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8EHQ-059613579

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L. S. Andrews, Manager
Health Sciences and Regulatory Programs

May 22, 1996

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401 M Street, S. W.
Washington, D. C.



8EHQ-96-13579
SP001 05/28/96

Attention: 8(e) Coordinator

RE: 8EHQ-0196-13579
DCN #88960000059
Follow-up Report

ORIGINAL



89960000139

Dear Sir:

On January 26, 1996, ARCO Chemical Company submitted, in accordance with the provisions of Section 8(e) of the Toxic Substances Control Act, preliminary results from a 14-day oral dose (gavage) range-finding study in rats with Propanol, [2-(1,1-dimethylethoxy)methylethoxy], also known as dipropylene glycol t-butyl ether (DPTB), CAS #132739-31-2. Final results from this study now have been received. The range finding study was conducted in preparation for a 90-day oral toxicity study with neurotoxicity parameters. The in-life phase of this latter study is complete, and results currently are being evaluated by the laboratory. The 90-day study was designed to meet the requirements of a U.S. EPA consent order (P93-193) and a new chemical notification in the European Community.

The final results from the range-finding study showed increases in liver weights in 4/10 animals at the high dose (1000 mg/kg/day). Only 2/10 animals receiving the middle dose (300 mg/kg/day) showed this response. No increase was seen at the low dose (100 mg/kg/day). Since plasma analysis showed no increases in levels of enzymes which act as markers for functional changes in the liver, the report concludes that this effect on liver weight most probably was an adaptive physiologic response to the high levels of exposure to the test material.

Histopathology on livers of high dose animals indicated a minor degree of centrilobular hepatocyte enlargement in 4/5 cases in males only; physiological adaptation again was considered the likely explanation by the report. The histopathology also showed eosinophilic droplets, presumed to be α_2 microglobulin, in renal cortical tubular epithelia in 5/5 cases. This is a response thought to be a condition specific to male rats and, therefore, not considered relevant to humans. There were no treatment-related changes noted for females receiving 1000 mg/kg/day. As a result of these changes, microscopic

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Dipropylene Glycol t-Butyl Ether
Page 2

examination of liver and kidneys was extended to low and intermediate group males, revealing that similar changes were confined to the kidneys in the majority of animals.

A slight increase in adrenal weight was noted for females receiving 1000 mg/kg/day. All other organ weights were normal and comparable to controls.

The statistically significant decrease in lymphocytes and leukocytes reported earlier in the preliminary analysis of results from high dose males only was confirmed in the final report. This effect was not considered to be biologically significant, based on intra and inter-group variations in the data. Preliminary results from the 90-day study confirm that no clear dose-response was seen in the data at any dose level. The indication of an effect on leukocyte counts from high dose males, identified in our January 26 letter on the basis of preliminary information, was not confirmed in the study report.

A copy of the final report from the 14-day study is attached. A copy of the 90-day study report will be submitted when available.

Sincerely,

A handwritten signature in cursive script that reads "L. S. Andrews". The signature is written in black ink and is positioned below the word "Sincerely,".

L. S. Andrews, Ph.D.

CC: Mr. Mark Howard
Office of Pollution Prevention & Toxics (7405)
U. S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Report

TOXICITY TO RATS BY REPEATED ORAL ADMINISTRATION FOR 14 DAYS

DPTB

BEST COPY AVAILABLE

Huntingdon
Life Sciences

DPTB

TOXICITY TO RATS BY REPEATED ORAL ADMINISTRATION FOR 14 DAYS

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

Sponsor

ARCO Chemical Company,
3801 West Chester Pike,
Newtown Square,
Pensylvania,
PA 19073,
U.S.A.

Testing facility

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

Report issued 16 May 1996

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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

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

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

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


.....

Helen Connick, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol.,
Study Director,
Huntingdon Life Sciences Ltd.

16th May 1996

Date -

QUALITY ASSURANCE STATEMENT

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

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

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	27 November 1995
Pre-experimental Period	-	-
Experimental Period	13 - 21 December 1995	2 January 1996
	10 January 1996	10 January 1996 -

Date of reporting audit findings to the Study Director and Management	28 February 1996
---	------------------



Rod Scammell,
 Audit Team Supervisor,
 Department of Quality Assurance,
 Huntingdon Life Sciences Ltd.

15.5.96

Date

RESPONSIBLE PERSONNEL

STUDY MANAGEMENT

Helen Connick, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol.,
Study Director.

Helen Connick

Nicola J. Watts, B.Sc. (Hons.),
Study Supervisor.

NJWatts

TOXICOLOGY

Audrey M. Bottomley, B.Sc. (Hons.), Dip.R.C.Path.,
Senior Toxicologist.

AMB Bottomley

PATHOLOGY

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

Chirukandath Gopinath

FORMULATION ANALYSIS

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

ISDawe

SUMMARY

The objective of this study was to assess the toxicity of dipropylene glycol t-butyl ether (DPTB), when administered to rats by once daily oral administration for 14 days. The test substance was administered to groups of 5 male and 5 female animals at dosages of 0 (Control), 100, 300 and 1000 mg/kg/day.

Clinical signs, bodyweight and food consumption data were recorded throughout the study; on Day 14 of treatment blood samples were collected for measurement of haematology and biochemistry parameters. Following the completion of two weeks (14 days) of treatment all surviving animals were killed and examined macroscopically. Specified organs were weighed and tissues processed for histopathological evaluation.

Principal study findings (especially concerning differences between animals of treated and control groups) are summarised below:

Mortality

There were no unscheduled deaths among treated animals during the study. A single control animal died during the study, from unknown causes.

Clinical signs

Salivation immediately after dosing was observed on occasions from Day 7 of treatment for most animals receiving 300 and all animals receiving 1000 mg/kg/day.

Bodyweights, food consumption and food conversion ratios

There were no obvious treatment-related effects at any dosage.

Haematology

A statistically significant reduction in lymphocyte count was observed in males receiving 1000 mg/kg/day.

Biochemistry

No statistically significant changes in blood chemistry were observed.

Organ weights

At 300 and 1000 mg/kg/day, marked increases in liver and kidney weights were seen in male and female animals, and slight increases in adrenal weights were seen in female animals.

Macroscopic pathology

Enlargement of the liver was seen in 4/10 animals receiving 1000 mg/kg/day and 2/10 animals receiving 300 mg/kg/day.

Microscopic pathology

Among male rats dosed at 1000 mg/kg/day, centrilobular hepatocyte enlargement was noted in 4/5 cases and eosinophilic droplets, presumed to be α_2 microglobulin, were seen in the renal cortical tubular epithelia in 5/5 cases. There were no treatment-related changes noted for females receiving 1000 mg/kg/day. As a result of the changes noted amongst males at the highest dosage level, microscopic examination of liver and kidneys was extended to low and intermediate group males: this revealed that similar changes were confined to the kidneys in the majority of animals.

Conclusion

At 1000 mg/kg/day, a statistically significant decrease in lymphocyte count was evident in males. In addition, at dosages of 1000 and 300 mg/kg/day there were marked increases in liver and kidney weights in both sexes: enlargement of the liver was also noted macroscopically for some animals. Microscopic examination revealed a minor degree of centrilobular hepatocyte enlargement in males receiving 1000 mg/kg/day.

Since plasma analysis showed no increases in levels of enzymes which act as markers for functional changes in the liver, the changes evident in the livers from animals receiving 1000 mg/kg/day are considered to be an adaptive response related to metabolism of the test material rather than a toxic change.

The eosinophilic droplet accumulation (considered to be α_2 microglobulin) seen in the renal cortical tubular epithelia of most treated males was not an unexpected finding after treatment with a test substance of this type. Disruption of protein recycling in the proximal tubule region of the male rat kidney, leading to localised accumulation of eosinophilic (hyaline-like) droplets, is a well-documented and probably species-specific response to repeated administration of various ethers and other hydrocarbons. It is not considered predictive of a hazard to human health.

The effects observed after 14 days of treatment were not of sufficient severity to preclude the use of dosages of up to 1000 mg/kg/day in a further study of 13 weeks duration.

The no-observed effect level (NOEL) on the basis of this study of 14 days duration was 100 mg/kg/day for females (based on increased liver and kidney weights): no absolute NOEL was found for males, due to the kidney changes recorded at the lowest test dosage. However, when this probably species-specific response is excluded from consideration, a male NOEL of 100 mg/kg/day was found (based on increased liver and kidney weights, liver enlargement noted at 1000 and 300 mg/kg/day and centrilobular hepatocyte enlargement noted at 1000 mg/kg/day). These effects on organ weights were probably adaptive since there were no concurrent microscopic changes. The NOEL for histopathological effects (excluding kidney tissue) was at least 1000 mg/kg/day in females and 300 mg/kg/day in males (based on centrilobular hepatocyte enlargement).

INTRODUCTION

The object of this study, performed at Huntingdon Life Sciences Ltd, England, was to assess the toxicity of the test substance, DPTB, to rats by repeated oral administration over a period of 14 days.

This study was conducted taking into account the objectives of the guidelines of the following regulatory bodies:

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

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

The high dosage (1000 mg/kg/day) employed in this study was selected on the basis of available toxicity data from a single dose study. A dosage of 1000 mg/kg/day is the limit dosage for repeat dose toxicity studies designed to the above guidelines. The low and intermediate dosage levels (100 and 300 mg/kg/day respectively) were selected as fractions of the maximum test dosage, and to allow evaluation of any dose-related response.

The rat is a recognised species of choice for regulatory requirement and the strain chosen on account of the availability of background data. Oral administration was chosen since this is a potential route of human exposure.

RELEVANT STUDY DATES

Protocol approval by:

Study Director	21 November 1995
HRC Management	21 November 1995
Study Sponsor	27 November 1995

Animal arrival at Huntingdon Life Sciences: 24 November 1995

Treatment commenced: 7 December 1995

Haematology and Biochemistry:

Week 2	20 December 1995
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Terminal kill (after completion of 14 days of treatment): 21 December 1995

TEST SUBSTANCE

Identity:	DPTB
Chemical name:	Dipropylene glycol t-butyl ether
Intended use:	Additive/solvent .
Appearance:	Clear, colourless liquid
Storage conditions:	Room temperature, in the dark, under nitrogen
Batch no.:	HRC - 1095
Expiry date#:	Assumed to be stable for at least the duration of this study
Purity:	>98%
Date received:	17 November 1995
Stability of formulations:	15 days at 4°C

The Sponsor is responsible for characterisation and stability of the test substance

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

A total of 31 male and 31 female Crl:CD BR rats approximately 28 days old and within a weight range of 17 g for males and 18 g for females, was received from Charles River Breeding Laboratories, Manston Road, Margate, Kent, England. For those animals selected for the study, their estimated age at the start of treatment was 6 weeks and their bodyweights were in the range 171 g to 208 g for males and 137 g to 172 g for females.

On arrival 5 males and 5 females selected at random were used for health check purposes. These animals were killed within 24 hours after arrival at HRC and subjected to routine macroscopic examination. Lungs, liver, kidneys, spleen and heart were preserved in fixative, but not processed further. No macroscopic abnormalities were detected.

The remaining rats were placed at random in suspended cages with wire mesh floors, according to sex, so that each cage contained, one rat. Each cage measured 21 cm high, 26 cm wide and 27 cm deep.

Animal room temperature and relative humidity controls were set at $21 \pm 2^\circ\text{C}$ and $55 \pm 10\%$. During the study, the values ranged from 17°C to 24°C and 40 to 58% respectively. The minor variations which occurred from these ranges were not considered to have affected the integrity of the study. Permanent weekly recordings of these parameters were made and these are archived with all other data for this study. Artificial lighting was controlled to give 12 hours continuous light and 12 hours continuous dark per 24 hours.

All rats had free access to tap water and pelleted SDS Rat and Mouse No. 1 maintenance diet. There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test substance was in the diet, or drinking water, both of which were routinely subjected to regular chemical analyses, results of which are lodged in Huntingdon Life Sciences Archives, Huntingdon.

After an acclimatisation period of six days, each animal was weighed and the required number of animals was selected by discarding those animals furthest from the mean bodyweight. The animals were then randomly assigned to cages, stratified by bodyweight. The appropriate number of cages were then allocated to each treatment group. This procedure assured that the initial group mean bodyweights were approximately equal.

A further period of acclimatisation of seven days was allowed between allocation of animals to groups and the commencement of treatment. During this period a review of animal health was undertaken by a veterinary officer. The spare animals were retained during this acclimatisation period to replace any rat showing signs of ill health. Following the commencement of treatment the spare rats were discarded with no further investigations performed.

Throughout the study the animals were housed in the Department of Rodent Toxicology, Barrired Rodent Building No. 1, Room 12.

ANIMAL IDENTIFICATION

Group	Animal numbers	
	♂	♀
1	1 - 5	21 - 25
2	6 - 10	26 - 30
3	11 - 15	31 - 35
4	16 - 20	36 - 40
Health check	41 - 45	46 - 50

The rats were housed individually. Each cage was identified by a coloured label according to group, each label was uniquely numbered with cage and study number and the animal number was tattooed on the leg of each rat. The cages constituting each group were dispersed in batteries so that possible environmental influences arising from their spatial distribution were equilibrated, as far as possible, for all treatments.

