CONFIDENTIAL

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ORAL TOXICITY STUDY IN RATS

(DAILY ADMINISTRATION FOR 4 WEEKS)

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

(b) (4)

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CONTENTS

Page

APPENDICES

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SUMMARY

Test compound:	(b) (4)
Test species:	SPF rats of the CFY strain (four groups 10♂ and 10♀).
Route of administration:	Oral (gavage) once each day, seven days a week.
Total duration of dosing:	4 weeks.
Dosage levels:	50 mg/kg/day 200 mg/kg/day 600 mg/kg/day

Results

Reaction to treatment at the various levels were:

600 mg/kg/day

1. Reduced grooming activity, salivation and resistance to being handled.
2. The death of 2 females.
3. Reduced rate of bodyweight gain.

200 mg/kg/day

Slightly reduced rate of bodyweight gain.

50 mg/kg/day

No reaction to treatment was seen.

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INTRODUCTION

This study, performed at the (b) (4) was part of a comparative study with five compounds coded (b) (4) The five compounds were tested using common control groups.

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PROCEDURE

Animal management and treatments

Eighty rats of the CFY strain were obtained from Carworth Europe, Huntingdon, England on 27 January 1972. The rats were allocated to 4 treatment groups as follows:

Group	Treatment level (b) (4) (mg/kg/day)	No. of rats ♂	♀
1A	Control	10	10
11	50	10	10
12	200	10	10
13	300	10	10

Following an initial quarantine and acclimatization period of 28 days to accustom the rats to the environmental conditions existing in our laboratories, each animal was weighed and allocated to one of several arbitrary weight ranges. This acclimatization period was unduly prolonged because no test compound was available. Equal numbers of animals from within each weight range were randomly allocated to each of the 4 treatment groups, and all animals then identified by earmark. In this way we ensured that each group contained a similar population of rats and initial mean bodyweights were also approximately equalized.

Accommodation

The rats were housed five to a cage (unless the number was reduced by mortality) in suspended cages fitted with wire-mesh floors. Animal-room temperature and relative humidity were controlled at 21 ± 2°C and 50 ± 5%, respectively.

Diet

All rats had free access to tap water and standard laboratory rat food, Spiller's Laboratory Small Animals Diet (autoclaved).

The efficiency with which food was utilized was assessed by calculation of mean food conversion ratios (FCR values), the ratio representing the weight of food consumed per unit gain in bodyweight.

Test compound

The sample of test compound used in this study was taken from a batch dated 17 February 1972.

Administration of test compound

(b) (4) was administered as a suspension in 0.5% sodium carboxymethyl cellulose.

A series of suspensions were prepared, the concentrations being chosen to give a constant volume-dosage of 5ml/kg. Control animals received the vehicle alone at the same volume-dosage.

All suspensions were prepared freshly each day and administered immediately afterwards through a rubber catheter (English numbers 3 or 4) inserted into the stomach.

Treatment in the manner described was continued, at approximately the same time each day, once a day, seven days a week, for a total period of four weeks.

Observations

All signs of ill-health or reaction to treatment were recorded.

Any rat showing signs of severe debility or intoxication was isolated. If death appeared imminent the rat was killed.

The quantity of food consumed by each cage of rats was recorded weekly.

Water consumption was assessed by inspection of the water bottles. Regular measurement of water intake was not introduced since there was no evidence of a treatment-related effect.

The weight of each rat was recorded initially and at twice weekly intervals subsequently.

Ophthalmoscopy

Before treatment commenced, and after 4 weeks, the eyes of all rats were examined using a Keeler indirect ophthalmoscope.

Laboratory investigations

(a) Urinalysis

After 3 weeks, urine samples were collected from 5 males and 5 females from groups 1A and 13. The estimations performed, together with the methods used, were as follows:

(i)	pH	-	by EIL meter
(ii)	Specific gravity (SG)	-	by refractometer calibrated against protein standards - high values were checked by pyknometric weighing
(iii)	Protein	-	using Albustix*. Positives were confirmed by precipitation with sulphosalicylic acid and quantitative estimation against Kingsway turbidimetric standards.
(iv)	Reducing substances	-	using Clinotest*
(v)	Glucose	-	using Clinistix*
(vi)	Ketones	-	using Acetest*. Positives were confirmed by Rothera's test
(vii)	Bile pigments	-	using Ictotest*. Positives were confirmed by Fouchet's test
(viii)	Urobilin	-	by Bogomolow's test

For tests (iv) to (viii) inclusive, results were graded as follows:

0	signifies negative
tr	signifies marginal positive (trace)
+	signifies positive

*Diagnostic reagent of Ames Company, Stoke Poges, England.

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000824

(ix) Microscopy of spun deposit - after centrifugation at 1000rev/minute for 10 minutes, the deposit was examined for:

epithelial cells	(E)
polymorphonuclear leucocytes	(P)
mononuclear leucocytes	(M)
erythrocytes	(R)
organisms	(O)
casts	(C)
abnormal constituents	(A)

Gradings of cell frequency in deposit were recorded as follows:

0	signifies none observed
1	signifies few in some fields
2	signifies few in all fields
3	signifies many in all fields

(b) Haematology

After 4 weeks, samples of blood were withdrawn from the orbital sinus of all rats, prior to treatment on the day of sampling.

The estimations performed, together with the methods used, were as follows:

Packed cell volume (PCV) - Estimated by Technicon SMA4A	% red cells
Haemoglobin (Hb) - Estimated by Technicon SMA4A	g/100ml blood
Red cell count (RBC) - Estimated by Technicon SMA4A	$x10^6$ cells/cmm
Absolute indices: Mean corpuscular haemoglobin concentration (MCHC) and mean cell volume (MCV):	
MCHC = Hb (g%) x 100 ÷ PCV (% red cells)	%
MCV = PCV (% red cells) x 10 ÷ RBC ($x10^6$/cmm)	cubic microns
Total white cell count (WBC) - Estimated by Technicon SMA4A	$x10^3$ cells/cmm
Differential count:	
(N) = Neutrophils	
(L) = Lymphocytes	
(E) = Eosinophils	
(B) = Basophils	$x10^3$ cells/cmm
(M) = Monocytes	
Platelet count - direct visual count (ammonium oxalate, 1% diluent) - Method of Brecher, G. and Cronkite, E.P. (J. Applied Physiology 1950, 3, 365)	$x10^3$ cells/cmm
Thrombotest - Owren, P.A. (Lancet, 1959, ii, 774)	seconds

(c) Blood chemistry

After treatment for 4 weeks, samples of blood were withdrawn from the orbital sinus of all rats, prior to treatment on the day of sampling.

The estimations performed, together with the methods used, were as follows:

Plasma Urea - Technicon Autoanalyser method (diacetyl monoxime)	mg%
Plasma Glucose - Technicon Autoanalyser method (Glucose Oxidase)	mg%
Serum alkaline phosphatase (SAP) - Technicon Autoanalyser method (4-amino-phenazone)	King Armstrong or KA units
Serum glutamic-pyruvic transaminase (SGPT) - Technicon Autoanalyser method sheet N.54 modified at HRC (fluorimetric)	Sigma Frankel or SF units

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000825

Terminal studies

On completion of the treatment period, all surviving rats were killed.

All rats were killed by carbon dioxide asphyxiation. The appearance of the tissues was then noted and the weights of the following organs recorded:

adrenals	heart	liver
brain	kidneys	testes

For intergroup comparison, relative organ weights were calculated as percentages of bodyweight, x 100.

Samples of the following tissues, (together with any other macroscopically abnormal entity) were preserved in 4% buffered formaldehyde (except eyes, which were preserved in Davidson's fixative):

adrenals	liver	skin*
aorta*	lungs	spleen
brain (medullary, cerebellar and cortical sections)	lymph nodes (cervical and mesenteric)	stomach (glandular and non-glandular)
caecum	oesophagus*	testes
colon*	ovaries	thymus
duodenum	pancreas	thyroid
eyes	pituitary	tongue*
femur*	prostate*	trachea*
heart	salivary glands*	urinary bladder
ileum	sciatic nerve*	uterus
jejunum*	body fat (if present)*	all tissues showing macroscopic abnormality
kidneys	seminal vesicles*	
mammary gland*	skeletal muscle*	

Femoral marrow smears were prepared and fixed in methyl alcohol. Tissues marked thus* were preserved but not processed further in the first instance.

Prior to microscopic examination tissues were embedded in paraffin wax, sections cut at 5μ and stained with haematoxylin and eosin. In addition, frozen sections of liver and kidney were cut at 12μ and stained for fat with Oil Red O, and for glycogen (liver) and basement membranes (kidney) with PAS.

In the first instance, microscopic examination was confined to:

(i) Macroscopically abnormal tissues from all rats that died, in order to elucidate the predominant pathology.

(ii) All rats of groups 1A and 13 killed after treatment for 4 weeks.

Statistical methods

Student's 't' test was employed to assess the significance of intergroup differences where the data suggested evidence of a response to treatment.

