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THE TOXICITY OF ORALLY AND INTRAVENOUSLY ADMINISTERED PARAQUAT DICHLORIDE IN CYNOMOLGUS MONKEYS

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SUMMARY

Test compound: Paraquat dichloride.

Test species: Cynomolgus monkey (Macaca fascicularis)

Sex: Male

Route of administration: Intravenous: 9 animals
Oral: 20 animals

Dose levels and deaths: *

Intravenous	No. dosed	Deaths	Survival time (days)
6 $mg.kg^{-1}$ (single dose)	1	0	
10 $mg.kg^{-1}$ (single dose)	1	1	2
16 $mg.kg^{-1}$ (single dose)	2	2	3,9
24 $mg.kg^{-1}$ (single dose)	2	2	1,2,
32 $mg.kg^{-1}$ (single dose)	2	2	2,2
40 $mg.kg^{-1}$ (single dose)	1	1	2
Oral			
45 $mg.kg^{-1}$ (single dose)	6	1	8
55 $mg.kg^{-1}$ (single dose)	2	0	
65 $mg.kg^{-1}$ (single dose)	6	3	2,3,4
85 $mg.kg^{-1}$ (single dose)	6	5	2,2,3,8,15

* Dose levels in $mg.kg^{-1}$ of paraquat ion (M.W. 257)

Clinical findings:

1. Intravenously administered paraquat caused death within 3 days in 7 animals.
2. The main symptoms resulting from paraquat administered by both routes were acute renal failure and lung congestion/oedema.

3. The following changes occurred in blood and urine constituents, and are considered to be significant:

uremia
polyuria followed by oliguria/anuria
high serum glutamic-puruvic transaminase levels
hypocalcemia
low blood and urine pH
haematuria
glycosuria (all indicative of renal damage)
high levels of serum leucine amino-peptidase
high levels of serum glutamic dehydrogenase
high levels of serum gamma glutamyl transpeptidase (all indicative of hepatic damage)

4. The toxicity of orally administed paraquat was dose related, but it was not possible to establish a clear LD 50.

5. Within any one dose group it was observed that the monkeys most likely to die were those excreting the greatest amounts of paraquat during the first 48 hours.

6. It is suggested that paraquat poisoning consists of an acute phase mainly due to renal failure, occurring within 48 hours of dosing, and a sub-acute phase caused by lung damage 7-15 days after dosing.

INTRODUCTION

The following report contains the results of 3 experiments on the toxicity of paraquat in cynomolgus monkeys. The general aim of the experiments was to study the effects produced by paraquat, administered by both intravenous and oral routes. The 3 experiments were:

ICI/40 The toxicity of intravenously administered paraquat

In a previous dosing test 2 male cynomolgus monkeys were given 6 $mg.kg^{-1}$* and 40 $mg.kg^{-1}$ of paraquat intravenously. The 6 $mg.kg^{-1}$ dose produced no detectable symptoms, while the 40 $mg.kg^{-1}$ dose caused severe respiratory distress and death after 48 hours. Following these results, it was decided to dose 3 groups of 2 animals with 32, 24 and 16 $mg.kg^{-1}$ of paraquat ion respectively.

ICI/50 The toxicity of orally administered paraquat

The results of the previous study (ICI/40) and previous experiments by other investigators (Murray and Gibson, 1972; Murray and Gibson, 1974) showed that there were considerable differences between the effects of paraquat administered intravenously, and the effects of the substance administered orally. In this series of experiments 4 oral dose levels of 85, 65, 55 and 45 $mg.kg^{-1}$ were used on 4 groups of 2 monkeys. In addition, one animal was dosed intravenously with 10 $mg.kg^{-1}$ as an extension of ICI/40.

ICI/52 Preliminary dose-range tests for paraquat in the male cynomolgus monkey

Two previous studies of paraquat administered orally to cynomolgus monkeys (ICI/50 and Murray and Gibson, 1972) failed to establish a clear LD 50. The purpose of this study was to provide further information towards this end, using 3 dose levels of 85, 65 and 45 $mg.kg^{-1}$ on 3 groups of 4 monkeys.

All dose levels are quoted as weights of paraquat ion (M.W. 257) unless stated otherwise.

MATERIALS AND METHODS

Animals

Twenty-nine cynomolgus monkeys (Macaca fascicularis) were obtained from a commercial supplier (Shamrock Farms Ltd). Animal bodyweights ranged from 3.4 kg to 5.5 kg, and in the case of the dose-range test project (ICI/52) animals were chosen so that each group mean weight was approximately the same, and each group contained the same number of light and heavy animals. Otherwise animals were allocated to groups at random.

On arrival at the Huntingdon Research Centre, and at monthly intervals thereafter, all animals were examined by our veterinary surgeon. Examination included intrapalpebral tuberculin tests (10,000 i.u. mammalian PPD) and chest X-ray.

Accommodation

The animals were housed in rack mounted stainless steel cages in a well ventilated holding area, maintained at a temperature of 71°F.

Diet

The animals were fed with a dry diet suitable for primates. A 1:1 ratio of 'FP1' (Dixon and Sons Ltd., Ware), and 'Laboratory Animal Diet No. 427/7' (Speciality Products, Witham, Essex). was used, each animal being offered 100 g of this diet and a 50 g 'Kennomeal' biscuit (Spratt's Patent Ltd., Central House, Barking, Essex) daily. In addition, fresh fruit or vegetable produce (approximately 75 g) and 35 g of bread were offered. The total amount of food available for each animal was therefore 260 g per day, except when haematological tests were to be performed on the following day, in which case food was witheld. Food consumption was monitored 3 times per week, and water was available at all times.

Test material

The test material was a fine white powder received from the Central Toxicology Laboratory, Imperial Chemical Industries Limited, Alderley Park, Cheshire. The substance was supplied as the herbicide paraquat (N,N' dimethyl 4, 4' bipyridilum) dichloride M.W. 257 (100.0 % pure).

The powder was dried in an oven at 110°C for 1 hour before use, and the required amounts were then weighed, dissolved and made up to volume in sterile, pyrogen-free, water for injections (May & Baker Ltd., Dagenham, Essex).