PREPARATION OF FORMULATIONS

The test substance, DPTB, was weighed out and stirred in with the vehicle, distilled deionised water, using a magnetic stirrer. Once up to volume, the formulation was homogenised to thoroughly mix the test substance and vehicle together. Any samples required were taken whilst the formulation was being stirred magnetically. Each concentration was prepared by direct dilution, on a weekly basis, the concentrations chosen to give a constant dosage volume of 10 ml/kg bodyweight. Following preparation daily aliquots were decanted and stored in amber glass bottles, sealed with plastic screw caps lined with waxed card and stored at 4°C when not in use.

Group/ colour code	Dosage (mg/kg/day)	Concentration (mg/ml)	Dose volume (ml/kg)
1: White	Control	0	10
2: Yellow	100	10	10
3: Green	300	30	10
4: Red	1000	100	10

FORMULATION SAMPLING AND ANALYSIS

Prior to the commencement of the study, the proposed formulation procedure was checked by chemical analysis to confirm that the method was acceptable and that formulations in a concentration range of 0.1 to 100 mg/ml were stable for 15 days at 4°C.

Samples of the solutions prepared for the first and second week of dosing (solutions were prepared on a weekly basis) were taken from all groups and were analysed to check the accuracy of preparation. Chemical analysis was carried out by Huntingdon Life Sciences Department of Analytical Chemistry and results are appended to this report.

ADMINISTRATION OF FORMULATIONS

The test substance, DPTB, was administered as a solution in distilled, deionised water. Control animals received the vehicle alone. The animals were dosed where possible, at approximately the same time each day using a suitably graduated syringe and a rubber catheter (Ch 8 or 10) inserted into the stomach. The dosage volume administered to each animal was calculated according to the most recent recorded bodyweight. A constant dosage volume of 10 ml/kg was used.

Treatment in this manner continued once a day for a period of 14 days.

OBSERVATIONS

Dated and signed records of all activities relating to the day by day running and maintenance of the study within the animal unit as well as to the group observations and examinations outlined in this procedure, were recorded in the Study Day Book.

The following observations were made during the course of the study:

Clinical signs and mortality

Individual animals were observed and palpated at least once daily for any signs of behavioural changes, reaction to treatment or ill health. These examinations were performed each day, at suitable intervals after dosing.

Dated and signed records of appearance, change and disappearance of clinical signs were maintained on clinical history sheets for individual animals.

Further checks were made early in each working day and again in the afternoon to look for dead or moribund animals. This allowed *post mortem* examination to be carried out during the working period of that day. At weekends a similar procedure was followed except that the final check was carried out at approximately mid-day.

The rat that was found dead during the study was subjected to a detailed macroscopic examination and a full spectrum of tissue samples were preserved in the appropriate fixative (see **MORTALITY**).

Bodyweight

The weight of each rat was recorded at the time of allocation of animals to groups (one week prior to commencement of treatment), on the day of commencement of treatment (Day 1), and twice a week thereafter, including the day of sacrifice.

Food consumption

The quantity of food consumed by each cage of rats was recorded on a weekly basis during the week prior to the commencement of treatment and on a twice weekly basis from commencement of treatment. Food intake per rat (g/rat/day) was calculated using the total amount of food given to and left by each rat in each group. The following formula was used:

Food consumption (g/rat/day) =

$$\frac{\text{Total food given} - \text{Total food left}}{\text{Number of animal days} *}$$

The results using this formula (presented in Appendix 2) were subject to rounding to the nearest whole number.

To provide a more accurate measure of the food consumed by each animal in a group, the following formula was used for group mean calculation:

Group mean consumption (g/rat/day) =

$$\frac{\text{Total food given to group} - \text{Total food left by group}}{\text{Number of animal days} * \text{ for the group}}$$

It is therefore inappropriate to attempt to calculate the stated group means (presented in Table 2) from the individual values in the appendix.

Efficiency of food utilisation

Food conversion ratios were calculated from the bodyweight and food consumption data as weight of food consumed per unit gain in bodyweight. The following formula was used:

$$\text{Food conversion ratio} = \frac{\text{Food consumed over the specified period}}{\text{Bodyweight gain over the specified period}}$$

The 'food consumed' was calculated as indicated in the **Food consumption** section and is not a mean of the individual cage means. The 'bodyweight gain' was calculated from the gain of each animal over the period specified and uses the mean gain in the formula.

- * The term 'animal day' counts one animal day for each animal alive for a whole day. It is assumed that on the day of death an animal does not eat.

Water consumption

Daily monitoring by visual appraisal of the water bottles was maintained throughout the study.

LABORATORY INVESTIGATIONS

On Day 14, samples of blood were withdrawn, under light ether anaesthesia, from the orbital sinus of all rats from each group.

The blood samples collected were divided into tubes as follows:

EDTA anticoagulant	for haematological investigations
Citrate anticoagulant	for coagulation tests
Heparin anticoagulant	for the remaining biochemical tests

Food was removed overnight from animals sampled for laboratory investigations.

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

Haematology	Units
The following estimations were performed with a Bayer-Technicon H1E haematology analyser:	
Packed cell volume (PCV)	%
Haemoglobin (Hb)	g/dl
Red cell count (RBC)	$\times 10^{12}/l$

Absolute indices (MCHC, MCV) were calculated as follows:

Mean corpuscular haemoglobin concentration (MCHC) $Hb (g/dl) \times 100 \div PCV (\%)$	g/dl
Mean corpuscular volume (MCV) $PCV (\%) \times 10 \div RBC (\times 10^{12}/l)$	fl
Mean corpuscular haemoglobin (MCH) $Hb (g/dl) \times 10 \div RBC (\times 10^{12}/l)$	pg
Total white cell count (WBC Total)	$\times 10^9/l$

Units

Differential WBC count

Neutrophils	(N))	
Lymphocytes	(L))	
Eosinophils	(E))	
Basophils	(B))	
Monocytes	(M))	
Large unstained cells	(LUC))	

× 10⁹/l

Cell Morphology: abbreviations used for cell morphology are indicated below:

Anisocytosis	Anis
Microcytosis	Micro
Macrocytosis	Macro
Variation in colour	Var
Hypochromasia	Hypo
Hyperchromasia	Hyper
Left shift	LS
Atypical cells	Atyp
Blast cells	Blast
No abnormalities detected	-
Slight	+
Moderate	++
Marked	+++

Platelet count (Plts)

The following was performed using the appropriate methodology, as described below:

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

Biochemistry

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

Total protein	g/dl
Albumin (Alb)	g/dl
Globulin (Glob): by subtraction Total protein (g/l) minus Albumin (g/l)	g/dl
Urea nitrogen (Urea Nitr)	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l

	Units
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic Phosphorus (P)	mEq/l
Chloride (Cl)	mEq/l
Cholesterol (Chol) - (enzymatic assay)	mg/dl
Alkaline phosphatase (AP) Reaction temperature 30°C	mU/ml
Glucose (Hexokinase mediated assay)	mg/dl
Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase' (ALT) Reaction temperature 30°C	mU/ml
Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase' (AST) Reaction temperature 30°C	mU/ml
Total bilirubin (Bilirubin)	mU/ml

TERMINAL STUDIES

Necropsy

On completion of 14 days of treatment, all surviving rats were killed.

All rats were killed by carbon dioxide asphyxiation and subjected to the necropsy procedure indicated below:

All superficial tissues were examined visually and by palpation and the cranial roof removed to allow observation of the brain, pituitary gland and cranial nerves. After ventral midline incision and skin reflection all subcutaneous tissues were examined. The condition of the thoracic viscera was noted with due attention to the thymus, lymph nodes and heart.

The abdominal viscera were examined before and after removal, the urinary bladder was examined externally and by palpation. The gastrointestinal tract was examined as a whole and the stomach and caecum were incised and examined. The lungs were removed and all pleural surfaces examined under suitable illumination. The liver was sectioned at intervals of a few millimetres; the kidneys were incised and examined. Any abnormalities in the appearance and size of the gonads, adrenals, uterus, intra-abdominal lymph nodes and accessory reproduction organs was recorded.

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

adrenals	liver	spleen
brain	ovaries	testes
epididymides	prostate	
kidneys	seminal vesicles	

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

Preservation of tissues

Samples of all tissues listed below from all animals were preserved. Eyes were preserved in Davidson's fixative. Testes/epididymides were fixed in Bouin's solution for 24 - 48 hours and were then transferred to 70% alcohol. All other tissues were preserved in buffered 10% formalin.

In addition, samples of any macroscopically abnormal tissues were routinely preserved, along with samples of adjacent tissue where appropriate.

adrenals*	heart*	skin
alimentary tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	kidneys*	spinal column (to preserve and examine sample of spinal cord from cervical level)
aorta	larynx and pharynx	spleen*
brain (medullary, cerebellar and cerebral sections)	liver*	sternum (for bone and marrow)
epididymides*	lungs (all lobes and mainstem bronchi)	testes*
eyes	lymph nodes (cervical and mesenteric)	thymus (where present)
femur (with joint)	mammary gland	thyroid (with parathyroid)
Harderian gland	ovaries	tongue
head (to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland)	other macroscopically abnormal tissue*	trachea
	pancreas	urinary bladder
	pituitary	uterus (corpus and cervix)
	prostate	vagina
	salivary gland	
	sciatic nerve	
	seminal vesicles	
	skeletal muscle	

* Tissues required for histopathological examination

Histopathological examination

Tissues required for microscopic examination in this study are marked '*' in the above list. For testes and epididymides, tissues were embedded in paraffin wax and sections were stained with PAS-haematoxylin. A transverse section of each testis and a full longitudinal section of each epididymis were cut as near as possible to 2 micrometres. Microscopic assessment of the testes was made with reference to the stages of the cycle of the seminiferous epithelium. Other tissues were embedded in paraffin wax and sections cut at 4 micrometres were stained with haematoxylin and eosin.

Frozen sections of liver, fixed in buffered formalin, were cut on a cryostat at 12 micrometres and stained for fat with Oil Red O (ORO). Additional transverse sections taken from the central area of each kidney for all males were immunostained for α_2 microglobulin.

The macroscopic and microscopic findings are presented in Appendix 6 by an automated data collection system. Particular care is taken during tissue removal and processing to ensure recovery and sectioning of all protocol-scheduled tissues. Understandably, omissions or irregularities can occasionally occur, in rodents the most vulnerable tissues in this regard being parathyroid, thymus, male mammary gland and autolysed portions of the gastrointestinal tract. For each animal, any tissue so affected is listed as not seen. WNL (within normal limits) indicates that a macroscopically abnormal tissue was within normal limits upon histopathological examination.

The microscopic examinations consisted of the following:

The specified list of tissue from all animals of the control group and high dosage group killed after 14 days of treatment.

Liver and kidneys from all male animals from the low and intermediate dosage levels (Groups 2 and 3).

Abnormal tissues from the sporadic animal.

Any macroscopically abnormal tissue in any animal.

STATISTICAL ANALYSIS

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

For all parameters the analyses were carried out using the individual animal as the basic experimental unit.

Food consumption data were analysed using cumulative totals. Bodyweight data were analysed using weight gains.

The following sequence of statistical tests was used for food consumption, bodyweight, haematology, biochemistry and organ weight data:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed, Fisher (1950) and Mantel (1963). Otherwise:

A test was applied to test for heterogeneity of variance between treatments, Bartlett (1937). Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, an analysis of ranks was used, Kruskal-Wallis (1952/3).

Analyses of variance were followed by Student's *t* test and Williams test (Williams 1971/2) for a dose-related response, although only the latter of these was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of this test (Shirley, 1977).

For organ weight data, analysis of covariance was used in place of analysis of variance in the above sequence. Analysis of covariance was performed using terminal bodyweight as covariate when the within group relationship between organ weight and bodyweight was significant at the 10% level.

LOCATION OF STUDY RECORDS

All specimens, raw data and other documents generated at Huntingdon Life Sciences during the course of this study, together with a copy of the final report, have been lodged in the Huntingdon Life Sciences Ltd Archives, Huntingdon, England.

Such specimens and records will be retained for a minimum period of 5 years from the date of issue of the final report. At the end of the 5-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.

Samples and data arising from any investigations made by the Sponsor, the findings of which are included in the Huntingdon Life Sciences final report, are retained and archived by the Sponsor.

PROCEDURE

The procedure used during the study were those documented in the relevant Huntingdon Life Sciences Procedures Manuals.

DEVIATIONS FROM PROTOCOL

Dosing solutions were prepared by direct dilution on a weekly basis and not freshly each day; this was considered acceptable since chemical stability of dose formulations was confirmed by analysis (see attached **FORMULATION ANALYSIS REPORT**).

RESULTS

ANALYTICAL CHEMISTRY

The achieved concentrations of formulations prepared for the first and second week of dosing were within acceptable limits around the nominal concentrations. These results are presented in this report (Addendum 1).

MORTALITY (Appendix 6)

There was one unscheduled death during the study, but this was a control animal.

Animal number 22 (Group 1♀) was found dead after the bleed on Day 14. There were no clinical signs noted during life-time. Macroscopic *post mortem* examination revealed the lungs were congested.

CLINICAL SIGNS (Appendix 6)

Transient post-dosing salivation was noted on occasions from Day 7 of treatment for all animals receiving 1000 mg/kg/day and all males and 4/5 females receiving 300 mg/kg/day.

There were no treatment-related clinical signs noted amongst animals receiving 100 mg/kg/day.

BODYWEIGHT (Figure 1, Table 1, Appendix 1)

Overall group mean bodyweight gains, noted for Days 1 to 12 and Days 1 to 15, for all treated groups of males were slightly lower than concurrent controls. Lower mean bodyweight gains were also noted for all groups from Days 12 to 15, however, this was mainly attributable to the period of food withdrawal associated with the terminal bleed procedure on Day 14. The pattern of lower overall mean bodyweight gain noted for treated groups of males was not dosage-related in degree and did not attain statistical significance and in view of the small group size no clear association with treatment was identified.