RESULTS

CLINICAL SIGNS

Rats receiving 600 mg/kg/day showed a reduction in grooming activity, the fur becoming stained with urine, and salivation from between 15 to 25 minutes after dosing. In addition, these animals, particularly the females, became difficult to handle when dosing.

Four males and seven females receiving 600 mg/kg/day showed signs of respiratory distress during weeks 3 and 4.

Rats receiving 200 mg/kg/day and 50 mg/kg/day remained normal throughout.

MORTALITIES (Appendix 1)

Two females receiving 600 mg/kg/day died during the night of day 8. Macroscopic examination revealed no abnormalities in one rat, while in the second only gaseous distension of the stomach and small intestine was observed.

One male control died during the night of day 17, having shown previous weight loss, and pallor of the extremities. Macroscopic examination revealed enlargment of the spleen and liver, the latter organ also appearing abnormally pale.

FOOD CONSUMPTION (Table 1, Appendix 2)

There was no marked adverse effect on food consumption.

BODYWEIGHT CHANGE (Table 2, Appendix 3, Figures 1 and 2)

There was a reduction in the rate of bodyweight gain in rats receiving 600 mg/kg/day, and to a lesser extent 200 mg/kg/day.

The rate of bodyweight gain was not adversely affected in rats receiving 50 mg/kg/day.

EFFICIENCY OF FOOD UTILISATION (Table 3)

There was an overall reduction in the efficiency of food utilisation in rats receiving 600 mg/kg/day, and to a lesser extent those receiving 200 mg/kg/day.

LABORATORY INVESTIGATIONS

a. Urinalysis (Table 4, Appendix 4)

All findings were considered to be within normal limits.

b. Haematology (Table 5, Appendix 5)

No differences were observed between control and treated values that were considered to be related to dosage with the test compound.

c. Blood chemistry (Table 6, Appendix 6)

No differences were observed between the values for treated and control animals that were considered to be related to dosage with the test compound.

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OPHTHALMOSCOPY (Table 7, Appendix 7)

No abnormalities of the eyes were observed.

TERMINAL STUDIES

a. Macroscopic post mortem examination (Appendix 8)

No abnormalities were observed that were considered to be related to treatment with the test compound.

A number of rats from both control and treated groups showed a minimal display of chronic respiratory disease, which was generally characterised by the presence of subpleural foci.

Other isolated findings observed were bilateral cortical scarring of the kidneys in Rat 118 (50 mg/kg/day), gaseous distension of the small intestine in Rats 132 and 136 (600 mg/kg/day), and in Rats 128 and 300 (200 mg/kg/day).

b. Organ weights (Tables 8 and 9, Appendices 9 and 10)

The only difference between mean values recorded for control and treated animals that was possibly related to treatment, related to the adrenal glands, which were slightly heavier in males receiving 600 mg/kg/day than in the remaining sub-groups of males. Since the values recorded for females receiving this dosage level were comparable with the control values, and since the histological picture was normal no significance can be attributed to this finding.

c. Histology (Appendix 12)

The microscopical abnormalities and variations from normal that were seen in the animals used in this experiment are described in detail. The following comments are made in summary:

Liver. Vacuolated and occasionally distended hepatocytes were seen in the centrilobular areas in a proportion of male animals from both control and treatment groups. Small foci of mononuclear cell infiltration were seen in the parenchyma in rats 2♂, 173♀, 179♀ (Control) 134♂, 306♀, 310♀(600mg/kg/day). These changes were considered to be those commonly seen in the livers of laboratory rats, were non-specific and hence were disregarded from the point of view of the experiment.

Kidney. Small groups of tubules characterised by their basophilic staining, a proportion of whi were distended with eosinophilic material were seen in association with minimal mononuclear cell infiltration of the renal cortex, in rats 1♂, 4♂ (Control) 133♂, 135♂, 136♂, 140♂, 308♀ (600mg/kg/day). Small foci of dystrophic mineralisation were seen in the medulla in rat 310♀ (600mg/kg/day). These changes were considered to be those commonly seen in laboratory rats and were not significant.

Other histopathological entities observed but not considered to be significant included:

Foci of extra-medullary haemopoiesis in the spleen of several animals from both control and treatment groups.

Minimal mononuclear cell infiltration in the ventricular myocardium in rats 5♂, 6♂, (Control) 303♀ (600mg/kg/day).

Plugs of eosinophilic material in the urinary bladder in rats 4♂, 7♂ (Control).

Distended seminiferous tubules in one testis in rat 5♂ (Control).

Telangiectasis in the parathyroid of rat 176♀ (Control).

Conclusion

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No histopathological change or variation from normal was seen in the tissues examined that was considered to be associated with the administration of the compound under test.

