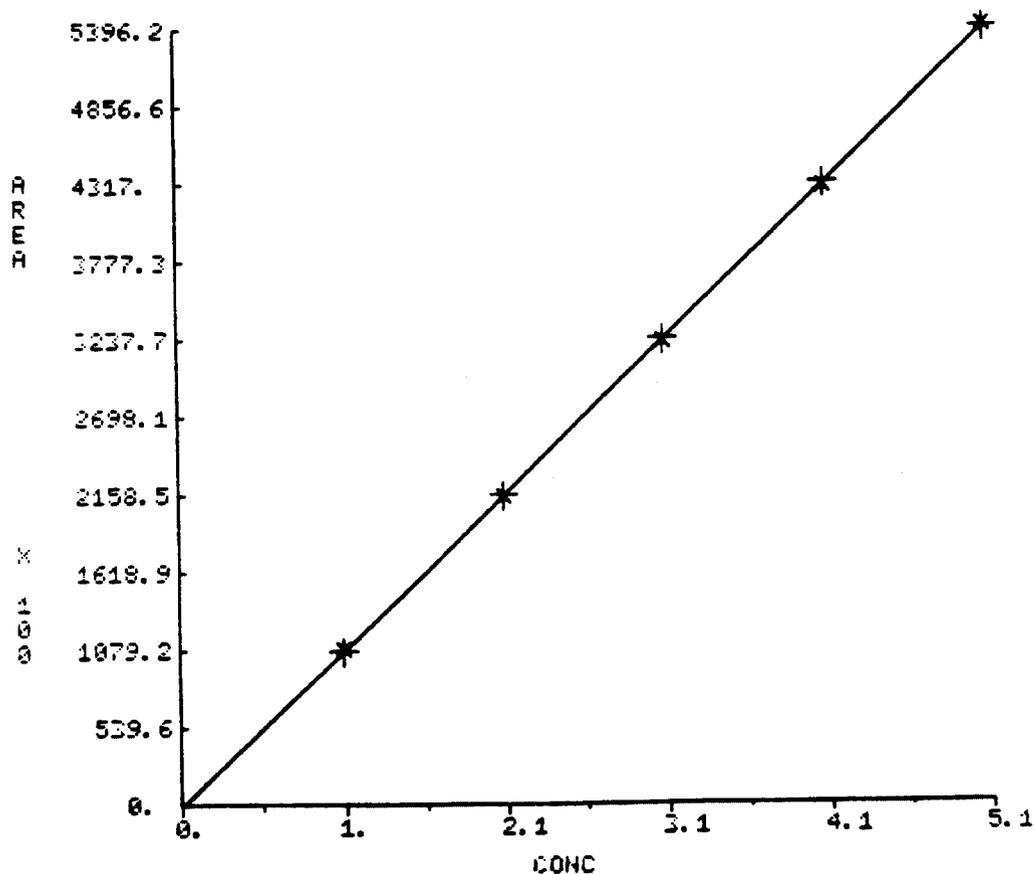
APPENDIX 8

(Chemical analysis - continued)

FIGURE 1

Typical calibration standard graph  
(Day 1)

With respect to isomer II



COEFFICIENTS OF LEAST SQUARES FIT TO A LINEAR EQUATION

KA= 0. KB= 105220.16 KC=-1632.8979

CORRELATION COEFFICIENT OF X-Y PAIRS = 0.9999423

COEFFICIENT OF DETERMINATION = 0.9998847

APPENDIX 8

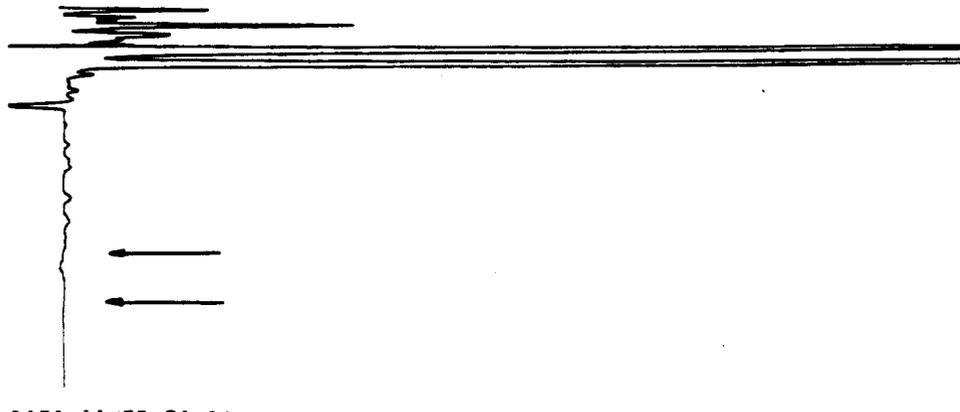
(Chemical analysis - continued)

FIGURE 2

Typical sample chromatograms  
(Day 1)

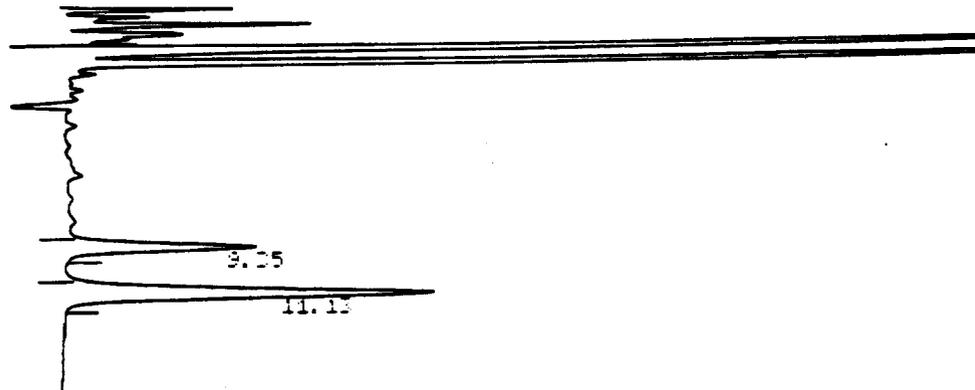
Group 1, Control (1 g/500 ml)

CHANNEL A INJECT 04-02-93 23:40:45 STORED TO BIN # 24



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

CHANNEL A INJECT 04-02-93 19:40:47 STORED TO BIN # 9



APPENDIX 8

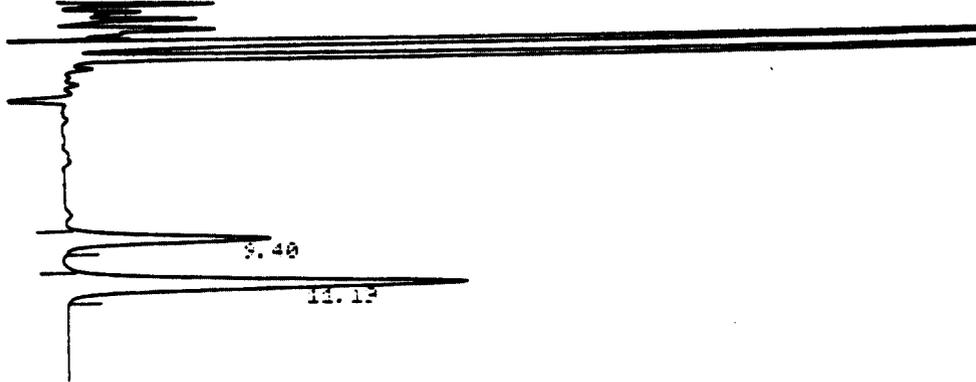
(Chemical analysis - continued)

FIGURE 2

(continued)

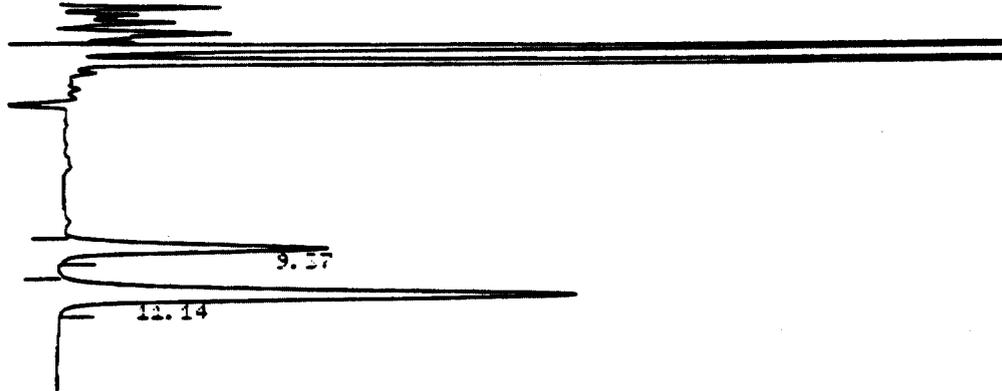
Group 3, 30 mg/ml (1 g/10000 ml)

CHANNEL A INJECT 04-02-93 21:00:46 STORED TO BIN # 14



Group 4, 100 mg/ml (1 g/25000 ml)

CHANNEL A INJECT 04-02-93 22:52:45 STORED TO BIN # 21



APPENDIX 8

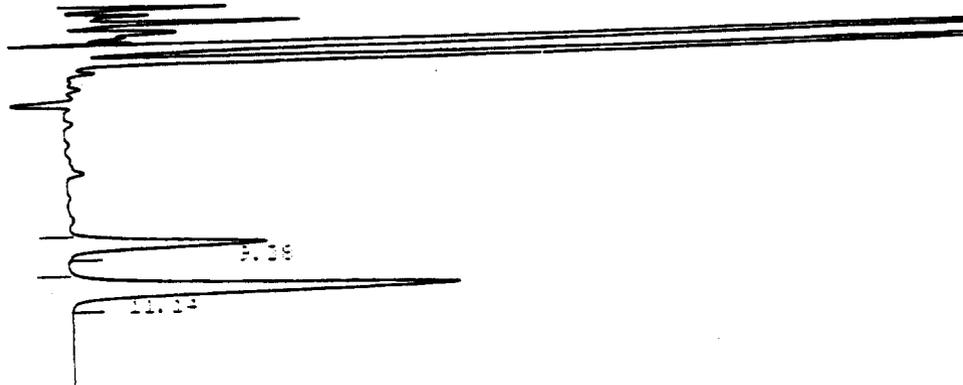
(Chemical analysis - continued)

FIGURE 3

Typical procedural recovery chromatograms  
(Day 1)

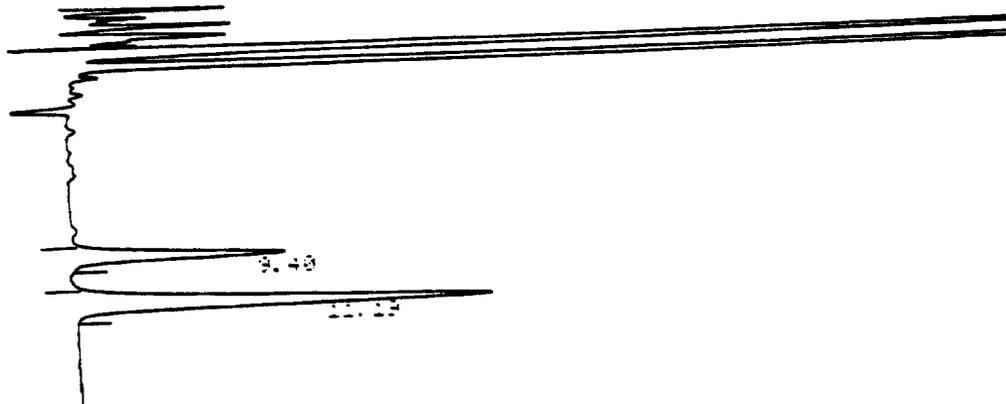
3 mg/ml (Isomer I 98.9%, Isomer II 100.1%)

CHANNEL A INJECT 04-02-90 13:08:47 STORED TO BIN # 7



30 mg/ml (Isomer I 100.2%, Isomer II 99.5%)

CHANNEL A INJECT 04-02-90 20:28:47 STORED TO BIN # 12



APPENDIX 8

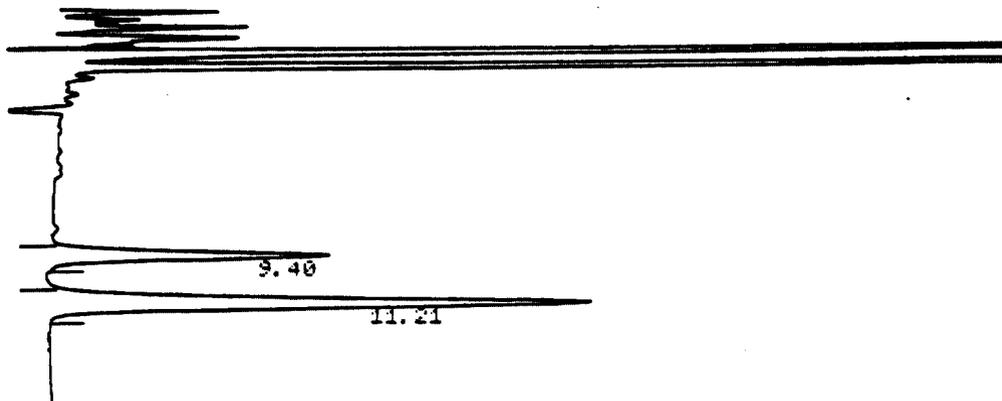
(Chemical analysis - continued)

FIGURE 3

(continued)

100 mg/ml, (Isomer I 100.5%, Isomer II 99.8%)

CHANNEL A INJECT 04-02-93 22:04:46 STORED TO BIN # 18





**APPENDIX 9**

**SEVEN-DAY PRELIMINARY ORAL TOXICITY**

**STUDY IN THE RAT**

**WITH**

**(HRC Schedule No.:**

**Authors:**

**Sarah A. Allan,  
David G. Coleman.**



## APPENDIX 9

(Preliminary toxicity study - continued)

### EXPERIMENTAL PROCEDURE

Groups of three male and three female rats were dosed by oral gavage with formulated 20, 10 and 5% w/v solutions in corn oil at a dose volume of 5 ml/kg/day to give dosages of 1000, 500 and 250 mg/kg/day respectively. A control group of six rats (three males and three females) received the vehicle alone at the same dose volume (5 ml/kg/day). Rats were dosed by oral gavage, once daily, for seven consecutive days.

### OBSERVATIONS

#### Mortalities

All rats receiving at 1000 mg/kg/day were sacrificed on humane grounds on Day 1 within 4 hours of dosing. Clinical signs seen prior to sacrifice included piloerection, hunched posture, abnormal gait (walking on toes), pallor of the extremities, lethargy and ataxia. Post mortem revealed a slight haemorrhage in the glandular region of the stomach for one female rat. No other macroscopic abnormalities were seen.

#### Clinical signs

For rats receiving 500 mg/kg/day increased salivation following dosing was noted on most occasions from Day 2 until termination (Day 8), this was generally associated with wet fur. On Day 1 piloerection and hunched posture were seen in both sexes along with abnormal gait (walking on toes) for females only. Fur loss was noted for one male and two females from Day 4 to termination.

Increased salivation, following dosing, was noted occasionally for rats treated at 250 mg/kg/day. No other clinical signs were noted for this low dosage group.

#### Bodyweight (Table 1)

There were no obvious differences from controls for actual bodyweight or bodyweight gains for rats treated at 250 or 500 mg/kg/day.

#### Food consumption (Table 2)

Food consumption for rats treated at 250 or 500 mg/kg/day with was similar to that of the controls.

## APPENDIX 9

(Preliminary toxicity study - continued)

### TERMINAL STUDIES

#### Organ weight (Table 3)

Slightly higher liver weights were recorded for rats dosed at 250 or 500 mg/kg/day than for control rats. This difference appeared to be dosage-related.

The spleen and kidney weights for rats treated at 250 or 500 mg/kg/day were comparable to those for the controls.

#### Macroscopic pathology

No macroscopic abnormalities were observed for rats treated at 250 or 500 mg/kg/day or for control rats.

### CONCLUSION

The results of this seven-day preliminary oral toxicity study with suggested that 500 mg/kg/day would be tolerated by the rat for the four-week study (HRC Schedule No.

A dosage sequence of 0, 15, 150 and 500 mg/kg/day was therefore proposed.

APPENDIX 9

(Preliminary toxicity study - continued)

TABLE 1

Bodyweight - group mean values (g)

Sex/treatment	Group/dosage (mg/kg/day)	Day		
		1	4	8
♂	Control	112	137 (25)	174 (37)
	250	124	151 (27)	186 (35)
	500	125	145 (20)	184 (39)
	1000	110	-	-
♀	Control	116	136 (20)	161 (25)
	250	121	141 (20)	167 (26)
	500	123	137 (14)	163 (26)
	1000	116	-	-

Bodyweight gains are given in parentheses

**APPENDIX 9**

**(Preliminary toxicity study - continued)**

**TABLE 2**

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

Sex/treatment	Group/dosage (mg/kg/day)	Food consumed (g/rat/week)
♂	Control	168
	250	181
	500	172
♀	Control	148
	250	155
	500	142

APPENDIX 9

(Preliminary toxicity study - continued)

TABLE 3

Organ weight - group mean values (g)

Sex/treatment	Group/dosage (mg/kg/day)	Organ		
		Liver	Spleen	Kidneys
♂	Control	8.09	0.67	1.48
	250	9.87	0.70	1.69
	500	10.50	0.66	1.60
♀	Control	7.30	0.49	1.43
	250	9.17	0.53	1.60
	500	10.53	0.56	1.60

# **HRC** Report

BEST COPY AVAILABLE

ACUTE ORAL TOXICITY  
TO THE RAT

## Huntingdon Research Centre



**CONFIDENTIAL**

**ACUTE ORAL TOXICITY  
TO THE RAT**

**Addressec:**

**Author:**

**Sarah A. Allan.**

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

**Report issued: 15 June 1992**

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## COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following appropriate Good Laboratory Practice Standards.

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

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register, 22 December 1978, and subsequent Amendments.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

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.

Japanese Ministry of Health and Welfare, Notification No. Yakuhatu 313 Pharmaceutical Affairs Bureau, 31 March 1982 and subsequent amendment Notification No. Yakuhatu 870, Pharmaceutical Affairs Bureau, 5 October 1988.

Japanese Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

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

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.



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Sarah A. Allan, B.Sc.,  
Study Director,  
Huntingdon Research Centre Ltd..

15-6-92  
Date

## QUALITY ASSURANCE STATEMENT

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, "process-based" inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of inspection

6-13.01.92

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

14.01.92

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

23.04.92



27.4.92

P. Watson,  
Systems Compliance Auditor,  
Department of Quality Assurance,  
Huntingdon Research Centre Ltd..

**AUTHOR'S SIGNATURE PAGE**

I the undersigned, hereby declare that the work was performed under my supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



**Sarah A. Allan, B.Sc.,  
Study Director,  
Department of Industrial Toxicology.**

## SUMMARY

A study was performed to assess the acute oral toxicity of \_\_\_\_\_ to the rat. The method followed was that described in:

EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.1. Acute toxicity (oral).

OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

A group of ten fasted rats (five males and five females) was given a single dose by gavage of the test substance, as supplied, at a dose level of 2.0 g/kg bodyweight. All animals were killed and examined macroscopically on Day 15, the end of the observation period.

There were no deaths. Clinical signs of reaction to treatment were pilo-erection, abnormal body carriage, abnormal gait, lethargy, decreased respiratory rate, pallor of the extremities, increased salivation and wet fur; recovery was complete by Day 3.

All rats achieved satisfactory bodyweight gains throughout the study.

No abnormalities were recorded at the macroscopic examination on Day 15.

The acute lethal oral dose to rats of \_\_\_\_\_ was found to be greater than 2.0 g/kg bodyweight.

\_\_\_\_\_ does not require labelling with the risk phrase R22 "Harmful if swallowed", in accordance with Council Directive 79/831/EEC Annex VI, Part II(D) as described in Commission Directive 91/325/EEC.

## INTRODUCTION

The study was designed to assess the toxicity of \_\_\_\_\_ following a single oral dose to the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with the following guidelines:

EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.1. Acute toxicity (oral).

OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

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

The dose level for the study was chosen on the basis of a preliminary investigation and in compliance with the guidelines.

The protocol was approved by the Study Director and HRC Management on 29 January 1992 and by the Sponsor on 10 February 1992.

The experimental phase of the study was undertaken between 3 and 26 March 1992.

**TEST SUBSTANCE**

**Identity:**

**Chemical name:**

**Lot number:**

**5364.**

**Expiry:**

**10 January 1993.**

**Purity:**

**>98%.**

**Appearance:**

**Clear colourless liquid.**

**Storage conditions:**

**Room temperature.**

**Date received:**

**3 January 1992.**

## EXPERIMENTAL PROCEDURE

### ANIMAL MANAGEMENT

Equal numbers of healthy male and female CD rats of Sprague-Dawley origin (Hsd/Ola:Sprague-Dawley(CD)) were obtained from Harlan Olac Ltd., Bicester, Oxon, England.

They were in the weight range of 111 to 143 g and approximately four to seven weeks of age prior to dosing (Day 1) in the main study. All the rats were acclimatised to the experimental environment for a period of seven days prior to the start of the main study.

The rats were allocated without conscious bias to cages within the treatment group. They were housed in groups of up to five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 6.

A standard laboratory rodent diet (Biosure LAD 1) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and approximately 4 hours after dosing.

The batch(es) of diet used for the study was analysed for certain nutrients, possible contaminants and micro-organisms (Appendix 1).

Results of 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 2).

The mean daily minimum and maximum temperatures of the animal room were 20°C and 22°C respectively and the mean daily relative humidity value was 58% R.H. Air exchange was maintained at 10 to 15 air changes per hour and lighting was controlled by means of a time switch to provide 12 hours of artificial light (0700 - 1900 hours) in each 24-hour period.