Overall mean bodyweight gain for all treated groups of females was generally comparable with concurrent controls.

FOOD CONSUMPTION (Table 2, Appendix 2)

Group mean food intake was generally comparable for all treated groups in comparison with concurrent controls.

FOOD CONVERSION RATIOS (Table 3)

Efficiency of food utilisation, as determined by food conversion ratios, was marginally inferior for treated groups of males and was associated with the slightly lower bodyweight gains noted for these animals, in comparison with controls.

Efficiency of food utilisation for males receiving 300 or 100 mg/kg/day and all treated groups of females were similar to concurrent controls.

LABORATORY INVESTIGATIONS**Haematology (Table 4, Appendix 3)**

Haematological investigations were performed on Day 14.

At 1000 mg/kg/day, a reduction in mean total white blood cell (WBC) and lymphocyte counts was seen in male and, to a lesser extent, in female animals compared with the controls; the difference noted in lymphocyte count for males attained statistical significance. Mean total WBC and lymphocyte counts for all groups fell within the historical control range given below.

There were no other findings considered to be treatment-related at any dosage.

Selected haematological parameters (group mean) were as follows:

Parameter	Sex	Group/dosage (mg/kg/day)				n	Background ranges				
		0	100	300	1000		5%	50%	95%	Mean	sd
Total white blood cells	♂	13.91	11.94	12.66	9.42	83	7.3	10.9	16.3	11.29	2.700
	♀	7.59	8.49	7.20	6.55	83	5.0	8.2	13.0	8.66	2.319
Lymphocytes	♂	12.16	9.96	10.53	7.96*	83	6.32	8.96	13.76	9.34	2.209
	♀	6.48	7.16	5.93	5.49	83	4.26	7.20	11.17	7.32	2.076

* $p < 0.05$

Background data drawn from studies performed June 1991 to November 1994 and based on rats aged 9 - 10 weeks

BIOCHEMISTRY (Table 5, Appendix 4)

Biochemical analyses of blood were performed on Day 14.

A slight reduction in mean alkaline phosphatase (AP) levels was noted for females receiving 1000 mg/kg/day. Amongst males receiving 1000 mg/kg/day, animal number 16 had an abnormally high AP level. With this animal excluded from calculation of the mean (to give a mean value for AP of 459 mU/ml) a slight reduction in AP in comparison with controls was also evident. These differences from controls for males and females did not however attain statistical significance. Mean values for all groups of males and females receiving 100 or 300 mg/kg/day were higher than the historical background control range.

There were no other changes in biochemistry parameters considered to be clearly attributable to treatment.

Group mean values for alkaline phosphatase were as follows:

Parameter	Sex	Group/dosage (mg/kg/day)				Background ranges					
		0	100	300	1000	n	5%	50%	95%	Mean	sd
Alkaline phosphatase	♂	533	619	545	530	106	220	328	501	339	84.19
	♀	323	392	347	282	106	144	216	335	225	63.01

No statistical significance ($p > 0.05$)

Background data drawn from studies performed June 1991 to November 1994 and based on rats aged 9 - 10 weeks

TERMINAL INVESTIGATIONS

Organ weights (Table 6, Appendix 5)

Analysis of organ weight data obtained after completion of 14 days of treatment revealed a marked increase in mean liver and kidney weights, following adjustment for bodyweight, in both male and female animals receiving 1000 or 300 mg/kg/day: these differences from controls attained statistical significance. In addition a slight increase in adrenal weight, following adjustment for bodyweight, was noted for males and females receiving 1000 and females receiving 300 mg/kg/day: statistical significance was attained in females receiving 1000 mg/kg/day.

Although absolute mean liver, kidney and adrenal weights generally fell within the historical control range for animals receiving 1000 or 300 mg/kg/day (see table below), the range of individual values for these groups was higher than the individual concurrent control group ranges seen in this study.

All other organ weights were generally unremarkable and comparable to the controls.

Selected organ weight parameters (group mean) based on absolute values were as follows:

Parameter	Sex	Group/dosage (mg/kg/day)				Background ranges					
		0	100	300	1000	n	5%	50%	95%	Mean	sd
Liver	♂	16.5	15.6	17.9	19.8	106	12.8	17.7	24.0	18.02	3.174
	♀	9.0	9.8	10.9	11.4	106	8.7	11.0	13.7	11.16	1.527
Kidneys	♂	2.33	2.20	2.58	2.51	101	2.32	2.91	3.57	2.924	0.3792
	♀	1.60	1.67	1.82	1.69	101	1.73	2.03	2.42	2.031	0.2097
Adrenals	♂	50.9	51.6	50.5	53.3	106	41.65	52.95	71.20	55.122	9.0424
	♀	52.2	54.5	65.4	64.0	106	51.90	66.25	87.62	67.727	11.6216

Statistical analysis performed on bodyweight adjusted values and therefore not presented

Background data drawn from studies performed June 1991 to November 1994 and based on rats aged 9 - 10 weeks

Macroscopic pathology (Table 7, Appendix 6)

Macroscopic examination of animals sacrificed after 14 days of treatment revealed enlargement of the liver in 2/5 male rats and 2/5 female rats treated receiving 1000 mg/kg/day, and in 1/5 male rats and 1/5 female rats receiving 300 mg/kg/day.

Microscopic pathology (Table 8, Appendix 6)

The following histopathological observations were made on the tissues from the animals examined for this study;

Treatment-related changes

There were no treatment-related changes in the female animals.

In the male animals, centrilobular hepatocyte (CL) enlargement was recorded in animals receiving 1000 mg/kg/day and eosinophilic, hyaline-like droplets were seen in the cortical tubular epithelia of a majority of treated animals from all dose groups. The group incidences seen were:

	Numbers of animals showing specific changes							
	1♂	2♂	3♂	4♂	1♀	2♀	3♀	4♀
Liver								
CL enlargement	0	0	0	4*	0	0	0	0
Kidneys								
Eosinophilic droplets	0	4*	5**	5**	0	0	0	0
Total number of animals examined	5	5	5	5	5	5	5	5

Statistical significance: * $p < 0.05$, ** $p < 0.01$

Attempts were made to use immunohistochemical techniques to confirm the α_2 -microglobulin content of the eosinophilic droplets seen in the kidney tubules of treated male rats, but this proved to be impossible. Suitable antibody reagent was not commercially available and samples obtained from non-commercial sources did not show adequate specificity.

The pattern of distribution of these granules within the renal cortical tubules and the fact that these droplets were only seen in male animals provides extremely strong evidence that this material is α_2 -microglobulin.

Incidental changes

The small number of other histological changes seen were considered to be spontaneous in origin and of no toxicological significance.

Conclusion

Exposure of male rats to DPTB in this study induced minor degrees of centrilobular hepatocyte enlargement at 1000 mg/kg/day and eosinophilic droplet accumulation in the renal cortical tubular epithelia of most animals at all dosage levels. These droplets may be α_2 -microglobulin.

CONCLUSION

At 1000 mg/kg/day, a statistically significant decrease in lymphocyte count was evident in males. In addition, at dosages of 1000 and 300 mg/kg/day there were marked increases in liver and kidney weights in both sexes; enlargement of the liver was also noted macroscopically for some animals. Microscopic examination revealed a minor degree of centrilobular hepatocyte enlargement in males receiving 1000 mg/kg/day.

Since plasma analysis showed no increases in levels of enzymes which act as markers for functional changes in the liver, the changes evident in the livers from animals receiving 1000 mg/kg/day are considered to be an adaptive response related to metabolism of the test material rather than a toxic change.

The eosinophilic droplet accumulation (considered to be α_2 microglobulin) seen in the renal cortical tubular epithelia of most treated males was not an unexpected finding after treatment with a test substance of this type. Disruption of protein recycling in the proximal tubule region of the male rat kidney, leading to localised accumulation of eosinophilic (hyaline-like) droplets, is a well-documented and probably species-specific response to repeated administration of various ethers and other hydrocarbons. It is not considered predictive of a hazard to human health. -

The effects observed after 14 days of treatment were not of sufficient severity to preclude the use of dosages of up to 1000 mg/kg/day in a further study of 13 weeks duration.

The no-observed effect level (NOEL) on the basis of this study of 14 days duration was 100 mg/kg/day for females (based on increased liver and kidney weights): no absolute NOEL was found for males, due to the kidney changes recorded at the lowest test dosage. However, when this probably species-specific response is excluded from consideration, a male NOEL of 100 mg/kg/day was found (based on increased liver and kidney weights, liver enlargement noted at 1000 and 300 mg/kg/day and centrilobular hepatocyte enlargement noted at 1000 mg/kg/day). These effects on organ weights were probably adaptive since there were no concurrent microscopic changes. The NOEL for histopathological effects (excluding kidney tissue) was at least 1000 mg/kg/day in females and 300 mg/kg/day in males (based on centrilobular hepatocyte enlargement).

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FIGURE 1
Bodyweights - group mean values

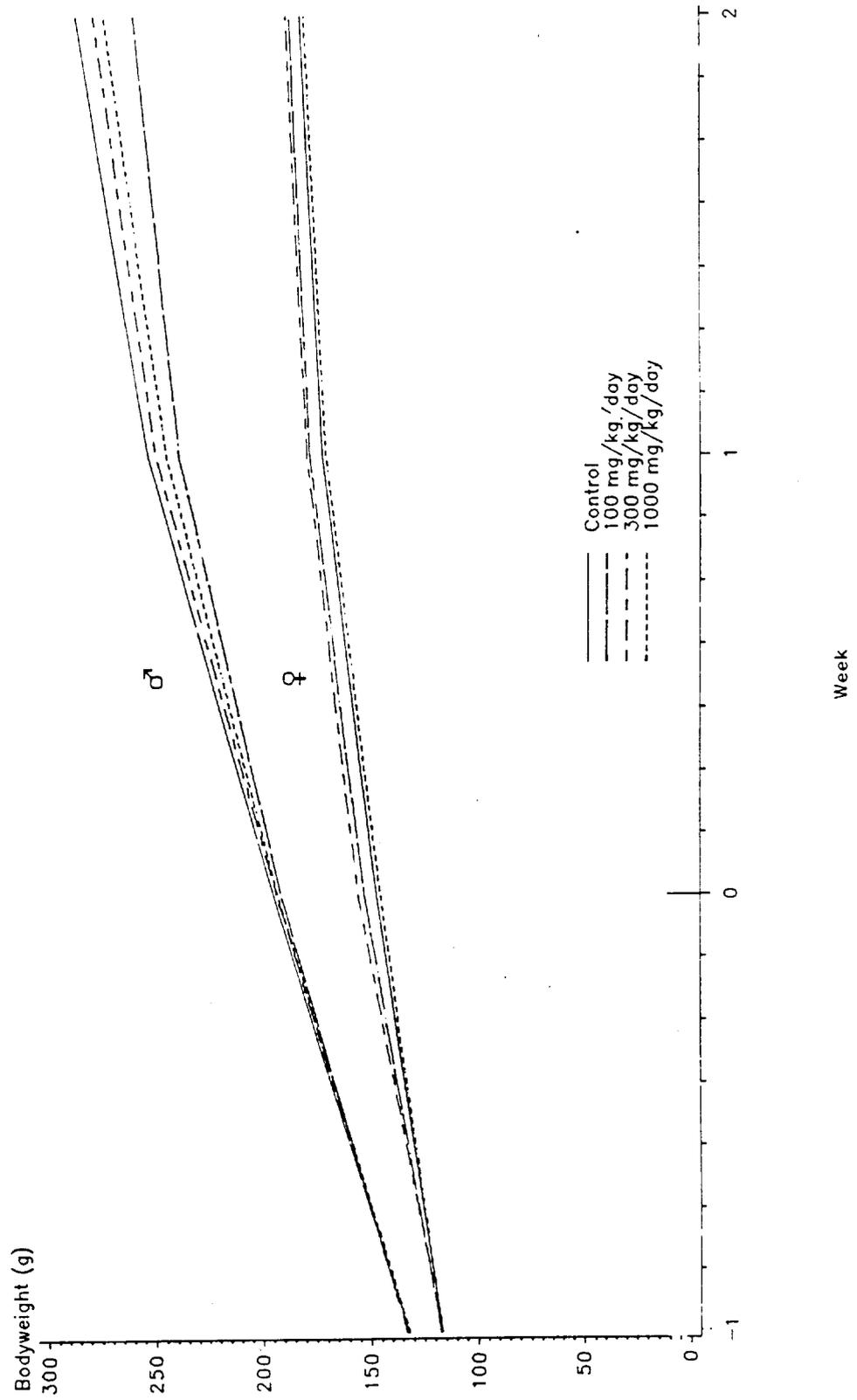


TABLE 1

Bodyweights - group mean values (g)

Day	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 100	3♂ 300	4♂ 1000	1♀ Control	2♀ 100	3♀ 300	4♀ 1000
-7	133	134	133	132	117	118	117	117
1	195	191	193	193	147	153	156	145
5	226	218	224	220	157	164	167	156
8	253	238	249	244	172	178	179	170
12	283	262	276	270	180	186	188	177
15	285	259	277	272	182	187	188	180
Gain (g)								
1 - 12	88.7	70.7	83.0	77.4	32.9	33.7	32.7	31.8
sd	11.1	9.2	7.4	12.7	8.4	8.2	6.1	3.2
% control	-	80	94	87	-	102	99	97
1 - 15	90.4	67.8	83.9	79.6	34.2	34.0	32.7	34.7
sd	11.3	10.0	9.1	12.3	8.3	5.2	6.4	10.3
% control	-	75	93	88	-	99	96	101

sd Standard deviation

No statistical significance ($p > 0.05$)