Dosing

Intravenous (ICI/40)

Six male Cynomolgus monkeys were allocated to 3 groups of 2 animals for ICI/40 and one animal was allocated in ICI/50. All were dosed with paraquat by intravenous injection lasting ten seconds; the substance being administered at 15.15 hours on day 0 according to the dose schedule shown below:

	Animal No.	Weight (g)	Dose Pqt. $kg.^{-1}$ (mg)	Wt. Pqt-$Cl_2.kg^{-1}$ (mg)	Total dose Pqt-Cl_2 (mg)
Group I	1	4150	32	44.2	184
	2	4600			203
Group II	6	3400	24	33.2	113
	8	3850			128
Group III	9	3400	16	22.1	75
	10	3850			85
Group IV	16	4500	10	13.8	62

Oral (ICI/50, ICI/52)

Twenty male Cynomolgus monkeys were allocated to 3 groups of 6 animals, and one group of 2 animals. They were dosed with paraquat by oral gavage in approximately 40 ml of water. In the case of animals Nos 4 and 7, 5 g of 'Complan' (Glaxo-Farley Foods, Plymouth) were added to the water immediately prior to dosing to 'buffer' the effects of paraquat on the stomach. All animals were anaesthetized with 'Saffan' (Glaxo Laboratories Ltd., Greenford)[1] during and (for at least 2 hours) after dosing (1 $ml.kg^{-1}$ i.v., followed by 0.5 $ml.kg^{-1}$ i.m. every 25 minutes), to reduce the risk of vomiting before the substance could be absorbed.

The doses were administered to 8 animals at 15.00 hours, and to 12 animals at 10.00 hours on day 0 according to the schedule shown below:

1. 'Saffan' contains - Alphaxalone (0.9 % w/v)
Alphadolone (0.3 % w/v)

	Animal No.	Time (hrs)	Weight (g)	Dose Pqt.kg^{-1} (mg)	Wt.Pqt-Cl_2.kg^{-1} (mg)	Total dose Pqt-Cl_2 (mg)
Group I	14	15.00	5300	85	117.5	623
	26	15.00	5000	85	117.5	587
	22	10.00	5550	85	117.5	652
	11	10.00	3950	85	117.5	464
	18	10.00	4600	85	117.5	540
Group II	4	15.00	3750	65	89.8	337
	7	15.00	3950	65	89.8	257
	21	10.00	5200	65	89.8	467
	13	10.00	4250	65	89.8	382
	20	10.00	4600	65	89.8	413
	5	10.00	3850	65	89.8	346
Group III	28	15.00	4500	55	76	259
	29	15.00	4000	55	76	220
Group IV	3	15.00	4750	45	62.2	296
	12	15.00	4950	45	62.2	308
	17	10.00	5300	45	62.2	330
	24	10.00	3450	45	62.2	215
	25	10.00	4050	45	62.2	252
	27	10.00	5150	45	62.2	320

Clinical investigations

(1) Clinical signs

The condition of the animals was observed during the day and at intervals during the night, any symptoms being recorded. Animals were killed only when they were found to be suffering pain which was either severe or likely to endure, and when the main result of the experiment had been attained.

(2) Food consumption

The quantity of food consumed overnight was recorded 3 times per week pre-exposure, and during the test period.

(3) Bodyweight

Bodyweights were recorded weekly.

(4) Radiographs

Lateral and antero-posterior radiographs were taken pre-exposure and whenever possible preterminally.

(5) Electrocardiography

Electrocardiograms (ECGs) were recorded on a Hewlet-Packard Model 1504 A electrocardiograph. The ECG was obtained from the fully conscious animal restrained in the supine position, using the standard limb leads (I, II and III), the augmented unipolar limb leads (aVR, aVL and aVF) and three chest leads (MVI, MVII, and MVIII). The chest leads corresponded to the 4th intercostal space approximately 3 cm to the right and left of the mid-sternal line and the 5th intercostal space in the mid-axillary line respectively (Atta and Vanace, 1960).

ECGs were recorded pre-exposure on all animals in ICI/40 and ICI/50, and pre-terminally whenever possible.

(6) Lung function tests

These consisted of:

(i) Lung mechanics

This test was used to assess the mechanical behaviour of the lungs and airways.

(ii) Lung ventilation

This test was used to assess the efficiency and distribution of pulmonary ventilation.

(iii) Blood gases

Measurements of pH, PCO_2 and PO_2 were made to assess the efficiency of gaseous exchange across the alveolar wall, and the body's acid-base balance.

Measurments of lung ventilation were made with the unanaesthetized animal seated quietly in a restraining chair and fitted with a face mask. The dead space within the face mask was minimized by using rubber liners especially moulded to fit the snouts of individual animals. The airtight seal around the face was achieved by using a rubber dam stretched tightly over the back of the mask, through which the snout of the animal was fitted. For ICI/40, lung mechanics were measured with the animals set up in the same way as above, but for ICI/50 the measurements were carried out under 'Saffan' anaesthesia (1 ml.kg^{-1} i.v.)

(i) Lung mechanics (Figure 1)

For unanesthetized animals, air flow into and out of the lungs was measured by means of a pneumotachograph fitted to the front of the mask, and a differential gas pressure transducer (model 270; Hewlett-Packard Equipment Ltd., 224 Bath Road, Slough, Bucks) which measured the pressure differential across the pneumotachograph.

Lung volume changes were derived by electrical integration of the flow signal with respect to time.

Intrapleural pressure was measured by means of a saline-filled nylon catheter of 0.7 mm internal diameter connected to a pressure transducer (Hewlett-Packard 268B). The catheter was inserted, under local anaesthesia, at a level of the fourth intercostal space, approximately 3 cm lateral to the mid-sternal line on the right side of the chest.

The mechanical properties of the lung were measured using an on-line digital computer system which gave almost instantaneous teletype presentation of the parameters. Data extraction was based on standard methods (Frank, Mead & Ferris, 1957; Amdur and Mead 1958) from simultaneous measurement of flow, volume and pressure changes during quiet respiration. A typical print-out from the monitoring system, and a list of the parameters measured, is shown in Figure 2.

Anaesthetized animals were intubated, and air flow was measured by connecting the endotracheal tube to a pneumotachograph. Pressure measurements were made with an oesophageal balloon, which was connected to the pressure transducer. Volume measurements and data extraction were carried out in the same way as for unanaesthetized animals.

The above technique was used on all the ICI/50 (orally dosed) animals, because in the previous study (ICI/40) the health of the animals was so poor that lung mechanics could be measured in only a few cases.