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FIGURE 1

Growth of male rats

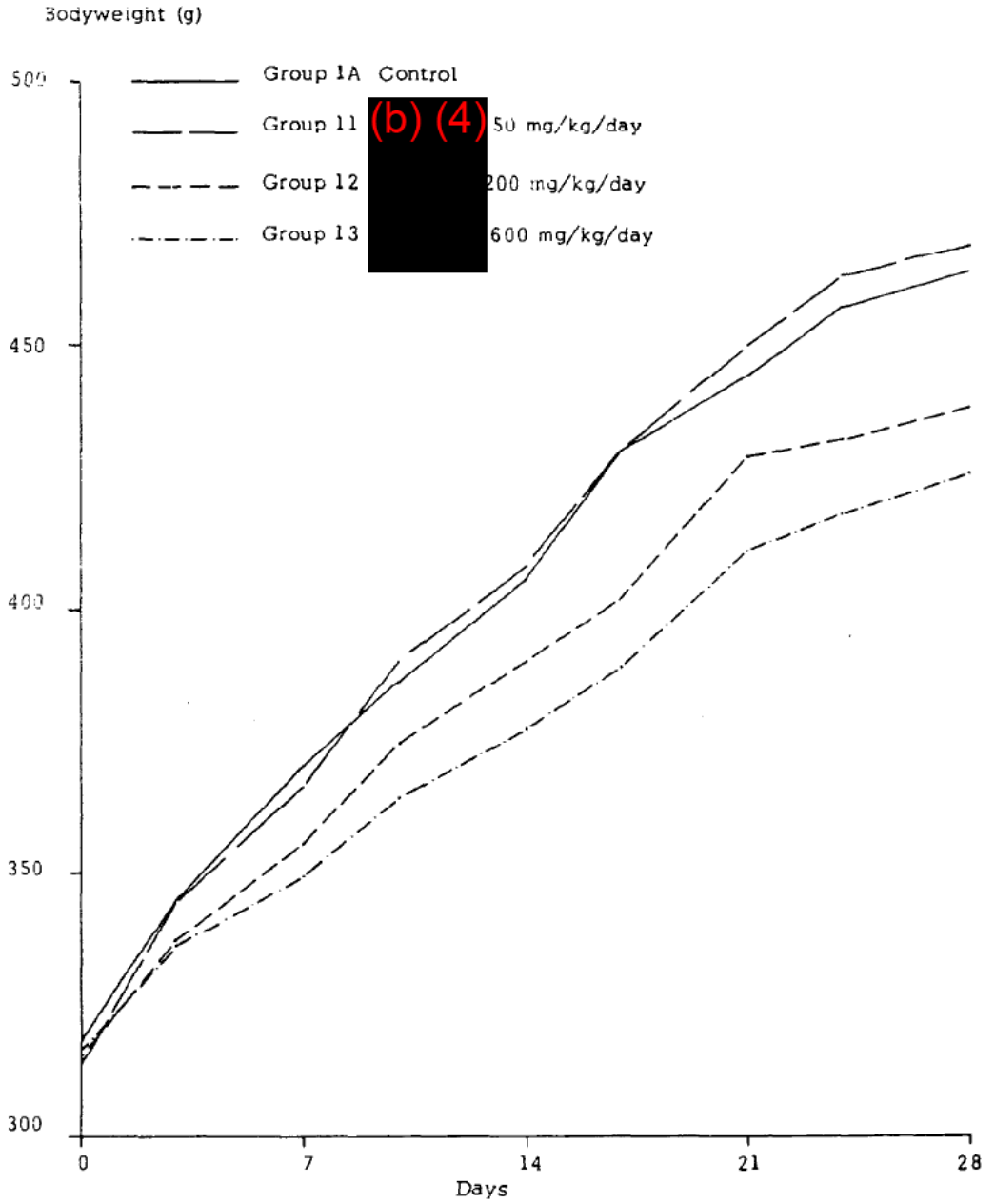

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FIGURE 2

Growth of female rats

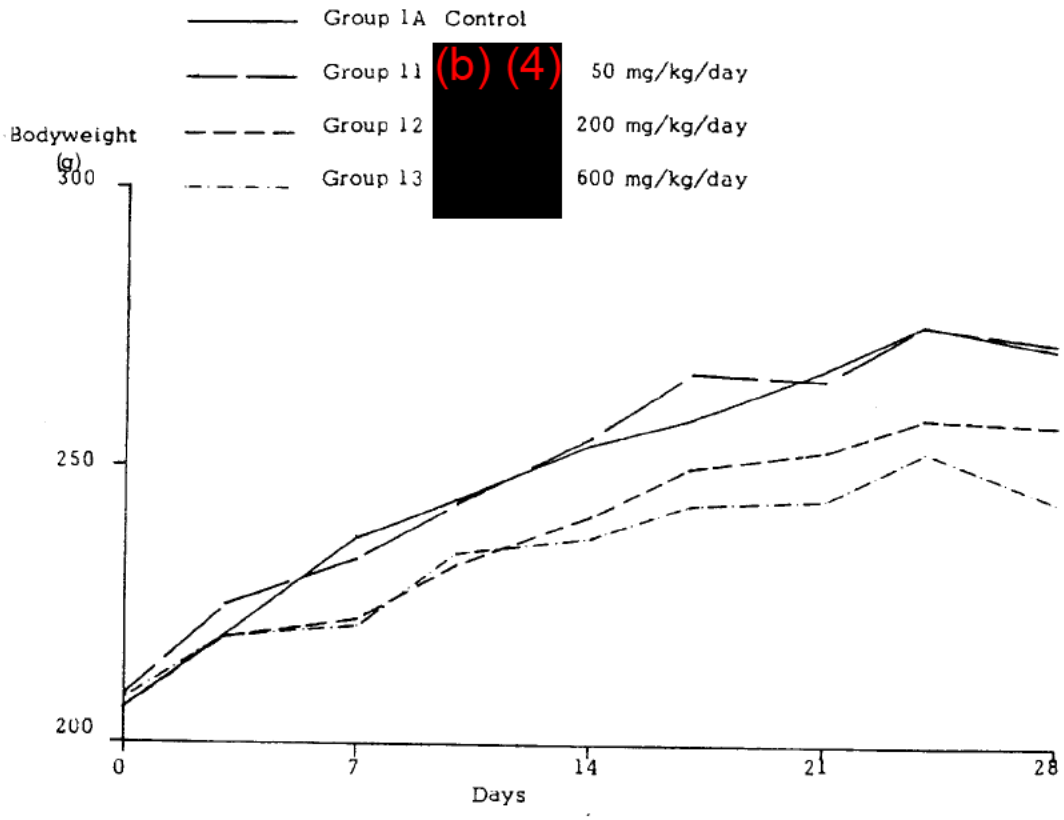

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TABLE 1

Group mean food consumption (g/rat/week)

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Week	♂				♀			
	1A	11	12	13	1A	11	12	13
1	186	197	180	183	136	142	122	122
2	186	197	196	191	129	142	129	133
3	181	190	180	180	144	149	141	126
4	209	205	189	196	154	145	131	142
Total	762	789	745	750	563	578	523	523
% control	-	104	98	98	-	103	93	93

000831

[redacted]

TABLE 2

Group mean bodyweights (g) and weight gains

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Initial weight	Weight at 28 days	Weight gain 0-28 days
1A♂	318	464	146
11	314	469	155
12	315	438	123
13	316	426	110**
1A♀	206	272	66
11	209	273	64
12	206	258	52*
13	208	244	36***

* P<0.5 in comparison with control values
** P<0.01 in comparison with control values
*** P<0.001 in comparison with control values

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[redacted]

TABLE 3

Group mean food conversion ratios

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Week	♂				♀			
	1A	11	12	13	1A	11	12	13
1	3.6	3.8	4.5	5.6	4.4	5.9	7.6	9.4
2	5.2	6.2	5.6	6.8	7.6	6.5	6.8	8.3
3	4.8	4.5	4.6	5.3	10.3	13.5	11.8	18.0
4	10.5	10.8	21.0	13.1	38.5	20.7	26.2	-
Overall	5.2	5.1	6.1	6.8	8.5	9.0	10.1	14.5

[redacted]

TABLE 4

Urinalysis - group mean values

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Week No.	Volume ml/rat	pH	SG	Protein mg%
1A♂	4	7.2	6.6	1034	68
13♂		6.7	6.6	1043	30
1A♀		5.1	6.5	1034	0
13♀		3.9	6.3	1041	0

[redacted]

TABLE 5

Haematology - Group mean values

Group:	1A	11	12	13
Compound:	-	[redacted]		
Level (mg/kg/day):	Control	50	200	600

Group	Week No	pcv %	Hb g%	RBC 10^6/cmm	MCHC	MCV cμ	WBC, 10^3/cmm						Thrombotest secs	Platelets 10^3/cmm
							Total	N	L	E	B	M		
1A♂	4	46	14.3	7.4	31	63	14.1	1.3	12.6	0.2	0.0	0.0	26	532
11		49	14.9	7.6	30	65	15.5	1.7	13.6	0.1	0.0	0.0	26	576
12		48	14.8	7.6	31	63	12.3	1.4	10.8	0.0	0.0	0.0	27	659
13		47	14.6	7.6	31	62	15.3	1.4	13.9	0.0	0.0	0.0	27	602
1A♀		44	13.6	7.2	31	61	10.6	0.5	10.1	0.0	0.0	0.0	25	740
11		47	14.4	7.3	31	64	9.3	1.1	8.0	0.2	0.0	0.0	27	552
12		46	14.0	7.4	31	62	13.3	1.2	12.0	0.1	0.0	0.0	25	740
13		46	14.1	7.4	31	62	14.0	1.4	12.5	0.1	0.0	0.0	27	717

TABLE 6

Blood Chemistry - Group mean values

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Week	Urea mg%	Total Glucose mg%	SAP KA units	SGPT SF units
1A♂	4	42	127	84	33
11		40	133	74	37
12		36	127	78	42
13		35	137	68	37
1A♀	4	39	128	71	40
11		40	128	53	40
12		37	111	66	40
13		38	129	79	34

[redacted]

TABLE 7

Ophthalmoscopy - summary of observations

Group:	1A	11	12	13
Compound:	-	(b) (4)		
Level (mg/kg/day):	Control	50	200	600

Group	Week	No of rats examined	Observed defects of		
			Lens	Cornea	Other
1A♂	0	10	2	-	-
11		10	2	-	-
12		10	2	-	-
13		10	2	-	-
1A♀		10	1	-	1
11		10	1	-	-
12		10	-	-	-
13		10	-	-	-
1A♂	4	9	3	-	-
11		10	2	-	-
12		10	2	-	-
13		10	2	-	-
1A♀		10	-	-	-
11		10	1	-	-
12		10	-	-	-
13		8	-	-	-

[redacted]

TABLE 8

Organ weights (absolute) - Group mean values (g)
for rats sacrificed at 4 weeks

Group:	1A	11	12	13
Compound:	-	[redacted]		
Level (mg/kg/day):	Control	50	200	600

Group	Body weight	Brain	Heart	Liver	Kidney	Adrenals x10^3	Gonads
1A♂	461	2.0	1.6	21.3	4.0	64	4.9
11	471	1.9	1.6	22.4	3.8	65	4.9
12	438	2.0	1.6	19.6	3.5	69	5.0
13	417	2.0	1.5	18.3	3.4	74*	4.5
1A♀	267	1.8	1.0	11.9	2.2	88	
11	279	1.9	1.1	12.6	2.2	82	
12	256	1.8	1.0	11.1	2.2	84	
13	242	1.8	1.0	11.0	2.1	82	

* $P < 0.05$ in comparison with control values

[redacted]

TABLE 9

Organ weights (relative+) - Group mean values
for rats sacrificed at 4 weeks

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Body weight	Brain	Heart	Liver	Kidney	Adrenals x10^3	Gonands
1A♂	461	43	35	463	86	1.4	107
11	471	41	34	477	81	1.4	106
12	438	45	36	449	81	1.6	114
13	417	47	35	440	81	1.8**	109
1A♀	267	67	39	447	82	3.3	
11	279	68	39	449	77	3.0	
12	256	71	39	434	85	3.3	
13	242	73	40	455	86	3.4	

+ as percentage control x 100
** F <0.01 in comparison with control values

[redacted]

APPENDIX 1

Mortalities with relevant ante and post mortem observations

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Rat No.	Day of death	Observations
1A♂	9	17	Found dead. Prior weight losses, pallor of extremities. Autopsy: Liver enlarged and pale, right lobe swollen; splenic enlargement (53 x 26mm). Histology: All tissues show evidence of advanced autolytic change. Heart: Many lymphoid cells are seen in the heart blood. Liver: Grossly infiltrated with lymphoma cells. Spleen: Grossly infiltrated with lymphoma cells. Diagnosis: Lymphocytic leukaemia.
13♀	304	8	Found dead. Autopsy: No abnormalities detected. Histology: Liver: Occasional small foci of mononuclear cell infiltration are noted in the parenchyma.
	309	8	Found dead. Autopsy: Gaseous distension of stomach and small intestine. Histology: Autolytic change obscures histological detail. Liver: Congested. Hepatic architecture within normal limits. Kidney: Congested. Adrenals: There is evidence of minimal congestion.