TABLE 2

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

Day	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 100	3♂ 300	4♂ 1000	1♀ Control	2♀ 100	3♀ 300	4♀ 1000
-7 - -1	28	27	27	27	21	21	21	20
1 - 4	30	28	28	29	22	21	21	20
5 - 7	31	28	30	31	22	22	21	22
8 - 11	32	29	31	31	22	22	22	21
12 - 14	26	23	24	25	17	19	20	18
Cumulative consumption (g/rat)								
1 - 14	418	383	399	408	295	293	295	283
sd	28.4	32.3	25.9	26.1	9.3	22.4	21.7	23.6
% control	-	92	95	98	-	99	100	96

sd Standard deviation

No statistical significance ($p > 0.05$)

TABLE 3

Food conversion ratios - group mean values

Day	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 100	3♂ 300	4♂ 1000	1♀ Control	2♀ 100	3♀ 300	4♀ 1000
1 - 4	3.8	4.2	3.7	4.3	8.8	7.5	6.9	7.0
5 - 7	3.4	4.1	3.6	3.8	4.4	4.6	5.5	4.8
8 - 11	4.2	5.0	4.5	4.8	10.8	10.2	9.5	12.3
12 - 14	47.7	n/a	79.8	33.3	19.8	188.9	n/a	19.0
1 - 14	4.6	5.7	4.8	5.1	8.7	8.6	9.0	8.2

n/a Insufficient bodyweight gain to give a meaningful ratio

Food conversion ratio = food consumption/bodyweight gain

TABLE 4

Haematology - group mean values

Day 14

Group/ dosage mg/kg/day	PCV %	Hb g/dl	RBC $10^{12}/l$	MCHC g/dl	MCV fl	MCH pg	TT s
1♂ Control	47.0	15.5	7.74	33.0	60.7	20.0	20
2♂ 100	49.4	16.2	8.27	32.8	59.7	19.6	22
3♂ 300	47.7	15.7	7.75	32.9	61.6	20.3	21
4♂ 1000	47.9	15.8	7.83	32.9	61.2	20.1	22
1♀ Control	47.1	16.1	8.01	34.1	58.9	20.1	19
2♀ 100	46.9	16.0	8.06	34.1	58.2	19.8	18
3♀ 300	46.1	15.6	7.76	33.9	59.4	20.1	19
4♀ 1000	46.9	16.0	8.02	34.1	58.4	19.9	19

No statistical significance ($p > 0.05$)

TABLE 4

(Haematology - continued)

Day 14

Group/ dosage mg/kg/day	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l	LUC 10 ⁹ /l	Plt 10 ⁹ /l
1♂ Control	13.91	1.03	12.16	0.08	0.04	0.26	0.35	1257
2♂ 100	11.94	1.20	9.96	0.07	0.03	0.34	0.33	1221
3♂ 300	12.66	1.40	10.53	0.09	0.04	0.30	0.31	1214
4♂ 1000	9.42	0.95	7.96*	0.05	0.02	0.22	0.22*	1235
1♀ Control	7.59	0.69	6.48	0.08	0.01	0.16	0.16	1204
2♀ 100	8.49	0.97	7.16	0.07	0.02	0.14	0.13	1271
3♀ 300	7.20	0.93	5.93	0.10	0.02	0.11	0.12	1190
4♀ 1000	6.55	0.76	5.49	0.08	0.01	0.10	0.10	1261

* $p \leq 0.05$

TABLE 5

Biochemistry - group mean values

Day 14

Group/ dosage mg/kg/day	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml
		Total	Alb	Glob					
1♂ Control	124	6.0	2.8	3.2	16	0.4	533	26	58
2♂ 100	129	6.1	2.9	3.1	15	0.5	619	28	66
3♂ 300	100	6.2	2.8	3.4	13	0.4	545	25	61
4♂ 1000	107	6.2	2.9	3.3	12*	0.5	530	28	59
1♀ Control	95	6.2	3.0	3.2	13	0.5	323	23	54
2♀ 100	110	6.2	3.0	3.2	13	0.5	392	26	55
3♀ 300	107	6.2	3.1	3.2	13	0.5	347	20	54
4♀ 1000	96	6.4	3.0	3.4	12	0.5	282	24	50

* $p \leq 0.05$

TABLE 5

(Biochemistry - continued)

Day 14

Group/ dosage mg/kg/day	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Control	<0.1	146	4.1	5.6	5.5	103	79
2♂ 100	0.1	147	4.4	5.6	5.5	103	68
3♂ 300	<0.1	146	4.3	5.6	5.2	102	75
4♂ 1000	<0.1	147	4.5	5.5	5.3	102	70
1♀ Control	0.1	145	4.0	5.4	4.3	104	91
2♀ 100	0.1	146	3.7	5.4	3.9	105	91
3♀ 300	0.1	146	3.8	5.4	4.3	105	96
4♀ 1000	<0.1	147	3.7	5.4	4.2	104	88

No statistical significance ($p > 0.05$)

TABLE 6

Organ weights - group mean values

Terminal

Group/ dosage mg/kg/day	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg
Unadjusted means						
1♂ Control	277	1.80	16.5	0.54	2.33	50.9
2♂ 100	253	1.76	15.6	0.55	2.20	51.6
3♂ 300	269	1.79	17.9	0.55	2.58	50.5
4♂ 1000	264	1.76	19.8	0.46	2.51	53.3
Adjusted means						
1♂	-	-	15.6	0.51	2.20	47.6
2♂	-	-	16.5	0.58	2.35	55.4
3♂	-	-	17.6*	0.55	2.54**	49.5
4♂	-	-	20.0*	0.47	2.53**	53.9

* $p \leq 0.05$, ** $p \leq 0.01$

TABLE 6
(Organ weights - continued)

Terminal

Group/ dosage mg/kg/day	Prostate g	Testes g	Seminal vesicle g	Epididymides g
Unadjusted means				
1♂ Control	0.464	2.77	0.68	0.480
2♂ 100	0.439	2.85	0.54	0.500
3♂ 300	0.531	2.91	0.65	0.480
4♂ 1000	0.452	2.74	0.59	0.473
Adjusted means				
1♂	-	-	-	0.462
2♂	-	-	-	0.521
3♂	-	-	-	0.474
4♂	-	-	-	0.476

No statistical significance ($p > 0.05$)

TABLE 6
(Organ weights - continued)

Terminal

Group/ dosage mg/kg/day	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
Unadjusted means							
1 ♀ Control	177	1.68	9.0	0.42	1.60	52.2	70.0
2 ♀ 100	181	1.65	9.8	0.45	1.67	54.5	79.6
3 ♀ 300	184	1.72	10.9	0.44	1.82	65.4	76.4
4 ♀ 1000	174	1.68	11.4	0.39	1.69	64.0	65.8
Adjusted means							
1 ♀	-	1.69	9.1	0.43	1.62	53.4	71.4
2 ♀	-	1.64	9.7	0.44	1.65	53.3	78.3
3 ♀	-	1.71	10.6 [*]	0.43	1.77 [*]	62.6	73.4
4 ♀	-	1.69	11.7 ^{**}	0.40	1.74 [*]	67.0 [*]	69.1

* $p \leq 0.05$, ** $p \leq 0.01$

TABLE 7
Macroscopic pathology incidence summary

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Removal reason: Terminal	1	2	3	4	1	2	3	4
Animals on study	5	5	5	5	5	5	5	5
Animals completed	5	5	5	5	4	5	5	5
Lymph Nodes - Cervical	0	1	0	0	0	0	0	0
Enlarged	0	0	2	0	0	0	0	0
Lungs	0	0	1	0	0	0	0	0
Petechiae	0	0	1	2	0	0	1	2
Liver	0	0	0	1	0	0	0	0
Median cleft, pale subcapsular area/s	0	0	1	2	0	0	1	2
Spleen	0	0	0	1	0	0	0	0
Small	0	0	0	1	0	0	0	0
Pale	0	0	0	1	0	0	0	0
Stomach Antrum Mucosa	0	0	0	0	2	1	1	1
White nodule, near to limiting ridge	0	0	0	0	0	0	2	0
Kidneys	0	0	0	0	0	0	2	0
Increased pelvic dilatation	0	0	0	0	0	0	0	0
Uterus	0	0	0	0	0	0	0	1
Fluid distension	0	0	0	0	0	0	0	1

TABLE 8
Microscopic pathology incidence summary

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Removal reason: Intercurrent	1	2	3	4	1	2	3	4
Animals on study	5	5	5	5	5	5	5	5
Animals completed	0	0	0	0	1	0	0	0
Lungs Examined	0	0	0	0	1	0	0	0
Vascular congestion	0	0	0	0	1	0	0	0
Factors Contributory To Death Examined	0	0	0	0	1	0	0	0
Unknown	0	0	0	0	1	0	0	0

TABLE 8
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	5	5	5	5	5	5	5	5
Animals on study	0	0	2	0	0	0	0	0
Animals completed	0	0	1	0	0	0	0	0
Lungs	0	0	0	0	0	0	0	0
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0
Vascular congestion	0	0	1	0	0	0	0	0
Heart	5	5	5	5	5	5	5	5
Examined	4	4	4	4	4	4	4	4
No abnormalities detected	1	0	0	0	0	0	0	0
Myocardial fibrosis (Total)	1	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Lymph Nodes - Cervical	0	1	0	0	0	0	0	0
Examined	0	1	0	0	0	0	0	0
Increased cellularity - generalised (Total)	0	1	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Spleen	5	5	5	5	5	5	5	5
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0
Liver	5	5	5	5	5	5	5	5
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0
Centrilobular hepatocyte enlargement (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0
Liver (ORO stain)	5	5	5	5	5	5	5	5
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0
Kidneys	5	5	5	5	5	5	5	5
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	0	0	0	0	0	0	0	0

TABLE 8
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study Animals completed	5 5	5 5	5 5	5 5	5 4	5 5	5 5	5 5
Kidneys	(Continued)							
Dystrophic mineralisation (Total)	0	0	1	0	1	0	1	1
Minimal	0	0	0	0	0	0	0	0
Moderate	0	0	1	0	1	0	1	1
Dilatation of the renal pelvis (Total)	0	0	1	0	0	0	2	0
Trace	0	0	0	0	0	0	1	0
Minimal	0	0	1	0	0	0	0	0
Moderate	0	0	0	0	0	0	1	0
Subcapsular inflammatory focus (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Eosinophilic droplets in cortical tubular epithelia (Total)	0	4	5	5	0	0	0	0
Minimal	0	2	0	1	0	0	0	0
Moderate	0	2	5	2	0	0	0	0
Marked	0	0	0	2	0	0	0	0
Uterus								
Examined	0	0	0	0	0	0	0	1
Luminal dilatation (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Epididymides								
Examined	5	0	0	5	0	0	0	0
No abnormalities detected	5	0	0	5	0	0	0	0
Testes								
Examined	5	0	0	5	0	0	0	0
No abnormalities detected	5	0	0	5	0	0	0	0
Adrenals								
Examined	5	0	0	5	4	0	0	5
No abnormalities detected	5	0	0	5	4	0	0	5
Stomach								
Examined	0	0	0	0	1	1	1	1

TABLE 8
(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group	Group	Group	Group	Group	Group	Group	Group
	1	2	3	4	1	2	3	4
Animals on study Animals completed	5	5	5	5	5	5	5	5
	5	5	5	5	4	5	5	5
Stomach Missing Focus of ectopic non-glandular epithelium within the glandular mucosa	(Continued)	0	0	0	1	0	0	0
	0	0	0	0	1	1	1	1

APPENDIX 1

Bodyweights - individual values (g)

Group 1♂:
Control

Animal number	Day					
	-7	1	5	8	12	15
1	124	190	229	264	297	298
2	132	194	223	250	283	285
3	132	189	219	242	275	278
4	134	193	220	246	271	275
5	142	208	240	263	292	288

Group 2♂:
100 mg/kg/day

Animal number	Day					
	-7	1	5	8	12	15
6	126	171	189	205	226	222
7	130	191	220	242	270	266
8	134	199	227	248	270	270
9	135	193	221	242	268	268
10	143	201	232	254	274	269

Group 3♂:
300 mg/kg/day

Animal number	Day					
	-7	1	5	8	12	15
11	125	181	219	245	271	274
12	130	194	226	251	279	271
13	134	195	218	243	268	269
14	135	194	227	252	284	288
15	140	201	230	254	278	283

Group 4♂:
1000 mg/kg/day

Animal number	Day					
	-7	1	5	8	12	15
16	127	183	215	239	266	271
17	128	193	213	236	251	254
18	134	197	223	244	271	270
19	135	193	219	246	274	279
20	136	197	228	255	289	287

APPENDIX 1

(Bodyweights - continued)

Group 1♀:
Control

Animal number	Day					
	-7	1	5	8	12	15
21	112	149	159	172	180	180
22	117	146	158	172	184	-
23	117	141	148	164	167	169
24	118	150	162	182	195	196
25	122	150	158	170	175	182

Group 2♀:
100 mg/kg/day

Animal number	Day					
	-7	1	5	8	12	15
26	113	156	165	181	182	192
27	116	152	163	179	189	187
28	117	152	164	178	192	188
29	120	159	176	187	200	197
30	122	144	150	163	169	169