FIGURE 1

Apparatus used for monitoring the mechanical characteristics of the lungs of a restrained, fully conscious, monkey

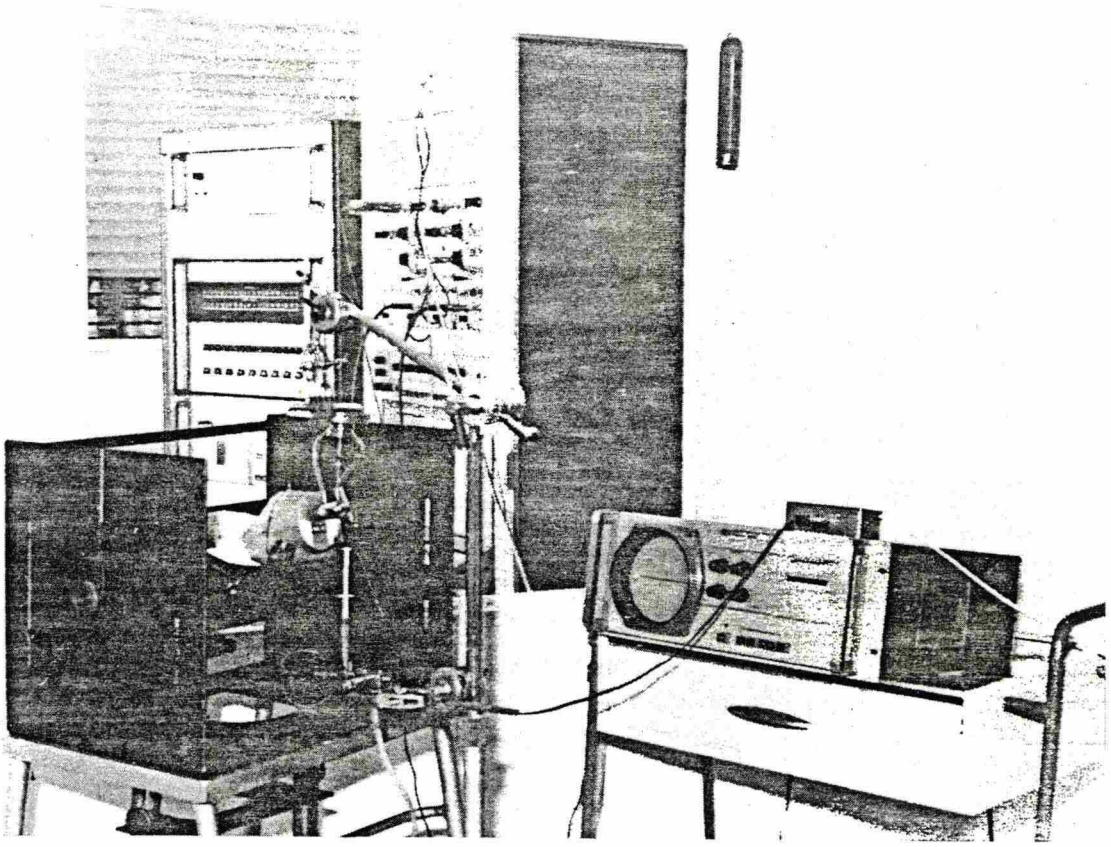


FIGURE 2

Male
6

A typical print-out from the lung mechanics system

3350

PRE-EXPOSURE

VT	RR	RMV	DEDC	VTP	CDYNL	RLI	RLE	RLI/RLE	RL
50.2	28	1422	55	4.6	10.84	.036	.044	82	.040
52.0	30	1545	57	6.1	8.46	.049	.028	178	.045
46.0	27	1231	55	3.5	13.08	.021	.045	46	.037
47.0	29	1370	55	4.7	9.99	.023	.067	34	.042
49.6	28	1403	58	4.8	10.37	.029	.049	60	.037
45.5	28	1263	54	5.1	8.96	.026	.049	53	.041
51.8	30	1539	57	6.1	8.53	.035	.038	94	.034
44.9	26	1181	56	5.3	8.52	.045	.055	82	.050
45.0	30	1349	55	6.8	6.61	.039	.036	111	.042
48.0	25	1210	50	6.3	7.60	.041	.031	133	.049
48.0	28	1351	55	5.3	9.30	.035	.044	87	.042
2.8	1.6	130	2.3	1.0	1.84	.010	.012	43.8	.005
6	6	10	4	19	20	28	27	50	12

The figures which appear on the top left hand side of the print-out refer to:

Project no.
Date
Species of animal
Sex
Animal No.
Wt of animal (gm)
Treatment :

VT — Tidal volume - ml (Vt)
RR — Respiratory rate - per min (f)
RMV — Respiratory minute volume - $ml.min^{-1}$
DEDC — Duration of expiratory phase as a percentage of the complete cycle
VTP — Tidal volume pressure swing (cm H_2O)
CDYNL — Dynamic lung compliance (Cdyn (I)) $ml.cm\ H_2O^{-1}$
RLI — Pulmonary resistance during inspiration (RI (i)) $cm\ H_2O.\ ml^{-1}.\ sec^{-1}$
RLE — Pulmonary resistance during expiration (RI (e)) $cm\ H_2O.\ ml^{-1}.\ sec^{-1}$
RI — Average pulmonary resistance (RI) $cm\ H_2O.ml^{-1}.sec.^{2}$

The final 3 lines of the printout indicate mean, standard deviation and coefficient of variation for each parameter.

(ii) Lung ventilation (Figure 3)

The distribution of pulmonary ventilation was assessed using a nitrogen washout technique based on the method of Darling, Fumana & Richards (1940).

After being fitted with a face mask and a suitable valve system, the animal breathed room air until a steady 'normal' respiratory pattern was obtained. To begin the test, at the end of a normal expiration, the animal began to breathe from a continuous stream of pure oxygen. Breath by breath analysis of the nitrogen content of respired air was made using a nitrogen analyser. The animal continued to breathe pure oxygen until the nitrogen content of the expired air was reduced to 2 %.