Each animal was identified by cage number and ear punching. Each cage was identified by a coloured label displaying the dose level, study schedule number, animal mark and the initials of the Study Director and Home Office licensee.

### TEST SUBSTANCE PREPARATION

was administered, as supplied by the Sponsor, at a volume not exceeding 2.10 ml/kg (specific gravity 0.9514) in the main study.

The absorption of the test substance was not determined.

The homogeneity, stability and purity of the test substance were the responsibility of the Sponsor.

## **TREATMENT PROCEDURE**

### **Preliminary study**

A preliminary study was carried out by dosing two male and two female rats at 1.0 g/kg bodyweight.

### **Main study**

A group of ten rats (five males and five females) was treated at 2.0 g/kg bodyweight.

### **Control animals**

No control animals were included in this study.

## **ADMINISTRATION OF TEST SUBSTANCE**

The appropriate dose volume of the test substance was administered to each rat by oral gavage using a syringe and plastic catheter (8 choke).

The day of dosing was designated Day 1.

## **OBSERVATIONS**

### **Mortality**

Cages of rats were checked at least twice daily for any mortalities.

### **Clinical signs**

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1 (a period of five hours). On subsequent days animals were observed once in the morning and again at the end of the experimental day. This latter observation was at approximately 16.30 hours on week days or 11.30 hours on Saturdays, Sundays and public holidays. The nature and severity of the clinical signs and time were recorded at each observation.

The animals on the preliminary and main studies were observed for 5 and 14 days respectively after dosing.

### **Bodyweight**

The bodyweight of each rat was recorded on Days 1 (prior to dosing), 8 and 15. Individual weekly bodyweight changes were calculated.

## **TERMINAL STUDIES**

### **Termination**

All animals on the main study were killed on Day 15 by cervical dislocation.

### **Macroscopic pathology**

All animals were subjected to a macroscopic examination which consisted of opening the cranial, abdominal and thoracic cavities. The macroscopic appearance of all tissues was recorded.

## **ARCHIVES**

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

## RESULTS

### PRELIMINARY STUDY (Table 1)

The results of the preliminary study indicated that the acute lethal oral dose to male and female rats of \_\_\_\_\_ was greater than 1.0 g/kg bodyweight.

### MAIN STUDY

A group of ten rats (five males and five females) was treated at 2.0 g/kg bodyweight.

### MORTALITY (Table 1)

There were no deaths following a single oral dose of \_\_\_\_\_ at 2.0 g/kg bodyweight.

### CLINICAL SIGNS (Table 2)

Pilo-erection and increased salivation were observed in all rats within five minutes of dosing. Pilo-erection persisted and was accompanied at later intervals on Day 1 by abnormal body carriage (hunched posture), abnormal gait (waddling), lethargy, pallor of the extremities and wet fur for all rats and also by decreased respiratory rate for one male and one female.

Recovery of all rats, as judged by external appearance and behaviour, was complete by Day 3.

### BODYWEIGHT (Tables 3 and 4)

All rats achieved satisfactory bodyweight gains throughout the study.

### MACROSCOPIC EXAMINATION

No macroscopic abnormalities were observed for animals killed on Day 15.

## CONCLUSION

The acute lethal oral dose to rats of \_\_\_\_\_ was found to be:  
greater than 2.0 g/kg bodyweight

**TABLE 1****Mortality data for groups of rats dosed orally with**

Study	Dose (g/kg)	Mortality ratio (No. of deaths) ( No. dosed )		
		♂	♀	Combined
Preliminary	1.0	0/2	0/2	0/4
Main	2.0	0/5	0/5	0/10

**TABLE 2****Signs of reaction to treatment observed in rats dosed orally with****Main study**

Signs	No. of rats in group of 5 showing signs	
	Dose (g/kg)	
	2.0	
	♂	♀
Pilo-erection	5	5
Abnormal body carriage (hunched posture)	5	5
Abnormal gait (waddling)	5	5
Lethargy	5	5
Decreased respiratory rate	1	1
Pallor of the extremities	5	5
Increased salivation	5	5
Wet fur	5	5

**TABLE 3**

**Individual bodyweights (g) of rats dosed orally with**

**Main study**

Sex	Dose (g/kg)	Animal number & ear mark	Bodyweight (g) at		
			Day 1	Day 8	Day 15
♂	2.0	1 RP	130	196	249
		2 LP	143	218	291
		3 RPLP	131	194	240
		4 RIRO	128	185	234
		5 LILO	131	189	244
♀	2.0	6 RP	122	164	189
		7 LP	120	156	174
		8 RPLP	111	149	160
		9 RIRO	116	162	186
		10 LILO	113	146	169

**TABLE 4**

**Individual bodyweight changes (g) of rats dosed orally with**

**Main study**

Sex	Dose (g/kg)	Animal number & ear mark	Bodyweight gains (g) at	
			Week 1	Week 2
♂	2.0	1 RP	66	53
		2 LP	75	73
		3 RPLP	63	46
		4 RIRO	57	49
		5 LILO	58	55
♀	2.0	6 RP	42	25
		7 LP	36	18
		8 RPLP	38	11
		9 RIRO	46	24
		10 LILO	33	23

## APPENDIX 1

### Biosure Laboratory Animal Diet No. 1

#### Composition and quality assurance aspects of diet

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

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

Nutrients	Target level	Tolerance %	Acceptable range	
Moisture	9.5	+10		10.5 % max
Crude fat	3.7	±15	3.1 -	4.3 %
Crude protein	21.5	±10	19.4 -	23.7 %
Crude fibre	2.0	±40	1.2 -	2.8 %
Ash	5.5	±15	4.7 -	6.3 %
Calcium	1.0	±20	0.8 -	1.2 %
Phosphorus	0.9	±20	0.7 -	1.1 %
Sodium	0.3	+100-50	0.15 -	0.60 %
Chloride	0.5	+100-50	0.25 -	1.0 %
Potassium	0.8	+100-50	0.4 -	1.6 %
Magnesium	0.15	±50	0.08 -	0.23 %
Iron	220	±50	110.0 -	330 mg/kg
Copper	15	±50	8.0 -	23 mg/kg
Manganese	70	±50	35.0 -	105 mg/kg
Zinc	60	±50	30.0 -	90 mg/kg
Vitamin A	12	+50-20	9.5 -	18 iu/g
Vitamin E	35	+150-20	28 -	88 mg/kg
<b>Contaminants</b>			<b>Maximum concentration</b>	
Fluoride			40	mg/kg
Nitrate (as NaNO <sub>3</sub> )			200	mg/kg
Nitrite (as NaNO <sub>2</sub> )			10	mg/kg
Lead			2.5	mg/kg
Arsenic			1.5	mg/kg
Cadmium			0.5	mg/kg
Mercury			0.1	mg/kg
Selenium			0.6	mg/kg
Total Aflatoxins			5	mcg/kg
Total P.C.B.			50	mcg/kg
Total D.D.T.			150	mcg/kg
Dieldrin			50	mcg/kg
Lindane			150	mcg/kg
Heptachlor			50	mcg/kg
Malathion			5000	mcg/kg

**APPENDIX 1**

**(continued)**

**Microbiological contents**

**Maximum concentration**

	LAD 1 (nuts)
Total viable organisms	10,000 per g diet
Mesophilic spores	30,000 per g diet
Salmonellae species	0 per g diet
Presumptive E. coli	0 per g diet
E. coli type 1	0 per g diet
Fungal units	1,000 per g diet
Antibiotic activity	0 per g diet

## APPENDIX 2

### 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.

CHCAVS DATA: Submission # BEIQ. 0893-12192 3 Seq A

CHCAVS TRAFFIC TRACKING: DBASE ENTRY FORM

TYPE: INT SUPP FLWP

SUBMITTER NAME: Confidential

INFORMATION REQUESTED: FLWP DATE:  
 0501 NO INFO REQUESTED  
 0502 INFO REQUESTED (TECH)  
 0503 INFO REQUESTED (VOL ACTIONS)  
 0504 INFO REQUESTED (REPORTING RATIONALE)  
 DISPOSITION:  
0503 REFER TO CHEMICAL SCREENING  
 0578 CAP NOTICE

SUB DATE: 08/16/93 OTS DATE: 08/17/93

CHEMICAL NAME: Benzenealkeneantile 4-alkyl-alpha  
alpha-di-alkyl

CSRAD DATE: 10/06/93

CASE #

Confident

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

INFORMATION TYPE	P F C	INFORMATION TYPE	P F C	INFORMATION TYPE	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (I VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUREL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0251 MSDS	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	0251 OTHER	01 02 04
0210 ACUTE TOX (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04		
0211 ACUTE TOX (ANIMAL)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 SUB ACUTE TOX (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04		
		0240 METAB/PHARMACO (HUMAN)	01 02 04		

TRIAL/AGE DATA: NON-CBI INVENTORY

YES (CONTINUE) YES (DROPP/RENE)

NO (DROP) NO (CONTINUE)

REFER:

SPECIES: RAT

TOXICOLOGICAL CONCERN:

LOW acute oral toxicity Med fragrance/cosmetic  
Med Subacute oral toxicity High household products

USE:

PRODUCTION:

COMMENTS: Non-Cop

Acute oral toxicity in the rat is low, based on 0% mortality after a single gavage of 2000mg/kg.  
 Neurotoxic signs in all rats included lethargy, piloerection + abnormal body carriage.  
 Subacute oral toxicity is

NOTE  
No observed  
toxic effects  
level

150 mg/kg/d

Medium concern in rats due to possible hepatotoxicity. Rats were gavaged for 28 days. Clinical signs at doses 2150 mg/kg/day included hunched posture + abnormal gait. Hepatic effects in the 2150 mg/kg/dose groups included ↑ relative liver weights and centrilobular or generalized minimal enlargement of hepatocytes. In the SDD mg/kg/day group ↑ urea nitrogen, ↑ glutamic-pyruvic transaminase + ↓ cholesterol levels were noted. Organ changes included liver enlargement and decreased absolute adrenal weight + relative spleen weight. A 7-day preliminary oral study was also conducted. Rats were gavaged at 250, SDD or 1000 mg/kg/day for 7 days. 6/6 rats dosed at 1000 mg/kg/day were sacrificed within 4 hours. Clinical signs prior to sacrifice include piloerection, hunched posture, abnormal gait, lethargy + ataxia. Rats dosed at SDD mg/kg/day exhibited piloerection + hunched posture.

8EHQ-0574-121725  
8EHQ-93-12192(1)  
SPOOL  
89940000193

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(B)

May 2, 1994

COMPANY SANITIZED

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Room 201, East Tower  
401 M Street, SW  
Washington, DC 20460

CONFIDENTIAL

Attn: TSCA §8(e) Submission

- Re: 1. TSCA §8(e) Submissions#: 8EHQ-9312192 submitted August 16, 1993
- 2. Telecon with EPA attorney Cynthia Lewis, April 21, 1994

Dear Sirs:

Please withdraw our original claim of confidentiality for the referenced TSCA 8(e) submission 8EHQ-9312192. We do not wish to maintain the chemical identity as confidential.

We have enclosed a sanitized and unsanitized version of a complete replacement TSCA 8(e) submission package (including a revised cover letter dated August 16, 1993) for CAS # 134123-93-6 Benzenepropanenitrile, 4-ethyl- $\alpha,\alpha$ -dimethyl-, and we request that you replace our original submission with this new version.

Please maintain the PMN number [ ], our company identity, references to company personnel and company synonyms and product codes of the chemical substance in the replacement version as Confidential Business Information.

If you have any questions, please contact me at [ ].

Sincerely,

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OPPT/CBIC  
94 MAY -6 AM 11:35

[ ]  
Enclosures

RECEIVED  
6-3-94

**SANITIZED COPY**

August 16, 1993

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Attn: TSCA §8(e) Submission

- Ref: 1. [ ]
- 2. [ ], Twenty-eight day Oral Toxicity Study in the Rat (draft report), received July 28, 1993
- 3. [ ], Acute Oral Toxicity to the Rat, report date 15 June 1992

Dear Sirs:

In compliance with the reporting requirements of TSCA Section 8(e) Substantial Risk Information, we are submitting the enclosed Draft Study Report (Ref. 2 & 3) for your review.

The draft report indicates the results of a twenty-eight day rat oral toxicity study performed on the following material manufactured by [ ] for which we filed a Premanufacture Notification [ ]:

Specific chemical name: Benzenepropanenitrile, 4-ethyl- $\alpha,\alpha$ -dimethyl-  
CAS#: 134123-93-6

The signs observed in the twenty-eight day study may indicate potential neurotoxic effects at high dose levels. Also observed were liver effects at high dose levels; however the significance of these effects are not known.

2

The potential neurotoxic signs observed in the twenty-eight day study are similar to the signs observed in the acute oral toxicity study. However, the signs in the acute oral study were transient. As a result, the acute oral toxicity study was not considered reportable under TSCA 8(e) at the time of receipt. Supported by the new information obtained from the twenty-eight day study, the results of the acute study suggest potential neurotoxicity, and we are submitting this study as required under TSCA Section 8(e).

We have changed the Material Safety Data Sheet to reflect the results of these studies. We have included additional statements related to the potential neurotoxicity and organ specific effects of the subject material. We will continue to advise the use of splash goggles or face shield when eye contact might occur, use of chemical resistant gloves, an use of a NIOSH approved respirator when inhalation of high concentrations may occur. The ventilation would meet ACGIH criteria.

As indicated in the PMN, the subject material will be used for fragrances to be used in cosmetic and household products. The maximum anticipated percent in a fragrance formulation was reported to be [ ] The fragrance formulation will be further diluted in the final product. For example, a typical level of 0.5% of a fragrance formulation is used in these soaps and detergents. Therefore, the final concentration of the subject material in a consumer product is anticipated to be very low. Because the potential neurotoxic effects were observed predominately at high dose level, we would not consider the levels to be used in the production of fragrance formulations and final products to represent a health risk to workers and consumers, respectively.

We have included sanitized and unsanitized versions of the Study Reports and request that you maintain this communication as "Confidential Business Information".

If you have any questions, please contact me at [ ].

Sincerely,  
[ ]

[ ]

Enclosures

**CONFIDENTIAL**

**NOTE**

This report is considered by the Study Director to be the 'final draft' and has been submitted to the HRC Quality Assurance Department Audit.

The sponsor is requested to review this document and communicate any comments to the Study Director as soon as possible. When these comments have been received and on completion of the QA audit, the FINAL REPORT containing Study Director and QA Statements will be issued.

**PLEASE NOTE**

In compliance with GLP any changes to the final report after the date of issue will be in the form of a separate amendment to the report.

Date: 27 July 1993

V.1.

**RECEIVED**

JUL 28 1993

**TWENTY-EIGHT DAY  
ORAL TOXICITY STUDY IN THE RAT**

**Addressee:**

**Testing facility:**

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

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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.

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.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

---

Stuart M. Denton, B.Sc.,  
Study Director,  
Huntingdon Research Centre Ltd.

---

Date

## QUALITY ASSURANCE STATEMENT

This report has been audited by the Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

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

---

Huntingdon Research Centre Ltd.

DRAFT

## QUALITY ASSURANCE STATEMENT

### DATES OF STUDY INSPECTIONS

Inspections were made by the Quality Assurance Department of the various phases of the study described in this report. The dates on which the inspections were made and the dates on which the 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		
Pre-experimental Period		
Experimental Period		

---

Huntingdon Research Centre Ltd.

**DRAFT**

**RESPONSIBLE PERSONNEL**

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Stuart M. Denton, B.Sc.,  
Study Director,  
Department of Industrial Toxicology.

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## SUMMARY

This study was performed to assess the systemic toxicity of \_\_\_\_\_ to the rat. The method followed was that outlined in Annex V, Part B, Method B7 in the EEC Directive 84/449/EEC and OECD Guideline for Testing of Chemicals No. 407 "Repeated dose oral toxicity - Rodent: 28-day or 14-day study".

a clear colourless liquid, intended for use as a fragrance enhancer, was administered by oral gavage, once daily, to groups of five male and five female rats for a period of not less than twenty-eight consecutive days, at fixed dosage levels of 15, 150 and 500 mg/kg/day. The test substance was prepared as suspensions in corn oil at concentrations of 0.3, 3.0 or 10% w/v.

A further group of rats (five males and five females) was used as a concurrent control receiving the vehicle (corn oil) alone.

Bodyweights, food consumption and clinical observations were recorded during the study. Blood samples for clinical investigations were taken on Day 27 and all animals were killed and examined macroscopically (males on Day 29 and females on Day 30). Histological examination of specified tissues was then initiated.

The following comments relating to real or possible treatment effects are made in summary:

**Clinical signs.** Hunched posture and abnormal gait were observed on most occasions throughout the study for rats receiving 500 mg/kg/day. Abnormal gait was seen during the last few days of the study in rats receiving 150 mg/kg/day. This sign was also evident in one male and one female accompanied by hunched posture on Day 2 at 150 mg/kg/day.

**Haematology.** Longer thrombotest times were recorded for male and female rats treated at 500 mg/kg/day.

**Biochemistry.** At the 500 mg/kg/day level, increased values were observed for glucose, urea nitrogen, chloride (in males), glutamic-pyruvic transaminase and phosphorus (in females) and decreased values for cholesterol.

**Organ weights.** Increased adjusted liver weights were seen in males and females and lower adjusted spleen and absolute adrenal weights in males only were recorded for rats treated at 500 mg/kg/day.

Increased adjusted liver weights were also recorded in male rats dosed at 150 mg/kg/day.

**Macroscopic pathology.** Liver enlargement was observed in all male rats and 4/5 female rats treated at 500 mg/kg/day.

## INTRODUCTION

The study was designed to assess the systemic toxicity to the rat of the test substance, intended for use as a fragrance enhancer, when repeatedly administered orally for a period of 28 consecutive days.

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

EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B7. Sub-acute toxicity (oral).

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

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 test substance was assessed for its toxicity to rats when administered orally, once daily for a minimum of twenty-eight consecutive days. Dosage levels of 15, 150 and 500 mg/kg/day were selected on the basis of available toxicity data (rat acute oral  $LD_{50} > 2$  g/kg, HRC Report no 131/AC) and a 7-day preliminary oral toxicity study in rats (HRC Schedule no Appendix 9) conducted at HRC. The results of the preliminary study indicated that the limit dose of 1000 mg/kg/day would not be tolerated by the rat, so the high level dosage was selected as 500 mg/kg/day.

The study protocol was approved by the Study Director and HRC Management on 26 January 1993 and by the Sponsor on 1 February 1993.

Rats were received from the supplier on 27 January 1993 and dosing commenced on 4 February 1993. Rats were killed following a four-week treatment period (males on 4 March and females on 5 March 1993).

The experimental phase of the study was conducted between 11 January 1993 and 14 July 1993.

**TEST SUBSTANCE**

**Identity:**

**Chemical name:**

**Benzenepropanenitrile, 4-ethyl-alpha, alpha-dimethyl:  
60 - 75%,  
Benzenepropanenitrile, 2-ethyl-alpha, alpha-dimethyl:  
1 - 5%,  
Benzenepropanenitrile, 3-ethyl-alpha, alpha-dimethyl:  
20 - 35%**

**Lot number:**

**5364**

**Expiry:**

**10 January 1993**

**Purity:**

**> 98%**

**Appearance:**

**Clear colourless liquid**

**Storage conditions:**

**Room temperature in the dark**

**Date received:**

**3 January 1992**

## EXPERIMENTAL PROCEDURE

### ANIMAL MANAGEMENT

A total of 22 male and 22 female healthy CD rats of Sprague-Dawley origin (CrI:CD® BR VAF PLUS™) was ordered from Charles River (U.K.) Ltd., Margate, Kent, England.

The rats were  $28 \pm 1$  days old, in a weight range of 65 to 91 g on arrival (27 January 1993). An eight-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 five 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 6).

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 7).

The rats were housed in Building R17, Room 7. Animal room temperature was controlled in the range 19 to 22°C and relative humidity was controlled in the range of 29 to 57% RH; the slightly low humidity value (29% RH) was recorded during the treatment period on Days 8, 9 and 12, however, the low humidity values were not considered to have affected the integrity of the study. 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.

Two days before the start of treatment each animal was weighed and forty rats were randomly allocated to four groups, each consisting of five males and five 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 five rats) constituting each group were distributed in batteries in such a manner that possible environmental influences arising from their spatial distribution were 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

The test substance was prepared initially in corn oil (high dose level). A series of formulations were prepared by further direct dilution of the high dose level.

Formulations were prepared freshly each day.

The chemical stability and homogeneity of test substance formulations in corn oil were assessed prior to the start of treatment by HRC Department of Analytical Chemistry and were found to be satisfactory (Appendix 8).

Concentration analyses of formulations prepared for administration on Day 1 were also performed by HRC Department of Analytical Chemistry. Results of these analyses were found to be satisfactory and are presented in Appendix 8.

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 the 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 this laboratory (HRC Schedule no. . . . Other levels were selected on the basis of the key dosages relative to EEC labelling requirements.

Groups of ten rats were dosed as follows:

Group	Cage label/ colour code	Treatment	Dosages (mg/kg/day)	No. of rats		Rat numbers	
				♂	♀	♂	♀
1	White	Control - corn oil	-	5	5	1-5	21-25
2	Yellow		15	5	5	6-10	26-30
3	Green		150	5	5	11-15	31-35
4	Red		500	5	5	16-20	36-40

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/...

Control animals received a similar dose volume.

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

Animals were treated once daily for at least twenty-eight consecutive days.

Prior to dosing, the test substance formulations were mixed by inversion ( $\times 20$ ).

## **OBSERVATIONS**

### **Clinical signs**

All animals were observed 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. On Saturdays and Sundays 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.

Gravimetric measurement of water consumption was initiated during Week 3 due to a suspected treatment-related effect on consumption seen early in the study.