APPENDIX 2

Food consumption - by cages (g/rat/week)

Group:	1A	11	12	13
Compound:	-	(b) (4)		
Level (mg/kg/day):	Control	50	200	600

Group	Week	Cage No		Group	Week	Cage No	
		1	2			35	36
1A♂	1	190	182	1A♀	1	138	133
	2	198	173		2	123	134
	3	174	190		3	139	148
	4	196	225		4	156	152
Group	Week	Cage No		Group	Week	Cage No.	
		23	24			57	58
11♂	1	199	194	11♀	1	125	148
	2	202	192		2	142	141
	3	188	192		3	155	143
	4	199	211		4	143	146
Group	Week	Cage No.		Group	Week	Cage No.	
		25	26			59	60
12♂	1	178	181	12♀	1	118	125
	2	191	201		2	128	129
	3	185	174		3	141	140
	4	185	192		4	133	129
Group	Week	Cage No.		Group	Week	Cage No.	
		27	28			61	62
13♂	1	172	193	13♀	1	119	125
	2	176	205		2	121	145
	3	162	198		3	133	120
	4	193	198		4	139	145

APPENDIX 3

Individual bodyweights (g)

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

APPENDIX 3
Individual bodyweights (g)

Group 1[A] male

Cage	Rat no.	...Day... 0	3	7	10	14	17	21	24	28
1	1	320	338	375	398	431	441	452	471	476
	2	346	370	395	414	433	451	458	472	484
	3	275	315	340	360	385	390	402	416	431
	4	328	361	381	402	422	432	428	450	450
	5	320	350	385	412	441	458	470	485	490
	Mean:	318	347	375	397	422	434	442	459	466
2	6	348	385	405	428	455	465	495	505	512
	7	320	355	371	396	424	446	470	477	477
	8	291	315	350	365	395	410	428	430	436
	9	335	345	380	340	307				
	10	295	312	322	340	371	381	394	410	420
	Mean:	318	342	366	374	390	426	447	456	461
Group size :		10	10	10	10	10	9	9	9	9
Group mean :		318	345	370	386	406	430	444	457	464
Mean change:			27	26	15	21	24	14	13	7

Group 11male

Cage	Rat no.	...Day... 0	3	7	10	14	17	21	24	28
23	111	305	330	352	375	390	415	430	440	437
	112	340	368	407	425	425	460	489	498	507
	113	322	355	380	415	435	450	472	491	498
	114	292	327	333	347	370	390	398	406	405
	115	315	347	367	390	400	420	435	442	455
	Mean:	315	345	368	390	404	427	445	455	460
24	116	325	360	379	383	400	417	428	440	440
	117	320	345	366	400	424	440	462	473	474
	118	300	330	364	395	420	445	474	498	515
	119	315	338	360	382	405	425	450	464	474
	120	310	340	356	392	415	435	460	480	488
	Mean:	314	343	365	390	413	432	455	471	478
Group size :		10	10	10	10	10	10	10	10	10
Group mean :		314	344	366	390	408	430	450	463	469
Mean change:			30	22	24	18	21	20	13	6

Group 12male

Cage	Rat no.	...Day... 0	3	7	10	14	17	21	24	28
25	121	300	317	346	353	365	368	395	395	402
	122	307	330	336	357	365	384	418	410	417
	123	322	330	352	376	382	390	415	416	421
	124	322	350	361	372	375	390	427	412	407
	125	324	348	368	391	415	430	468	480	487
	Mean:	315	335	353	370	380	392	425	423	427
26	126	300	330	342	342	375	395	410	426	435
	127	290	314	334	350	370	372	395	407	410
	128	344	380	384	415	437	455	470	478	491
	129	346	375	404	430	450	450	482	486	497
	130	298	300	323	355	368	385	410	412	412
	Mean:	316	340	357	378	400	411	433	442	449
Group size :		10	10	10	10	10	10	10	10	10
Group mean :		315	337	355	374	390	402	429	432	438
Mean change:			22	18	19	16	12	27	3	6

APPENDIX 3
Individual bodyweights (g)

Group 13 male

Cage	Rat no.	0	3	7	10	14	17	21	24	28
		...Day...								
27	131	337	345	369	380	390	395	412	390	414
	132	275	280	317	335	342	355	380	384	386
	133	314	337	345	370	375	385	398	406	416
	134	336	362	382	385	398	405	412	437	434
	135	316	346	382	350	373	387	410	422	434
	Mean:	316	334	359	364	376	385	402	408	417
28	136	305	320	287	325	320	345	365	366	376
	137	328	355	389	405	440	450	485	510	523
	138	307	327	306	335	350	368	390	397	408
	139	325	358	357	380	395	405	440	447	461
	140	320	330	357	370	385	394	415	425	412
	Mean:	317	338	339	363	378	392	419	429	436
Group size :		10	10	10	10	10	10	10	10	10
Group mean :		316	336	349	364	377	389	411	418	426
Mean change:			20	13	14	13	12	22	8	8

Group 1A female

Cage	Rat no.	0	3	7	10	14	17	21	24	28
		...Day...								
35	171	220	230	245	256	272	271	272	290	281
	172	240	255	271	284	293	296	298	320	307
	173	185	195	215	218	233	233	240	255	248
	174	195	210	220	222	235	235	240	251	253
	175	180	205	220	214	223	231	255	255	250
	Mean:	204	219	234	239	251	253	261	274	268
36	176	215	221	242	240	254	265	268	271	264
	177	200	222	240	249	256	258	272	276	280
	178	180	185	200	214	220	228	240	241	235
	179	230	245	272	280	290	306	321	320	319
	180	210	225	245	261	262	270	275	285	278
	Mean:	207	220	240	249	256	265	275	279	275
Group size :		10	10	10	10	10	10	10	10	10
Group mean :		206	219	237	244	254	259	268	276	272
Mean change:			14	18	7	10	6	9	8	-5

Group 11 female

Cage	Rat no.	0	3	7	10	14	17	21	24	28
		...Day...								
57	281	217	237	256	242	265	267	265	270	260
	282	183	193	190	202	215	225	237	248	245
	283	234	240	250	264	270	290	280	290	296
	284	225	245	253	261	275	295	295	310	305
	285	200	215	224	237	250	250	258	266	260
	Mean:	212	226	235	241	255	265	267	277	273
58	286	200	210	223	236	257	263	258	265	260
	287	222	235	239	250	255	275	275	286	290
	288	188	198	200	214	222	240	230	248	240
	289	210	250	261	274	280	300	295	306	295
	290	212	230	233	251	259	265	265	275	275
	Mean:	206	225	231	245	255	269	265	276	272
Group size :		10	10	10	10	10	10	10	10	10
Group mean :		209	225	233	243	255	267	266	276	273
Mean change:			16	8	10	12	12	-1	11	-4

[redacted]

APPENDIX 3
Individual bodyweights (g)

Group 12 female

Cage	Rat no.	0	3	7	10	14	17	21	24	28
		...Day...								
59	291	198	217	228	233	252	255	262	270	260
	292	212	227	234	241	255	270	280	280	280
	293	210	220	226	233	245	262	262	265	267
	294	200	205	207	210	215	220	225	231	228
	295	214	225	225	240	246	260	270	268	260
	Mean:	207	219	224	231	243	253	260	263	259
60	296	220	244	232	248	250	262	250	262	275
	297	220	230	230	243	250	260	252	268	255
	298	195	210	211	226	230	250	242	255	285
	299	194	215	223	232	245	240	260	253	230
	300	198	200	202	212	222	220	230	240	240
	Mean:	205	220	220	232	239	246	247	256	257
Group size :		10	10	10	10	10	10	10	10	10
Group mean :		206	219	222	232	241	250	253	259	258
Mean change:			13	3	10	9	9	3	6	-1

Group 13 female

Cage	Rat no.	0	3	7	10	14	17	21	24	28
		...Day...								
61	301	215	228	236	240	230	255	275	293	250
	302	185	197	196	199	210	210	216	226	225
	303	238	255	254	265	268	270	274	281	235
	304	200	214	204						
	305	204	210	196	214	212	230	229	245	245
	Mean:	208	221	217	230	230	241	249	261	239
62	306	220	233	236	232	228	240	231	250	255
	307	205	230	235	234	240	240	220	196	212
	308	217	229	234	243	255	257	265	273	270
	309	185	168	193						
	310	215	230	230	241	250	240	240	263	262
	Mean:	208	218	226	238	243	244	239	246	250
Group size :		10	10	10	8	8	8	8	8	8
Group mean :		208	219	221	234	237	243	244	253	244
Mean change:			11	2	12	3	6	1	10	-9

[redacted]

APPENDIX 4

Urinalysis - individual values

Group: 1A 11 12 13

Compound: - (b) (4)