From this test the following parameters were determined:

Tidal volume (VT)

Respiratory Rate (RR)

Respiratory minute volume (RMV)

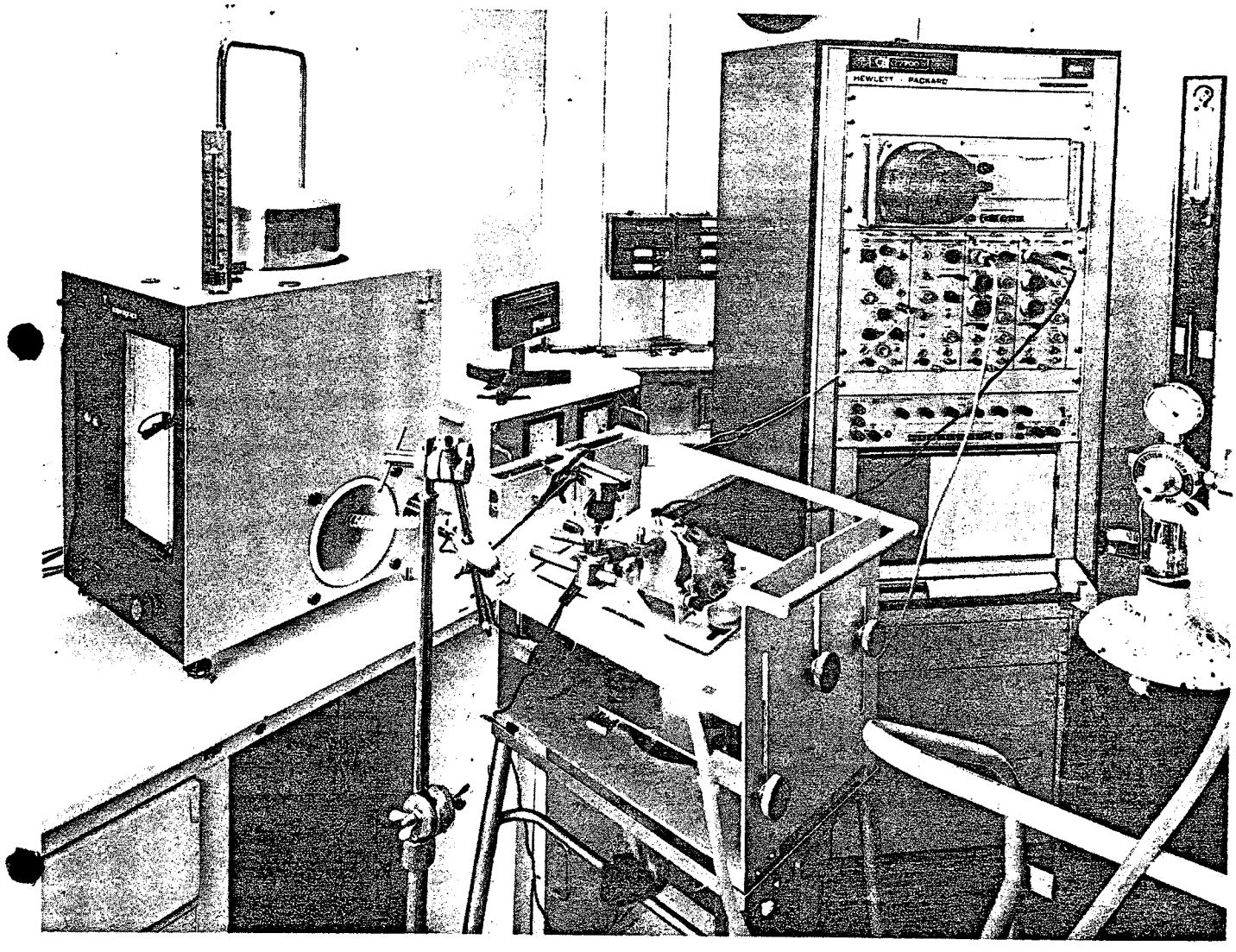
Total time breathing pure oxygen to reach 2 % nitrogen in expired air (T - 2 %).

Total number of breaths of pure oxygen to reach 2 % nitrogen in expired air (N - 2 %).

Cumulative volume of expired air to reach 2 % nitrogen in expired air (CVT - 2 %)

FIGURE 3

Measurement of distribution of pulmonary ventilation



(iii) Blood gases

Measurements of blood gases, pH and base excess (Siggard-Andersen, 1971), were made on 200 µl samples of blood taken from the femoral artery of a restrained, fully conscious, supine animal. Analyses were carried out immediately after sampling, using a BMS3 blood microsystem (Radiometer A/S Emdrupvej 72, DK 2400, Copenhagen NV, Denmark) after the methods of Severinghaus (1968) and Siggard-Andersen (1963).

Base excess (B.E) measured in $mEq.l^{-1}$ indicates the accumulation of non-volatile acid or base in the blood. A positive value indicates a base excess (non-volative acid deficit), a negative value indicates a base deficit (non-volatile acid excess).

The partial pressure of carbon dioxide in arterial blood (PCO_2), measured in mm Hg, is determined directly by means of the PCO_2 electrode. The partial pressure of oxygen (PO_2), measured in mm Hg, is determined 2 and 4 minutes after withdrawal of the blood sample by a PO_2 electrode and the PO_2 at the time of sampling calculated by extrapolation.

Example of calculation:

PO_2 (2 min) mm Hg	PO_2 (4 min) mm Hg	PO_2 (0 min) mm Hg	Corrected PO_2 mm Hg
90	80	100	100 x K = 104

K = Correction Factor (1.04).

The correction factor is applied to account for the calibration of the electrodes with gas mixtures in which the rate of diffusion differs from that of liquids by a factor of 1.04.

(7) Haematology

Ten ml of blood was obtained from the femoral vein of the restrained animal in the supine position. Food was withdrawn at 17.00 hours on the day before sampling.

Brief details of the investigations performed, methods used and appropriate units for the parameters measured are given below:

		Units
(1)	Erythrocyte sedimentation rate (ESR) - Method of Wintrobe	$mm.hr^{-1}$
(2)	Packed cell volume (PCV) - Estimated by Technicon SMA4A	%
(3)	Haemoglobin (Hb) - Estimated by Technicon SMA4A	g.100 ml $blood^{-1}$
(4)	Red cell count (RBC) - Estimated by Technicon SMA4A	millions. μl^{-1}

(5)	Reticulocyte count (Retics) - brilliant cresyl blue and new methylene blue	% red cells
(6)	Total white blood cell count (WBC) - Estimated by Technicon SMA4A	$x10^3$ cells μl^{-1}
(7)	Differential count	
	(N) = Neutrophils	
	(L) = Lymphocytes	
	(E) = Eosinophils	
	(B) = Basophils	
	(M) = Monocytes	%
(8)	Platelet count - direct visual count (ammonium oxalate 1 % diluent) - method of Brecher, G & Cronkite, E.P (1950) (J. Appl. Physiol. 3, 365).	$x10^3$ μl^{-1}
(9)	Prothrombin time - Quick's one-stage method using Simplastin	seconds