Group 3♀:
300 mg/kg/day

Animal number	Day					
	-7	1	5	8	12	15
31	112	152	166	177	184	191
32	116	164	181	196	206	204
33	117	142	149	164	168	170
34	119	149	161	166	180	177
35	121	172	181	191	202	200

Group 4♀:
1000 mg/kg/day

Animal number	Day					
	-7	1	5	8	12	15
36	113	153	160	171	183	183
37	114	139	152	174	175	192
38	118	137	148	162	167	166
39	120	141	152	164	169	170
40	120	156	169	179	190	188

+ Animal number 22♀ found dead Day 14

APPENDIX 2

Food consumption - individual values (g/rat/day)

Group 1♂:
Control

Day	Animal				
	1	2	3	4	5
-7 - -1	29	26	27	30	29
1 - 4	33	30	27	30	31
5 - 7	34	30	27	31	30
8 - 11	35	33	29	33	31
12 - 14	28	26	26	27	23

Group 2♂:
100 mg/kg/day

Day	Animal				
	6	7	8	9	10
-7 - -1	24	26	30	27	27
1 - 4	25	28	32	29	29
5 - 7	25	28	30	28	29
8 - 11	25	31	31	31	30
12 - 14	19	25	25	24	23

Group 3♂:
300 mg/kg/day

Day	Animal				
	11	12	13	14	15
-7 - -1	27	29	27	28	26
1 - 4	31	29	26	29	28
5 - 7	32	29	28	30	30
8 - 11	32	30	28	33	31
12 - 14	25	22	21	29	26

Group 4♂:
1000 mg/kg/day

Day	Animal				
	16	17	18	19	20
-7 - -1	26	26	27	27	26
1 - 4	29	31	28	29	30
5 - 7	31	32	26	31	33
8 - 11	32	29	28	33	36
12 - 14	26	23	22	27	26

APPENDIX 2

(Food consumption - continued)

Group 1 ♀:
Control

Day	Animal				
	21	22	23	24	25
-7 - -1	21	20	20	21	22
1 - 4	21	20	28	21	22
5 - 7	21	22	20	24	22
8 - 11	22	23	20	25	22
12 - 14	17	(16)	16	19	19

Group 2 ♀:
100 mg/kg/day

Day	Animal				
	26	27	28	29	30
-7 - -1	20	22	21	22	19
1 - 4	20	22	21	22	19
5 - 7	21	22	22	23	20
8 - 11	21	23	23	24	19
12 - 14	21	18	17	22	16

Group 3 ♀:
300 mg/kg/day

Day	Animal				
	31	32	33	34	35
-7 - -1	21	22	18	20	24
1 - 4	21	23	18	20	22
5 - 7	21	23	20	19	21
8 - 11	23	25	19	21	23
12 - 14	21	19	18	26	18

Group 4 ♀:
1000 mg/kg/day

Day	Animal				
	36	37	38	39	40
-7 - -1	21	20	17	18	22
1 - 4	21	20	18	19	22
5 - 7	22	25	20	20	23
8 - 11	22	21	18	20	23
12 - 14	19	20	15	17	18

() Value excluded from means and statistical analysis, animal no. 22♀ found dead Day 14

APPENDIX 3

Haematology - individual values

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	PCV Hb		RBC	MCHC	MCV	MCH	TT
		%	g/dl	10 ¹² /l	g/dl	fl	pg	s
1♂ Control	1	47.6	15.7	7.60	32.9	62.6	20.6	21
	2	49.1	16.0	7.73	32.6	63.5	20.7	20
	3	45.6	15.2	7.73	33.4	59.0	19.7	20
	4	46.0	15.0	7.71	32.7	59.7	19.5	20
	5	46.6	15.6	7.92	33.5	58.9	19.7	20
	Mean sd	47.0 1.40	15.5 0.40	7.74 0.115	33.0 0.41	60.7 2.15	20.0 0.56	20 0.6
2♂ 100	6	48.7	15.8	8.38	32.5	58.1	18.9	21
	7	49.5	16.3	8.32	32.9	59.6	19.6	22
	8	50.3	16.3	8.55	32.5	58.9	19.1	24
	9	47.2	15.8	7.75	33.5	60.9	20.4	20
	10	51.2	16.7	8.36	32.6	61.2	20.0	22
	Mean sd	49.4 1.53	16.2 0.38	8.27 0.305	32.8 0.42	59.7 1.31	19.6 0.62	22 1.5
3♂ 300	11	48.4	15.8	7.86	32.6	61.6	20.1	21
	12	46.8	15.5	7.81	33.0	59.9	19.8	21
	13	49.7	16.3	8.11	32.9	61.3	20.1	21
	14	46.4	15.3	7.69	33.0	60.3	19.9	21
	15	47.4	15.7	7.29	33.2	65.0	21.6	21
	Mean sd	47.7 1.33	15.7 0.38	7.75 0.300	32.9 0.22	61.6 2.01	20.3 0.74	21 0.2
4♂ 1000	16	49.0	15.9	7.97	32.4	61.5	19.9	ctd
	17	50.2	16.5	8.33	32.9	60.3	19.9	27
	18	48.9	16.1	7.80	33.0	62.6	20.7	22
	19	46.6	15.5	7.84	33.2	59.4	19.7	19
	20	45.0	14.8	7.23	32.9	62.2	20.5	19
	Mean sd	47.9 2.10	15.8 0.65	7.83 0.397	32.9 0.29	61.2 1.33	20.1 0.43	22 3.9

sd Standard deviation

ctd Clotted sample

APPENDIX 3

(Haematology - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l	LUC 10 ⁹ /l	Plt 10 ⁹ /l
1♂ Control	1	8.09	0.70	6.81	0.04	0.02	0.26	0.26	1213
	2	11.70	0.86	10.33	0.05	0.03	0.15	0.27	1334
	3	14.77	0.73	13.42	0.07	0.05	0.19	0.31	1429
	4	18.01	1.72	15.19	0.16	0.05	0.37	0.52	1053
	5	17.00	1.12	15.04	0.10	0.03	0.31	0.39	1256
	Mean sd	13.91 4.058	1.03 0.422	12.16 3.572	0.08 0.048	0.04 0.013	0.26 0.089	0.35 0.108	1257 140.6
2♂ 100	6	13.67	1.23	11.79	0.02	0.05	0.26	0.33	1423
	7	13.83	1.90	10.99	0.18	0.03	0.36	0.37	1192
	8	9.29	0.72	7.91	0.05	0.02	0.31	0.26	991
	9	6.92	0.67	5.81	0.03	0.02	0.21	0.18	1363
	10	15.99	1.47	13.32	0.08	0.05	0.58	0.49	1134
	Mean sd	11.94 3.715	1.20 0.518	9.96 3.046	0.07 0.065	0.03 0.015	0.34 0.143	0.33 0.117	1221 174.8
3♂ 300	11	10.95	1.80	8.45	0.09	0.03	0.25	0.34	1016
	12	12.51	1.55	10.45	0.06	0.03	0.20	0.22	1242
	13	13.64	1.51	11.06	0.16	0.04	0.49	0.38	1282
	14	9.89	0.84	8.48	0.06	0.03	0.23	0.25	1146
	15	16.29	1.28	14.19	0.07	0.05	0.33	0.36	1386
	Mean sd	12.66 2.488	1.40 0.361	10.53 2.356	0.09 0.042	0.04 0.009	0.30 0.117	0.31 0.071	1214 140.4
4♂ 1000	16	7.17	1.38	5.32	0.03	0.01	0.23	0.19	1025
	17	6.20	0.65	5.22	0.02	0.01	0.13	0.17	1280
	18	12.00	0.78	10.78	0.04	0.03	0.14	0.23	1369
	19	9.94	0.98	8.48	0.07	0.02	0.21	0.19	1194
	20	11.81	0.95	10.01	0.10	0.04	0.39	0.32	1308
	Mean sd	9.42 2.649	0.95 0.276	7.96 2.593	0.05 0.033	0.02 0.013	0.22 0.104	0.22 0.060	1235 133.3

sd Standard deviation

APPENDIX 3

(Haematology - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast
1♂ Control	1	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-
	Mean sd									
2♂ 100	6	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-
	8	-	+	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-
	Mean sd									
3♂ 300	11	-	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-
	Mean sd									
4♂ 1000	16	-	-	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-
	19	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-
	Mean sd									

sd Standard deviation

APPENDIX 3

(Haematology - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	PCV Hb		RBC	MCHC	MCV	MCH	TT
		%	g/dl	$10^{12}/l$	g/dl	fl	pg	s
1♀ Control	21	45.5	15.5	8.04	34.1	56.6	19.3	19
	22	49.4	16.7	8.26	33.8	59.8	20.2	18
	23	46.4	15.9	7.83	34.3	59.3	20.4	19
	24	47.2	15.9	8.08	33.6	58.4	19.6	18
	25	47.2	16.3	7.85	34.5	60.2	20.8	21
	Mean sd	47.1 1.44	16.1 0.46	8.01 0.178	34.1 0.36	58.9 1.43	20.1 0.61	19 1.5
2♀ 100	26	46.1	15.9	8.17	34.6	56.4	19.5	ctd
	27	46.1	15.6	7.97	33.8	57.8	19.5	17
	28	47.9	16.3	8.23	33.9	58.2	19.8	ctd
	29	47.5	16.2	8.06	34.0	59.0	20.1	19
	30	46.7	15.9	7.86	34.0	59.4	20.2	18
	Mean sd	46.9 0.82	16.0 0.28	8.06 0.149	34.1 0.31	58.2 1.17	19.8 0.33	18 1.0
3♀ 300	31	44.2	15.0	7.47	33.9	59.2	20.1	19
	32	45.6	15.4	7.65	33.8	59.6	20.2	17
	33	44.6	15.4	7.62	34.5	58.5	20.2	18
	34	48.4	16.2	8.17	33.5	59.2	19.8	20
	35	47.6	16.0	7.89	33.7	60.4	20.3	19
	Mean sd	46.1 1.85	15.6 0.49	7.76 0.274	33.9 0.38	59.4 0.69	20.1 0.19	19 0.9
4♀ 1000	36	47.1	15.8	8.07	33.6	58.3	19.6	20
	37	45.7	15.6	7.99	34.1	57.1	19.5	20
	38	46.4	16.0	7.97	34.5	58.2	20.1	19
	39	48.5	16.7	8.18	34.5	59.3	20.5	18
	40	46.7	15.8	7.90	33.8	59.2	20.0	20
	Mean sd	46.9 1.04	16.0 0.43	8.02 0.107	34.1 0.41	58.4 0.89	19.9 0.40	19 0.8

sd Standard deviation

ctd Clotted sample

APPENDIX 3

(Haematology - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	WBC Total 10 ⁹ /l	N 10 ⁹ /l	L 10 ⁹ /l	E 10 ⁹ /l	B 10 ⁹ /l	M 10 ⁹ /l	LUC 10 ⁹ /l	Plt 10 ⁹ /l
1♀ Control	21	8.31	0.53	7.22	0.14	0.01	-0.21	0.20	1378
	22	8.51	1.02	7.06	0.06	0.02	0.19	0.16	1105
	23	6.44	0.76	5.40	0.06	0.01	0.11	0.09	1071
	24	9.56	0.70	8.20	0.09	0.02	0.25	0.30	1292
	25	5.12	0.43	4.50	0.07	0.01	0.06	0.05	1174
	Mean sd	7.59 1.780	0.69 0.228	6.48 1.494	0.08 0.034	0.01 0.005	0.16 0.077	0.16 0.098	1204 128.9
2♀ 100	26	6.00	0.58	5.19	0.08	0.01	0.07	0.07	1150
	27	6.32	1.10	5.02	0.06	0.01	0.05	0.08	1273
	28	10.81	0.91	9.37	0.10	0.02	0.20	0.21	1404
	29	11.57	1.13	9.97	0.07	0.03	0.21	0.17	1473
	30	7.75	1.12	6.26	0.06	0.02	0.16	0.13	1056
	Mean sd	8.49 2.565	0.97 0.235	7.16 2.348	0.07 0.017	0.02 0.008	0.14 0.074	0.13 0.059	1271 172.7
3♀ 300	31	7.95	1.28	6.38	0.10	0.01	0.07	0.12	1146
	32	8.76	1.07	7.29	0.10	0.02	0.13	0.15	1426
	33	6.17	0.83	5.03	0.10	0.02	0.11	0.09	1202
	34	5.90	0.54	4.99	0.08	0.02	0.14	0.14	1085
	35	7.20	0.91	5.97	0.11	0.01	0.11	0.10	1092
	Mean sd	7.20 1.199	0.93 0.276	5.93 0.968	0.10 0.011	0.02 0.005	0.11 0.027	0.12 0.025	1190 140.0
4♀ 1000	36	6.10	0.85	4.85	0.12	0.01	0.16	0.12	1190
	37	5.32	0.61	4.51	0.06	0.00	0.05	0.08	1264
	38	9.00	1.37	7.23	0.13	0.02	0.11	0.14	1217
	39	6.45	0.49	5.74	0.04	0.02	0.06	0.10	1417
	40	5.86	0.47	5.12	0.06	0.01	0.13	0.08	1217
	Mean sd	6.55 1.432	0.76 0.374	5.49 1.072	0.08 0.040	0.01 0.008	0.10 0.047	0.10 0.026	1261 91.2