Level (mg/kg/day): Control 50 200 600

Group	Rat No.	Week No.	Volume ml/rat	pH	SG	Protein mg%	Total red. subs.	Glucose	Ketones	Bile pigments	Urobilinogen	Microscopy E	P	M	R	O	C	A
1A♂	1	4	7.0	6.4	1036	20	0	0	0	0	0	1	0	0	0	3	0	1sp
	2		7.5	6.6	1032	40	0	0	0	0	0	0	0	0	0	2	0	1sp
	3		6.5	6.7	1035	80	0	0	0	0	0	1	0	0	0	2	0	0
	4		6.0	6.7	1037	100	0	0	0	0	0	0	0	0	0	2	0	0
	5		9.0	6.8	1032	100	0	0	0	0	0	1	0	0	0	2	0	1sp
13♂	131	4	6.0	6.5	1048	50	0	0	0	0	0	0	0	0	0	2	0	3sp
	132		6.5	6.4	1042	40	tr	0	0	0	0	0	0	0	0	2	0	0
	133		9.0	6.8	1036	30	0	0	0	0	0	0	0	0	0	2	0	0
	134		7.0	6.7	1041	10	0	0	0	0	0	0	0	0	0	3	0	1sp
	135		5.0	6.5	1046	20	0	0	0	0	0	0	0	0	0	3	0	0
1A♀	171	4	4.0	6.6	1034	0	0	0	0	0	0	0	0	0	0	1	0	0
	172		7.0	6.5	1031	0	0	0	0	0	0	0	0	0	0	2	0	0
	173		5.5	6.4	1030	0	0	0	0	0	0	0	1	0	0	2	0	0
	174		3.5	6.5	1042	0	0	0	0	0	0	1	0	0	0	2	0	0
	175		5.5	6.4	1034	0	0	0	0	0	0	0	0	0	0	1	0	0
13♀	301	4	5.0	6.6	1045	0	0	0	0	0	0	0	0	0	0	1	0	0
	302		3.0	6.3	1041	0	0	0	0	0	0	1	0	0	0	1	0	0
	303		4.5	6.1	1036	0	0	0	0	0	0	0	0	0	0	1	0	0
	305		4.0	6.5	1036	0	0	0	0	0	0	0	0	0	0	1	0	0
	306		3.0	6.1	1047	0	0	0	0	0	0	0	0	0	0	1	0	0

tr = trace sp = sperm

APPENDIX 5

Haematology - individual values

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Week 4

Group No.	Animal no.	PCV %	Hb g%	RBC mill. /cmm	MCHC %	MCV cu	WBC (1000/cmm)						Platelets 1000/cmm	Thrombotest secs.
							Total	N	L	E	B	M		
1Am	1	46	14.0	7.4	30	62	14.2	2.6	11.6	0.0	0.0	0.0	400	25
	2	45	13.8	7.4	31	61	14.7	1.5	12.6	0.6	0.0	0.0	420	29
	3	47	14.8	7.6	31	62	15.6	1.6	14.0	0.0	0.0	0.0	450	27
	4	48	15.0	7.6	31	63	15.2	1.2	13.4	0.3	0.0	0.3	540	27
	5	44	13.4	7.0	30	63	12.8	1.0	11.5	0.3	0.0	0.0	780	27
	6	46	14.2	7.2	31	64	14.8	2.1	12.4	0.3	0.0	0.0	790	23
	7	45	13.8	7.2	31	62	9.6	0.4	9.2	0.0	0.0	0.0	420	29
	8	49	14.8	7.6	30	64	16.2	1.0	15.2	0.0	0.0	0.0	510	23
	10	47	14.6	7.6	31	62	13.4	0.5	12.9	0.0	0.0	0.0	480	27
	Mean	46	14.3	7.4	31	63	14.1	1.3	12.6	0.2	0.0	0.0	532	26
11m	111	48	14.9	7.6	31	63	18.6	2.6	15.6	0.0	0.0	0.0	560	27
	112	50	15.4	7.5	31	67	14.0	1.1	12.9	0.0	0.0	0.0	730	28
	113	51	15.2	7.6	30	67	11.6	1.2	10.4	0.0	0.0	0.0	450	26
	114	47	14.6	7.5	31	63	10.8	0.2	10.6	0.0	0.0	0.0	340	26
	115	52	14.9	7.8	29	67	7.6	1.7	5.9	0.0	0.0	0.0	700	24
	116	48	14.6	7.6	30	63	11.2	1.3	9.6	0.2	0.0	0.0	720	27
	117	50	15.0	7.6	30	66	24.6	3.0	21.6	0.0	0.0	0.0	580	27
	118	47	14.7	7.6	31	62	13.8	2.2	11.6	0.0	0.0	0.0	600	27
	119	48	14.9	7.6	31	63	22.6	1.8	20.3	0.5	0.0	0.0	530	26
	120	49	14.9	7.4	30	66	19.8	2.0	17.8	0.0	0.0	0.0	550	27
	Mean	49	14.9	7.6	30	65	15.5	1.7	13.6	0.1	0.0	0.0	576	26
12m	121	47	14.7	7.3	31	64	16.4	2.0	14.4	0.0	0.0	0.0	750	24
	122	48	14.9	8.0	31	60	12.2	1.5	10.7	0.0	0.0	0.0	880	32
	123	47	14.6	7.3	31	64	13.6	2.2	11.4	0.0	0.0	0.0	540	26
	124	49	14.8	7.6	30	64	9.8	0.6	9.2	0.0	0.0	0.0	530	25
	125	48	14.8	7.4	31	65	8.4	1.0	7.2	0.2	0.0	0.0	610	28
	126	48	15.1	7.4	31	65	9.8	1.4	8.4	0.0	0.0	0.0	860	31
	127	48	15.0	8.0	31	60	13.4	1.3	12.1	0.0	0.0	0.0	640	26
	128	49	14.8	7.6	30	64	18.0	2.9	15.1	0.0	0.0	0.0	660	26
	129	46	14.6	7.6	32	61	13.8	0.6	13.2	0.0	0.0	0.0	460	26
	130	47	14.7	7.6	31	62	7.6	0.9	6.5	0.2	0.0	0.0	660	27
	Mean	48	14.8	7.6	31	63	12.3	1.4	10.8	0.0	0.0	0.0	659	27
13m	131	50	15.4	7.8	31	64	20.4	2.0	18.4	0.0	0.0	0.0	580	30
	132	46	14.6	7.4	32	62	14.0	2.0	11.8	0.3	0.0	0.0	490	30
	133	48	14.8	7.8	31	62	17.0	1.0	16.0	0.0	0.0	0.0	540	23
	134	48	14.8	7.8	31	62	15.4	1.2	14.2	0.0	0.0	0.0	480	26
	135	46	14.6	7.4	32	62	14.6	1.2	13.4	0.0	0.0	0.0	420	26
	136	46	14.6	7.4	32	62	13.6	1.4	12.2	0.0	0.0	0.0	580	26
	137	44	13.6	7.1	31	62	11.8	1.2	10.6	0.0	0.0	0.0	510	28
	138	47	14.6	7.7	31	61	11.9	1.2	10.7	0.0	0.0	0.0	880	28
	139	48	14.8	7.6	31	63	17.4	2.4	15.0	0.0	0.0	0.0	750	27
	140	47	14.6	7.7	31	61	16.8	0.3	16.5	0.0	0.0	0.0	790	27
	Mean	47	14.6	7.6	31	62	15.3	1.4	13.9	0.0	0.0	0.0	602	27

[redacted]

APPENDIX 5

(continued)