## 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 all rats prior to termination (Week 4).

The collected blood samples were divided as follows:

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

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

All 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 counts (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) Calculated: $\text{PCV (\%)} \times 10 \div \text{RBC } (\times 10^6/\text{mm}^3)$	fl
Platelet counts (Plts)	$\times 10^3/\text{mm}^3$
Total white blood cell count (WBC)	$\times 10^3/\text{mm}^3$

**Units**

The following estimations were measured using the appropriate methodology:

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

s

Differential white blood cell count (Diff) - namely:

Neutrophils (N)

Lymphocytes (L)

Eosinophils (E)

Basophils (B)

Monocytes (M)

$\times 10^3/\text{mm}^3$

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.

Additional blood film slides were prepared and examined for morphological abnormalities. Abnormal cells (see below) observed when examining the stained slides were recorded and included in the haematology appendix.

- 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) Calculated: Total protein (g/dl) minus Alb (g/dl)	g/dl
Albumin/Globulin ratio (A/G) Calculated from Total protein and Albumin concentrations	g/dl
Urea nitrogen (Urea Nitr)	mg/dl
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 a minimum of 28 days of treatment all animals were killed (males Day 29, females Day 30) by carbon dioxide asphyxiation and a complete autopsy undertaken. The order of sacrifice was determined using pre-set cage sequence. Specified organs were weighed and relevant tissue samples were fixed for microscopic examination.

### Organ weight

The following organs from each animal were dissected free of fat and weighed:

adrenals	liver
brain	ovaries
kidneys	spleen
testes (with epididymides)	

### Macroscopic pathology

The macroscopic appearance of the tissues of all rats was recorded and samples of the following 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)
duodenum	ovaries	thymus (where present)
eyes (Davidson's fluid as fixative)	pancreas	thyroid (with parathyroid)
femur (with joint)	pharynx	tongue
head	pituitary	trachea
heart*	prostate	urinary bladder
ileum	rectum	uterus
jejunum	salivary gland	vagina
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 µm and stained with haematoxylin and eosin.

Microscopic examination of prepared slides (from tissues indicated under Macroscopic pathology) was carried out for all rats of Group 1 (control group) and Group 4 (high dosage group) killed on Day 29 or 30.

Following documented approval from the Sponsor, microscopic examinations were extended to include the livers of all male and female rats of the low and intermediate 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, organ weight and clinical pathology data:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 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 are expressed at the 5% (\*  $P < 0.05$ ) or 1% (\*\*  $P < 0.01$ ) level.

## REFERENCES

1. FISHER, R.A. (1932) *Statistical methods for research workers*, 4th ed., Oliver and Boyd.
2. MANTEL, N. (1963) *J. Amer. Statist. Ass.*, 58, 690.
3. BARTLETT, M.S. (1937) *Proc. Roy. Soc., A*, 160, 268.
4. KRUSKAL, W.H. and WALLIS, W.A. (1952/3) *J. Amer. Statist. Ass.*, 47, 583 and 48, 907.
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.

## GOOD LABORATORY PRACTICE

### ~~Regulations~~

The study was conducted in compliance with the principles of Good Laboratory Practice as set forth in:

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.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

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

## **QUALITY ASSURANCE DEPARTMENT REVIEW**

The Department of Quality Assurance reviewed the study protocol prior to commencement of treatment and conducted inspections of the various phases of the study as required by the above Good Laboratory Practice principles. The dates on which the findings of these inspections were reported to the Study Director and to HRC Management are specified earlier in this report.

The final report was reviewed by HRC's Department of Quality Assurance comparing individual findings against raw data and comparing the statements and results presented in the report with individual data presented in the appendices of the report.

## **PROCEDURES**

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

## **ARCHIVES**

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

Such 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.

### **Water consumption (Figure 4, Table 4)**

Gravimetric measurement during the third week of the study revealed increased consumption for males and females receiving 150 or 500 mg/kg/day in comparison with controls. This probably reflects the increased salivation seen in these animals and may arise from unpalatability of the test substance.

## **CLINICAL PATHOLOGY**

### **Haematology (Table 5, Appendix 2)**

Significantly longer thrombotest times ( $P < 0.05$  or  $0.01$ ) were recorded for male and female rats treated at 500 mg/kg/day in comparison with controls. The remaining statistical differences from controls were marginal, within the expected range and were therefore considered to be of no toxicological importance.

The occurrence of polychromasia and anisocytosis among young laboratory rats is not uncommon and at the incidence seen in this study (on one occasion for each condition) was not considered to be toxicologically significant.

### **Biochemistry (Table 6, Appendix 3)**

At the 500 mg/kg/day level, slight but statistically significant increases in urea nitrogen levels (males) and glutamic-pyruvic transaminase and phosphorus levels (females) and a decrease in cholesterol (males) were considered to be related to treatment.

The marginally lower alkaline phosphatase activities noted for females receiving 500 mg/kg/day were considered to be of no toxicological importance in the absence of a dosage relationship within the treated female groups.

Other statistically significant differences, namely glucose (males), chlorine (males), alkaline phosphatase (females) were marginal, within the expected range and therefore of no toxicological importance.

There were no statistically significant differences in biochemical parameters between controls and rats treated at 150 or 15 mg/kg/day.

## **TERMINAL PROCEDURES**

### **Organ weights (Table 7, Appendix 4)**

Statistically significantly higher ( $P < 0.01$ ) liver weights (adjusted to take bodyweight into consideration) in males and females and lower ( $P < 0.01$ ) adjusted spleen and ( $P < 0.05$ ) absolute adrenal weights in males only were observed for rats treated 500 mg/kg/day in comparison with controls.

Statistically significantly higher ( $P < 0.01$ ) adjusted liver weights were recorded in male rats dosed at 150 mg/kg/day in comparison with controls.

## RESULTS

### OBSERVATIONS

#### Clinical signs (Table 1, Appendix 5)

For rats receiving 500 mg/kg/day, hunched posture and abnormal gait were observed on most occasions throughout the study.

Abnormal gait was seen during the last few days of the study in rats receiving 150 mg/kg/day. This sign was also evident in one male and one female, accompanied by hunched posture on Day 2.

Additional signs were related to the mode of dosing or the nature of the dose formulation. Increased salivation frequently seen in all rats dosed at 500 and 150 mg/kg/day, was generally accompanied by wet fur. This sign is commonly observed in gavage studies and may be related to test substance palatability. Greasy fur, commonly seen during the latter half of the study in all rats dosed at 500 and 150 mg/kg/day, is usually observed when corn oil is used as a vehicle and may indicate the spreading of dose residue during grooming. Red/brown staining around the mouth, seen in all animals dosed at 500 mg/kg/day and the majority of animals at 150 mg/kg/day is related to the stress of the dosing procedure. Loose faeces were observed in the cage tray for all animals receiving 500 or 150 mg/kg/day on Days 28 and 29 and probably result from the anaesthesia administered during the blood sampling procedure. A swollen area around the right eye was noted for one male rat treated at 15 mg/kg/day from Day 27 to termination (Day 29); this observation was related to trauma caused during the blood sampling procedure on Day 27. No other signs were seen at this dosage level. None of these signs are considered to be toxicologically important.

No clinical observations were observed in the control group.

#### Bodyweights (Figure 2, Table 2, Appendix 1)

There were no apparent differences in actual bodyweights and no significant difference in overall bodyweight gains between control and treated rats of both sexes.

#### Food consumption (Figure 3, Table 3)

The food consumption fluctuated amongst the groups during the study. Notably a slight increase was observed during Weeks 3 and 4 for male rats receiving 500 and 150 mg/kg/day and for female rats receiving 500 mg/kg/day. However, in the absence of any effect on bodyweight, this change was considered to be coincidental.

Covariate analysis revealed no other statistically significant differences between the organ weights of treated and control rats.

**Macroscopic pathology (Table 8, Appendix 5)**

The macroscopic examination performed at the terminal kill revealed the following change:

**Liver** - Enlargement was observed in all male rats and 4/5 female rats treated with 500 mg/kg/day compared with 1/10 control rats.

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

**Microscopic pathology (Table 9, Appendix 5)**

**Treatment-related findings** - Centrilobular or generalised minimal enlargement of hepatocytes was seen in 5/5 males and 4/5 females receiving the high dosage level and 2/5 males receiving the intermediate dosage level. This was considered to be related to treatment. No similar changes were seen in males at the low dosage level or in controls, nor in any females from either control or other treated groups.

**Incidental findings** - The other histological changes seen were within the normally observed range of changes for animals of this species and age and were not considered to be of any toxicological significance.

A minimally increased cellularity of the splenic white pulp of 2/5 male animals at the high dosage level. This spleen change is within the normal spectrum of splenic histological diversity and is not considered to be related to treatment. No changes were seen that might account for the weight decrease recorded for the spleen in male rats of the highest dosage group.

No changes were seen that might account for the weight decrease recorded for adrenal glands in male rats of the highest dosage group.



## DISCUSSION

In this rat subacute study with . , there was evidence of a treatment-related effect on the liver. Among rats dosed at 500 mg/kg/day, increased liver weights, macroscopically enlarged livers and centrilobular or generalised hepatocyte enlargement were noted. This is considered to be a toxic effect on the liver and is seen further in disturbances in biochemical parameters namely cholesterol level (males) and glutamic-pyruvic transaminase level (females). The low incidence of the microscopic liver change and, in the absence of any disturbances in biochemical parameters, the effect seen in the intermediate dosage group (150 mg/kg/day), namely increased liver weight, was considered to be a reduced continuation of the treatment-related effect seen for the high dosage animals and was considered to be an adaptive response.

The clinical signs observed in rats receiving 500 mg/kg/day (hunched posture and abnormal gait) were indicative of discomfort and poor health. The high dosage of 500 mg/kg/day was rejected as the dosage level at which no-toxic effects were observed.

As the only treatment-related effects seen among rats treated at 150 mg/kg/day was adaptive, this dosage level was chosen as the no-observed toxic effect level (NOTEL) for in the rat.

No treatment-related effects were seen among rats treated at 15 mg/kg/day.



## CONCLUSION

Based on the results of this study it was considered that administration of \_\_\_\_\_ at 150 mg/kg/day represents a no-observed toxic effect level (NOTEL) in the rat and that in accordance with EEC Council Directive 79/831/EEC, Annex VI, Part 11(D), as described in Commission Directive 91/325/EEC, labelling with the R48 risk phrase is not required.

**FIGURE 1**

**Group and cage arrangement in batteries**

Group	Cage label/ colour code	Treatment	Dosages (mg/kg/day)	No. of rats		Rat numbers	
				♂	♀	♂	♀
1	White	Control - corn oil	-	5	5	1-5	21-25
2	Yellow		15	5	5	6-10	26-30
3	Green		150	5	5	11-15	31-35
4	Red		500	5	5	16-20	36-40

♂

1	2
3	4

♀

1	2
3	4

FIGURE 2

Bodyweights - group mean values

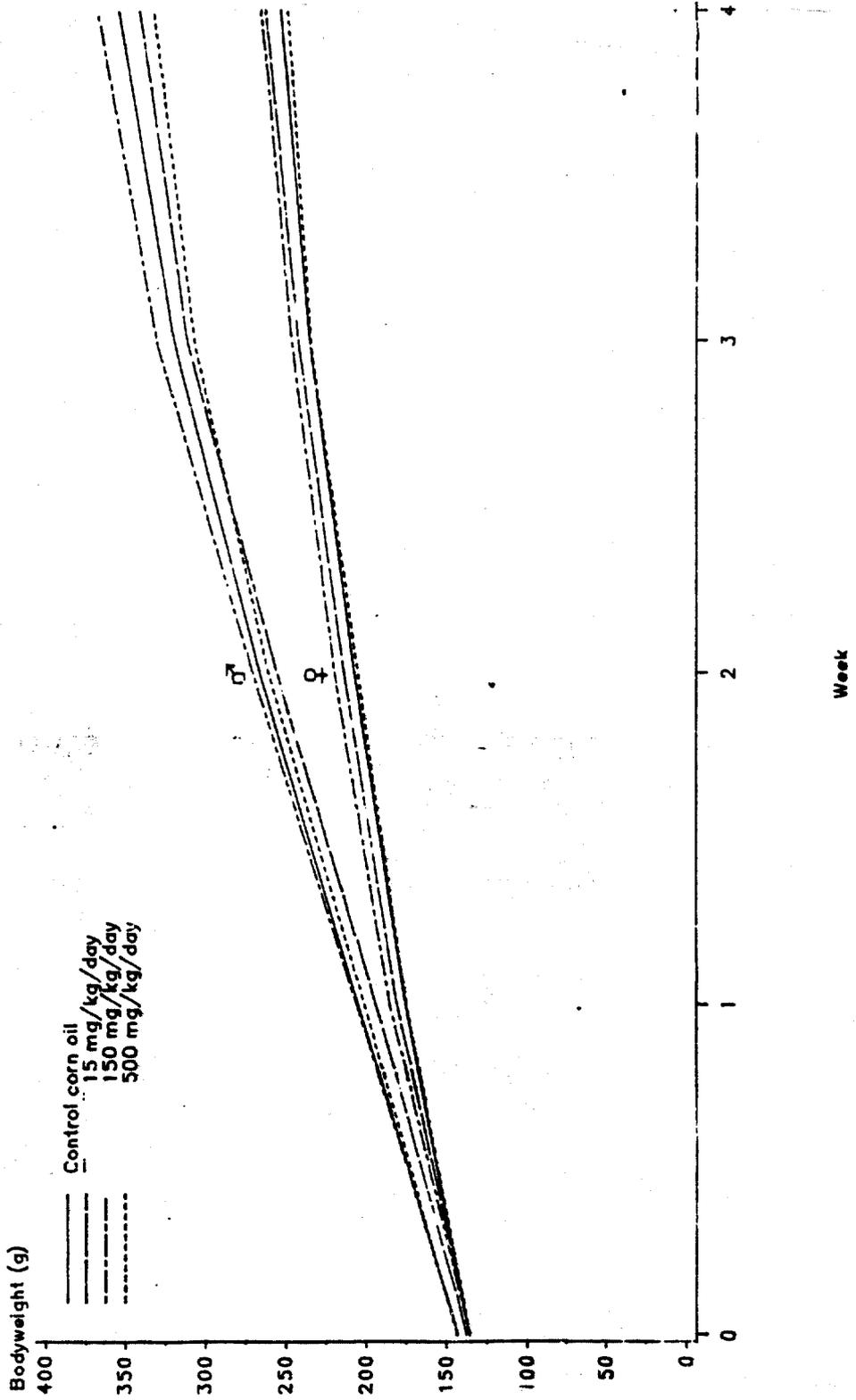


FIGURE 3

Food consumption - group mean values

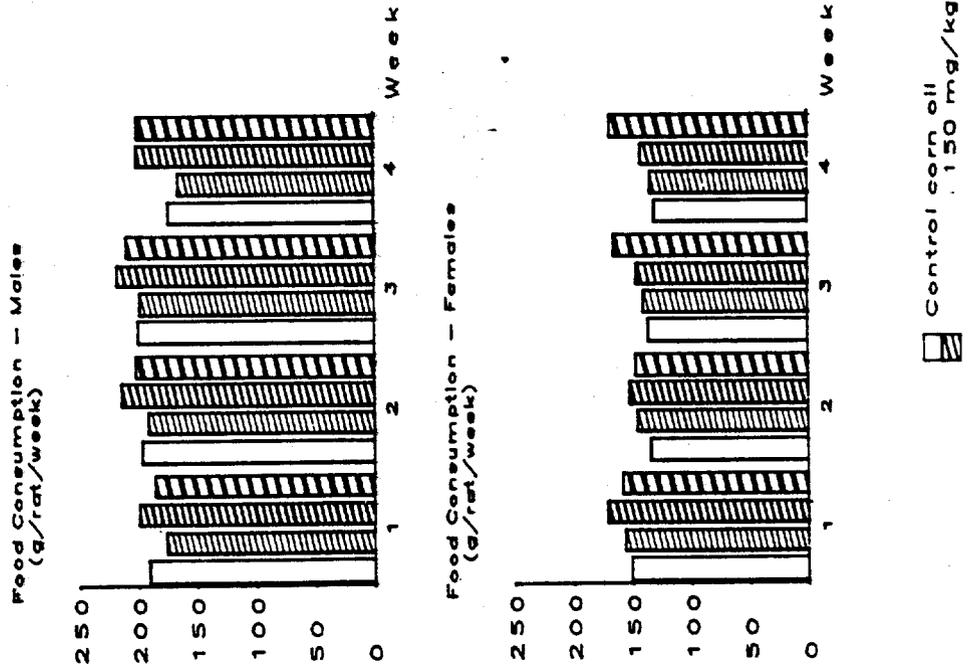
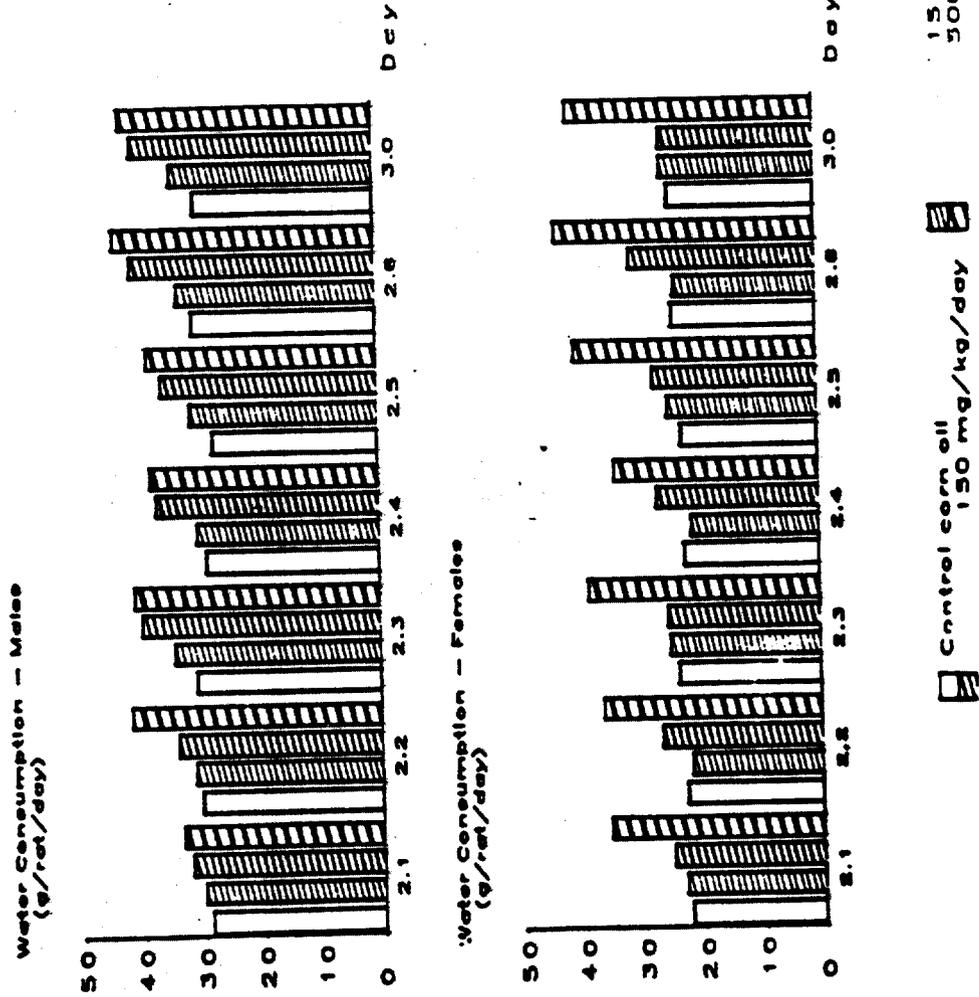


FIGURE 4

Water consumption - group mean values





**TABLE I**

**Signs of reaction to treatment following oral administration of**

Group/ dosage mg/kg/day	Clinical signs	Earliest appearance (Day)	Last appearance (Day)	Maximum consecutive duration (days)	Animal number				
					Maximum severity of observed signs				
1♂ Corn oil	-	-	-	-	(1) -	(2) -	(3) -	(4) -	(5) -
2♂ 15	-	-	-	-	(6) -	(7) -	(8) -	(9) -	(10) -
3♂ 150	Increased salivation	2	28	11	(11) B	(12) B	(13) B	(14) B	(15) B
	Greasy fur	21	29	9	A	A	A	A	A
	Wet fur	23	28	6	A	A	A	A	A
	Hunched posture	2	2	1	-	-	-	-	A
	Abnormal gait - walking on toes	2	29	3	+	+	+	+	+
	Red/brown staining around mouth	4	9	1	-	+	+	+	+
	Loose faeces on cage tray	28	28	1	20% loose faeces/ 80% normal faeces. Individuals not identified.				
4♂ 500	Increased salivation	2	28	27	(16) B	(17) B	(18) B	(19) B	(20) B
	Greasy fur	20	29	10	B	B	B	B	B
	Wet fur	13	28	16	B	B	B	B	B
	Hunched posture	2	29	21	A	A	A	A	A
	Abnormal gait - walking on toes	2	29	21	+	+	+	+	+
	Red/brown staining around mouth	2	14	3	+	+	+	+	+
	Loose faeces on cage tray	28	29	2	40% loose faeces/ 60% normal faeces. Individuals not identified.				