sd Standard deviation

APPENDIX 3

(Haematology - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	Anis	Micro	Macro	Var	Hypo	Hyper	LS	Atyp	Blast
1♀ Control	21	-	-	-	-	-	-	-	-	-
	22	-	-	-	-	-	-	-	-	-
	23	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-
	Mean sd									
2♀ 100	26	-	-	-	-	-	-	-	-	-
	27	-	-	-	-	-	-	-	-	-
	28	-	-	-	-	-	-	-	-	-
	29	-	-	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-	-	-
	Mean sd									
3♀ 300	31	-	-	-	-	-	-	-	-	-
	32	-	-	-	-	-	-	-	-	-
	33	-	-	-	-	-	-	-	-	-
	34	-	-	-	-	-	-	-	-	-
	35	-	-	-	-	-	-	-	-	-
	Mean sd									
4♀ 1000	36	-	-	-	-	-	-	-	-	-
	37	-	-	-	-	-	-	-	-	-
	38	-	-	-	-	-	-	-	-	-
	39	-	-	-	-	-	-	-	-	-
	40	-	-	-	-	-	-	-	-	-
	Mean sd									

sd Standard deviation

APPENDIX 4

Biochemistry - individual values

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml
			Total	Alb	Glob					
1♂ Control	1	128	6.2	2.9	3.3	14	0.5	584	22	51
	2	131	6.1	2.8	3.3	19	0.5	484	26	54
	3	117	5.8	2.7	3.1	13	0.4	545	25	65
	4	113	6.0	2.8	3.2	19	0.4	531	29	61
	5	129	5.9	2.7	3.2	14	0.4	521	29	59
	Mean sd	124 8.0	6.0 0.16	2.8 0.08	3.2 0.08	16 2.9	0.4 0.05	533 36.4	26 2.9	58 5.6
2♂ 100	6	155	5.9	2.9	3.0	15	0.4	592	31	79
	7	105	6.2	3.0	3.2	14	0.5	552	31	59
	8	122	6.2	2.9	3.3	14	0.5	710	26	62
	9	116	6.1	3.0	3.1	14	0.5	671	31	70
	10	146	6.0	2.9	3.1	17	0.5	571	21	58
	Mean sd	129 21.0	6.1 0.13	2.9 0.05	3.1 0.11	15 1.3	0.5 0.04	619 68.0	28 4.5	66 8.8
3♂ 300	11	99	6.1	2.7	3.4	14	0.4	637	31	83
	12	101	6.3	2.8	3.5	14	0.4	556	22	72
	13	110	6.0	2.7	3.3	16	0.4	584	20	50
	14	86	6.5	3.0	3.5	11	0.5	418	23	49
	15	105	6.1	2.7	3.4	11	0.5	529	29	52
	Mean sd	100 9.0	6.2 0.20	2.8 0.13	3.4 0.08	13 2.2	0.4 0.05	545 81.4	25 4.7	61 15.4
4♂ 1000	16	93	6.1	2.8	3.3	12	0.5	812	34	78
	17	137	6.4	2.9	3.5	12	0.5	474	21	49
	18	107	6.1	2.8	3.3	14	0.4	408	28	59
	19	98	6.1	2.9	3.2	9	0.4	505	28	47
	20	100	6.2	3.0	3.2	12	0.5	449	27	60
	Mean sd	107 17.5	6.2 0.13	2.9 0.08	3.3 0.12	12 1.8	0.5 0.05	530 161.8	28 4.6	59 12.3

sd Standard deviation

APPENDIX 4

(Biochemistry - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Control	1	0.1	147	3.5	5.8	5.6	103	67
	2	0.1	147	4.2	5.5	5.8	100	61
	3	0.1	146	4.1	5.7	5.4	103	89
	4	0.1	146	4.6	5.5	5.6	104	105
	5	<0.1	145	4.2	5.5	5.2	105	73
	Mean sd	<0.1 0.8	146 0.8	4.1 0.40	5.6 0.14	5.5 0.23	103 1.9	79 17.9
2♂ 100	6	0.1	146	5.2	5.6	6.0	103	68
	7	0.1	147	3.9	5.6	4.9	103	54
	8	0.2	147	3.9	5.5	5.4	104	49
	9	0.1	147	4.0	5.7	5.6	102	92
	10	0.2	146	5.1	5.5	5.5	104	76
	Mean sd	0.1 0.05	147 0.5	4.4 0.67	5.6 0.08	5.5 0.40	103 0.8	68 17.3
3♂ 300	11	0.1	144	4.5	5.6	5.1	101	73
	12	0.1	147	4.3	5.5	4.8	103	73
	13	0.1	147	4.3	5.6	5.3	102	79
	14	0.1	146	4.8	5.9	5.5	101	83
	15	<0.1	148	3.8	5.6	5.5	103	67
	Mean sd	<0.1 1.5	146 1.5	4.3 0.36	5.6 0.15	5.2 0.30	102 1.0	75 6.2
4♂ 1000	16	0.1	149	4.2	5.4	5.8	103	69
	17	0.1	147	4.0	5.7	5.5	102	90
	18	<0.1	146	5.6	5.3	5.2	103	58
	19	0.1	145	4.6	5.5	4.8	101	67
	20	0.1	146	4.3	5.6	5.1	100	65
	Mean sd	<0.1 1.5	147 1.5	4.5 0.63	5.5 0.16	5.3 0.38	102 1.3	70 12.0

sd Standard deviation

APPENDIX 4

(Biochemistry - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	Glu- cose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GPT mU/ ml	GOT mU/ ml
			Total	Alb	Glob					
1♀ Control	21	98	6.2	3.0	3.2	13	0.5	312	21	59
	22	96	6.5	3.2	3.3	15	0.5	338	23	50
	23	94	5.9	3.0	2.9	11	0.4	345	21	52
	24	80	6.1	2.8	3.3	15	0.5	317	22	52
	25	107	6.4	3.0	3.4	10	0.4	305	29	58
	Mean sd	95 9.7	6.2 0.24	3.0 0.14	3.2 0.19	13 2.3	0.5 0.05	323 17.2	23 3.3	54 4.0
2♀ 100	26	105	6.2	3.0	3.2	11	0.5	348	24	52
	27	114	6.6	2.9	3.7	15	0.6	458	26	58
	28	110	6.3	3.1	3.2	10	0.5	402	24	50
	29	125	6.2	3.0	3.2	13	0.4	384	27	53
	30	98	5.8	2.9	2.9	14	0.4	366	27	63
	Mean sd	110 10.1	6.2 0.29	3.0 0.08	3.2 0.29	13 2.1	0.5 0.08	392 42.2	26 1.5	55 5.3
3♀ 300	31	97	6.5	3.1	3.4	17	0.5	284	26	62
	32	119	6.2	3.0	3.2	13	0.5	341	22	55
	33	106	6.0	3.1	2.9	11	0.4	318	21	48
	34	94	6.0	2.9	3.1	14	0.5	408	17	51
	35	121	6.4	3.2	3.2	11	0.5	382	16	52
	Mean sd	107 12.3	6.2 0.23	3.1 0.11	3.2 0.18	13 2.5	0.5 0.04	347 49.5	20 4.0	54 5.3
4♀ 1000	36	87	6.5	3.0	3.5	12	0.4	248	24	53
	37	96	6.0	2.6	3.4	14	0.5	356	34	53
	38	89	6.7	3.2	3.5	10	0.5	217	23	46
	39	107	6.9	3.3	3.6	13	0.5	286	23	50
	40	102	6.0	2.8	3.2	13	0.5	301	18	49
	Mean sd	96 8.5	6.4 0.41	3.0 0.29	3.4 0.15	12 1.5	0.5 0.04	282 53.0	24 5.9	50 2.9

sd Standard deviation

APPENDIX 4

(Biochemistry - continued)

Day 14 (20 December 1995)

Group/ dosage mg/kg/day	Animal no.	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1 ♀ Control	21	0.1	145	3.8	5.6	4.3	104	109
	22	0.1	147	4.5	5.6	4.8	103	105
	23	0.1	146	3.7	5.1	3.8	106	78
	24	0.1	144	4.3	5.5	5.0	104	90
	25	0.1	144	3.5	5.1	3.4	104	72
	Mean sd	0.1 0.00	145 1.3	4.0 0.42	5.4 0.26	4.3 0.67	104 1.1	91 16.2
2 ♀ 100	26	0.1	146	3.5	5.4	4.0	105	76
	27	0.1	146	3.7	5.4	3.8	105	95
	28	0.1	148	3.7	5.5	4.0	106	89
	29	0.1	145	4.1	5.4	3.9	103	112
	30	0.1	146	3.7	5.1	3.9	105	81
	Mean sd	0.1 0.00	146 1.1	3.7 0.22	5.4 0.15	3.9 0.08	105 1.1	91 14.0
3 ♀ 300	31	0.1	145	3.5	5.5	4.3	103	112
	32	0.1	145	3.2	5.4	4.0	103	98
	33	0.1	146	3.8	5.2	3.7	106	101
	34	0.1	147	4.8	5.4	5.0	106	93
	35	0.1	148	3.8	5.4	4.3	107	76
	Mean sd	0.1 0.00	146 1.3	3.8 0.60	5.4 0.11	4.3 0.48	105 1.9	96 13.2
4 ♀ 1000	36	0.1	148	4.0	5.5	4.6	105	73
	37	0.1	145	4.0	5.3	4.3	103	81
	38	0.1	146	3.6	5.4	3.9	104	88
	39	<0.1	147	3.4	5.4	3.8	105	102
	40	0.1	148	3.7	5.3	4.6	105	97
	Mean sd	<0.1 1.3	147 1.3	3.7 0.26	5.4 0.08	4.2 0.38	104 0.9	88 11.7

sd Standard deviation

APPENDIX 5

Organ weights - individual values

Terminal

Group/ dosage mg/kg/day	Animal no.	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg
1♂ Control	1	289	1.77	16.5	0.55	2.44	53.6
	2	279	1.76	16.7	0.52	2.45	51.0
	3	270	1.79	15.7	0.55	2.31	49.1
	4	267	1.85	17.5	0.53	2.29	52.8
	5	281	1.82	16.0	0.54	2.15	48.1
	Mean sd		277 9.0	1.80 0.035	16.5 0.70	0.54 0.012	2.33 0.123
2♂ 100	6	217	1.79	13.3	0.47	1.77	40.9
	7	261	1.79	16.4	0.57	2.21	55.3
	8	262	1.65	16.0	0.61	2.36	44.3
	9	264	1.79	17.1	0.50	2.32	54.7
	10	261	1.79	15.0	0.57	2.36	62.9
	Mean sd		253 20.0	1.76 0.062	15.6 1.47	0.55 0.056	2.20 0.252
3♂ 300	11	268	1.79	17.0	0.56	2.56	39.7
	12	266	1.85	17.2	0.49	2.56	53.3
	13	259	1.80	17.3	0.59	2.35	49.3
	14	281	1.77	19.7	0.51	2.63	53.8
	15	272	1.77	18.3	0.63	2.80	56.5
	Mean sd		269 8.3	1.79 0.034	17.9 1.13	0.55 0.059	2.58 0.162
4♂ 1000	16	266	1.64	18.4	0.44	2.24	48.9
	17	247	1.67	18.4	0.32	2.37	47.7
	18	265	1.79	18.9	0.50	2.55	56.8
	19	265	1.82	20.2	0.52	2.57	50.6
	20	277	1.85	23.3	0.53	2.82	62.6
	Mean sd		264 10.8	1.76 0.092	19.8 2.06	0.46 0.089	2.51 0.220

sd Standard deviation

APPENDIX 5

(Organ weights - continued)

Terminal

Group/ dosage mg/kg/day	Animal no.	Prostate g	Testes		Seminal vesicle g	Epididymides	
			Left g	Right g		Left g	Right g
1♂ Control	1	0.539	1.51	1.56	0.69	0.235	0.271
	2	0.446	1.40	1.47	0.59	0.228	0.235
	3	0.452	1.38	1.38	0.58	0.228	0.198
	4	0.555	1.11	1.11	0.84	0.224	0.225
	5	0.330	1.48	1.47	0.68	0.273	0.282
	Mean sd	0.464 0.0899	1.37 0.155	1.40 0.172	0.68 0.105	0.238 0.0202	0.242 0.0343
2♂ 100	6	0.289	1.15	1.10	0.40	0.212	0.217
	7	0.543	1.43	1.39	0.78	0.242	0.279
	8	0.489	1.56	1.50	0.47	0.244	0.235
	9	0.267	1.51	1.56	0.55	0.261	0.271
	10	0.607	1.52	1.54	0.51	0.287	0.255
	Mean sd	0.439 0.1530	1.43 0.165	1.42 0.188	0.54 0.145	0.249 0.0275	0.251 0.0256
3♂ 300	11	0.408	1.34	1.33	0.89	0.228	0.255
	12	0.670	1.55	1.52	0.69	0.253	0.239
	13	0.546	1.66	1.86	0.52	0.209	0.222
	14	0.534	1.39	1.46	0.40	0.234	0.235
	15	0.497	1.18	1.25	0.76	0.261	0.262
	Mean sd	0.531 0.0947	1.42 0.185	1.48 0.237	0.65 0.194	0.237 0.0207	0.243 0.0160
4♂ 1000	16	0.378	1.39	1.39	0.39	0.235	0.217
	17	0.414	1.34	1.33	0.87	0.258	0.271
	18	0.586	1.38	1.38	0.66	0.241	0.236
	19	0.485	1.37	1.38	0.49	0.208	0.206
	20	0.398	1.38	1.38	0.53	0.247	0.245
	Mean sd	0.452 0.0850	1.37 0.018	1.37 0.024	0.59 0.185	0.238 0.0187	0.235 0.0253

sd Standard deviation

APPENDIX 5

(Organ weights - continued)