Week 4

Group No.	Animal no.	PCV %	Hb g%	RBC mill./cmm	MCHC %	MCV cu	WBC (1000/cmm)						Platelets 1000/cmm	Thrombotest secs.
							Total	N	L	E	B	M		
1Af	171	45	13.8	7.4	31	61	7.9	0.3	7.6	0.0	0.0	0.0	910	25
	172	42	12.9	6.8	31	62	10.6	0.6	10.0	0.0	0.0	0.0	760	27
	173	42	13.0	6.8	31	62	10.6	0.6	10.0	0.0	0.0	0.0	650	22
	174	43	12.8	7.0	30	61	8.6	0.3	8.3	0.0	0.0	0.0	740	24
	175	43	13.3	7.0	31	61	9.6	1.0	8.6	0.0	0.0	0.0	920	25
	176	43	13.4	6.9	31	62	17.6	0.4	16.9	0.4	0.0	0.0	750	31
	177	45	13.9	7.2	31	62	9.0	0.5	8.5	0.0	0.0	0.0	640	29
	178	47	14.6	7.6	31	62	11.6	0.2	11.4	0.0	0.0	0.0	580	22
	179	46	14.6	7.7	32	60	9.8	0.6	9.2	0.0	0.0	0.0	700	24
	180	45	14.0	7.4	31	61	10.6	0.4	10.2	0.0	0.0	0.0	710	23
	Mean	44	13.6	7.2	31	61	10.6	0.5	10.1	0.0	0.0	0.0	736	25
11f	281	47	14.3	7.3	30	64	7.6	0.6	7.0	0.0	0.0	0.0	530	25
	282	47	14.4	7.4	31	64	8.6	0.5	8.1	0.0	0.0	0.0	450	27
	283	47	14.6	7.3	31	64	8.0	0.6	7.2	0.2	0.0	0.0	440	24
	284	47	14.4	7.4	31	64	8.4	1.0	7.2	0.2	0.0	0.0	600	26
	285	47	14.6	7.3	31	64	10.8	1.3	9.3	0.2	0.0	0.0	600	27
	286	46	14.6	7.4	32	62	9.8	1.8	7.8	0.2	0.0	0.0	530	27
	287	44	13.6	7.2	31	61	10.6	2.5	8.1	0.0	0.0	0.0	640	27
	288	47	14.0	7.4	30	64	7.6	0.9	6.5	0.2	0.0	0.0	610	29
	289	48	14.8	7.3	31	66	11.6	0.9	10.2	0.5	0.0	0.0	700	30
	290	47	14.4	7.2	31	65	9.8	0.6	8.8	0.4	0.0	0.0	420	25
	Mean	47	14.4	7.3	31	64	9.3	1.1	8.0	0.2	0.0	0.0	552	27
12f	291	48	14.9	8.0	31	60	15.0	1.8	12.9	0.3	0.0	0.0	770	24
	292	45	13.6	7 2	30	62	14.4	0.9	13.5	0.0	0.0	0.0	900	26
	293	45	13.6	7.1	30	63	7.3	1.0	6.1	0.1	0.0	0.0	680	26
	294	48	14.9	7.8	31	62	15.2	0.9	14.3	0.0	0.0	0.0	820	26
	295	45	13.6	7.2	30	62	13.6	1.1	12.5	0.0	0.0	0.0	740	25
	296	45	14.0	7.2	31	62	13.4	1.6	11.8	0.0	0.0	0.0	710	26
	297	44	13.4	7.1	30	62	11.8	1.4	10.1	0.2	0.0	0.0	600	28
	298	46	14.6	7.3	32	63	14.6	1.5	12.8	0.3	0.0	0.0	880	25
	299	47	14.6	7.6	31	62	11.6	1.4	10.2	0.0	0.0	0.0	680	23
	300	43	13.0	7.3	30	59	15.8	0.3	15.5	0.0	0.0	0.0	620	23
	Mean	46	14.0	7.4	31	62	13.3	1.2	12.0	0.1	0.0	0.0	740	25
13f	301	46	14.6	7.5	32	61	11.6	0.2	11.4	0.0	0.0	0.0	770	27
	302	46	14.7	7.7	32	60	12.6	0.8	11.6	0.3	0.0	0.0	640	25
	303	45	13.8	7.5	31	60	14.6	1.2	13.4	0.0	0.0	0.0	790	26
	305	40	12.0	6.5	30	62	11.9	1.0	10.9	0.0	0.0	0.0	910	29
	306	44	13.8	7.0	31	63	14.9	0.6	14.3	0.0	0.0	0.0	600	25
	307	50	15.2	7.8	30	64	17.6	4.2	13.0	0.4	0.0	0.0	690	32
	308	49	14.9	7.5	30	65	15.6	1.6	14.0	0.0	0.0	0.0	610	29
	310	45	13.7	7.4	30	61	12.9	1.5	11.4	0.0	0.0	0.0	730	26
	Mean	46	14.1	7.4	31	62	14.0	1.4	12.5	0.1	0.0	0.0	717	27

APPENDIX 6

Blood Chemistry - Individual values

Group: 1A 11 12 13

Compound: - (b) (4)

Level (mg/kg/day): Control 50 200 600

Week 4

Group No.	Animal no.	Urea mg%	Glucose mg%	SAP KA units	SGPT SF units
1Am	1	46	121	67	36
	2	43	121	94	34
	3	40	132	105	39
	4	40	122	92	31
	5	45	122	106	28
	6	44	110	94	27
	7	37	141	62	35
	8	43	132	67	31
	10	43	145	69	40
	Mean	42	127	84	33
11m	111	42	123	90	35
	112	42	147	81	28
	113	40	124	74	31
	114	33	137	81	36
	115	40	138	69	29
	116	31	134	71	35
	117	32	128	74	37
	118	54	135	85	45
	119	40	132	48	40
	120	44	136	71	57
	Mean	40	133	74	37
12m	121	41	117	91	36
	122	36	118	77	36
	123	34	139	72	37
	124	39	117	72	39
	125	41	120	70	41
	126	32	110	78	45
	127	39	135	86	43
	128	32	142	99	55
	129	33	133	68	41
	130	30	140	63	46
	Mean	36	127	78	42
13m	131	32	154	51	39
	132	32	115	51	42
	133	42	127	90	48
	134	33	146	74	41
	135	34	134	74	31
	136	38	130	46	37
	137	39	133	83	33
	138	34	128	74	32
	139	32	160	64	28
	140	33	138	76	35
	Mean	35	136	68	37

[redacted]

APPENDIX 6

(continued)

Week 4

Group No.	Animal no.	Urea mg%	Glucose mg%	SAP KA units	SGPT SF units
1Af	171	33	133	60	36
	172	46	128	55	39
	173	46	119	64	45
	174	37	114	76	38
	175	46	136	83	31
	176	36	135	64	41
	177	39	120	83	55
	178	32	122	87	39
	179	38	146	67	37
	180	33	128	67	40
	Mean	39	128	71	40
11f	281	36	123	51	38
	282	38	110	62	37
	283	44	139	60	35
	284	48	124	51	50
	285	35	130	39	46
	286	35	130	39	41
	287	43	137	55	40
	288	43	135	58	40
	289	32	116	48	41
	290	46	138	64	37
	Mean	40	128	53	40
12f	291	36	93	53	28
	292	32	123	53	35
	293	37	115	70	41
	294	33	107	68	40
	295	38	105	72	40
	296	40	109	65	40
	297	40	109	53	41
	298	33	134	81	37
	299	44	97	72	45
	300	40	114	70	51
	Mean	37	111	66	40
13f	301	36	124	58	36
	302	37	126	81	31
	303	31	136	55	35
	305	30	144	53	30
	306	37	128	90	25
	307	44	130	48	29
	308	45	132	127	41
	310	42	116	122	46
	Mean	38	129	79	34

APPENDIX 7

Ophthalmoscopy - individual observations

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Week	Rat No	Eye/	Observation
1A♂	0	6,9	B	Suture lines
11♂		112,118	B	Suture lines
12♂		121,129	B	Suture lines
13♂		134,137	B	Suture lines
1A♀		172	B	Suture lines
		180	L	Conjunctivitis
11♀		249	B	Suture lines
12♀		-	-	-
13♀		-	-	-
1A♂	4	6	B	Suture lines
		7	L	Hyaloid remnant with blood
		9	B	Suture lines
11♂		111,113	B	Suture lines
12♂		121	L	Suture line
		125	R	Hyaloids
13♂		135	B/L	Suture lines/Post. polar opacity
		138	B	Suture lines
1A♀		-	-	-
11♀		285	R	Post. polar opacity
12♀		-	-	-
13♀		-	-	-

/ R = right, L = left, B = both

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APPENDIX 8

Macroscopic pathology - individual observations
in rats sacrificed at 4 weeks

Group:	1A	11	12	13
Compcund:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

Group	Rat No	Observations
1A♂	1	No abnormality is detected.
	2	Lungs: Occasional subpleural punctate grey green foci.
	3	Lungs: Occasional subpleural punctate grey green foci.
	4,5	No abnormality is detected.
	6	Lungs: Occasional subpleural punctate grey green foci.
	7	No abnormality is detected.
	8	Lungs: Occasional subpleural grey green foci 1mm diameter.
	10	Lungs: Occasional subpleural punctate grey green foci on costal surface of right posterior lobe.
11♂	111	No abnormality is detected.
	112	Lungs: Anterior border of right posterior lobe consolidated.
	113,114,115,116	No abnormality is detected.
	117	Lungs: Occasional subpleural punctate grey green foci.
	118	Kidneys: Minimal bilateral cortical scarring.
	119,120	No abnormality is detected.
12♂	121	No abnormality is detected.
	122	Lungs: Scattered subpleural punctate grey green foci.
	123	Lungs: Occasional subpleural punctate grey green foci.
	124, 125	No abnormality is detected.
	126	Lungs: Occasional subpleural punctate grey foci left lobe.
	127	Lungs: Occasional subpleural punctate grey green foci.

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APPENDIX 8

(continued)

Group	Rat No	Observations
12♂ (cont)	128	Small Intestine: Gaseous distension.
	129	No abnormality is detected.
	130	Lungs: Occasional subpleural punctate grey green foci.
13♂	131	No abnormality is detected.
	132	Lungs: Right middle lobe consolidated.
		Small Intestine: Gaseous distension.
	133,134	No abnormality is detected.
	135	Lungs: Occasional subpleural grey green foci 1mm diameter.
	136	Small Intestine: Gaseous distension.
	137	No abnormality is detected.
	138	Lungs: Occasional subpleural punctate grey green foci.
	139,140	No abnormality is detected.