A Slight response  
 B Moderate response  
 +/- Sign present or absent

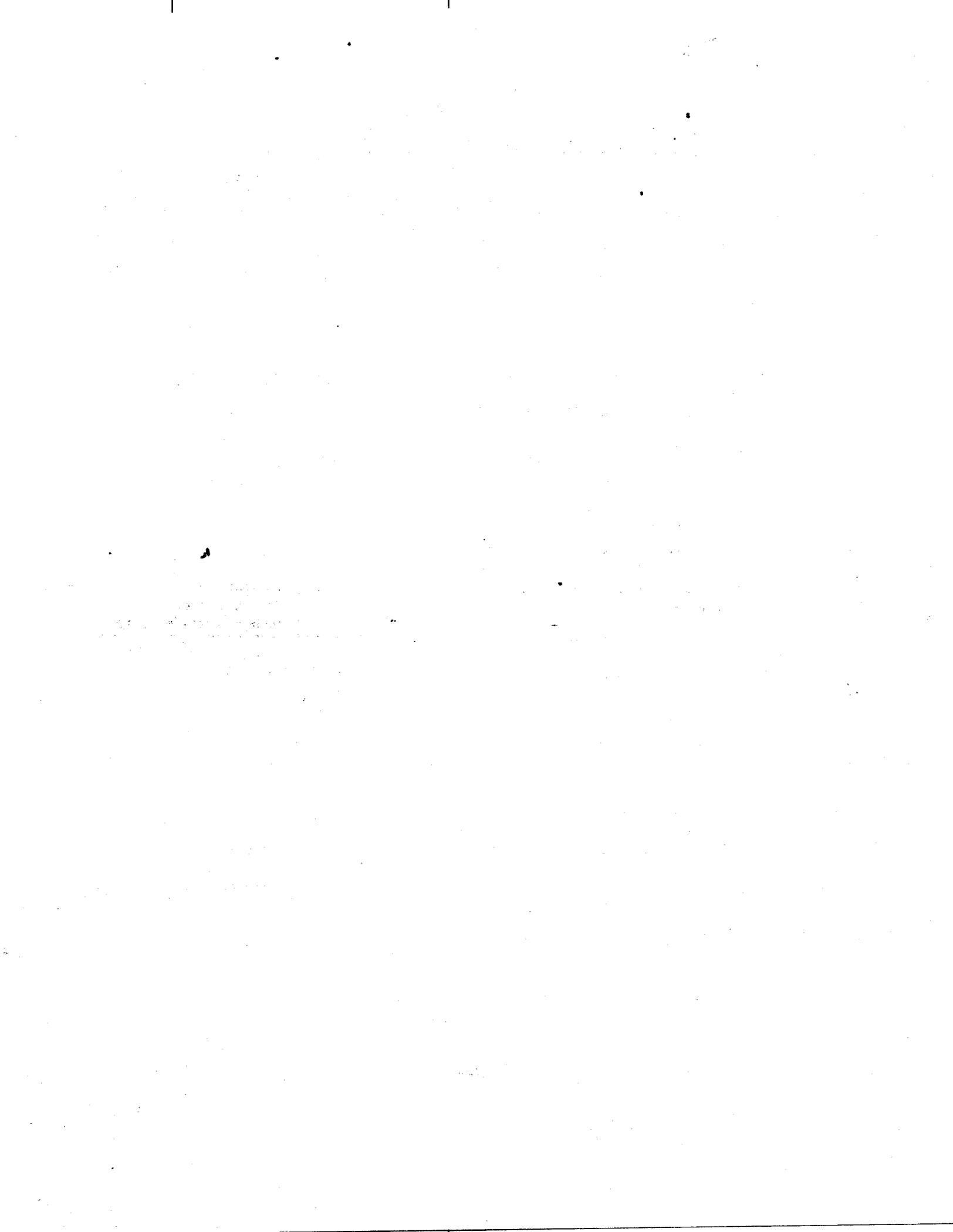


TABLE 1

(Signs of reaction to treatment - continued)

Group/ dosage mg/kg/day	Clinical signs	Earliest appearance (Day)	Last appearance (Day)	Maximum consecutive duration (days)	Animal number				
					Maximum severity of observed signs				
1♀ Corn oil	-	-	-	-	(21)	(22)	(23)	(24)	(25)
2♀ 15	-	-	-	-	(26)	(27)	(28)	(29)	(30)
3♀ 150	Increased salivation	2	29	13	(31)	(32)	(33)	(34)	(35)
	Greasy fur	21	30	10	B	A	A	A	B
	Wet fur	23	28	6	A	A	A	A	A
	Hunched posture	2	2	1	-	-	-	-	A
	Abnormal gait - walking on toes	2	30	4	+	+	+	+	+
	Red/brown staining around mouth	2	9	1	-	+	+	+	+
	Diarrhoea on cage tray	28	28	1	20% diarrhoea/ 80% normal faeces. Individuals not identified.				
4♀ 500	Increased salivation	2	29	28	(36)	(37)	(38)	(39)	(40)
	Greasy fur	20	30	11	B	B	B	B	B
	Wet fur	13	29	17	B	B	B	B	B
	Hunched posture	2	30	22	A	A	A	A	A
	Abnormal gait - walking on toes	2	30	22	+	+	+	+	+
	Diarrhoea on cage tray	28	28	1	40% diarrhoea/ 60% normal faeces. Individuals not identified.				

A Slight response

B Moderate response

+/- Sign present or absent

**TABLE 2**

**Bodyweights - group mean values (g)**

Week	Group and dosage (mg/kg/day)							
	1♂ Corn oil	2♂ 15	3♂ 150	4♂ 500	1♀ Corn oil	2♀ 15	3♀ 150	4♀ 500
0	142	137	142	143	135	135	135	135
1	205	194	206	201	175	181	185	175
2	265	255	270	260	206	213	219	205
3	319	310	329	306	234	241	245	233
4	352	340	366	331	252	252	264	248
<b>Gain Weeks 0-4</b>	<b>210</b>	<b>203</b>	<b>223</b>	<b>188</b>	<b>117</b>	<b>127</b>	<b>129</b>	<b>113</b>

**Blank Non-significant**

**TABLE 3**

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

Week	Group and dosage (mg/kg/day)							
	1♂ Corn oil	2♂ 15	3♂ 150	4♂ 500	1♀ Corn oil	2♀ 15	3♀ 150	4♀ 500
1	190	176	200	186	151	156	171	159
2	197	191	214	202	134	145	152	147
3	200	198	218	210	137	140	146	166
4	174	165	201	201	131	134	142	168
Group mean cumulative value	761	730	833	799	553	575	611	640
% of control value	-	96	109	105	-	104	110	116

Statistical analysis not performed as only 1 cage/sex/group

TABLE 4

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

Week 3	Group and dosage (mg/kg/day)							
	1♂ Corn oil	2♂ 15	3♂ 150	4♂ 500	1♀ Corn oil	2♀ 15	3♀ 150	4♀ 500
Day 15	28.8	29.8	31.8	33.2	22.2	23.0	25.0	35.4
Day 16	30.0	31.0	33.8	41.4	22.6	21.6	26.6	36.4
Day 17	30.6	34.2	39.6	40.8	23.6	25.0	25.4	38.6
Day 18	28.8	30.2	37.0	38.0	22.4	21.2	27.0	34.0
Day 19	27.4	31.2	36.0	38.2	22.8	25.0	27.4	40.6
Day 20	30.6	33.0	40.8	43.6	24.0	23.6	31.0	43.4
Day 21	30.0	33.8	40.4	42.2	24.4	25.6	25.8	41.2
Group mean cumulative value	206.2	223.2	259.4	277.4	162.0	165.0	188.2	269.6
% of control value	-	108	126	135	-	102	116	166

Statistical analysis not performed as only 1 cage/sex/group

TABLE 5

Haematology - group mean values

Week 4 (2 March 1993)

Group/ dosage mg/kg/day	PCV %	Hb g/dl	RBC $\times 10^9/mm^3$	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♂ Corn oil	53	15.6	6.8	29.3	78	13.6	2.52	10.82	0.00	0.00	0.28	1032	21
2♂ 15	52	15.5	6.8	29.6	77	13.4	3.09	10.03	0.10	0.00	0.18	1125	22
3♂ 150	53	15.8	6.8	29.7	78	16.5	2.89	13.52	0.00	0.00	0.13	1088	22
4♂ 500	52	15.7	6.9	30.2	76	14.4	1.74	12.58	0.09	0.00	0.00	1055	27
1♀ Corn oil	52	15.0	6.7	29.1	77	9.7	0.77	8.90	0.05	0.00	0.00	1143	20
2♀ 15	52	15.2	6.7	29.2	77	10.7	1.60	9.09	0.03	0.00	0.02	1022	21
3♀ 150	52	15.1	6.8	29.2	76	10.4	0.92	9.47	0.02	0.00	0.01	1069	21
4♀ 500	52	15.4	6.8	29.5	77	10.2	1.29	8.79	0.04	0.00	0.10	971	23

Blank Non-significant

\* P<0.05

\*\* P<0.01

K Kruskal-Wallis analysis

NA No analysis necessary, all data values the same

TABLE 6

Biochemistry - group mean values

Week 4 (2 March 1993)

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♂ Corn oil	104	6.2	2.9	3.3	0.88	10	0.5	496	28	57	0.1	142	3.8	5.2	5.0	95	74
2♂ 15	110	6.3	2.9	3.4	0.87	10	0.5	525	31	57	0.1	142	3.9	5.2	5.2	96	76
3♂ 150	112	6.2	2.9	3.3	0.88	14	0.5	511	33	58	0.1	143	4.1	5.2	5.5	96	70
4♂ 500	** 130	6.5	2.9	3.6	0.82	20	0.5	513	31	67	0.2	141	4.1	5.2	5.1	98	** 39
1♀ Corn oil	116	6.3	3.1	3.2	0.96	12	0.5	367	18	46	0.1	142	3.8	5.4	4.2	98	77
2♀ 15	107	6.1	3.0	3.1	0.97	12	0.5	252	19	48	0.1	142	3.9	5.4	4.2	97	75
3♀ 150	119	6.4	3.1	3.3	0.94	13	0.5	326	21	51	0.1	142	3.8	5.3	4.2	99	70
4♀ 500	134	6.4	3.1	3.3	0.94	15	0.6	264	25	51	0.2	143	3.7	5.4	4.8	98	62

Blank Non-significant

\* P<0.05

\*\* P<0.01

K Kruskal-Wallis analysis

F Frequency analysis

TABLE 7

Organ weights - group mean values

Week 5 (4 March 1993)

Group/ dosage mg/kg/day	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Corn oil	347	A 1.95 (1.94)	A 18.5 (18.0)	A 0.91 (0.89)	A 2.98 (2.94)	54.0	3.91
2♂ 15	335	1.94 (1.95)	19.0 (19.6)	0.75 (0.77)	2.92 (2.96)	48.6	3.96
3♂ 150	359	1.93 (1.91)	19.6 (18.2)	0.81 (0.77)	3.31 (3.21)	50.8	3.71
4♂ 500	326	1.90 (1.92)	25.9 ** (27.2)	0.62 ** (0.62)	3.13 (3.22)	* 46.9	3.95

Blank Non-significant

\* P<0.05

\*\* P<0.01

A Values, adjusted for final bodyweight, given in parentheses

TABLE 7

(Organ weights - continued)

Week 5 (5 March 1993)

Group/ dosage mg/kg/day	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Corn oil	250	A 1.81 (1.81)	A 12.4 (12.6)	A 0.61 (0.62)	A 2.27 (2.29)	67.2	92.5
2♀ 15	258	1.87 (1.86)	13.8 (13.4)	0.66 (0.64)	2.26 (2.23)	64.5	96.8
3♀ 150	262	1.88 (1.86)	15.5 ** (14.8)	0.62 (0.60)	2.16 (2.18)	66.8	102.5
4♀ 500	243	1.86 (1.88)	18.5 ** (19.3)	0.56 (0.59)	2.36 (2.42)	76.4	99.1

Blank Non-significant

\*\* P<0.01

A Values, adjusted for final bodyweight given in parentheses

TABLE 8

Macroscopic pathology incidence summary

Removal reason: Terminal	Group	Group	Group	Group	Group	Group	Group	Group
	1	2	3	4	1	2	3	4
Animals on study	5	5	5	5	5	5	5	5
Animals completed	5	5	5	5	5	5	5	5
	----- Males -----				----- Females -----			
<b>Fur</b>								
Stained	1	0	0	0	0	0	0	0
<b>Skin</b>								
Scab/s	0	2	0	0	0	0	0	0
<b>Eyes</b>								
Damaged	0	1	0	0	0	0	0	0
Congested	0	0	0	1	0	0	0	0
<b>Incisors</b>								
Pale	0	0	1	0	0	0	1	1
<b>Lymph Nodes - Cervical</b>								
Enlarged	5	5	3	5	1	3	1	2
<b>Thymus</b>								
Enlarged	0	0	0	0	0	0	1	0
<b>Lungs</b>								
Petechiac	2	2	0	1	0	1	2	2
Not collapsed	1	0	0	0	0	0	0	0
Congested	0	0	0	0	2	0	0	0
<b>Liver</b>								
Pale subcapsular area/s - median cleft	1	0	0	0	0	1	0	1
Enlarged	1	1	1	5	0	0	1	4
Pale subcapsular area/s	0	0	1	1	0	0	0	0

**TABLE 8**  
**(Macroscopic 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 Animals completed	5 5							
<b>Spleen</b> Capsule thickened area/s Torsioned	0 0	0 1	1 0	2 0	2 0	1 0	0 0	1 0
<b>Stomach</b> White nodule/s, near to limiting ridge	0	3	0	0	0	0	0	0
<b>Adrenals</b> Enlarged	0	0	0	0	0	0	0	1
<b>Kidneys</b> Increased pelvic dilatation	0	0	1	0	0	0	0	0
<b>Uterus</b> Fluid distension	0	0	0	0	1	2	2	3

TABLE 2

Microscopic pathology incidence summary

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	5	5	5	5	5	5	5	5
<b>Heart</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5
<b>Spleen</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5
Extramedullary haemopoiesis (Total)	3	3	3	3	3	3	3	3
Minimal	3	3	3	3	3	3	3	3
Increased cellularity of the white pulp (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Trace	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Capular thickening (Total)	0	0	0	0	0	0	0	0
Trace	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
<b>Liver</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5
Parenchymal inflammatory cells (Total)	4	4	4	4	4	4	4	4
Trace	1	1	1	1	1	1	1	1
Centrilobular hepatocyte enlargement (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Congested sinuses	1	1	1	1	1	1	1	1
Generalised hepatocyte enlargement (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
<b>Kidney</b>								
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	5	5	5	5	5	5	5	5

**TABLE 9.**  
(Microscopic pathology incidence summary - continued)

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	5	5	5	5	5	5	5	5
Animals completed	5	5	5	5	5	5	5	5
(Continued)								
<b>Kidneys</b>	0	0	0	0	0	0	0	1
Mineral foci at the corticomedullary junction (Total)	0	0	0	0	0	0	0	1
Minimal	1	0	0	2	0	0	0	0
Eosinophilic droplets in cortical tubular epithelia (Total)	1	0	0	2	0	0	0	0
Minimal	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	2	0	0	1	2	0	0	2
<b>Lungs</b>	1	0	0	0	1	0	0	1
Examined	1	0	0	0	1	0	0	1
No abnormalities detected	1	0	0	1	1	0	0	1
Congestion	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	2	0	0	1	2	0	0	2
<b>Lymph Nodes - Cervical</b>	5	0	0	5	5	0	0	5
Examined	5	0	0	5	5	0	0	5
No abnormalities detected	2	0	0	2	2	0	0	2
Plasmacytosis	0	0	0	1	0	0	0	0
Lymphoid proliferation	0	0	0	1	0	0	0	0
Prominent germinal centres	4	0	0	0	1	0	0	1
Prominent distended venules containing small lymphocytes	2	0	0	2	0	0	0	1
<b>Uterus</b>	0	0	0	0	1	0	0	3
Examined	0	0	0	0	1	0	0	3
Luminal dilatation (Total)	0	0	0	0	1	0	0	3
Minimal	0	0	0	0	0	0	0	0
Examined	0	0	0	0	0	0	0	0
No abnormalities detected	0	0	0	1	0	0	0	0

## APPENDIX 1

### Bodyweights - individual values (g)

**Group 1♂**  
Corn oil

Cage number	Animal number	Week				
		0	1	2	3	4
1	1	149	208	270	323	356
	2	128	181	236	282	313
	3	139	200	254	294	320
	4	155	220	281	338	374
	5	139	215	286	356	399

**Group 2♂**  
15 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
2	5	134	191	243	296	327
	7	133	200	264	318	351
	8	123	173	242	299	314
	9	131	179	234	284	315
	10	152	225	291	353	391

**Group 3♂**  
150 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
3	11	141	209	278	348	385
	12	130	187	245	303	337
	13	149	218	284	347	397
	14	137	192	248	292	318
	15	154	223	293	356	391

**Group 4♂**  
500 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
4	16	148	196	251	297	320
	17	146	204	261	295	311
	18	134	196	265	330	369
	19	155	219	278	320	340
	20	132	190	245	287	314

## APPENDIX 1

### (Bodyweights - continued)

Group 1♀  
Corn oil

Cage number	Animal number	Week				
		0	1	2	3	4
5	21	136	187	225	254	277
	22	129	167	195	215	227
	23	135	181	215	245	268
	24	140	174	203	240	260
	25	137	167	194	217	229

Group 2♀  
15 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
6	26	134	179	215	240	259
	27	133	179	209	237	256
	28	138	185	225	256	278
	29	126	165	188	214	231
	30	143	196	228	259	284

Group 3♀  
150 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
7	31	133	184	217	246	272
	32	128	171	207	233	248
	33	140	186	215	239	257
	34	134	183	217	248	265
	35	140	202	238	261	279

Group 4♀  
500 mg/kg/day

Cage number	Animal number	Week				
		0	1	2	3	4
8	36	134	168	193	219	232
	37	134	172	200	229	242
	38	135	179	208	236	245
	39	142	193	234	268	285
	40	130	163	188	215	235

## APPENDIX 2

### Haematology - individual values

Week 4 (2 March 1993)

Group/ dosage mg/kg/day	Animal no.	PCV %	Hb g/dl	RBC x10 <sup>6</sup> / mm <sup>3</sup>	MCHC %	MCV fl	WBC + Diff x10 <sup>3</sup> /mm <sup>3</sup>					Plts x10 <sup>9</sup> / mm <sup>3</sup>	TT s	
							Total	N	L	E	B			M
1♂ Corn oil	1	52	15.2	6.6	29.2	79	11.4	1.82	9.35	0.00	0.00	0.23	904	21
	2	56	16.1	7.2	28.8	78	9.4	2.07	7.14	0.00	0.00	0.19	1008	21
	3	52	15.2	6.6	29.2	79	14.3	1.14	13.01	0.00	0.00	0.14	1091	21
	4	55	16.1	7.1	29.3	77	16.2	4.37	11.50	0.00	0.00	0.32	1116	20
	5	51	15.2	6.6	29.8	77	16.8	3.19	13.10	0.00	0.00	0.50	1039	20
	Mean SD		53 2.2	15.6 0.49	6.8 0.30	29.3 0.36	78 1.0	13.6 3.16	2.52 1.272	10.82 2.557	0.00 0.000	0.00 0.000	0.28 0.142	1032 83.0
2♂ 15	6	51	15.2	6.6	29.8	77	12.0	2.76	8.76	0.24	0.00	0.24	1077	22
	7	55	16.1	7.3	29.3	75	13.8	2.48	10.90	0.00	0.00	0.41	1023	21
	8A	50	14.6	6.4	29.2	78	12.0	3.00	8.64	0.12	0.00	0.24	1042	22
	9	52	15.6	6.7	30.0	78	13.0	2.86	10.01	0.13	0.00	0.00	1290	20
	10	53	15.8	6.9	29.8	77	16.2	4.37	11.83	0.00	0.00	0.00	1191	23
	Mean SD		52 1.9	15.5 0.58	6.8 0.34	29.6 0.35	77 1.2	13.4 1.74	3.09 0.738	10.03 1.373	0.10 0.101	0.00 0.000	0.18 0.177	1125 113.1
3♂ 150 <sup>A</sup>	11	51	15.4	6.4	30.2	80	18.4	6.62	11.78	0.00	0.00	0.00	1206	23
	12	55	16.4	7.1	29.8	77	14.4	1.44	12.82	0.00	0.00	0.14	1232	22
	13	55	16.4	6.9	29.8	80	20.8	4.16	16.43	0.00	0.00	0.21	1026	23
	14	53	15.2	6.9	28.7	77	12.6	1.26	11.21	0.00	0.00	0.13	1073	20
	15	52	15.6	6.8	30.0	76	16.5	0.99	15.35	0.00	0.00	0.17	903	21
	Mean SD		53 1.8	15.8 0.57	6.8 0.26	29.7 0.58	78 1.9	16.5 3.23	2.89 2.444	13.52 2.273	0.00 0.000	0.00 0.000	0.13 0.079	1088 135.0
4♂ 500	16	54	16.1	7.2	29.8	75	14.0	1.40	12.60	0.00	0.00	0.00	952	26
	17	53	15.8	6.9	29.8	77	14.0	1.54	12.32	0.14	0.00	0.00	910	33
	18P	49	15.2	6.4	31.0	77	14.3	1.29	12.87	0.14	0.00	0.00	1247	24
	19	52	15.8	6.9	30.4	75	15.0	2.25	12.60	0.15	0.00	0.00	1002	28
	20	53	15.8	6.9	29.8	77	14.7	2.21	12.50	0.00	0.00	0.00	1164	25
	Mean SD		52 1.9	15.7 0.33	6.9 0.29	30.2 0.54	76 1.1	14.4 0.44	1.74 0.458	12.58 0.199	0.09 0.079	0.00 0.000	0.00 0.000	1055 144.2

SD Standard deviation

A Anisocytosis

P Polychromasia

APPENDIX 2

(Haematology - continued)

Week 4 (2 March 1993)