Terminal

Group/ dosage mg/kg/day	Animal no.	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1 ♀ Control	21	176	1.79	8.7	0.44	1.65	47.7	64.5
	23	163	1.58	7.4	0.35	1.47	41.4	62.2
	24	196	1.77	10.2	0.49	1.74	66.0	75.7
	25	172	1.58	9.8	0.41	1.56	53.5	77.5
	Mean sd	177 14.0	1.68 0.112	9.0 1.27	0.42 0.057	1.60 0.116	52.2 10.47	70.0 7.74
2 ♀ 100	26	186	1.62	9.6	0.50	1.72	57.8	88.3
	27	184	1.61	9.4	0.42	1.67	60.1	91.0
	28	180	1.60	9.5	0.46	1.57	53.0	70.0
	29	191	1.73	11.5	0.44	1.90	56.0	79.6
	30	163	1.67	9.3	0.42	1.49	45.5	68.9
Mean sd	181 10.6	1.65 0.056	9.8 0.92	0.45 0.031	1.67 0.157	54.5 5.65	79.6 10.15	
3 ♀ 300	31	189	1.69	9.6	0.39	1.93	57.8	64.8
	32	198	1.84	11.9	0.51	2.05	72.0	95.9
	33	164	1.60	11.0	0.46	1.68	58.8	67.1
	34	174	1.71	9.6	0.39	1.70	69.6	61.8
	35	192	1.78	12.5	0.46	1.74	68.9	92.6
Mean sd	184 14.1	1.72 0.093	10.9 1.33	0.44 0.051	1.82 0.158	65.4 6.61	76.4 16.41	
4 ♀ 1000	36	176	1.81	11.0	0.37	1.77	75.9	61.7
	37	186	1.62	12.1	0.45	1.81	78.0	82.3
	38	161	1.68	9.7	0.39	1.56	47.6	65.1
	39	163	1.58	10.6	0.34	1.53	50.1	51.5
	40	183	1.69	13.4	0.41	1.77	68.3	68.3
Mean sd	174 11.5	1.68 0.087	11.4 1.42	0.39 0.042	1.69 0.132	64.0 14.30	65.8 11.18	

sd Standard deviation

APPENDIX 6**Individual clinical and pathological findings**

In this appendix the clinical, macroscopic and microscopic findings relating to each animal are listed.

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

Study pathologist: Richard L. Gregson, M.Phil., Ph.D., C. Biol., M.I.Biol.,
Senior Pathologist
Department of Pathology

Peer review: John M. Offer, Ph.D., C.Biol., M.I.Biol.,
Consultant Pathologist
Department of Pathology

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 1♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 2♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 3♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 4♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 5♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocardial fibrosis: (Minimal)

The following tissues were considered normal:

Spleen; Liver; Liver (ORO stain); Kidneys; Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 6♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Minimal)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 7♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 8♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 9♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Minimal)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 10♂ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Increased cellularity - generalised: (Minimal)

The following tissues were considered normal:

Liver; Kidneys

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 11♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on one occasion on Day 14.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Dystrophic mineralisation: (Minimal)
Dilatation of the renal pelvis: (Minimal)
Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 12♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on one occasion on Day 9.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 13♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on two occasions from Day 8.

MACROSCOPIC FINDINGS

Lungs

Petechiae: (A few)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Lungs : (W.N.L.); Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 14♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on three occasions from Day 8.

MACROSCOPIC FINDINGS

Lungs

Petechiae: (A few)

Liver

Enlarged: 19.732g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Vascular congestion

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Liver : (W.N.L.)

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 15♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on four occasions from Day 10.

MACROSCOPIC FINDINGS

Liver

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

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Liver : (W.N.L.)

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 16♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on seven occasions from Day 7.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte enlargement: (Minimal)

Kidneys

Subcapsular inflammatory focus: (Minimal)

Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Heart; Spleen; Liver (ORO stain); Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 17♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on six occasions from Day 7.

MACROSCOPIC FINDINGS

Spleen
Small
Pale

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver
Centrilobular hepatocyte enlargement: (Minimal)

Kidneys
Eosinophilic droplets in cortical tubular epithelia: (Moderate)

The following tissues were considered normal:

Heart; Spleen : (W.N.L.); Liver (ORO stain); Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 18♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on five occasions from Day 7.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Minimal)

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 19♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on six occasions from Day 7.

MACROSCOPIC FINDINGS

Liver

Enlarged: 20.179g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte enlargement: (Minimal)

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Marked)

The following tissues were considered normal:

Heart; Spleen; Liver (ORO stain); Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 20♂ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on seven occasions from Day 7.

MACROSCOPIC FINDINGS

Liver

Enlarged: 23.296g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver

Centrilobular hepatocyte enlargement: (Moderate)

Kidneys

Eosinophilic droplets in cortical tubular epithelia: (Marked)

The following tissues were considered normal:

Heart; Spleen; Liver (ORO stain); Epididymides; Testes; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 21 ♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Dystrophic mineralisation: (Moderate)

Stomach

Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 22♀ (Intercurrent)

CLINICAL FINDINGS

Animal found dead after the routine laboratory investigations bleed on Day 14.
There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

Found dead

Lungs

Congested

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs

Vascular congestion

Factors Contributory To Death

Unknown

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 23♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 24♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: Control
Rat No/Sex: 25♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health or behavioural change.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Adrenals

Tissues not available for examination were:

Stomach : (Not seen)

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 26♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Stomach

Focus of ectopic non-glandular epithelium within the glandular mucosa

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 27♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 28♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 29♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 100 mg/kg/day
Rat No/Sex: 30♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

No abnormalities detected

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 31 ♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on one occasion on Day 10.

MACROSCOPIC FINDINGS

Kidneys

Increased pelvic dilatation: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Dilatation of the renal pelvis: (Trace)

The following tissues were considered normal:

Liver

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 32♀ (Terminal)

CLINICAL FINDINGS

There were no signs of ill health, behavioural change or reaction to treatment.

MACROSCOPIC FINDINGS

Kidneys

Increased pelvic dilatation: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Dystrophic mineralisation: (Minimal)

Dilatation of the renal pelvis: (Moderate)

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 33♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on one occasion on Day 12.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Stomach

Focus of ectopic non-glandular epithelium within the glandular mucosa

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 34 ♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on one occasion on Day 9.

MACROSCOPIC FINDINGS

No abnormalities detected

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 300 mg/kg/day
Rat No/Sex: 35♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on five occasions from Day 7.

MACROSCOPIC FINDINGS

Liver

Enlarged: 12.538g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Liver : (W.N.L.)

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 36♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on five occasions from Day 10.

MACROSCOPIC FINDINGS

Uterus

Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Kidneys

Dystrophic mineralisation: (Moderate)

Uterus

Luminal dilatation: (Minimal)

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Adrenals

Pathologist: R.Gregson

APPENDIX 6
(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 37♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on five occasions from Day 8.

MACROSCOPIC FINDINGS

Liver

Enlarged: 12.146g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

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

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 38♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on six occasions from Day 7.

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Stomach

Focus of ectopic non-glandular epithelium within the glandular mucosa

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 39♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on four occasions from Day 7.

MACROSCOPIC FINDINGS

No abnormalities detected

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Heart; Spleen; Liver; Liver (ORO stain); Kidneys; Adrenals

Pathologist: R.Gregson

APPENDIX 6

(Pathology - continued)

Compound: DPTB
Dosage Level: 1000 mg/kg/day
Rat No/Sex: 40♀ (Terminal)

CLINICAL FINDINGS

Salivation immediately after dosing was noted on eight occasions from Day 7.

MACROSCOPIC FINDINGS

Liver
Enlarged: 13.383g

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

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

Pathologist: R.Gregson

CERTIFICATE OF ANALYSIS

ARCO CHEMICAL COMPANY
 3801 West Chester Pike
 Newtown Square, Pennsylvania 19703



Contact: Frank Liotta
Phone: 610-359-2308

CERTIFICATE OF ANALYSIS

DIPROPYLENE GLYCOL t-BUTYL ETHER

Lot No.: HRC-1095

Date: 11/08/95

Property	Analysis
Assay as Dipropylene Glycol t-Butyl Ether <i>(by GC, copy attached)</i>	>98%
Impurities <i>(by GC)</i>	
Dipropylene Glycol	wt. %
Dipropylene Glycol Di-t-butyl Ether	wt. %
Water Content <i>(by Karl Fisher)</i>	%
APHA Color	5
Specific Gravity (@ 20°C)	0.9121
Acidity	meq/g
Distillation (@ 760 mm-Hg)	
IBP	
50% Point	
DP	

DIPROPYLENE GLYCOL T-BUTYL ETHER (DPTB)
FORMULATION ANALYSIS

Authors:

I Suzanne Dawe,
Steven F Johnson,
B Shenaz Nunhuck.

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INTRODUCTION

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

the determination of concentrations of DPTB in test formulations analysed during the toxicity study.

the determination of the homogeneity and stability of DPTB in aqueous formulations.

the validation of the analytical procedure for the determination of DPTB in aqueous formulations.

The formulations for this study were prepared as solutions of DPTB in distilled water by Pharmacy personnel at Huntingdon Life Sciences Ltd. The formulations appeared hazy at the higher concentrations, therefore the homogeneity was assessed.

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

Gas liquid chromatograph (GLC): As detailed in the chromatographic section.

Balance: Mettler AT261, fitted with an LC-P45 printer.

General laboratory glassware.

Reagents

Test substance: DPTB.
 Supplier: Arco Chemical Company.
 Batch no: HRC 1095.
 Stated purity: > 97%.

Water: Elgastat UHP-4, deionised reverse osmosis.

Sample extraction

A representative sample of test formulation was appropriately diluted using water to provide a solution containing DPTB at an expected concentration of 100 µg/ml.

The concentration of DPTB in the final solution was quantified by gas liquid chromatography using flame ionisation detection as detailed in the following section.

Typical chromatographic conditions

Gas liquid chromatograph (GLC):
 Instrument: Hewlett Packard 5890.
 Autosampler: Hewlett Packard 6890.
 Detector: Flame ionisation.
 Data handling: Perkin-Elmer Nelson Access*Chrom.

Analytical column: Fused silica.
 Configuration: 30 m × 0.53 mm id.
 Liquid phase: DB-wax.
 Film thickness: 1 µm.

Temperatures:
 Injection port: 200°C.
 Oven: 75°C (1 minute),
 ramped at 20°C/minute to 135°C (5 minutes),
 ramped at 30°C/minute to 225°C (2 minutes).
 275°C.

Detector:

Gases:	
Carrier:	Helium, 10 ml/minute.
Detector:	Air, 450 ml/minute.
	Hydrogen, 40 ml/minute.
Injection volume:	1 μ l.
Injection mode:	Splitless for 1 minute then purged at a split ratio of 1 : 10.
Retention times:	Peak 1 5.8 minutes,
	Peak 2 6.0 minutes,
	Peak 3 6.5 minutes.
Integration sensitivity:	80 mV.

Calibration

A primary standard solution was prepared for each analytical occasion by dissolving an accurately weighed quantity (50 mg) of DPTB in water. A solution for instrument calibration at a concentration of 100 μ g/ml was prepared by appropriate dilution of the primary standard using water.

The calibration standard was injected onto the GLC, at regular intervals, alternating with every two samples throughout the injection sequence.

At each analytical occasion, duplicate calibration standards were prepared to confirm the accuracy of preparation.

Linearity solutions, containing DPTB at concentrations in the range 40 μ g/ml - 200 μ g/ml, were injected onto the GLC to confirm the linearity of detector response.

Calculation

The peak area response of the 3 major peaks at the characteristic retention times for DPTB in each calibration and sample chromatogram were measured. Since the response of each peak was consistent the concentration of DPTB was determined with respect to peak 1 using the following equation:

$$\text{Concentration, mg/ml} = \frac{R_s}{R_c} \times C \times V \times 10^{-3}$$

Where	R_s	=	Mean peak area response for DPTB (peak 1) in test chromatogram
	R_c	=	Mean peak area response for DPTB (peak 1) in the calibration standard
	C	=	Concentration of calibration standard (μ g/ml)
	V	=	Dilution factor of sample

Limit of detection

The limit of detection, defined as the concentration of DPTB in aqueous solution producing a peak response equivalent to $3 \times$ baseline noise, was determined by preparing solutions containing DPTB at decreasing concentrations to provide a peak of suitable size.

VALIDATION OF THE METHOD OF ANALYSIS

Prior to the start of the study, specimen aqueous formulations of DPTB were prepared at nominal concentrations of 0.1 mg/ml and 100 mg/ml by Analytical Chemistry personnel. The analytical procedure was validated at the low and high inclusion levels by determining the accuracy and precision of analytical results generated for the analysis of six replicate samples from the prepared formulations.

HOMOGENEITY AND STABILITY IN AQUEOUS FORMULATIONS

Specimen formulations (2000 ml), containing DPTB at nominal concentrations of 0.1 mg/ml and 100 mg/ml, were prepared and sub-divided ($250 \text{ ml} \times 8$) by Pharmacy personnel to simulate the anticipated preparation, distribution and sampling of doses for analysis during the toxicity study.

A bottle of each formulation was thoroughly mixed by inversion and magnetically stirred. After magnetic stirring for 5 minutes (0 hour), 2 hours and 4 hours, samples (1 ml) were removed for analysis from points at approximately one-quarter, one-half and three-quarters the depth (representing the top, middle and bottom) of the formulation.