APPENDIX 8

(continued)

Group	Rat No	Observations
1A♀	171,172	No abnormality is detected.
	173	Lungs: Occasional subpleural punctate grey green foci.
	174,175,176	No abnormality is detected.
	177	Lungs: Occasional subpleural punctate grey green foci.
	178	No abnormality is detected.
	179	Lungs: Azygos lobe collapsed. Occasional subpleural punctate grey green foci. All lobes.
		Small Intestine: Ileum: Jejunum: Pale.
	180	Lungs: Occasional subpleural punctate grey green foci.
		Small Intestine: Ileum: Jejunum: Pale
11♀	281,282	No abnormality is detected.
	283	Lungs: Scattered subpleural punctate grey green foci.
	284	Lungs: Occasional subpleural punctate grey green foci.
	285	Lungs: Scattered subpleural grey green foci 1mm diameter.
	286	Lungs: Scattered subpleural punctate grey green foci.
	287	No abnormality is detected.
	288	Lungs: Scattered subpleural punctate grey green foci.
	289,290	No abnormality is detected.
12♀	291,292,293, 294	No abnormality is detected.
	295	Lungs: Scattered subpleural punctate grey green foci.
	296	No abnormality is detected.
	297	Lungs: Occasional subpleural punctate grey green foci.
	298,299	No abnormality is detected.
	300	Small Intestine: Gaseous distension.

APPENDIX 8

(continued)

Group	Rat No	Observations
13♀ (cont.)	301	Lungs: Scattered subpleural punctate grey green foci.
	302	Lungs: Scattered subpleural punctate grey green foci.
	303,305,306 307,308,310	No abnormality is detected.

[redacted]

APPENDIX 9

Organ weights (absolute) - individual values
for rats sacrificed at 4 weeks

Group: 1A 11 12 13

Compound: - (b) (4)

Level (mg/kg/day): Control 50 200 600

Group No.	Rat No.	Body wt.	Brain	Heart	Liver	Kidney	Adrenal mg	Gonads
1Am	1	475	1.9	1.6	19.0	3.8	60	4.8
	2	483	1.8	1.4	23.5	4.0	75	4.8
	3	432	2.0	1.4	20.2	3.7	57	4.7
	4	450	2.0	2.4	18.7	4.0	77	5.2
	5	488	2.2	1.5	25.4	4.6	65	6.2
	6	505	1.9	1.6	25.0	4.4	56	4.7
	7	471	2.0	1.5	19.9	4.0	58	5.0
	8	432	1.8	1.4	18.8	3.6	62	4.8
	10	412	2.0	1.5	21.6	3.7	65	4.3
	Mean	461	2.0	1.6	21.3	4.0	64	4.9
11m	111	447	1.9	1.2	24.3	4.1	61	5.2
	112	519	2.0	1.6	25.0	4.3	85	4.8
	113	508	1.9	2.6	24.8	4.3	65	5.0
	114	418	1.9	1.2	19.3	3.3	50	4.8
	115	464	2.0	1.6	23.4	4.0	70	5.1
	116	432	2.0	1.8	20.0	3.5	62	4.9
	117	467	2.0	1.5	21.2	3.7	68	5.1
	118	509	2.0	1.5	21.6	3.8	55	4.9
	119	470	1.8	1.5	22.5	3.3	75	4.8
	120	477	1.9	1.5	22.3	3.8	60	4.9
	Mean	471	1.9	1.6	22.4	3.8	65	4.9
12m	121	408	1.7	1.2	19.1	3.3	80	4.3
	122	422	2.0	1.5	18.0	3.4	65	5.0
	123	415	2.0	1.5	20.6	3.5	75	4.6
	124	419	2.0	1.6	17.2	3.3	72	5.3
	125	482	2.0	1.6	22.9	3.9	74	5.2
	126	435	1.9	1.7	20.4	3.5	65	5.4
	127	416	2.0	1.7	20.1	3.6	74	5.2
	128	482	2.2	1.7	18.2	3.7	62	5.2
	129	492	2.0	2.0	21.6	4.1	70	5.0
	130	411	2.0	1.5	18.1	3.2	48	4.7
	Mean	438	2.0	1.6	19.6	3.5	69	5.0
13m	131	402	1.9	1.4	16.2	3.6	71	4.6
	132	374	1.8	1.3	16.3	2.8	71	4.3
	133	410	2.0	1.3	15.5	3.2	78	4.6
	134	422	2.1	1.5	20.0	4.0	82	4.6
	135	430	2.0	1.4	18.4	3.1	54	4.4
	136	368	1.9	1.4	15.4	2.9	87	3.8
	137	510	2.1	1.6	19.0	3.8	82	4.8
	138	395	1.9	1.4	18.2	3.2	67	4.6
	139	449	2.0	1.7	24.2	3.9	87	5.2
	140	407	1.9	1.6	19.8	3.4	63	4.5
	Mean	417	2.0	1.5	18.3	3.4	74	4.5

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APPENDIX 9

(continued)

Group No.	Rat No.	Body wt.	Brain	Heart	Liver	Kidney	Adrenal
							mg
1Bf	171	276	1.8	1.1	12.8	2.1	80
	172	306	1.8	1.2	13.6	2.3	108
	173	245	1.8	1.0	12.1	1.8	80
	174	250	1.8	0.9	11.4	2.3	86
	175	244	1.8	0.9	10.0	1.9	86
	176	259	1.7	1.0	13.2	2.3	77
	177	277	1.8	1.1	11.3	2.3	78
	178	231	1.7	0.9	10.0	2.0	96
	179	316	1.8	1.1	13.7	2.5	107
	180	270	1.8	1.1	11.4	2.4	84
	Mean	267	1.8	1.0	11.9	2.2	88
11f	281	272	1.9	1.2	13.1	2.4	84
	282	246	1.8	0.9	11.0	1.8	85
	283	300	1.9	1.2	15.6	2.0	86
	284	308	2.0	1.0	13.2	2.4	110
	285	277	1.9	1.1	11.4	1.9	62
	286	268	1.9	1.1	11.2	2.2	80
	287	290	1.9	1.0	11.8	2.2	76
	288	238	1.9	1.2	9.5	1.8	80
	289	312	1.9	1.0	13.4	2.5	72
	290	281	1.8	1.1	15.5	2.4	85
	Mean	279	1.9	1.1	12.6	2.2	82
12f	291	260	1.8	1.0	9.9	2.1	67
	292	280	1.9	1.0	12.5	2.5	92
	293	258	1.8	1.0	10.3	1.9	90
	294	226	1.7	0.8	8.6	1.8	70
	295	262	1.8	1.0	10.6	2.0	76
	296	275	1.8	1.1	13.6	2.9	91
	297	253	1.9	1.0	12.2	2.2	98
	298	282	1.9	1.1	12.6	2.8	100
	299	230	1.7	1.0	9.8	1.7	70
	300	231	1.8	0.9	11.0	2.0	90
	Mean	256	1.8	1.0	11.1	2.2	84
13f	301	252	1.8	1.1	11.6	2.2	62
	302	222	1.7	0.8	10.5	1.9	75
	303	238	1.9	1.1	11.0	2.0	
	305	238	1.7	1.1	10.9	2.0	100
	306	250	1.8	0.9	12.7	2.3	74
	307	212	1.6	0.9	10.1	2.1	83
	308	266	1.7	0.9	9.6	1.9	82
	310	259	1.9	1.0	11.5	2.2	93
	Mean	242	1.8	1.0	11.0	2.1	82

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APPENDIX 10

Organ weights (relative+) - individual values
for rats sacrificed at 4 weeks

Group: 1A 11 12 13

Compound: - (b) (4)

Level (mg/kg/day): Control 50 200 600

Group No.	Rat No.	Body wt. (g)	Brain	Heart	Liver	Kidney	Adrenal	Gonads
1Am	1	475	40	34	400	80	1.3	101
	2	483	37	29	487	83	1.6	99
	3	432	46	32	468	86	1.3	109
	4	450	44	53	416	89	1.7	116
	5	488	45	31	520	94	1.3	127
	6	505	38	32	495	87	1.1	93
	7	471	42	32	423	85	1.2	106
	8	432	42	32	435	83	1.4	111
	10	412	49	36	524	90	1.6	104
	Mean	461	43	35	463	86	1.4	107
11m	111	447	43	27	544	92	1.4	116
	112	519	39	31	482	83	1.6	92
	113	508	37	51	488	85	1.3	98
	114	418	45	29	462	79	1.2	115
	115	464	43	34	504	86	1.5	110
	116	432	46	42	463	81	1.4	113
	117	467	43	32	454	79	1.5	109
	118	509	39	29	424	75	1.1	96
	119	470	38	32	479	70	1.6	102
	120	477	40	31	468	80	1.3	103
	Mean	471	41	34	477	81	1.4	106
12m	121	408	42	29	468	81	2.0	105
	122	422	47	36	427	81	1.5	118
	123	415	48	36	496	84	1.8	111
	124	419	48	38	411	79	1.7	126
	125	482	41	33	475	81	1.5	108
	126	435	44	39	469	80	1.5	124
	127	416	48	41	483	87	1.8	125
	128	482	46	35	378	77	1.3	108
	129	492	41	41	439	83	1.4	102
	130	411	49	36	440	78	1.2	114
	Mean	438	45	36	449	81	1.6	114
13m	131	402	47	35	403	90	1.8	114
	132	374	48	35	436	75	1.9	115
	133	410	49	32	378	78	1.9	112
	134	422	50	36	474	95	1.9	109
	135	430	47	33	428	72	1.3	102
	136	368	52	38	418	79	2.4	103
	137	510	41	31	373	75	1.6	94
	138	395	48	35	461	81	1.7	116
	139	449	45	38	539	87	1.9	116
	140	407	47	39	486	84	1.5	111
	Mean	417	47	35	440	81	1.8	109