Group/ dosage mg/kg/day	Animal no.	PCV	Hb	RBC	MCHC	MCV	WBC + Diff x10 <sup>3</sup> /mm <sup>3</sup>					Plts	TT	
		%	g/dl	x10 <sup>6</sup> / mm <sup>3</sup>	%	fl	Total	N	L	E	B	M	x10 <sup>3</sup> / mm <sup>3</sup>	s
1♀ Corn oil	21	51	14.6	6.7	28.6	76	8.1	0.73	7.37	0.00	0.00	0.00	1073	20
	22	55	15.4	7.2	28.0	76	6.8	0.75	5.92	0.14	0.00	0.00	1110	21
	23	50	14.8	6.4	29.6	78	10.5	0.95	9.56	0.00	0.00	0.00	1185	20
	24	53	15.4	6.8	29.1	78	13.4	0.94	12.33	0.13	0.00	0.00	1217	20
	25	50	15.0	6.3	30.0	79	9.8	0.49	9.31	0.00	0.00	0.00	1130	21
	Mean SD	52 2.2	15.0 0.36	6.7 0.36	29.1 0.79	77 1.3	9.7 2.52	0.77 0.188	8.90 2.428	0.05 0.074	0.00 0.000	0.00 0.000	1143 57.9	20 0.5
2♀ 15	26	53	15.6	6.8	29.4	78	8.2	1.80	6.31	0.00	0.00	0.08	1155	20
	27	51	15.2	6.6	29.8	77	12.1	2.42	9.68	0.00	0.00	0.00	817	25
	28	54	15.8	6.9	29.3	78	7.2	0.43	6.77	0.00	0.00	0.00	1083	19
	29	51	14.3	6.7	28.0	76	13.7	1.37	12.33	0.00	0.00	0.00	933	21
	30	51	15.0	6.5	29.4	78	12.5	2.00	10.38	0.13	0.00	0.00	1121	21
	Mean SD	52 1.4	15.2 0.58	6.7 0.16	29.2 0.69	77 0.9	10.7 2.86	1.60 0.757	9.09 2.531	0.03 0.058	0.00 0.000	0.02 0.036	1022 142.4	21 2.3
3♀ 150	31	49	14.6	6.4	29.8	77	13.6	1.63	11.97	0.00	0.00	0.00	1025	22
	32	53	15.2	7.0	28.7	76	8.2	0.74	7.38	0.08	0.00	0.00	1090	20
	33	51	15.2	6.5	29.8	78	11.1	0.78	10.32	0.00	0.00	0.00	823	21
	34	52	15.1	7.0	29.0	74	7.4	0.52	6.81	0.00	0.00	0.07	860	20
	35	53	15.2	6.9	28.7	77	11.8	0.94	10.86	0.00	0.00	0.00	1549	21
	Mean SD	52 1.7	15.1 0.26	6.8 0.29	29.2 0.56	76 1.5	10.4 2.58	0.92 0.423	9.47 2.255	0.02 0.036	0.00 0.000	0.01 0.031	1069 290.2	21 0.8
4♀ 500	36	51	14.9	6.5	29.2	78	10.8	0.97	9.72	0.11	0.00	0.00	907	22
	37	52	15.4	6.7	29.6	78	10.6	0.85	9.65	0.00	0.00	0.11	923	23
	38	55	16.1	7.2	29.3	76	10.1	1.21	8.59	0.10	0.00	0.20	1034	28
	39	49	15.0	6.2	30.6	79	10.1	1.72	8.28	0.00	0.00	0.10	1009	23
	40	54	15.5	7.2	28.7	75	9.5	1.71	7.70	0.00	0.00	0.10	982	21
	Mean SD	52 2.4	15.4 0.48	6.8 0.44	29.5 0.70	77 1.6	10.2 0.51	1.29 0.407	8.79 0.879	0.04 0.058	0.00 0.000	0.10 0.071	971 54.6	23 2.7

SD Standard deviation

APPENDIX 3

Biochemistry - individual values

Week 4 (2 March 1993)

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/l	Ca mEq/ mEq/l	P mEq/ mEq/l	Cl mEq/ mEq/l	Chol mg/dl	
			Total	Alb														Glob
1d Corn oil	1	91	5.8	2.7	3.1	0.87	12	0.4	347	19	48	0.1	142	3.9	5.3	4.8	97	91
	2	93	6.5	3.0	3.5	0.86	9	0.5	608	39	59	0.1	142	3.8	5.3	5.0	96	66
	3	108	5.8	2.9	2.9	1.00	11	0.5	503	29	67	0.1	143	4.1	5.0	4.9	97	55
	4	133	6.3	2.9	3.4	0.85	9	0.4	430	27	53	0.1	142	3.8	5.3	5.0	94	85
	5	93	6.4	2.9	3.5	0.83	11	0.5	591	27	57	0.1	143	3.4	5.3	5.4	93	75
	Mean	104	6.2	2.9	3.3	0.88	10	0.5	496	28	57	0.1	142	3.8	5.2	5.0	95	74
	SD	17.8	0.34	0.11	0.27	0.068	1.3	0.05	109.7	7.2	7.1	0.00	0.5	0.25	0.13	0.23	1.8	14.4
2d 15	6	93	6.4	3.0	3.4	0.88	12	0.5	453	28	51	0.1	141	3.8	5.3	5.1	95	84
	7	123	6.5	2.9	3.6	0.81	8	0.5	511	37	56	0.1	144	3.8	5.4	4.9	97	85
	8	113	6.1	2.8	3.3	0.85	10	0.5	516	27	47	0.1	141	4.4	5.2	5.0	98	58
	9	98	6.2	2.9	3.3	0.88	12	0.4	528	33	64	0.1	142	3.8	5.0	5.2	95	81
	10	125	6.3	3.0	3.3	0.91	10	0.6	617	31	66	0.1	143	3.5	5.1	6.0	96	70
	Mean	110	6.3	2.9	3.4	0.87	10	0.5	525	31	57	0.1	142	3.9	5.2	5.2	96	76
	SD	14.4	0.16	0.08	0.13	0.038	1.7	0.07	59.0	4.0	8.2	0.00	1.3	0.33	0.16	0.44	1.3	11.5
3d 150	11	119	6.3	2.8	3.5	0.80	18	0.4	511	26	47	0.1	142	3.4	5.3	5.6	96	76
	12	129	6.5	3.0	3.5	0.86	12	0.5	606	32	66	0.2	142	4.0	5.2	4.9	96	67
	13	102	6.4	3.0	3.4	0.88	14	0.5	508	33	55	0.1	143	5.2	5.3	5.9	95	74
	14	97	6.0	2.8	3.2	0.88	8	0.4	470	32	62	0.1	142	3.7	5.1	5.1	95	95
	15	115	5.9	2.9	3.0	0.97	18	0.5	461	41	62	0.1	144	4.4	5.3	5.9	99	44
	Mean	112	6.2	2.9	3.3	0.88	14	0.5	511	33	58	0.1	143	4.1	5.2	5.5	96	70
	SD	13.0	0.26	0.10	0.22	0.061	4.2	0.05	57.5	5.4	7.5	0.04	0.9	0.70	0.09	0.46	1.6	18.7
4d 500	16	139	6.4	2.8	3.6	0.78	20	0.5	539	36	65	0.1	140	4.0	5.1	5.3	97	23
	17	130	6.0	3.0	3.0	1.00	26	0.6	431	29	62	0.1	141	1.7	5.1	5.4	99	33
	18	128	6.7	2.9	3.8	0.76	18	0.5	504	24	61	0.2	142	4.2	5.3	4.8	95	63
	19	129	6.8	3.0	3.8	0.79	19	0.6	499	33	67	0.1	141	4.5	5.1	5.1	98	34
	20	123	6.8	3.0	3.8	0.79	18	0.5	590	35	80	0.4	142	4.2	5.3	5.1	100	40
	Mean	130	6.5	2.9	3.6	0.82	20	0.5	513	31	67	0.2	141	4.1	5.2	5.1	98	39
	SD	5.8	0.34	0.09	0.35	0.099	3.3	0.05	58.3	4.9	7.6	0.13	0.8	0.29	0.11	0.23	1.9	14.9

SD Standard deviation

APPENDIX 3

(Biochemistry - continued)

Week 4 (2 March 1993)

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/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
			Total	Alb													
1♀ Corn oil	21	127	6.3	3.0	3.3	0.91	9	0.4	377	18	0.1	143	3.9	5.4	4.1	98	92
	22	105	6.6	3.2	3.4	0.94	9	0.4	283	20	0.1	143	3.7	5.5	3.9	98	67
	23	112	6.0	2.9	3.1	0.94	13	0.4	464	18	0.2	141	4.2	5.3	4.1	99	70
	24	141	6.6	3.2	3.4	0.94	9	0.4	287	19	0.1	142	3.7	5.4	4.4	96	78
	25	93	5.8	3.0	2.8	1.07	19	0.7	424	13	0.1	143	3.5	5.2	4.3	100	78
	Mean SD	116 18.8	6.3 0.36	3.1 0.13	3.2 0.063	0.96 4.4	0.5 0.13	367 81.0	18 2.7	46 3.4	0.1 0.04	142 0.9	3.8 0.26	5.4 0.11	4.2 0.19	98 1.5	77 9.7
2♀ 15	26	103	6.0	2.9	3.1	0.94	14	0.6	251	20	0.1	144	3.8	5.5	4.3	96	82
	27	99	5.8	2.9	2.9	1.00	13	0.5	220	20	0.1	141	4.2	5.3	4.3	99	54
	28	118	6.6	3.1	3.5	0.89	11	0.4	226	20	0.1	140	4.1	5.5	4.2	94	105
	29	101	6.1	3.2	2.9	1.10	12	0.6	238	12	0.1	140	3.3	5.3	4.3	97	67
	30	113	6.1	2.9	3.2	0.91	11	0.5	327	24	0.1	143	3.9	5.3	4.1	100	65
	Mean SD	107 8.3	6.1 0.29	3.0 0.14	3.1 0.085	0.97 1.3	0.5 0.08	252 43.4	19 4.4	48 6.5	0.1 0.00	142 1.8	3.9 0.35	5.4 0.11	4.2 0.09	97 2.4	75 19.7
3♀ 150	31	115	6.5	3.3	3.2	1.03	15	0.6	306	22	0.1	140	4.0	5.3	4.4	98	63
	32	112	6.5	3.1	3.4	0.91	11	0.3	381	21	0.1	143	4.0	5.2	4.1	100	95
	33	121	6.3	3.0	3.3	0.91	14	0.5	328	16	0.1	143	3.6	5.3	4.4	99	61
	34	138	6.3	3.1	3.2	0.97	15	0.5	262	21	0.1	141	3.7	5.3	4.1	97	55
	35	109	6.5	3.0	3.5	0.86	10	0.6	353	23	0.1	141	3.9	5.4	4.2	99	76
	Mean SD	119 11.5	6.4 0.11	3.1 0.12	3.3 0.065	0.94 2.3	0.5 0.12	326 45.4	21 2.7	51 5.9	0.1 0.00	142 1.3	3.8 0.18	5.3 0.07	4.2 0.15	99 1.1	70 15.9
4♀ 500	36	113	6.9	3.2	3.7	0.86	12	0.5	217	25	0.2	143	3.8	5.4	4.4	97	62
	37	127	6.1	3.2	2.9	1.10	14	0.5	236	21	0.1	142	3.3	5.3	4.3	100	59
	38	145	6.2	3.1	3.1	1.00	16	0.6	323	28	0.2	144	3.7	5.4	6.0	97	51
	39	159	6.5	3.0	3.5	0.86	15	0.6	317	20	0.1	141	4.5	5.5	5.0	96	50
	40	125	6.2	2.9	3.3	0.88	19	0.6	227	33	0.2	143	3.2	5.2	4.4	100	86
	Mean SD	134 18.1	6.4 0.33	3.1 0.13	3.3 0.107	0.94 2.6	0.6 0.05	264 51.6	25 5.3	51 7.4	0.2 0.05	143 1.1	3.7 0.51	5.4 0.11	4.8 0.72	98 1.9	62 14.6

SD Standard deviation

**APPENDIX 4**

**Organ weights - individual values**

Week 5 (4 March 1993)

Group/ dosage mg/kg/day	Animal no.	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Corn oil	1	353	1.82	15.9	0.88	2.83	49.8	3.91
	2	311	1.94	16.8	0.67	2.88	48.1	3.82
	3	313	1.90	16.6	0.70	2.83	54.2	4.06
	4	368	1.95	19.9	1.03	3.27	60.7	3.87
	5	392	2.12	23.3	1.25	3.07	57.2	3.90
	Mean SD	347 35.1	1.95 0.109	18.5 3.09	0.91 0.242	2.98 0.193	54.0 5.19	3.91 0.091
2♂ 15	6	324	1.99	17.0	0.85	2.67	45.0	3.72
	7	347	1.99	19.3	0.73	2.72	44.3	3.93
	8	309	1.86	16.0	0.78	2.53	55.1	4.19
	9	311	1.92	18.6	0.73	3.25	46.3	3.98
	10	383	1.96	24.2	0.68	3.43	52.3	3.98
	Mean SD	335 30.8	1.94 0.057	19.0 3.16	0.75 0.064	2.92 0.395	48.6 4.81	3.96 0.167
3♂ 150	11	379	1.84	20.6	0.87	3.18	50.5	3.54
	12	328	1.88	18.8	0.85	2.87	45.5	3.85
	13	391	2.00	24.2	0.83	3.57	49.9	3.88
	14	315	1.95	15.5	0.65	3.26	54.6	3.30
	15	381	1.98	19.1	0.85	3.68	53.4	3.97
	Mean SD	359 34.5	1.93 0.068	19.6 3.13	0.81 0.089	3.31 0.320	50.8 3.54	3.71 0.281
4♂ 500	16	314	1.88	23.1	0.59	2.97	46.8	4.03
	17	303	1.90	25.6	0.62	2.96	49.1	3.90
	18	367	2.04	30.4	0.72	3.46	50.3	4.13
	19	340	1.89	27.1	0.57	3.28	44.4	3.92
	20	308	1.79	23.1	0.59	2.96	43.9	3.76
	Mean SD	326 26.6	1.90 0.089	25.9 3.07	0.62 0.062	3.13 0.232	46.9 2.81	3.95 0.141

SD Standard deviation

APPENDIX 4

(Organ weights - continued)

Week 5 (5 March 1993)

Group/ dosage mg/kg/day	Animal no.	Body wt. g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Corn oil	21	278	1.85	13.2	0.55	2.15	72.0	70.9
	22	223	1.80	9.7	0.46	2.15	75.0	87.0
	23	264	1.80	13.1	0.77	2.43	62.6	102.6
	24	256	1.79	13.7	0.75	2.55	65.6	110.6
	25	231	1.80	12.3	0.53	2.09	61.0	91.6
	Mean SD	250 23.1	1.81 0.024	12.4 1.57	0.61 0.139	2.27 0.204	67.2 6.04	92.5 15.23
2♀ 15	26	251	1.86	12.9	0.54	2.10	62.9	92.0
	27	250	1.77	12.2	0.80	2.27	69.7	109.3
	28	278	2.02	15.6	0.51	2.32	72.5	86.8
	29	229	1.73	12.2	0.60	2.17	57.7	76.7
	30	283	1.97	16.2	0.83	2.45	59.8	119.3
	Mean SD	258 22.4	1.87 0.125	13.8 1.94	0.66 0.149	2.26 0.136	64.5 6.36	96.8 17.24
3♀ 150	31	268	1.82	15.6	0.64	2.21	53.5	111.9
	32	250	1.81	14.3	0.48	1.86	69.8	106.2
	33	254	1.90	15.2	0.74	2.09	70.8	100.6
	34	263	2.03	15.1	0.48	2.28	69.4	93.5
	35	273	1.82	17.1	0.76	2.37	70.4	100.5
	Mean SD	262 9.5	1.88 0.092	15.5 1.04	0.62 0.138	2.16 0.196	66.8 7.44	102.5 6.90
4♀ 500	36	231	1.78	18.5	0.56	2.15	69.9	110.3
	37	240	1.86	17.1	0.53	2.52	61.7	79.8
	38	240	1.80	18.8	0.52	2.57	103.7	98.6
	39	283	1.92	22.6	0.72	2.49	71.4	102.7
	40	223	1.92	15.4	0.49	2.09	75.3	104.1
	Mean SD	243 23.3	1.86 0.065	18.5 2.66	0.56 0.091	2.36 0.229	76.4 16.05	99.1 11.58

SD Standard deviation

**Clinical and pathological data relating to rats killed at termination**

<b>Group:</b>	1	2	3	4
<b>Compound:</b>	Control			
<b>Level (mg/kg/day):</b>	0	15	150	500

In this appendix the clinical, macroscopic and microscopic findings relating to each animal are listed on one page. These findings are presented by an automated data collation system.

The following abbreviation is used:

**W.N.L. - Within normal limits (macroscopic abnormality)**



## APPENDIX 5

(Pathology - continu...)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 1♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

#### Fur

Stained - periorbital region/s: (Right , Red , Brown , Minimal)

#### Lymph Nodes - Cervical

Enlarged: 8mm

#### Liver

Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Extramedullary haemopoiesis: (Minimal)

#### Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Minimal)

#### Lymph Nodes - Cervical

Plasmacytosis

Prominent germinal centres

Prominent distended venules containing small lymphocytes

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 1♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Liver : (W.N.L.); Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 2♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 9mm

**Lungs**

Petechiae: (A few)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Lymph Nodes - Cervical**

Plasmacytosis

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals; Lungs : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:        "  
Dosage Level:    Corn oil  
Rat No/Sex:      3♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 7mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Extramedullary haemopoiesis: (Minimal)

**Lymph Nodes - Cervical**  
Prominent germinal centres

The following tissues were considered normal:

Heart; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 4♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 8mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Lymph Nodes - Cervical**

Prominent germinal centres

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 5♂ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 10mm

**Lungs**

Petechiae: (A few)

Not collapsed

**Liver**

Enlarged: 23.292g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Extramedullary haemopoiesis: (Minimal)

**Liver**

Parenchymal inflammatory cells: (Trace , Foci)

Congested sinuses

**Lungs**

Congestion

**Lymph Nodes - Cervical**

Prominent germinal centres

Prominent distended venules containing small lymphocytes

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 5♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Kidneys; Adrenals

Pathologist: R.L.Gregson

**APPENDIX 5**

**(Pathology - continued)**

**Compound:**

**Dosage Level:** 15 mg/kg/day

**Rat No/Sex:** 6♂ (Terminal)

**CLINICAL FINDINGS**

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

**MACROSCOPIC FINDINGS**

**Lymph Nodes - Cervical**

Enlarged: 8mm

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver

**Pathologist:** R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound: .....

Dosage Level: 15 mg/kg/day

Rat No/Sex: 7♂ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

#### Skin Scabs

Infra-auricular region/s: (Right , One) 1mm

#### Lymph Nodes - Cervical

Enlarged: 9mm

#### Lungs

Petechiae: (A few)

#### Spleen

Torsioned: (Severe)

#### Stomach Antrum Mucosa

White nodule/s, near to limiting ridge: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 8♂ (Terminal)

### CLINICAL FINDINGS

The right eye was swollen following the blood sampling procedure on Day 27, 28 and 29. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Skin Scabs

Lip/s: (Right , Upper , One) 3mm

#### Eyes

Damaged: (Right)

#### Lymph Nodes - Cervical

Enlarged: 10mm

#### Stomach Antrum Mucosa

White nodule/s, near to limiting ridge: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

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

**Lymph Nodes - Cervical**

Enlarged: 8mm

**Stomach Antrum Mucosa**

White nodule/s, near to limiting ridge: (One) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

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: 8mm

**Lungs**

Petechiae: (A few)

**Liver**

Enlarged: 24.193g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**

Congested sinuses

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 11♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of the study and abnormal gait (walking on toes) on Day 27 to 29. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 10mm

**Spleen**

Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**

Centrilobular hepatocyte enlargement: (Minimal)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 12♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of the study and abnormal gait (walking on toes) on Day 27 to 29 and red/brown staining around the mouth after dosing on Day 4. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Incisors**

Pale: (Right , Lower)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 13♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of dosing, abnormal gait (walking on toes) on Day 27 to 29 and red/brown staining around the mouth after dosing on Day 4. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 9mm

**Liver**

Enlarged: 24.174g

Pale subcapsular area/s: (One) median lobe, 2mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 14♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of dosing, abnormal gait (walking on toes) on Day 27 to 29 and red/brown staining around the mouth after dosing on Day 4, 7 and 9. Loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 15♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted intermittently during the first half of the study and on most occasions during the second half of the study. This was accompanied by greasy and wet fur during the last week of dosing, abnormal gait (walking on toes) on Day 27 to 29 (and on Day 2 accompanied by hunched posture). Red/brown staining around the mouth was seen after dosing on Day 9 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

Lymph Nodes - Cervical  
Enlarged: 7mm

Kidneys

Increased pelvic dilatation: (Right , Minimal)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte enlargement: (Minimal)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 16♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 7mm

**Liver**  
Enlarged: 23.098g  
Pale subcapsular area/s: (A few, Punctate) left lobe

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Increased cellularity of the white pulp: (Minimal)

**Liver**  
Congested sinuses  
Generalised hepatocyte enlargement: (Minimal)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 16♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Kidneys; Adrenals; Lymph Nodes - Cervical : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound: -  
Dosage Level: 500 mg/kg/day  
Rat No/Sex: 17♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 8mm

**Liver**  
Enlarged: 25.614g

**Spleen**  
Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Capsular thickening: (Trace)

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

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 17♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Kidneys; Adrenals; Lymph Nodes - Cervical : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX B

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 18♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**  
Enlarged: 8mm

**Liver**  
Enlarged: 30.430g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Extramedullary haemopoiesis: (Minimal)  
Increased cellularity of the white pulp: (Minimal)

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

**Kidneys**  
Eosinophilic droplets in cortical tubular epithelia: (Minimal)

**Lymph Nodes - Cervical**  
Lymphoid proliferation

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 18♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 19♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

**Eyes**  
Congested: (Right , Minimal)

**Lymph Nodes - Cervical**  
Enlarged: 9mm

**Lungs**  
Petechiae: (A few)

**Liver**  
Enlarged: 27.069g

**Spleen**  
Capsule thickened area/s: (A few) 1mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**  
Capsular thickening: (Trace)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 19♂ - continued

**MICROSCOPIC FINDINGS - continued**

**Liver**

Congested sinuses  
Generalised hepatocyte enlargement: (Minimal)

**Lungs**

Congestion

**Lymph Nodes - Cervical**

Plasmacytosis  
Prominent distended venules containing small lymphocytes

The following tissues were considered normal:

Heart, Kidneys; Adrenals; Eyes : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 20♂ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation accompanied by abnormal gait (walking on toes) and slight hunched posture was noted throughout the majority of the study. Greasy and wet fur was observed on the majority of occasions during the second half of the study and red/brown staining around the mouth after dosing intermittently during the first two weeks of the study. Loose faeces were seen on the cage tray on Day 28 and 29.