The remaining bottles were refrigerated (nominally $+4^{\circ}\text{C}$) in the dark. At time-points representing 2 days, 8 days and 15 days storage, a fresh bottle of each formulation was equilibrated to room temperature for 1 hour, mixed by inversion, magnetically stirred (5 minutes) and sampled at 0 hour as detailed above.

At each occasion, the three samples from each formulation were analysed in accordance with the analytical procedure. The samples removed after stirring for 2 hours were retained frozen (-20°C) for contingency.

CONCENTRATIONS IN TEST FORMULATIONS

At specified time-points during the study, freshly-prepared formulations (20 ml) were sampled by Pharmacy personnel and submitted for analysis. On receipt, samples (20 ml) of each test formulation were thoroughly mixed by vigorous shaking and duplicate sub-samples were analysed in accordance with the analytical procedure.

RESULTS AND DISCUSSION

The concentrations of DPTB in test formulations analysed during the study and the deviation of mean results from nominal values are summarised in Table 1. Mean results were within 4% of nominal concentrations.

The homogeneity and stability of DPTB in aqueous solution, containing DPTB at nominal concentrations of 0.1 mg/ml and 100 mg/ml, was confirmed after ambient temperature storage for up to 4 hours and refrigerated storage for up to 15 days. However, the mean results obtained for the 0.1 mg/ml formulation were approximately 13% above nominal. Since this formulation had been serially diluted from the 100 mg/ml formulation, a second formulation was prepared at 0.1 mg/ml by direct weighing and dilution which was evaluated for stability. Mean results were within 4% of nominal concentrations, confirming accuracy of formulation and remained constant throughout the storage period. The results obtained are presented in Table 2.

Procedural recovery data obtained during method validation confirm the accuracy of the analytical procedure: a mean procedural recovery value of 96.7% (CV=0.47, n=6) was obtained at 0.1 mg/ml and 100.1% (CV=0.40, n=6) was obtained at 100 mg/ml. The results obtained are presented in Table 5.

The limit of detection was determined as 0.004 mg/ml.

A typical calibration standard graph confirming the linearity of detector response for DPTB over the concentration range 40 - 200 $\mu\text{g/ml}$ is presented in Figure 1.

Typical analytical chromatograms are presented in Figure 2. In Figure 2, the absence of a peak at the characteristic retention volumes for DPTB in the control sample chromatogram demonstrates the specificity of the GLC assay.

CONCLUSION

The mean concentrations of DPTB in test formulations analysed during the study were within 4% of nominal concentrations confirming the accuracy of formulation.

The results also confirm that formulations were both homogeneous and stable during ambient temperature storage for 4 hours and refrigerated storage for 15 days, a period of time exceeding the time from preparation to completion of dosing.

TABLE 1
Concentrations of DPTB in test formulations

Week of study	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			RME (%)
			Analysis 1	Analysis 2	Mean	
1	Control	0	ND	ND	ND	-
	2	10	9.97	10.0	9.99	-0.1
	3	30	30.3	30.2	30.2	+0.7
	4	100	104	104	104	+4.0
2	Control	0	ND	ND	ND	-
	2	10	9.77	9.85	9.81	-1.9
	3	30	29.8	29.8	29.8	-0.7
	4	100	102	103	103	+3.0

ND None detected (<0.004 mg/ml).

RME Relative mean error

TABLE 2

Homogeneity and stability of DPTB in aqueous formulations

Nominal inclusion (mg/ml)	Trial	Storage conditions			Analysed concentration (mg/ml)				RME (%)
		Days	Hours	Temp, °C	Top	Middle	Bottom	Mean	
0.1	1	0	0	+21	0.112	0.114	0.112	0.113	-
			4	+21	0.114	0.112	0.112	0.113	0.0
		2	0	+4	0.111	0.113	0.112	0.112	-0.9
			8	+4	0.113	0.113	0.113	0.113	0.0
			15	+4	0.113	0.113	0.113	0.113	0.0
0.1	2	0	0	+21	0.100	0.0997	0.100	0.100	-
			4	+21	0.0997	0.103	0.103	0.102	+2.0
		2	0	+4	0.104	0.103	0.101	0.103	+3.0
			8	+4	0.0999	0.0991	0.0979	0.0990	-1.0
			15	+4	0.101	0.102	0.0999	0.101	+1.0
100	1	0	0	+21	101	100	101	101	-
			4	+21	102	103	102	102	+1.0
		2	0	+4	102	101	101	101	0.0
			8	+4	102	102	102	102	+1.0
			15	+4	104	104	103	103	+2.0

CV Coefficient of variation

RME Relative mean error, representing the deviation from time zero.

Trial 1 Formulations prepared by serial dilution from the 100 mg/ml formulation

Trial 2 Formulation prepared by direct weighing and dilution

Results were calculated using unrounded figures.

TABLE 3

Procedural recovery data for DPTB in aqueous formulation

Analytical phase	Nominal fortification (mg/ml)	
	0.1	100
Validation	96.9	99.8
	97.0	100.6
	97.1	100.0
	96.6	100.5
	96.4	99.7
	95.9	99.7
Mean	96.7	100.1
CV (%)	0.47	0.40
Range	95.9 - 97.1	99.7 - 100.6
n	6	6

CV Coefficient of variation

n Number of determinations

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

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

FIGURE 1

Typical linearity standard graph
(Week 1)

Method: DKB200:[D1.FORM.ARO.ARO25]DPTB_LIQ_E.MET;1
 Component: PEAK 1
 Date: 20-FEB-1996 11:09:53.86
 Linear fit, Origin Treatment....Ignore.
 K0: -9.7594E+01 K1: 2.5480E+03
 Coeff. of determination: 1.0000

Standard Sample	Component Area	Component Mass	‡ Rel. St. Dev.
STD 1	102143	39.976	
STD 2	202736	79.952	
STD 3	306219	119.93	
STD 4	406183	159.90	
STD 5	510348	199.88	
STD 1A	102227	39.976	
STD 2A	203639	79.952	
STD 3A	305849	119.93	
STD 4A	405494	159.90	
STD 5A	509953	199.88	

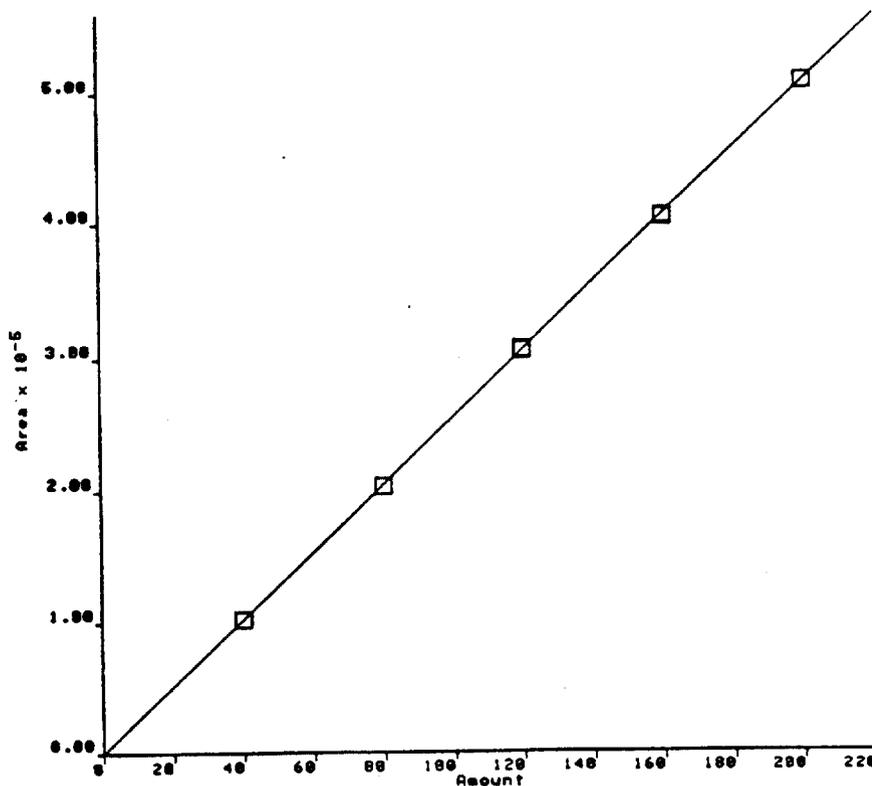
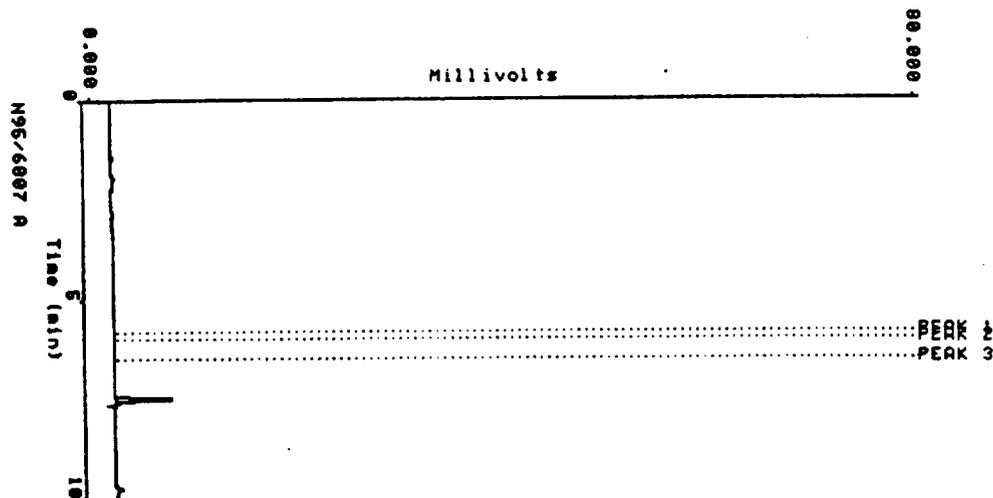


FIGURE 2

Typical sample chromatograms
(Week 1)

Group 1, Control (dilution factor 1 : 1)



Group 2, 10 mg/ml (dilution factor 1 : 100)

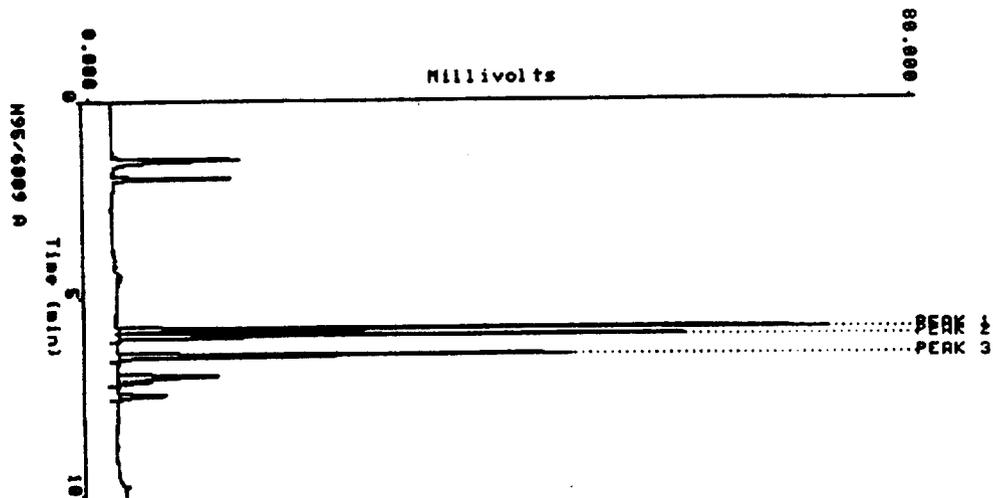
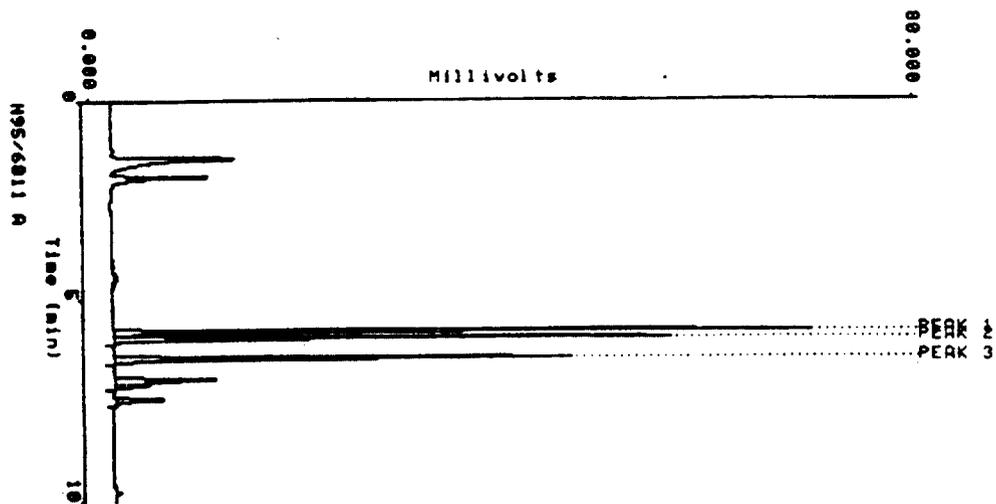


FIGURE 2

(continued)

Group 3, 30 mg/ml (dilution factor 1 : 300)



Group 4, 100 mg/ml (dilution factor 1 : 1000)