(8) Biochemistry

Plasma Urea - Urease/Berthelot Reaction, Fawcett, J.K. and Scott J.F. (1960) (J. Clin. Path. 13, 156)	mg %
Plasma glucose - Technicon autoanalyser method (glucose oxidase)	mg %
Total serum proteins - Technicon autoanalyser method (Biuret)	g %
Serum protein electrophoresis and AG ratio - electrophoretic breakdown of albumin, $\alpha 1$, $\alpha 2$, β and γ globulins - using millipore phoroslides, staining with ponceau S.	%
Serum glutamic - pyruvic transaminase (SGPT) - LKB 8600 Reaction Rate Analyser diamed test kit (J.T. Baker)	$mU.ml^{-1}$
Serum leucine amino-peptidase (LAP) - (Sigma Technical Bulletin 251)	Goldberg & Rutenburg or GR units
Serum Bilirubin- Method of Malloy, H.T. & Evelyn, K.A. (J. Biol. , chem. 1937, 119, 480) as modified by H. Vanden Bossche (Clinica.Chim.Acta. 1965, 11, 379) on Technicon Autoanalyser	mg %
Gamma Glutamyl Transpeptidase (γGT)	$mU\ ml^{-1}$
Glutamic Dehydrogenase (GLDH)	$mU\ ml^{-1}$
Creatine Phosphokinase (CPK) LKB 8600 Reaction Rate Analyser Boehringer test kit 15721	$mU\ ml^{-1}$
Sodium (Na^+) - Flame photometer (E.E.L.)	$mEq.l^{-1}$
Potassium (K^+) - Flame photometer (E.E.L.)	$mEq.l^{-1}$
Calcium (Ca^{++}) - Technicon Autoanalyser method (Cresophthalein complexone)	$mEq.l^{-1}$

(9) Urinalysis

(a) Collection

Normal procedure for urine samples collected for routine analysis is to take an overnight sample from animals whose drinking water has been witheld. However in the present study all urine was required for paraquat level analysis as well as urinalysis. For this reason water was not withheld, urine volumes were measured every 24 hours, and samples retained for paraquat analysis and urinalysis.

(b) Specific gravity of urine (SG)

This is a standard test, but of little value in the present experiment since water was not withheld, and also because water spilled by the animal from the automatic watering system is collected with the urine and false values may be introduced. For the same reason total urine volumes, especially very high ones, are suspect.

(c) Quantitative tests

pH (by pH meter) and protein (by sulphosalicylic acid test).

(d) Qualitative tests

Reducing substances ('Clinitest' * +++ = orange)
Glucose ('Clinistix' *)
Ketones ('Acetest'*)- confirmed by Rothera's test when positive)
Bile pigments (Ictotest* ++ = strongly positive)
Urobilinogen (Bogomolow's test)
Haemoglobin ('Haemistix'*)
* Diagnostic reagents of Ames Company, Stoke Poges, England

(e) Microscopy

The specimen of urine was centrifuged at 3,000 r.p.m. for 10 minutes and then the deposit was microscopically examined for:

Epithelial cells	(E)
Polymorphonuclear leucocytes	(P)
Mononuclear leucocytes	(M)
Erythrocytes	(R)
Organisms	(O)
Casts	(C)
Abnormal constituents	(A)

The grading of cell frequency in the spun deposit was as follows:

0	=	Nil
1	=	few in some fields examined
2	=	few in all fields examined
3	=	many in all fields examined

(f) Estimation of urinary paraquat levels

Standard solutions were made up by adding known amounts of paraquat to 5 ml samples of cynomolgus urine. These were then treated with 2 ml of a fresh solution containing 0.05 g. ml^{-1} sodium dithionite, and 0.05 g ml^{-1} sodium bicarbonate. The optical density of the free radical of paraquat formed by this procedure was then measured at 604 nm against a blank containing the same proportions of urine and reducing solution as the test samples, and a standard curve (of optical density against concentration) was constructed.

Urinary paraquat levels were estimated by measuring the optical density of the samples treated in the same way as above, and obtaining the paraquat concentrations from the standard curve.

(10) Bacteriology

Samples of the cardiac lobe of the lung were taken aseptically during autopsy, and were incubated in aerobic media (trypticase soy broth), and anaerobic media (fluid thioglycollate), for 24 hours at 37°C. Bacterial growth in the tubes was examined microscopically after Gram staining and motility was observed by phase contrast microscopy. Samples were then subcultured onto the surface of blood agar plates (ICI/50). Antibiotic sensitivity discs were placed on the plates which were then incubated at 37°C overnight and examined for the presence of zones of inhibited growth around the sensitivity discs.

(11) Autopsies

All animals were subjected to detailed macroscopic examination, and all abnormalities were noted. Selected tissues/organs were fixed in formalin (10 %) and are available for examination (Kidneys, liver, heart, lungs and adrenals).

Test Programme

Clinical investigations were performed as far as possible at the following times for ICI/40 and ICI/50:

ECG and Radiography	Pre-exposure Pre-terminally
Lung Mechanics	Pre-exposure 48 hours after dosing Pre-terminally
Lung Ventilation and Blood gases	Pre-exposure 48 hours after dosing 4 days after dosing 7 days after dosing 12 days after dosing Pre-terminally
Haematology, Blood Biochemistry and Urinalysis	Pre-exposure 24 hours after dosing 48 hours after dosing 7 days after dosing Pre-terminally

RESULTS OF INTRAVENOUS DOSING STUDY (ICI/40)

The symptoms shown by the six animals, together with the results of all the clinical investigations carried out, are summarized in the following Clinical signs sheets(Appendix 1). The detailed results of all tests are given in Appendices 2- 7.

The condition of all animals declined rapidly after dosing, 5 being dead by the third day, and one surviving 8 days (Figure 4). The results of the clinical investigations were as follows:

Clinical signs

Animals No. 2 and 6 began to show adverse symptoms within 2 hours of dosing, becoming lethargic and eating very little. Within the next 24 hours all the animals except No. 9 developed similar symptoms, consisting of vomiting, anorexia, dyspnoea and hypothermia. A copious oily secretion was also produced, which saturated the animals' body fur. An initial polyuria during the first 24 hours was followed by a marked reduction in urine volumes by 48 hours after dosing.

Food consumption

The animals were fed 2 hours after dosing; 2 failed to eat and 4 others vomited during the night. All animals showed a marked decrease in food consumption throughout the test period.

Bodyweight

A fall in bodyweights was recorded during the test period.