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APPENDIX 10

(continued)

Group No.	Rat No.	Body wt. (g)	Brain	Heart	Liver	Kidney	Adrenal
1Bf	171	276	65	40	464	76	2.9
	172	306	59	39	444	75	3.5
	173	245	73	41	494	73	3.3
	174	250	72	36	456	92	3.4
	175	244	74	37	410	78	3.5
	176	259	66	39	510	89	3.0
	177	277	65	40	408	83	2.8
	178	231	74	39	433	87	4.2
	179	316	57	35	434	79	3.4
	180	270	67	41	422	89	3.1
	Mean	267	67	39	447	82	3.3
11f	281	272	70	44	482	88	3.1
	282	246	73	37	447	73	3.5
	283	300	63	40	520	67	2.9
	284	308	65	32	429	78	3.6
	285	277	69	40	412	69	2.2
	286	268	71	41	418	82	3.0
	287	290	66	34	407	76	2.6
	288	238	80	50	399	76	3.4
	289	312	61	32	429	80	2.3
	290	281	64	39	552	85	3.0
	Mean	279	68	39	449	77	3.0
12f	291	260	69	38	381	81	2.6
	292	280	68	36	446	89	3.3
	293	258	70	39	399	74	3.5
	294	226	75	35	381	80	3.1
	295	262	69	38	405	76	2.9
	296	275	65	40	495	105	3.3
	297	253	75	40	482	87	3.9
	298	282	67	39	447	99	3.5
	299	230	74	43	426	74	3.0
	300	231	78	39	476	87	3.9
	Mean	256	71	39	434	85	3.3
13f	301	252	71	44	460	87	2.5
	302	222	77	36	473	86	3.4
	303	238	80	46	462	84	
	305	238	71	46	458	84	4.2
	306	250	72	36	508	92	3.0
	307	212	75	42	476	99	4.2
	308	266	64	34	361	71	3.1
	310	259	73	39	444	85	3.6
	Mean	242	73	40	455	86	3.4

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APPENDIX 11

Histology - Individual observations in rats sacrificed at 4 weeks

Group:	1A	11	12	13
Compound:	-		(b) (4)	
Level (mg/kg/day):	Control	50	200	600

The following organs were weighed:

Liver	Adrenals
Testes	Brain
Kidney	Heart

Samples of the following tissues from all rats were preserved in 10% buffered formalin:

Adrenals	Pituitary*
Aorta*	Prostate*
Body fat*	Salivary gland*
Brain*	Sciatic nerve*
Caecum*	Seminal vesicles*
Colon*	Skeletal muscle*
Duodenum*	Skin*
Eyes*	Spleen
Femur*	Stomach*
Heart	Testes
Ileum*	Thymus*
Jejunum*	Thyroids
Kidneys	Trachea*
Liver	Tongue*
Lung*	Urinary bladder
Lymph nodes*	Uterus*
Mammary gland*	Any abnormal tissue
Oesophagus*	
Ovaries*	
Pancreas*	

Femoral marrow smears were prepared and fixed in methyl alcohol. Tissues marked thus* were preserved but not processed further in the first instance.

Tissues for histopathological examination were fixed from 1-2 weeks in 10% buffered formalin, routinely processed in 56°C M.P. paraffin wax, sectioned at 5μ and stained with haematoxylin and eosin.

APPENDIX 11

(continued)

GROUP 1A♂

Rat 1

Spleen: Small foci of extra-medullary haemopoiesis are noted.

Liver: Vacuolated and occasional distended hepatocytes are noted in the centrilobular areas.

Kidney: Small groups of tubules characterized by their basophilic staining, a proportion of which are seen to be distended and contain eosinophilic material are seen in association with minimal mononuclear cell infiltration of the cortex.

Rat 2

Liver: Occasional small foci of mononuclear cell infiltration are noted in the parenchyma. Vacuolated hepatocytes are seen in centrilobular areas.

Spleen: Foci of extra-medullary haemopoiesis are noted.

Rat 3

Liver: Occasional vacuolated hepatocytes are noted in centrilobular areas.

Rat 4

Liver: Vacuolated hepatocytes are noted in centrilobular areas.

Kidney: Occasional small groups of tubules characterized by their basophilic staining, a proportion of which are seen to be distended and to contain granular eosinophilic material are seen in association with minimal mononuclear cell infiltration of the cortex.

Urinary bladder: An eosinophilic plug is seen in the lumen.

Rat 5

Heart: Small foci of mononuclear cell infiltration are noted in the ventricular musculature.

Spleen: Occasional foci of extra-medullary haemopoiesis are noted.

Liver: Vacuolated hepatocytes are seen in centrilobular areas.

Testes: The majority of seminiferous tubules in one testis are distended.

APPENDIX 11

(continued)

Rat 6	
Heart:	A focus of mononuclear cell infiltration is seen in the ventricular musculature.
Liver:	Occasional vacuolated hepatocytes are noted in centrilobular areas.
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Rat 7	
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Occasional vacuolated hepatocytes are seen in centrilobular areas.
Urinary bladder:	An eosinophilic plug is noted in the lumen.
Rat 8	
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.
Rat 10	
Liver:	Vacuolated and distended hepatocytes are prominent in centrilobular areas.

APPENDIX 11

(continued)

GROUP 13♂

Rat 131

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 132

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 133

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Occasional vacuolated hepatocytes are noted in centrilobular areas.
Kidney:	Minimal mononuclear cell infiltration is noted in the cortex.

Rat 134

Liver:	Vacuolated hepatocytes are seen in centrilobular areas. Small foci of mononuclear cell infiltration are noted in the parenchyma.

Rat 135

Liver:	Vacuolated hepatocytes are noted in centrilobular areas.
Kidney:	Small groups of tubules that are characterised by their basophilic staining are noted in the cortex.

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APPENDIX 11

(continued)

Rat 136	
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.
Kidney:	Occasional small groups of tubules characterized by their basophilic staining are seen in association with minimal mononuclear cell infiltration of the cortex.
Rat 137	
Liver:	Occasional vacuolated hepatocytes are noted in centrilobular areas.
Rat 138	
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Vacuolated hepatocytes are seen in centrilobular areas.
Rat 139	
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Vacuolated hepatocytes are noted in occasional centrilobular areas.
Rat 140	
Liver:	Vacuolated hepatocytes are seen in centrilobular areas.
Kidney:	Occasional small groups of tubules characterized by their basophilic staining, a proportion of which are distended with eosinophilic material, are seen in association with minimal mononuclear cell infiltration of the cortex.

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APPENDIX II

(continued)

GROUP 1A♀

Rat 171

Spleen:	Occasional foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 172

Liver.	Occasional vacuolated hepatocytes are noted in centrilobular areas.

Rat 173

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Small foci of mononuclear cell infiltration are noted in the parenchyma.

Rat 174

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 175

Spleen:	Occasional foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 176

Liver:	Hepatic architecture within normal limits.
Parathyroid:	A telangiectatic focus is seen in one gland.

Rat 177

Spleen:	Occasional foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

APPENDIX 11

(continued)

Rat 178

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 179

Liver:	Occasional small lymphoid foci are noted in the parenchyma.

Rat 180

Liver:	Hepatic architecture within normal limits.

APPENDIX 11

(continued)

GROUP 13?

Rat 301

Spleen:	Occasional foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 302

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 303

Heart:	Small foci of mononuclear cell infiltration are noted in the ventricular myocardium.
Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 305

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.

Rat 306

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Occasional small foci of mononuclear cell infiltration are noted in the parenchyma.

Rat 307

Spleen:	Occasional macrophages containing brown pigment are noted.
Liver:	Hepatic architecture within normal limits.

[redacted]

APPENDIX II

(continued)

Rat 308

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Hepatic architecture within normal limits.
Kidney:	Occasional tubules, which are characterized by their basophilic staining are seen in association with minimal mononuclear cell infiltration in the cortex.

Rat 310

Spleen:	Foci of extra-medullary haemopoiesis are noted.
Liver:	Occasional foci of mononuclear cell infiltration are noted in the parenchyma.
Kidney:	Small foci of dystrophic mineralisation are noted in the medulla.