### MACROSCOPIC FINDINGS

#### Lymph Nodes - Cervical

Enlarged: 9mm

#### Liver

Enlarged: 23.104g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Liver

Centrilobular hepatocyte enlargement: (Minimal)  
Congested sinuses

#### Kidneys

Eosinophilic droplets in corticl tubular epithelia: (Minimal)

#### Lymph Nodes - Cervical

Plasmacytosis  
Prominent distended venules containing small lymphocytes

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 20♂ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Adrenals

Pathologist: R.L.Gregson

**APPENDIX 5**

**(Pathology - continued)**

**Compound:**

**Dosage Level:** Corn oil

**Rat No/Sex:** 21 ♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

No abnormalities were seen in the animal

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals

**Pathologist:** R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 22♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

#### Lungs

Congested

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Liver

Parenchymal inflammatory cells: (Trace , Foci)

The following tissues were considered normal:

Heart; Spleen; Kidneys; Adrenals; Lungs : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: Corn oil

Rat No/Sex: 23♀ (Terminal)

### CLINICAL FINDINGS

No signs of ill health or behavioural change were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 7mm

**Spleen**

Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Extramedullary haemopoiesis: (Minimal)

Capsular thickening: (Moderate , Area)

**Lymph Nodes - Cervical**

Prominent germinal centres

The following tissues were considered normal:

Heart; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

**APPENDIX 5**

**(Pathology - continued)**

Compound:

Dosage Level: Corn oil

Rat No/Sex: 24♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Uterus**

Luminal dilatation: (Minimal)

The following tissues were considered normal:

Heart; Spleen; Liver; Kidneys; Adrenals

Pathologist: R.L.Gregson

**APPENDIX 5**

**(Pathology - continued)**

Compound:

Dosage Level: Corn oil

Rat No/Sex: 25♀ (Terminal)

**CLINICAL FINDINGS**

No signs of ill health or behavioural change were noted.

**MACROSCOPIC FINDINGS**

**Lungs**  
Congested

**Spleen**  
Capsule thickened area/s: (A few) 1mm

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following observations were noted:

**Spleen**  
Capsular thickening: (Trace)

**Liver**  
Congested sinuses

**Lungs**  
Congestion

The following tissues were considered normal:

Heart; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 26♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 7mm

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 27♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 9mm

**Liver**

Pale subcapsular area/s - median cleft: (One , Punctate)

**Spleen**

Capsule thickened area/s: (A few , Diffuse)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 28♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

#### Lungs

Petechiae: (A few)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

**APPENDIX 5**

**(Pathology - continued)**

**Compound:**

**Dosage Level:** 15 mg/kg/day

**Rat No/Sex:** 29♀ (Terminal)

**CLINICAL FINDINGS**

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

**MACROSCOPIC FINDINGS**

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

**MICROSCOPIC FINDINGS**

The following tissues were considered normal:

Liver

**Pathologist:** R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 15 mg/kg/day

Rat No/Sex: 30♀ (Terminal)

### CLINICAL FINDINGS

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

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 10mm

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 31 ♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

Lungs

Petechiae: (A few)

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 32♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, red/brown staining around the mouth on Day 4 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 33♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, red/brown staining around the mouth on Day 4 and 9 and loose faeces were seen on cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: (Minimal) 6mm

**Thymus**

Enlarged

**Lungs**

Petechiae: (A few)

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 34♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30, red/brown staining around the mouth on Day 4 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Incisors

Pale: (Left, Lower)

#### Uterus

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 150 mg/kg/day

Rat No/Sex: 35♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted on the majority of occasions throughout the study, this was associated with wet fur on Day 23 to 28. Greasy fur was observed from the beginning of Week 3 everyday until termination (Day 30), abnormal gait (walking on toes) on Day 27 to 30 (and on Day 2 accompanied by hunched posture) red/brown staining around the mouth on Day 2, 4 and 9 and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Liver**  
Enlarged: (Minimal) 17.106g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**  
Congested sinuses

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 36♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 3. Abnormal gait (walking on toes) and hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

#### Lungs

Petechiae: (A few)

#### Liver

Enlarged: 18.507g

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

#### Spleen

Extramedullary haemopoiesis: (Minimal)

#### Liver

Generalised hepatocyte enlargement: (Minimal)

The following tissues were considered normal:

Heart; Kidneys; Adrenals; Lungs : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 37♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and hunched posture was observed on the majority of occasions throughout the study and loose faeces on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Incisors**

Pale: (Lower)

**Liver**

Enlarged: (Minimal) 17.057g

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**

Generalised hepatocyte enlargement: (Minimal)

**Uterus**

Luminal dilatation: (Minimal)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 37♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Spleen; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 38♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and slight hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 7mm

**Lungs**

Petechiae: (A few)

**Liver**

Enlarged: 18.823g

**Spleen**

Capsule thickened area/s: (A few) 1mm

**Adrenals**

Enlarged: 103.7mg

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Capsular thickening: (Trace)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 38♀ - continued

**MICROSCOPIC FINDINGS - continued**

**Liver**

Congested sinuses  
Generalised hepatocyte enlargement: (Minimal)

**Kidneys**

Mineral foci at the corticomedullary junction: (Minimal)

**Lungs**

Congestion

**Lymph Nodes - Cervical**

Plasmacytosis  
Prominent distended venules containing small lymphocytes

The following tissues were considered normal:

Heart; Adrenals : (W.N.L.)

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 39♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and slight hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Lymph Nodes - Cervical**

Enlarged: 8mm

**Liver**

Pale subcapsular area/s - median cleft: (One) 1mm

Enlarged: 22.577g

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Spleen**

Extramedullary haemopoiesis: (Minimal)

**Lymph Nodes - Cervical**

Prominent germinal centres

**Uterus**

Luminal dilatation: (Minimal)

**APPENDIX 5**

**(Pathology - continued)**

Rat No/Sex: 39♀ - continued

**MICROSCOPIC FINDINGS - continued**

The following tissues were considered normal:

Heart; Liver : (W.N.L.); Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 5

(Pathology - continued)

Compound:

Dosage Level: 500 mg/kg/day

Rat No/Sex: 40♀ (Terminal)

### CLINICAL FINDINGS

A post dose increase in salivation was noted everyday (except Day 1) throughout the study, this was associated with wet fur during the second half of the study and greasy fur during Week 4. Abnormal gait (walking on toes) and slight hunched posture was observed on the majority of occasions throughout the study and loose faeces were seen on the cage tray on Day 28. No other signs of ill health, behavioural change or reaction to treatment were noted.

### MACROSCOPIC FINDINGS

**Uterus**

Fluid distension

All the other organs and tissues appeared normal.

### MICROSCOPIC FINDINGS

The following observations were noted:

**Liver**

Generalised hepatocyte enlargement: (Minimal)

**Uterus**

Luminal dilatation: (Minimal)

The following tissues were considered normal:

Heart; Spleen; Kidneys; Adrenals

Pathologist: R.L.Gregson

## APPENDIX 6

### Special Diet Services Rat and Mouse Maintenance Diet

#### Composition and quality assurance aspects of diet

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

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

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

#### Contaminants

#### Maximum concentration

Fluoride	20	mg/kg
Nitrate (as NaNO <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 6

(Aspects of diet - 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 7

### Quality assurance aspects of drinking water

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

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

These results include levels of:

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

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



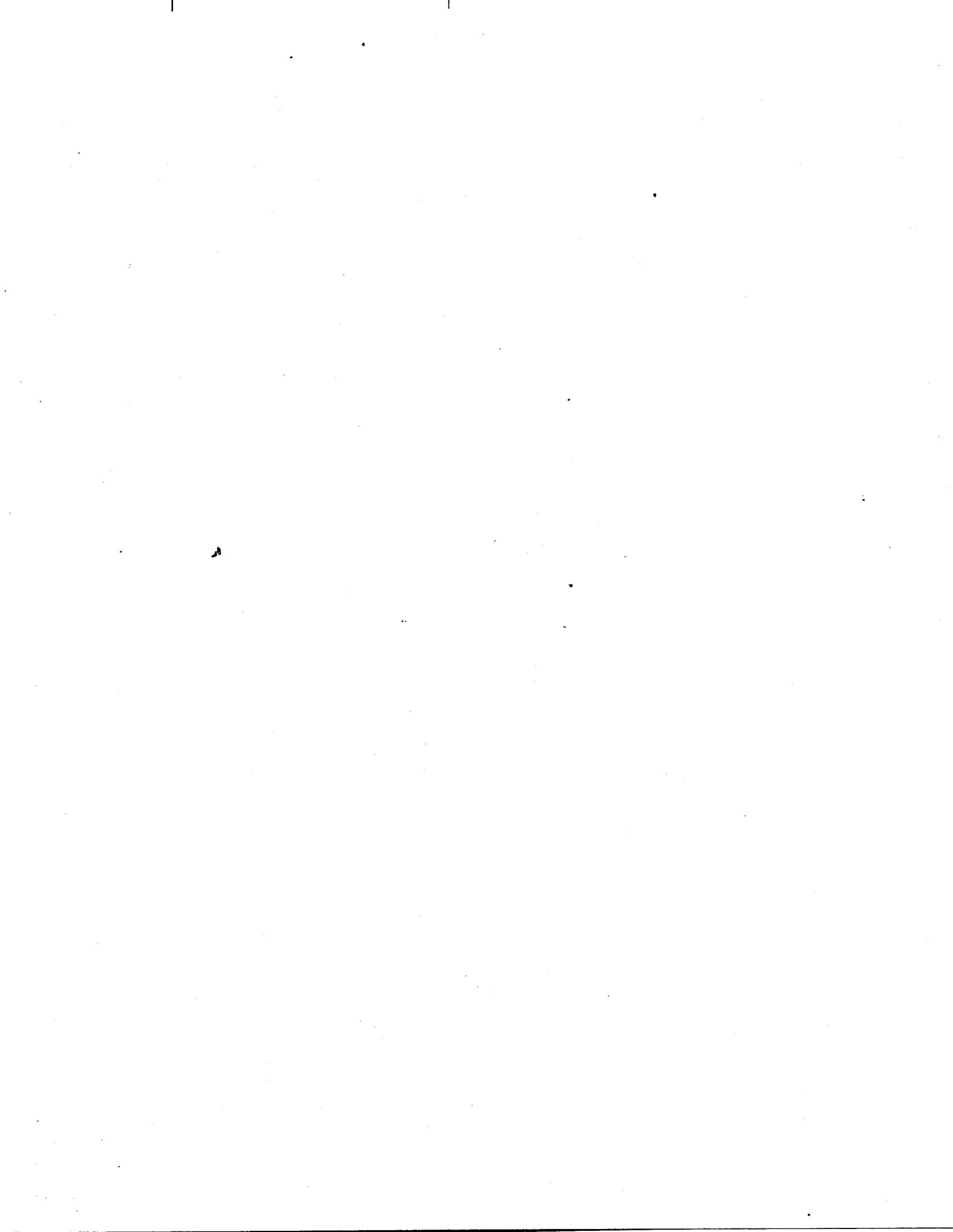
**APPENDIX 8**

**The analysis of                      in corn oil**

**THE ANALYSIS OF  
IN CORN OIL FORMULATIONS**

**Authors:**

**Alan Anderson,  
I. Suzanne Dawe,  
S. Patel.**



## APPENDIX 8

(Chemical analysis - continued)

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## APPENDIX 8

(Chemical analysis - continued)

### INTRODUCTION

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

The determination of concentrations of                      n dose formulations prepared for Day 1 of of the study.

The determination of the chemical stability of                      in corn oil formulations.

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

The formulations for this study were prepared as solutions of                      in corn oil by Formulation personnel at Huntingdon Research Centre Limited.

## APPENDIX 8

(Chemical analysis - continued)

### EXPERIMENTAL PROCEDURE

#### ANALYTICAL PROCEDURE

##### Apparatus and instrumentation

High performance liquid chromatograph (HPLC):

Pump:

Autosampler:

Detector:

Integrator:

As detailed below or suitable alternative.

Spectra-Physics SP8770.

Waters Associates WISP model 710B.

Waters Associates model 481.

Spectra-Physics SP4270.

General laboratory glassware.

##### Reagents

Test material:

Supplier:

Batch no.:

Stated purity:

5364.

>98%.

Acetonitrile:

Merck Ltd., Far UV HiPerSolv for HPLC

Tetrahydrofuran:

Rathburn Chemicals Ltd., HPLC grade

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 (50 or 100 ml) of tetrahydrofuran. The extract was appropriately diluted, initially using tetrahydrofuran and finally using mobile phase, to provide a solution containing the expected concentration range 2 - 4  $\mu\text{g/ml}$ .

The final solution was filtered (Whatman PURADISC™ 25PP, 0.2 $\mu\text{m}$ ) and the concentration of was quantified by high performance liquid chromatography using ultraviolet detection as detailed in the following section.

**APPENDIX 8****(Chemical analysis - continued)****Typical chromatographic conditions**

Analytical column:	Merck Limited, LiChrospher 100 RP-18e, 5 $\mu$ m, 125 mm x 4 mm ID.
Guard column:	Merck Limited, LiChrospher 100 RP-18e, 5 $\mu$ m, 4 mm x 4 mm ID.
Mobile phase:	Acetonitrile/water (1/1 v/v).
Flow rate:	1.0 ml/minute.
Detector wavelength:	UV, 215 nm.
Injection volume:	30 $\mu$ l.
Integrator attenuation:	16.
Retention volume:	Isomer I 9.5 ml. Isomer II 11.0 ml.

**Calibration**

A primary standard solution was prepared for each analytical occasion by dissolving an accurately weighed quantity (50 mg) of \_\_\_\_\_ in acetonitrile. Solutions for instrument calibration, containing \_\_\_\_\_ in the concentration range 1 - 5  $\mu$ 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.

## APPENDIX 8

### (Chemical analysis - continued)

#### Calculation

The peak response for \_\_\_\_\_ in each calibration chromatogram was measured with respect to each isomer 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 each isomer of \_\_\_\_\_ in sample and procedural recovery chromatograms was measured and the concentration of \_\_\_\_\_ was determined with respect to each isomer using the following equation:

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

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)

Results were corrected for the appropriate mean procedural recovery value at analysis.

#### Limit of detection

The limit of detection, defined as the concentration of \_\_\_\_\_ 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

The analytical procedure was validated by fortifying a minimum of six samples (1 ml) of control vehicle with \_\_\_\_\_ to concentrations of 1 mg/ml and 200 mg/ml, which were analysed in accordance with the analytical procedure. The test substance, \_\_\_\_\_, was added either as a solution in tetrahydrofuran (inclusion levels  $< 20$  mg/ml) or as neat test material (inclusion levels  $\geq 20$  mg/ml).

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

## APPENDIX 8

(Chemical analysis - continued)

### DETERMINATION OF CONCENTRATIONS OF [REDACTED] IN DOSE FORMULATIONS ANALYSED DURING 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 CHEMICAL STABILITY OF [REDACTED] IN CORN OIL FORMULATIONS

Representative samples (approximately 20 ml) of freshly-prepared specimen formulations, containing [REDACTED] at nominal concentrations of 1 mg/ml and 200 mg/ml, were thoroughly mixed and duplicate sub-samples (approximately 1ml) were removed for analysis (0 hour).

The formulations were stored at ambient temperature in the dark. At time-points representing 4 hours and 24 hours storage, the formulations were mixed and sampled for analysis as above.

At each occasion, two sub-samples of each formulation were analysed in accordance with the analytical procedure.

## APPENDIX 8

(Chemical analysis - continued)

### RESULTS

The mean concentrations of \_\_\_\_\_ in dose formulations analysed on Day 1 of the study and the deviation of mean results from nominal values are summarised, with respect to both isomers, in Table 1. Mean results were within the range +2%/-6% of nominal concentrations. Individual analytical results and associated procedural recovery data are detailed in Table 2.

The results in Table 3 confirm that, at nominal concentrations of 1 mg/ml and 200 mg/ml, \_\_\_\_\_ is chemically stable in the corn oil formulation during storage in the dark at ambient temperature for 24 hours.

Procedural recovery data obtained during method validation and the determination of stability are presented in Table 4. The data confirm the precision and accuracy of the analytical method with respect to both isomers. Results for the analysis of the test sample are corrected for the appropriate mean procedural recovery value at analysis.

Typical calibration standard graphs are presented in Figure 1 which confirms the linearity of detector response for \_\_\_\_\_ over the concentration range 1 - 5 µg/ml. Typical analytical chromatograms are presented in Figures 2 and 3. In Figure 2, the absence of a peak at the characteristic retention volumes for each isomer of \_\_\_\_\_ in the control sample chromatogram demonstrates the specificity of the HPLC assay.

### CONCLUSION

The analytical results confirm that the dose formulations were accurately prepared and were stable from the time of preparation to the completion of dosing.

**APPENDIX 8**

**(Chemical analysis - continued)**

**TABLE 1**

**Summary: mean concentrations of                    n dose formulations**

**1.1 With respect to isomer I**

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
1	Control	0	ND	-
	2	3	2.84	-5.3
	3	30	29.3	-2.3
	4	100	99.4	-0.6

**1.2 With respect to isomer II**

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
1	Control	0	ND	-
	2	3	2.93	-2.3
	3	30	30.0	0.0
	4	100	102	+2.0

**ND**    None detected (<0.075 mg/ml)

**RME**    Relative mean error, representing the deviation from nominal

**APPENDIX 8**

(Chemical analysis - continued)

**TABLE 2**

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

1.1 With respect to isomer I

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean
1	Control	0	ND	ND	ND		
	2	3	2.86	2.82	2.84	98.9	
	3	30	29.2	29.4	29.3	100.2	100.7
	4	100	99.2	99.5	99.4	100.5	

2.2 With respect to isomer II

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recoveries (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Mean
1	Control	0	ND	ND	ND		
	2	3	2.91	2.94	2.93	100.1	
	3	30	29.9	30.0	30.0	99.5	98.3
	4	100	104	101	102	99.8	

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

**APPENDIX 8**

**(Chemical analysis - continued)**

**TABLE 3**

**Chemical stability of 1 in corn oil formulations**

**3.1 With respect to isomer I**

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)			RME (%)
		Analysis 1	Analysis 2	Mean	
1	0	0.980	0.972	0.976	-
	4	0.962	0.986	0.974	-0.2
	24	1.01	0.995	1.00	+2.5
200	0	199	199	199	-
	4	198	199	199	0.0
	24	194	194	194	-2.5

**3.2 With respect to isomer II**

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)			RME (%)
		Analysis 1	Analysis 2	Mean	
1	0	0.990	0.991	0.990	-
	4	0.979	0.998	0.988	-0.2
	24	0.994	0.989	0.991	+0.1
200	0	202	203	203	-
	4	202	203	202	-0.5
	24	201	201	201	-1.0

ND None detected (<0.075 mg/ml)

RME Relative mean error, representing the deviation from time zero

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

**APPENDIX 8**

(Chemical analysis - continued)

**TABLE 4**

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

(results are expressed as percent recovery)

4.1 With respect to isomer I

Analytical phase	Nominal level of fortification (mg/ml)	
	1	200
Validation	100.1	102.2
	100.6	101.8
	99.7	102.1
	100.0	102.0
	99.2	101.0
	100.0	101.6
Stability	100.0	102.0
	102.6	97.9
Mean	100.3	101.3
SD (±)	1.02	1.44
Range	99.2 - 102.6	97.9 - 102.2
n	8	8

SD Standard deviation  
n Number of determinations

APPENDIX 8

(Chemical analysis - continued)

TABLE 4

(continued)

(results are expressed as percent recovery)

4.2 With respect to isomer II

Analytical phase	Nominal level of fortification (mg/ml)	
	1	200
Validation	97.2	98.4
	97.5	97.6
	96.8	98.2
	96.9	98.1
	97.1	98.0
	97.5	97.7
Stability	98.4	101.0
	98.9	98.9
Mean	97.5	98.5
SD (±)	0.74	1.09
Range	96.8 - 98.9	97.6 - 101.0
n	8	8