Radiography

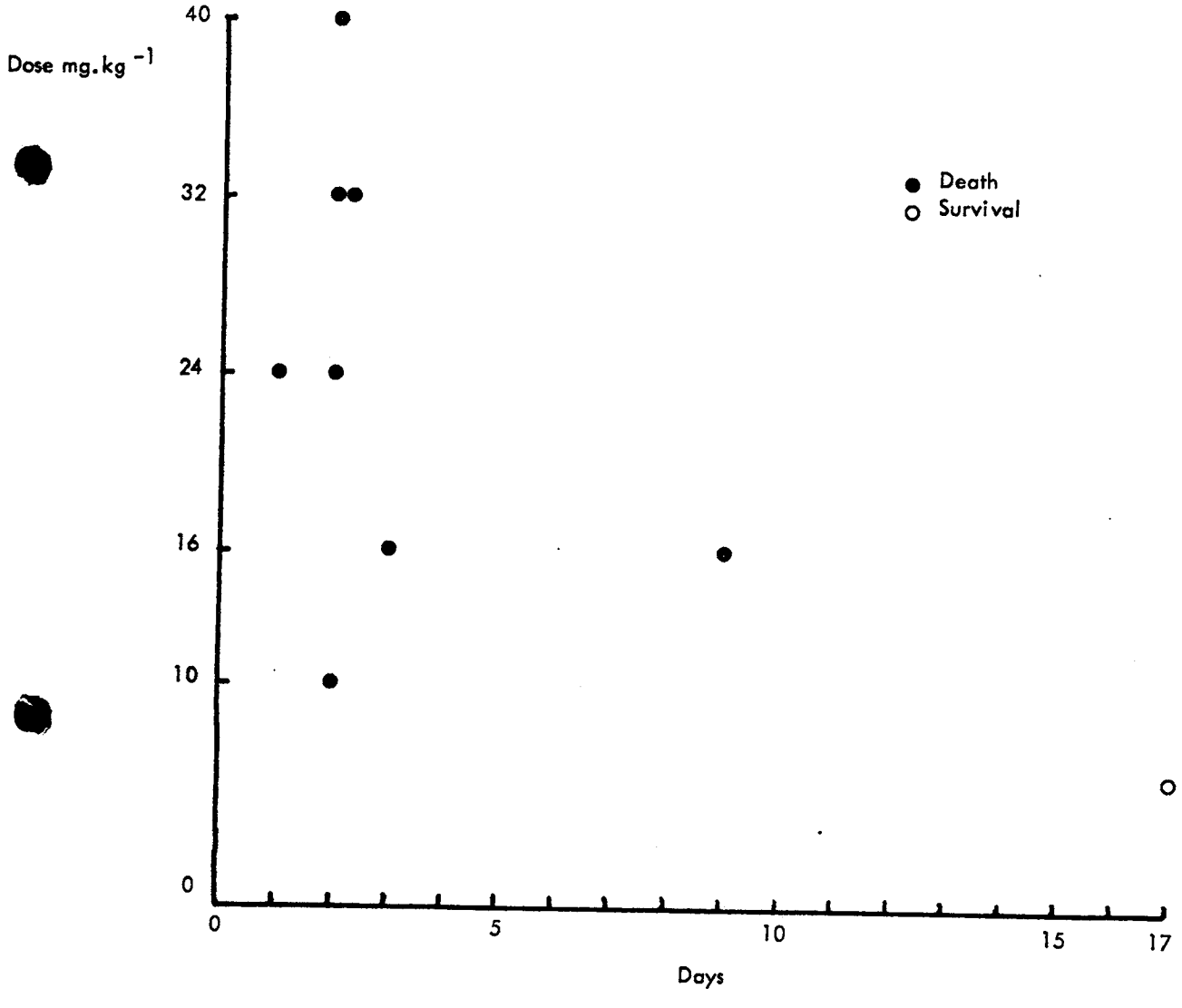
No changes were detected.

Electrocardiography

Four of the 5 animals tested after dosing showed tall, narrow-peaked T-waves on leads V_1 and V_2. In addition 1 and 8 had prominent notched P-waves on V_1 and V_2, while No. 6 had inverted T-waves and broad flattened P-waves.

FIGURE 4

A comparison of dose level with survival time for monkeys dosed intravenously with paraquat



Lung function tests

The condition of the animals was so poor that only one (No. 9) could be used successfully for lung mechanics testing, and 3 for lung ventilation (Nos 6, 9 and 10). All three had high respiratory rates, low tidal volumes and large respiratory minute volumes.

Lung mechanics (Appendix 2)

No. 9 showed a marked fall in lung compliance on the two occasions after dosing when it was tested.

Lung ventilation (Appendix 3)

All 3 showed increases in the cumulative tidal volumes to 2 % nitrogen, after dosing. No. 9 was tested on 3 occasions and exhibited a progressive increase in respiratory minute volume, although the CVT - 2 % reached a maximum on day 2 and some improvement was apparent on days 4 and 8 (see Figure 5).

Blood gases (Appendix 4)

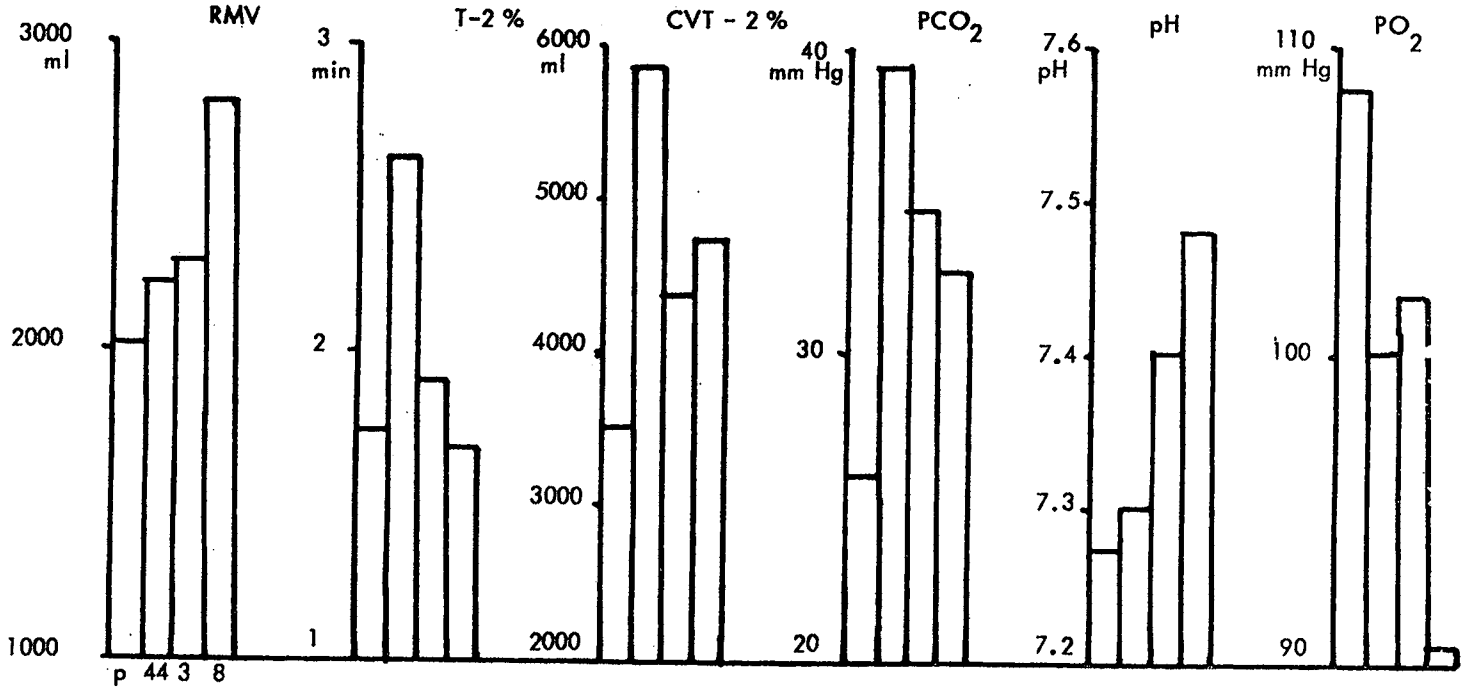
The 6 animals tested all had low pH, low PCO_2 and high PO_2 when tested pre-exposure. After dosing all except No. 9 showed a further fall in pH, accompanied by a further drop in PCO_2 in Nos. 1, 8 and 16. A marked increase in PO_2 occurred in Nos 6, 8 and 16. In the case of Nos 9 and 10, which were tested several times, the pH rose progressively.

Haematology, Biochemistry and Urinalysis (see(Appendix 5-7)

The results of the pre-exposure blood analyses of 3/6/74 on the 6 animals then intended for dosing tests showed some slight abnormalities. The experiment was postponed for one week and 10 animals were then bled on 11/6/74. All 10 animals were slightly abnormal, but since the results of the experiment were urgently required, the study was started on 17/6/74 and 4 animals were used as controls for the blood parameters throughout the study. These 4 animals subsequently showed very few readings outside the range considered to be normal.

FIGURE 5

Changes in lung ventilation and blood gas data
obtained from i.v. dosed animal No. 9 during the test period



The 4 columns shown for each test represent the following:

Column 1	Pre-exposure levels
Column 2	44 hours after dosing
Column 3	3 days after dosing
Column 4	8 days after dosing

The test animals developed abnormal values in several parameters, particularly in plasma urea, SGPT, Ca^{++}, urinary pH and urinary volume, as shown in Figure 6. Glucose, reducing substances and blood pigments appeared in the urine, and there was an increase in urinary protein.

Paraquat was detected in the urine after dosing, and urinary paraquat levels and volumes are shown in Table 1.

Bacteriology

The cultures from animals Nos 1, 6 & 9, contained Gram positive Cocci in chains and Gram positive motile rods. The cultures from No. 10 contained Gram positive motile rods.

Autopsies

Group I Monkey No. 1m

Lung:	left lung - occasional areas of moderate congestion.
Liver:	marked generalized pallor
Kidneys:	moderate bilaterally uniform pallor
Spleen:	large and darkly discoloured.
Pancreas:	multiple areas of marked congestion.
Stomach:	gross gaesous distension
Duodenum:	moderate gaseous distension
Colon:	moderate gaseous distension
Descending Colon:	scattered areas of moderate mucosal congestion, probably due to the presence of a parasitic infestation by an Oesophagostomum sp.
Ileo-caecal valve:	congested. Probably due to the presence of a parasitic infestation by an Oesophagostomum sp.

Monkey No. 2m

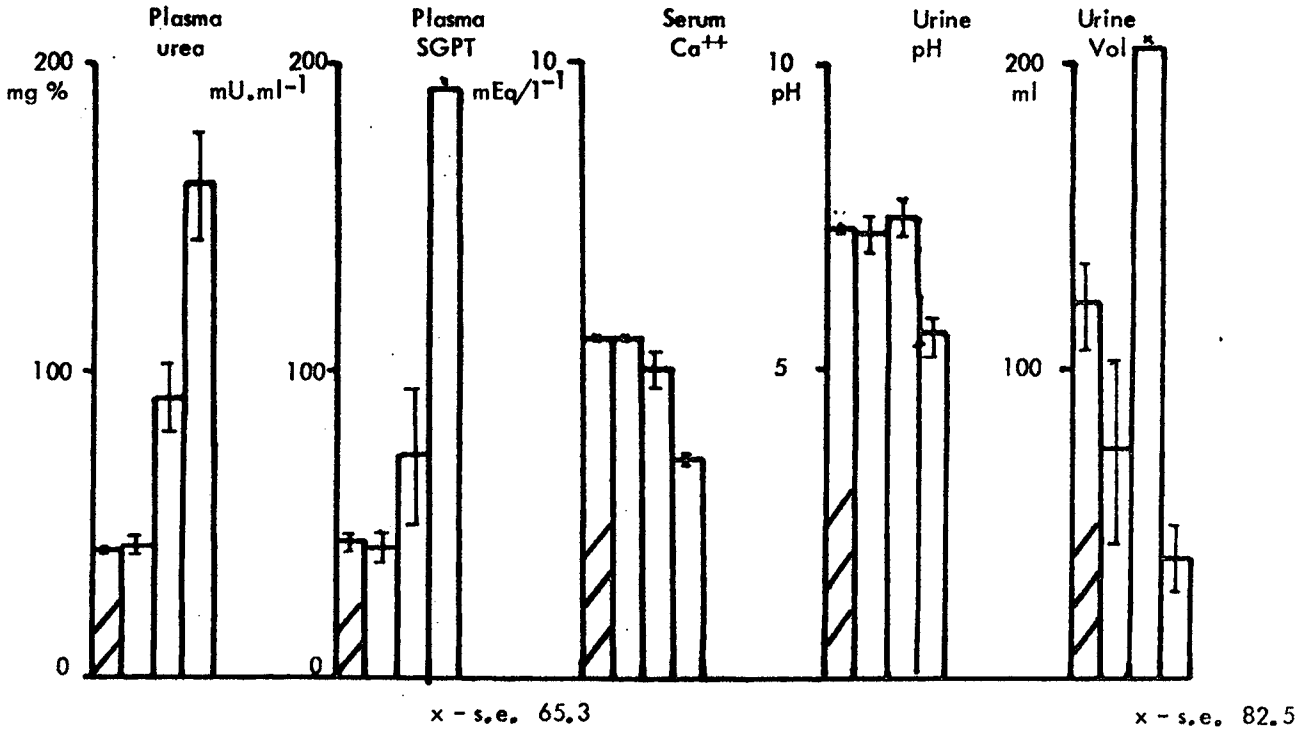
Lung :	congestion/consolidation
Liver:	generalised pallor
Kidneys:	generalised pallor