SD Standard deviation  
n Number of determinations

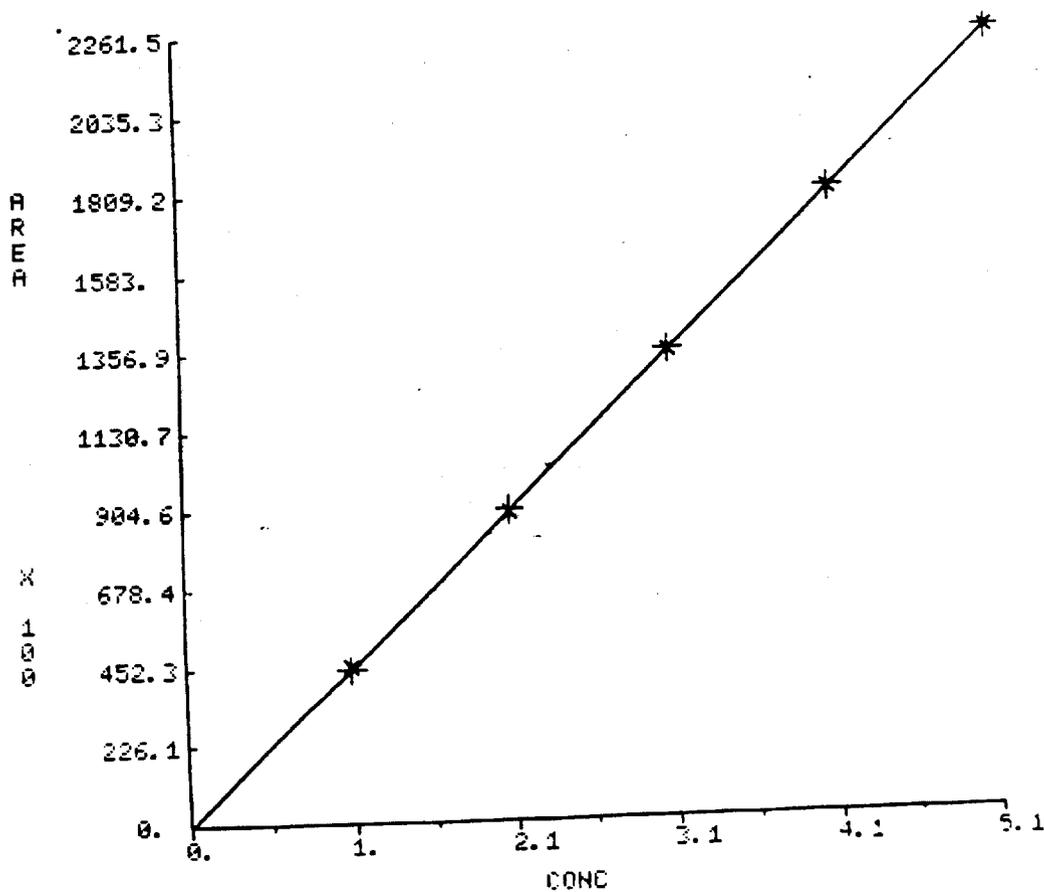
APPENDIX 8

(Chemical analysis - continued)

FIGURE 1

Typical calibration standard graph  
(Day 1)

With respect to isomer I



COEFFICIENTS OF LEAST SQUARES FIT TO A LINEAR EQUATION  
KA= 0. KB= 44065.805 KC=-528.29951  
CORRELATION COEFFICIENT OF X-Y PAIRS = 0.9999668  
COEFFICIENT OF DETERMINATION = 0.9999336

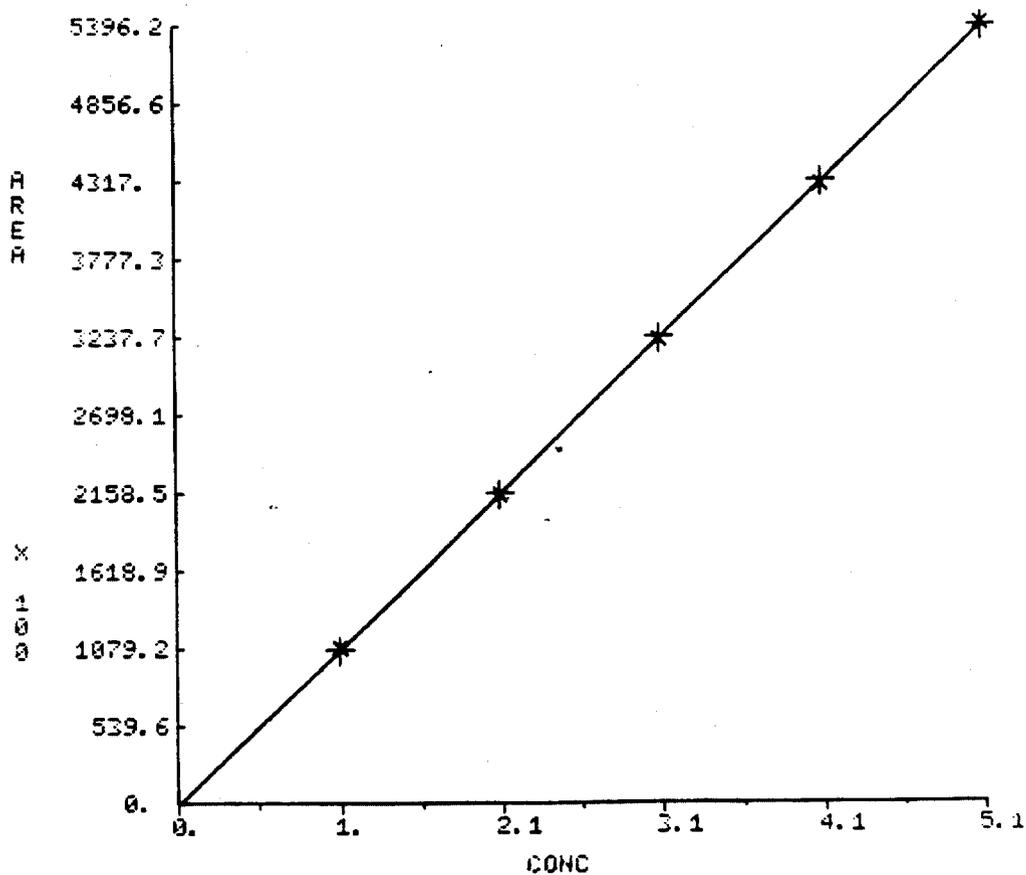
APPENDIX 8

(Chemical analysis - continued)

FIGURE 1

Typical calibration standard graph  
(Day 1)

With respect to isomer II



COEFFICIENTS OF LEAST SQUARES FIT TO A LINEAR EQUATION

KA= 0.                    KB= 105220.16            KC=-1632.8979

CORRELATION COEFFICIENT OF X-Y PAIRS = 0.9999423

COEFFICIENT OF DETERMINATION = 0.9998847

APPENDIX 8

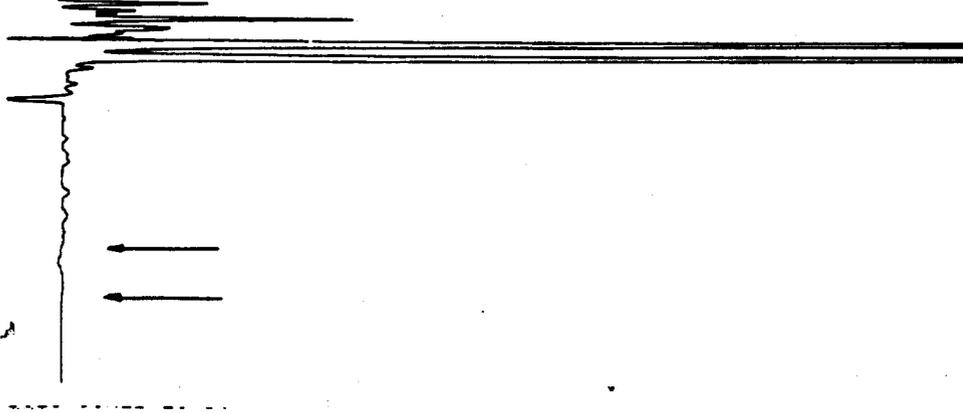
(Chemical analysis - continued)

FIGURE 2

Typical sample chromatograms  
(Day 1)

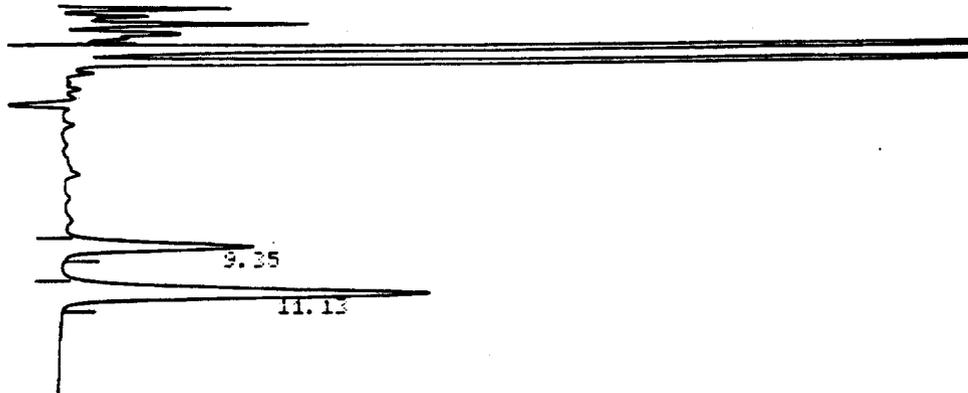
Group 1, Control (1 g/500 ml)

CHANNEL A INJECT 04-02-93 22:40:45 STORED TO BIN # 24



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

CHANNEL A INJECT 04-02-93 19:40:47 STORED TO BIN # 9



APPENDIX 8

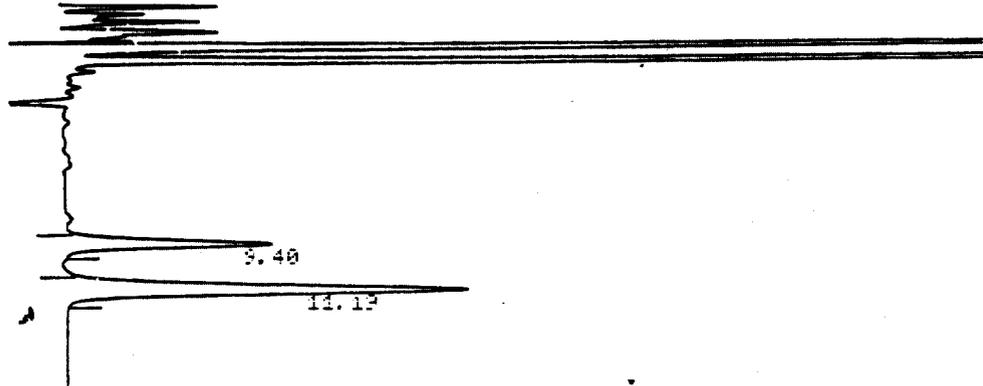
(Chemical analysis - continued)

FIGURE 2

(continued)

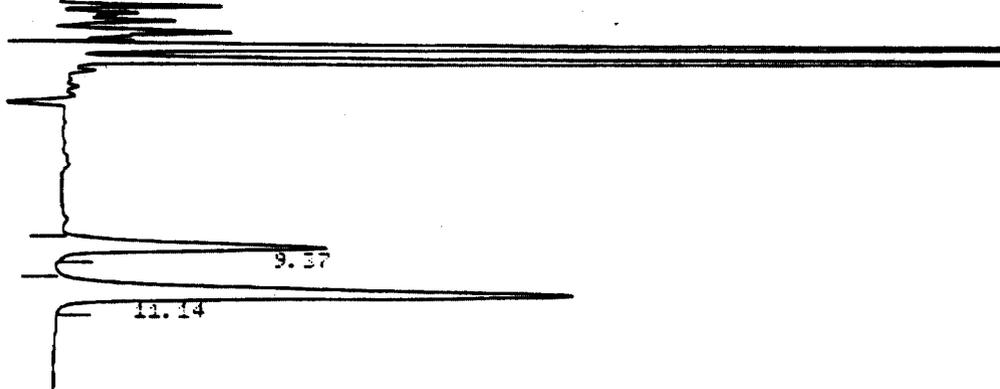
Group 3, 30 mg/ml (1 g/10000 ml)

CHANNEL A INJECT 04-02-93 21:00:46 STORED TO BIN # 14



Group 4, 100 mg/ml (1 g/25000 ml)

CHANNEL A INJECT 04-02-93 22:52:45 STORED TO BIN # 21



APPENDIX 8

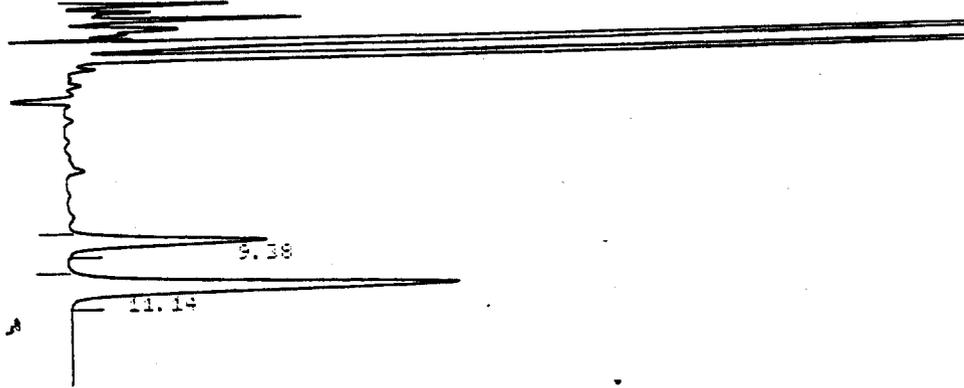
(Chemical analysis continued)

FIGURE 3

Typical procedural recovery chromatograms  
(Day 1)

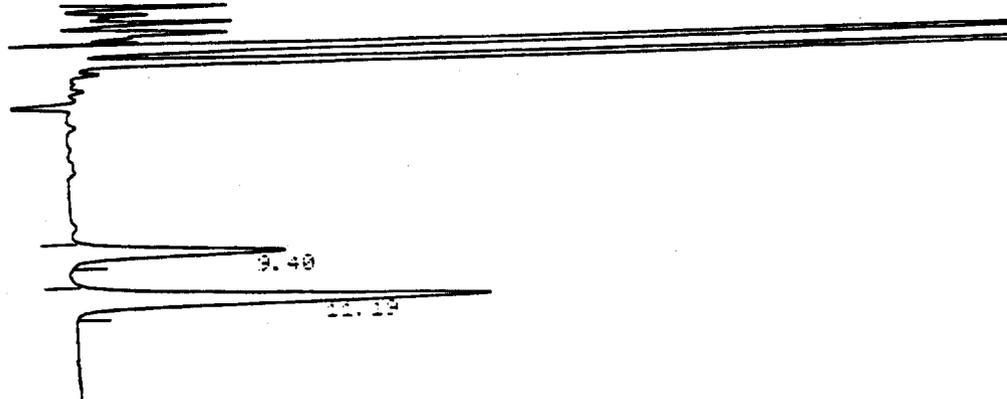
3 mg/ml (Isomer I 98.9%, Isomer II 100.1%)

CHANNEL A INJECT 04-02-93 19:08:47 STORED TO BIN # 7



30 mg/ml (Isomer I 100.2%, Isomer II 99.5%)

CHANNEL A INJECT 04-02-93 20:28:47 STORED TO BIN # 12



APPENDIX 8

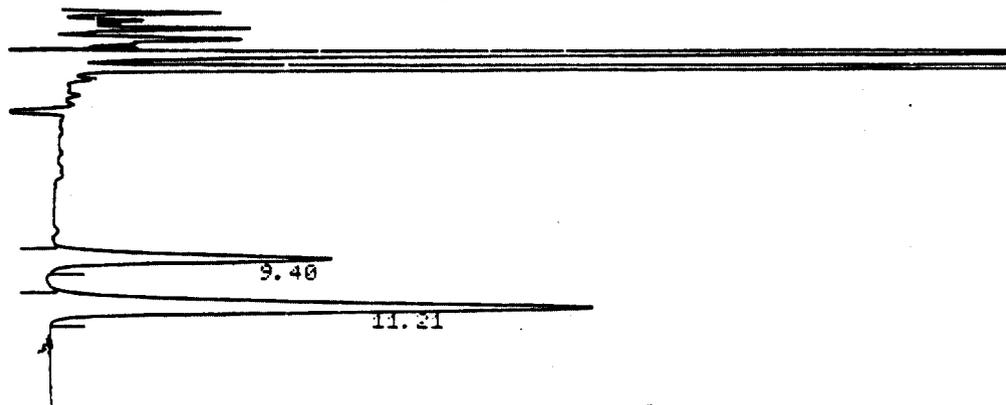
(Chemical analysis - continued)

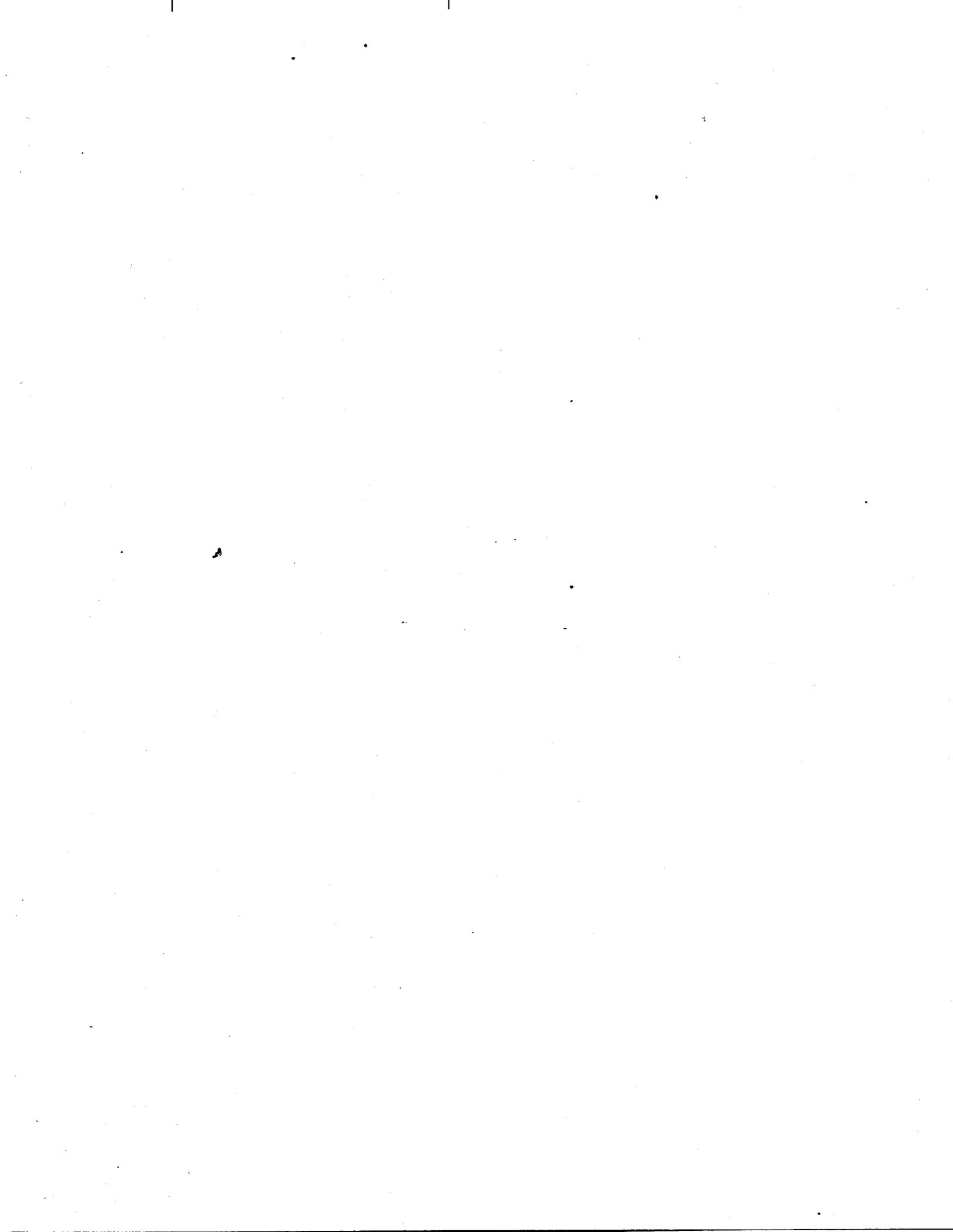
FIGURE 3

(continued)

100 mg/ml, (Isomer I 100.5%, Isomer II 99.8%)

CHANNEL A INJECT 04-02-93 22:04:46 STORED TO BIN # 18

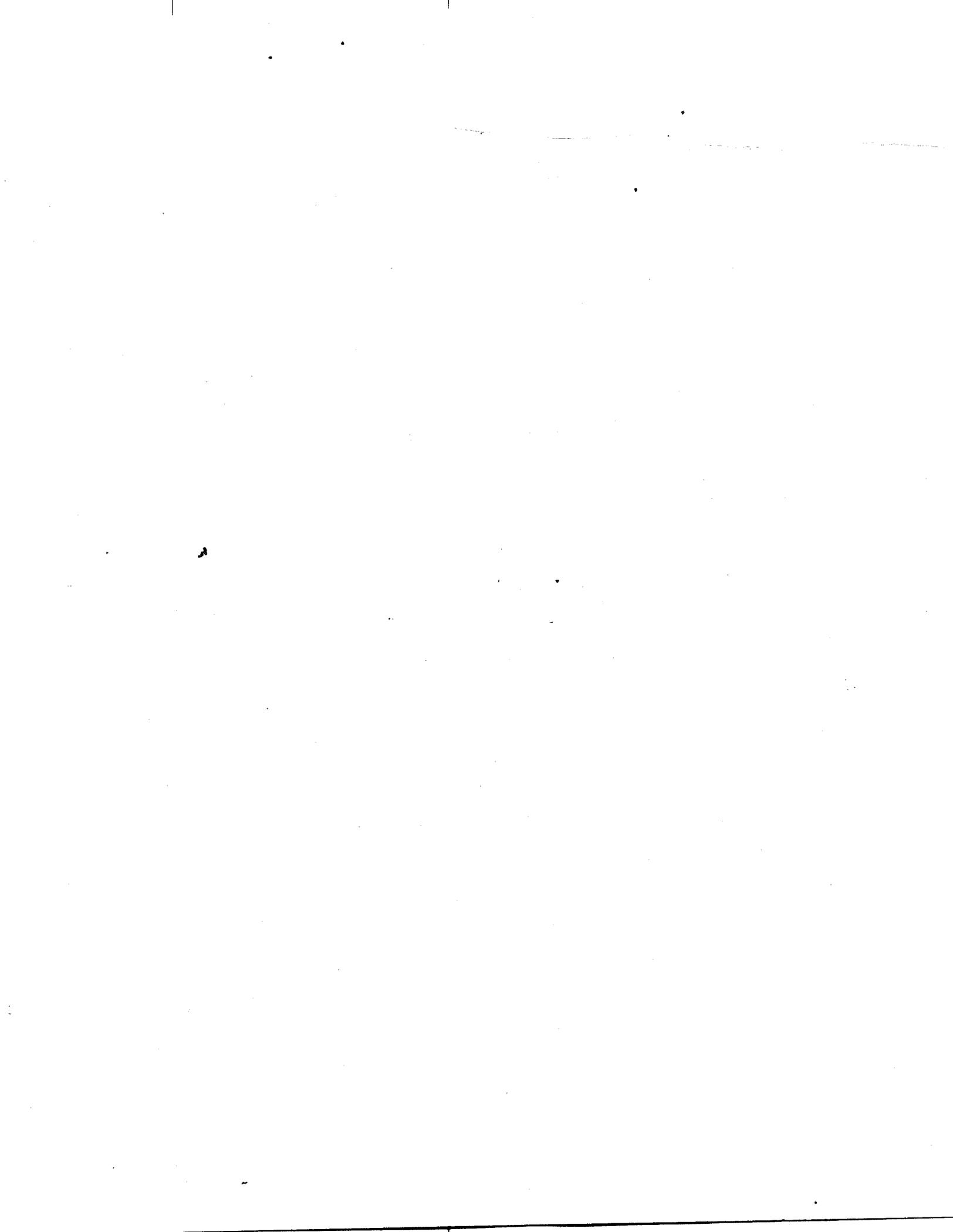




**SEVEN-DAY PRELIMINARY ORAL TOXICITY  
STUDY IN THE RAT  
WITH  
(HRC Schedule No.:**

**Authors:**

Sarah A. Allan,  
David G. Coleman.



## APPENDIX 9

### (Preliminary toxicity study - continued)

#### EXPERIMENTAL PROCEDURE

Groups of three male and three female rats were dosed by oral gavage with formulated 20, 10 and 5% w/v solutions in corn oil at a dose volume of 5 ml/kg/day to give dosages of 1000, 500 and 250 mg/kg/day respectively. A control group of six rats (three males and three females) received the vehicle alone at the same dose volume (5 ml/kg/day). Rats were dosed by oral gavage, once daily, for seven consecutive days.

#### OBSERVATIONS

##### Mortalities

All rats receiving at 1000 mg/kg/day were sacrificed on humane grounds on Day 1 within 4 hours of dosing. Clinical signs seen prior to sacrifice included piloerection, hunched posture, abnormal gait (walking on toes), pallor of the extremities, lethargy and ataxia. Post mortem revealed a slight haemorrhage in the glandular region of the stomach for one female rat. No other macroscopic abnormalities were seen.

##### Clinical signs

For rats receiving 500 mg/kg/day increased salivation followed dosing was noted on most occasions from Day 2 until termination (Day 8), this was generally associated with wet fur. On Day 1 piloerection and hunched posture were seen in both sexes along with abnormal gait (walking on toes) for females only. Fur loss was noted for one male and two females from Day 4 to termination.

Increased salivation, following dosing, was noted occasionally for rats treated at 250 mg/kg/day. No other clinical signs were noted for this low dosage group.

##### Bodyweight (Table 1)

There were no obvious differences from controls for actual bodyweight or bodyweight gains for rats treated at 250 or 500 mg/kg/day.

##### Food consumption (Table 2)

Food consumption for rats treated at 250 or 500 mg/kg/day with was similar to that of the controls.

## APPENDIX 9

(Preliminary toxicity study - continued)

### TERMINAL STUDIES

#### Organ weight (Table 3)

Slightly higher liver weights were recorded for rats dosed at 250 or 500 mg/kg/day than for control rats. This difference appeared to be dosage-related.

The spleen and kidney weights for rats treated at 250 or 500 mg/kg/day were comparable to those for the controls.

#### Macroscopic pathology

No macroscopic abnormalities were observed for rats treated at 250 or 500 mg/kg/day or for control rats.

### CONCLUSION

The results of this seven-day preliminary oral toxicity study with \_\_\_\_\_ suggested that 500 mg/kg/day would be tolerated by the rat for the four-week study (HRC Schedule No. \_\_\_\_\_).

A dosage sequence of 0, 15, 150 and 500 mg/kg/day was therefore proposed.

APPENDIX 9

(Preliminary toxicity study - continued)

TABLE 1

Bodyweight - group mean values (g)

Sex/treatment	Group/dosage (mg/kg/day)	Day		
		1	4	8
♂	Control	112	137 (25)	174 (37)
	250	124	151 (27)	186 (35)
	500	125	145 (20)	184 (39)
	1000	110	-	-
♀	Control	116	136 (20)	161 (25)
	250	121	141 (20)	167 (26)
	500	123	137 (14)	163 (26)
	1000	116	-	-

Bodyweight gains are given in parentheses

APPENDIX 9

(Preliminary toxicity study - continued)

TABLE 2

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

Sex/treatment	Group/dosage (mg/kg/day)	Food consumed (g/rat/week)
♂	Control	168
	250	181
	500	172
♀	Control	148
	250	155
	500	142

APPENDIX 9

(Preliminary toxicity study - continued)

TABLE 3

Organ weight - group mean values (g)

Sex/treatment	Group/dosage (mg/kg/day)	Organ		
		Liver	Spleen	Kidneys
♂	Control	8.09	0.67	1.48
	250	9.87	0.70	1.69
	500	10.50	0.66	1.60
♀	Control	7.30	0.49	1.43
	250	9.17	0.53	1.60
	500	10.53	0.56	1.60

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# **HRC** Report

**COMPANY SANITIZED**

**ACUTE ORAL TOXICITY  
TO THE RAT**

## **Huntingdon Research Centre**



20 pgs.

**CONFIDENTIAL**

**ACUTE ORAL TOXICITY  
TO THE RAT**

**Addressee:**

**Author:**

**Sarah A. Allan.**

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

**Report issued: 15 June 1992**

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## COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following appropriate Good Laboratory Practice Standards.

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

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register, 22 December 1978, and subsequent Amendments.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

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.

Japanese Ministry of Health and Welfare, Notification No. Yakuhatsu 313 Pharmaceutical Affairs Bureau, 31 March 1982 and subsequent amendment Notification No. Yakuhatsu 870, Pharmaceutical Affairs Bureau, 5 October 1988.

Japanese Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

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

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.



---

Sarah A. Allan, B.Sc.,  
Study Director,  
Huntingdon Research Centre Ltd..

15-6-92  
Date

## QUALITY ASSURANCE STATEMENT

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, "process-based" inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of inspection

6-13.01.92

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

14.01.92

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

23.04.92



P. Watson,  
Systems Compliance Auditor,  
Department of Quality Assurance,  
Huntingdon Research Centre Ltd..

27.4.92

**AUTHOR'S SIGNATURE PAGE**

I the undersigned, hereby declare that the work was performed under my supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



Sarah A. Allan, B.Sc.,  
Study Director,  
Department of Industrial Toxicology.

## SUMMARY

A study was performed to assess the acute oral toxicity of \_\_\_\_\_ to the rat. The method followed was that described in:

EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.1. Acute toxicity (oral).

OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

A group of ten fasted rats (five males and five females) was given a single dose by gavage of the test substance, as supplied, at a dose level of 2.0 g/kg bodyweight. All animals were killed and examined macroscopically on Day 15, the end of the observation period.

There were no deaths. Clinical signs of reaction to treatment were pilo-erection, abnormal body carriage, abnormal gait, lethargy, decreased respiratory rate, pallor of the extremities, increased salivation and wet fur; recovery was complete by Day 3.

All rats achieved satisfactory bodyweight gains throughout the study.

No abnormalities were recorded at the macroscopic examination on Day 15.

The acute lethal oral dose to rats of \_\_\_\_\_ was found to be greater than 2.0 g/kg bodyweight.

\_\_\_\_\_ does not require labelling with the risk phrase R22 "Harmful if swallowed", in accordance with Council Directive 79/831/EEC Annex VI, Part II(D) as described in Commission Directive 91/325/EEC.

## INTRODUCTION

The study was designed to assess the toxicity of \_\_\_\_\_ following a single oral dose to the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with the following guidelines:

EEC Methods for the determination of toxicity, Directive 84/449/EEC (OJ No. L251, 19.9.84), Part B, Method B.1. Acute toxicity (oral).

OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

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

The dose level for the study was chosen on the basis of a preliminary investigation and in compliance with the guidelines.

The protocol was approved by the Study Director and HRC Management on 29 January 1992 and by the Sponsor on 10 February 1992.

The experimental phase of the study was undertaken between 3 and 26 March 1992.

**TEST SUBSTANCE**

**Identity:**

**Chemical name:**

Benzenepropanenitrile, 4-ethyl-alpha, alpha  
-dimethyl: 60-75%,  
Benzenepropanenitrile, 2-ethyl-alpha, alpha  
-dimethyl: 1-5%,  
Benzenepropanenitrile, 3-ethyl-alpha, alpha  
-dimethyl: 20-35%.

**Lot number:**

5364.

**Expiry:**

10 January 1993.

**Purity:**

> 98%.

**Appearance:**

Clear colourless liquid.

**Storage conditions:**

Room temperature.

**Date received:**

3 January 1992.

## EXPERIMENTAL PROCEDURE

### ANIMAL MANAGEMENT

Equal numbers of healthy male and female CD rats of Sprague-Dawley origin (Hsd/Ola:Sprague-Dawley(CD)) were obtained from Harlan Olac Ltd., Bicester, Oxon, England.

They were in the weight range of 111 to 143 g and approximately four to seven weeks of age prior to dosing (Day 1) in the main study. All the rats were acclimatised to the experimental environment for a period of seven days prior to the start of the main study.

The rats were allocated without conscious bias to cages within the treatment group. They were housed in groups of up to five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 6.

A standard laboratory rodent diet (Biosure LAD 1) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and approximately 4 hours after dosing.

The batch(es) of diet used for the study was analysed for certain nutrients, possible contaminants and micro-organisms (Appendix 1).

Results of 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 2).

The mean daily minimum and maximum temperatures of the animal room were 20°C and 22°C respectively and the mean daily relative humidity value was 58% R.H. Air exchange was maintained at 10 to 15 air changes per hour and lighting was controlled by means of a time switch to provide 12 hours of artificial light (0700 - 1900 hours) in each 24-hour period.

Each animal was identified by cage number and ear punching. Each cage was identified by a coloured label displaying the dose level, study schedule number, animal mark and the initials of the Study Director and Home Office licensee.

### TEST SUBSTANCE PREPARATION

was administered, as supplied by the Sponsor, at a volume not exceeding 2.10 ml/kg (specific gravity 0.9514) in the main study.

The absorption of the test substance was not determined.

The homogeneity, stability and purity of the test substance were the responsibility of the Sponsor.

## **TREATMENT PROCEDURE**

### **Preliminary study**

A preliminary study was carried out by dosing two male and two female rats at 1.0 g/kg bodyweight.

### **Main study**

A group of ten rats (five males and five females) was treated at 2.0 g/kg bodyweight.

### **Control animals**

No control animals were included in this study.

## **ADMINISTRATION OF TEST SUBSTANCE**

The appropriate dose volume of the test substance was administered to each rat by oral gavage using a syringe and plastic catheter (8 choke).

The day of dosing was designated Day 1.

## **OBSERVATIONS**

### **Mortality**

Cages of rats were checked at least twice daily for any mortalities.

### **Clinical signs**

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1 (a period of five hours). On subsequent days animals were observed once in the morning and again at the end of the experimental day. This latter observation was at approximately 16.30 hours on week days or 11.30 hours on Saturdays, Sundays and public holidays. The nature and severity of the clinical signs and time were recorded at each observation.

The animals on the preliminary and main studies were observed for 5 and 14 days respectively after dosing.

### **Bodyweight**

The bodyweight of each rat was recorded on Days 1 (prior to dosing), 8 and 15. Individual weekly bodyweight changes were calculated.

## **TERMINAL STUDIES**

### **Termination**

All animals on the main study were killed on Day 15 by cervical dislocation.

### **Macroscopic pathology**

All animals were subjected to a macroscopic examination which consisted of opening the cranial, abdominal and thoracic cavities. The macroscopic appearance of all tissues was recorded.

## **ARCHIVES**

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

## **RESULTS**

### **PRELIMINARY STUDY (Table 1)**

The results of the preliminary study indicated that the acute lethal oral dose to male and female rats of \_\_\_\_\_ was greater than 1.0 g/kg bodyweight.

### **MAIN STUDY**

A group of ten rats (five males and five females) was treated at 2.0 g/kg bodyweight.

### **MORTALITY (Table 1)**

There were no deaths following a single oral dose of \_\_\_\_\_ at 2.0 g/kg bodyweight.

### **CLINICAL SIGNS (Table 2)**

Pilo-erection and increased salivation were observed in all rats within five minutes of dosing. Pilo-erection persisted and was accompanied at later intervals on Day 1 by abnormal body carriage (hunched posture), abnormal gait (waddling), lethargy, pallor of the extremities and wet fur for all rats and also by decreased respiratory rate for one male and one female.

Recovery of all rats, as judged by external appearance and behaviour, was complete by Day 3.

### **BODYWEIGHT (Tables 3 and 4)**

All rats achieved satisfactory bodyweight gains throughout the study.

### **MACROSCOPIC EXAMINATION**

No macroscopic abnormalities were observed for animals killed on Day 15.

## CONCLUSION

The acute lethal oral dose to rats of \_\_\_\_\_ was found to be:  
greater than 2.0 g/kg bodyweight

**TABLE 1**

**Mortality data for groups of rats dosed orally with**

Study	Dose (g/kg)	Mortality ratio (No. of deaths) ( No. dosed )		
		♂	♀	Combined
Preliminary	1.0	0/2	0/2	0/4
Main	2.0	0/5	0/5	0/10

**TABLE 2**

**Signs of reaction to treatment observed in rats dosed orally with**

**Main study**

Signs	No. of rats in group of 5 showing signs	
	Dose (g/kg)	
	2.0	
	♂	♀
Pilo-erection	5	5
Abnormal body carriage (hunched posture)	5	5
Abnormal gait (waddling)	5	5
Lethargy	5	5
Decreased respiratory rate	1	1
Pallor of the extremities	5	5
Increased salivation	5	5
Wet fur	5	5

**TABLE 3**

**Individual bodyweights (g) of rats dosed orally with**

**Main study**

Sex	Dose (g/kg)	Animal number & ear mark	Bodyweight (g) at		
			Day 1	Day 8	Day 15
♂	2.0	1 RP	130	196	249
		2 LP	143	218	291
		3 RPLP	131	194	240
		4 RIRO	128	185	234
		5 LILO	131	189	244
♀	2.0	6 RP	122	164	189
		7 LP	120	156	174
		8 RPLP	111	149	160
		9 RIRO	116	162	186
		10 LILO	113	146	169

**TABLE 4**

**Individual bodyweight changes (g) of rats dosed orally with**

**Main study**

Sex	Dose (g/kg)	Animal number & ear mark	Bodyweight gains (g) at	
			Week 1	Week 2
♂	2.0	1 RP	66	53
		2 LP	75	73
		3 RPLP	63	46
		4 RIRO	57	49
		5 LILO	58	55
♀	2.0	6 RP	42	25
		7 LP	36	18
		8 RPLP	38	11
		9 RIRO	46	24
		10 LILO	33	23

## APPENDIX 1

### Biosure Laboratory Animal Diet No. 1

#### Composition and quality assurance aspects of diet

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

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

Nutrients	Target level	Tolerance %	Acceptable range	
Moisture	9.5	+10		10.5 % max
Crude fat	3.7	±15	3.1	- 4.3 %
Crude protein	21.5	±10	19.4	- 23.7 %
Crude fibre	2.0	±40	1.2	- 2.8 %
Ash	5.5	±15	4.7	- 6.3 %
Calcium	1.0	±20	0.8	- 1.2 %
Phosphorus	0.9	±20	0.7	- 1.1 %
Sodium	0.3	+100-50	0.15	- 0.60 %
Chloride	0.5	+100-50	0.25	- 1.0 %
Potassium	0.8	+100-50	0.4	- 1.6 %
Magnesium	0.15	±50	0.08	- 0.23 %
Iron	220	±50	110.0	- 330 mg/kg
Copper	15	±50	8.0	- 23 mg/kg
Manganese	70	±50	35.0	- 105 mg/kg
Zinc	60	±50	30.0	- 90 mg/kg
Vitamin A	12	+50-20	9.5	- 18 iu/g
Vitamin E	35	+150-20	28	- 88 mg/kg
<b>Contaminants</b>			<b>Maximum concentration</b>	
Fluoride			40	mg/kg
Nitrate (as NaNO <sub>3</sub> )			200	mg/kg
Nitrite (as NaNO <sub>2</sub> )			10	mg/kg
Lead			2.5	mg/kg
Arsenic			1.5	mg/kg
Cadmium			0.5	mg/kg
Mercury			0.1	mg/kg
Selenium			0.6	mg/kg
Total Aflatoxins			5	mcg/kg
Total P.C.B.			50	mcg/kg
Total D.D.T.			150	mcg/kg
Dieldrin			50	mcg/kg
Lindane			150	mcg/kg
Heptachlor			50	mcg/kg
Malathion			5000	mcg/kg

**APPENDIX 1**

**(continued)**

**Microbiological contents**

**Maximum concentration**

	<b>LAD 1 (nuts)</b>
<b>Total viable organisms</b>	<b>10,000 per g diet</b>
<b>Mesophilic spores</b>	<b>30,000 per g diet</b>
<b>Salmonellae species</b>	<b>0 per g diet</b>
<b>Presumptive E. coli</b>	<b>0 per g diet</b>
<b>E. coli type 1</b>	<b>0 per g diet</b>
<b>Fungal units</b>	<b>1,000 per g diet</b>
<b>Antibiotic activity</b>	<b>0 per g diet</b>

## APPENDIX 2

### 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.

**Triage of 8(e) Submissions**

Date sent to triage: \_\_\_\_\_

**NON-CAP**

CAP

Submission number: 12192 B

TSCA Inventory:

Y

N

**D**

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

**ATOX**

**SBTOX**

SEN

**w/NEUR**

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): \_\_\_\_\_

Notes:

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

<b>For Contractor Use Only</b>	
entire document: <b>0</b> 1 2 pages <u>1-3</u>	pages <u>1-3, tabs</u>
<b>Notes:</b>	
Contractor reviewer: <u>UPG</u>	Date: <u>12/13/94</u>

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Submission # SEHQ. 0594-12192 SEQ. B

TYPE: INT Supp FLWP

SUBMITTER NAME: Confidential

INFORMATION REQUESTED: FLWP DATE: \_\_\_\_\_  
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 0502 INFO REQUESTED (TECH)  
 0503 INFO REQUESTED (VOL ACTIONS)  
 0504 INFO REQUESTED (REPORTING RATIONALE)  
 DISPOSITION:  
0639 REFER TO CHEMICAL SCREENING  
 0678 CAP NOTICE

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

SUB DATE: 05/02/94 OTS DATE: 05/06/94 CSRAD DATE: 06/03/94

CHEMICAL NAME: Benzene propenenitrile, 4-ethyl-alpha,  
alpha-dimethyl-  
Benzene propenenitrile, 2-ethyl-alpha, alpha-dimethyl-  
alpha-dimethyl-

CAS# 134123-93-6  
134123-91-4

Benzene propenenitrile, 3-ethyl-  
alpha, alpha-dimethyl-  
134123-92-5

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
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0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCC/REL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	MSDS	01 02 04
0210 ACUTE TOX (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	OTHER	01 02 04
0211 CHR. TOX (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

NON-CBI INVENTORY YES (CONTINUE) NO (DROP)  
ONGOING REVIEW YES (DROP/REFER) NO (CONTINUE)  
SPECIES RAT  
TOXICOLOGICAL CONCERN: LOW Subst orl Ac. ora  
USE: Fragrances in  
cosmetics; household  
products  
PRODUCTION:

DETERMINE

CAS SR  
COMMENTS: Non-Cap

12192B

L

Subacute oral toxicity in the rat is of low concern. Sprague-Dawley rats (5/sex/dose) received daily gavage doses of 0, 15, 150, or 500 mg/kg/day for 28 days. No rats died. No clinical signs were observed at 15 mg/kg. At the higher doses, clinical signs included salivation, greasy and wet fur, hunched posture, abnormal gait, red/brown staining around mouth, and loose feces, all of which showed a dose-dependent severity. At 500 mg/kg, animals exhibited slight but significant changes in urea nitrogen, glutamic-pyruvic transaminase, phosphorus, and cholesterol. Spleen and adrenal weights were significantly decreased at 500 mg/kg/day. Relative liver weight was significantly increased in males at 150 mg/kg/day and greater and 500 mg/kg/day females, which corresponded to hepatocyte enlargement in these animals (2/5 and 5/5 males and 4/5 females).

L

Acute oral toxicity in the rat is of low concern based on no mortality at a dose of 2000 mg/kg. Sprague-Dawley rats (5/sex) received a single gavage dose of 2000 mg/kg. Clinical signs included piloerection, abnormal posture and gait, lethargy, decreased breathing, pallor of extremities, salivation, and wet fur. No treatment-related abnormalities were noted at necropsy.