Group II Monkey No. 6m

Lung:	left anterior lobe, hilar region: an area of congestion/consolidation (15 x 8 mm). Posterior margin of left posterior lobe - a similar area (15 x 12 mm). Right mid-lobe, anterior border - an area of congestion/consolidation (20 x 10 mm). Right anterior lobe - and adhesion to the fourth and fifth intercostal spaces, probably due to lung function procedures.
Liver:	marked generalized pallor
Kidneys:	moderately severe bilaterally uniform pallor.
Stomach:	moderate gaseous distension
Ileum:	moderate gaseous distension. Some serosanguineous fluid present.
Tongue:	lateral aspects of the root - two areas of localized congestion.

FIGURE 6

Mean changes in blood and urine parameters from all i.v. dosed animals



The 4 columns shown for each test represent the following:

Column 1	(hatched at base) mean values of 19 untreated cynomolgus monkeys, tested on one occasion
Column 2	Pre-exposure levels of test animals
Column 3	24 hours after dosing
Column 4	48 hours after dosing

Standard Error bars cre shown

TABLE 1

Urine volumes and paraquat levels - i.v. dosed animals - ICI/40

Days after dosing	Group I				Group II				Group III				Group IV	
	Animal No. 1		2		6.		8		9		10		16	
	Vol	PQT	Vol	PQT	Vol	PQT	Vol	PQT	Vol	PQT	Vol	PQT	Vol	PQT
1	355	103	105	95	165	74	55	41	605	17	85	44	68	15
2	80	0	2	0	1	0	Dead		33	tr	57	tr	61	tr
	Dead		Dead		Dead				46					
									98	tr	43	0	Dead	
									50					
									50	0	Dead			
									50					
									150					
									113					
									Dead					

Urine volumes are in ml, and paraquat levels in mg (= total amount in urine)

Monkey No. 8

Trachea:	frothy exudate
Lung:	consolidation
Liver:	pale, slight yellow discolouration
Kidneys:	pallid
Stomach:	moderate gaseous distension
Colon:	moderate gaseous distension

Group III Monkey No. 9

Lung:	all lobes, gross consolidation and congestion
Heart:	enlarged, bubbles of gas in pericardium
Liver:	yellow tinge and pale spots
Kidneys:	pale and speckled
Fat:	fat deposits in mesenteries and pericardium bright yellow
Stomach:	moderate gaseous distension
Ileum	oesophagostomum infestation
Gall bladder:	light green

Monkey No. 10

Lung:	congestion/consolidation
Liver:	pale, all lobes spotted, particularly on underside
Kidneys:	pale
Gall bladder:	blue, filled with copious green viscous fluid
Spleen:	pale and bluish colouration
Gut:	oesophagostomum infestation

Group IV Monkey No. 16

Lung:	left and right lobes marked intralobar adhesions. Dependent borders of left lobes and costal aspect of right lobes, multiple fine costal to parietal pleural adhesions. Left and right posterior lobes, posterior margins, firmly adherent to the diaphragm. Right anterior lobe costal aspect, an area of consolidation 1.5 x 1.0 cm. The dorsal margin of the right anterior lobe was markedly congested. Dorsal aspect of left and right lung, scattered yellow subpleural nodules of up to 5 mm diameter, probably due to a lung mite infestation.
Liver:	marked generalized pallor
Kidneys:	marked bilaterally uniform pallor. Left kidney mid-central aspect, a subcapsular cyst 6 mm diameter.
Thyroid:	small
Stomach:	scattered areas of mucosal ulceration, punctate up to 5 mm diameter, mainly in the fundic region.

RESULTS OF ORAL DOSING STUDY (ICI/50 & ICI/52)

The symptoms shown by each animal, together with the results of all clinical investigations are summarised in the clinical signs sheets (Appendix 8). For the results of the ICI/50 study, where detailed investigations were made, a separate sheet is provided for each animal, while for the ICI/52 survival study, the results for each group are summarised. The results of the clinical investigations were as follows:

Clinical signs (Appendix 8)

All animals developed some symptoms, including an unpleasant ammoniacal odour and some degree of increased apocrine and sebaceous secretions. All of the Group I animals, 4 from Group II and 2 from Group IV had diarrhoea. Vomiting occurred overnight after dosing in Nos 22, 21, 28, 29, 17, 25 and 27 and only one of these No. 22 (Group I) subsequently died. Three animals from Group I and 3 from Group II rapidly became ill and died within 3 days (see Figure 7). Two further Group I animals died later, together with one animal from Group IV (No. 12); a second Group IV animal (No. 17) became very ill, with marked dyspnoea, at 10 days, but subsequently recovered. The usual terminal symptoms were that the animal became lethargic, with some dyspnoea, especially when any stress was involved, such as removing the animal from its cage for bleeding. Obtaining arterial and venous blood samples was also very difficult at this stage. Within any one dose group the animals which died tended to be those with the highest urinary paraquat levels (see Figure 8 and Table II). Of the 3 animals surviving in Group II, 2 were dosed with a small amount of 'Complan' in the paraquat solution; these had the lowest urinary paraquat levels of the 6 animals in this group.

Radiography

No changes were detected.

Electrocardiography

Some abnormalities occurred in the T-waves which were not present pre-exposure. Just prior to death No. 12 had very high narrow T-waves, and No. 14 had inverted T-waves. Slight T-wave abnormalities were also observed in Nos 4, 7 and 26.

FIGURE 7

A comparison of dose level with survival time for monkeys dosed orally with paraquat

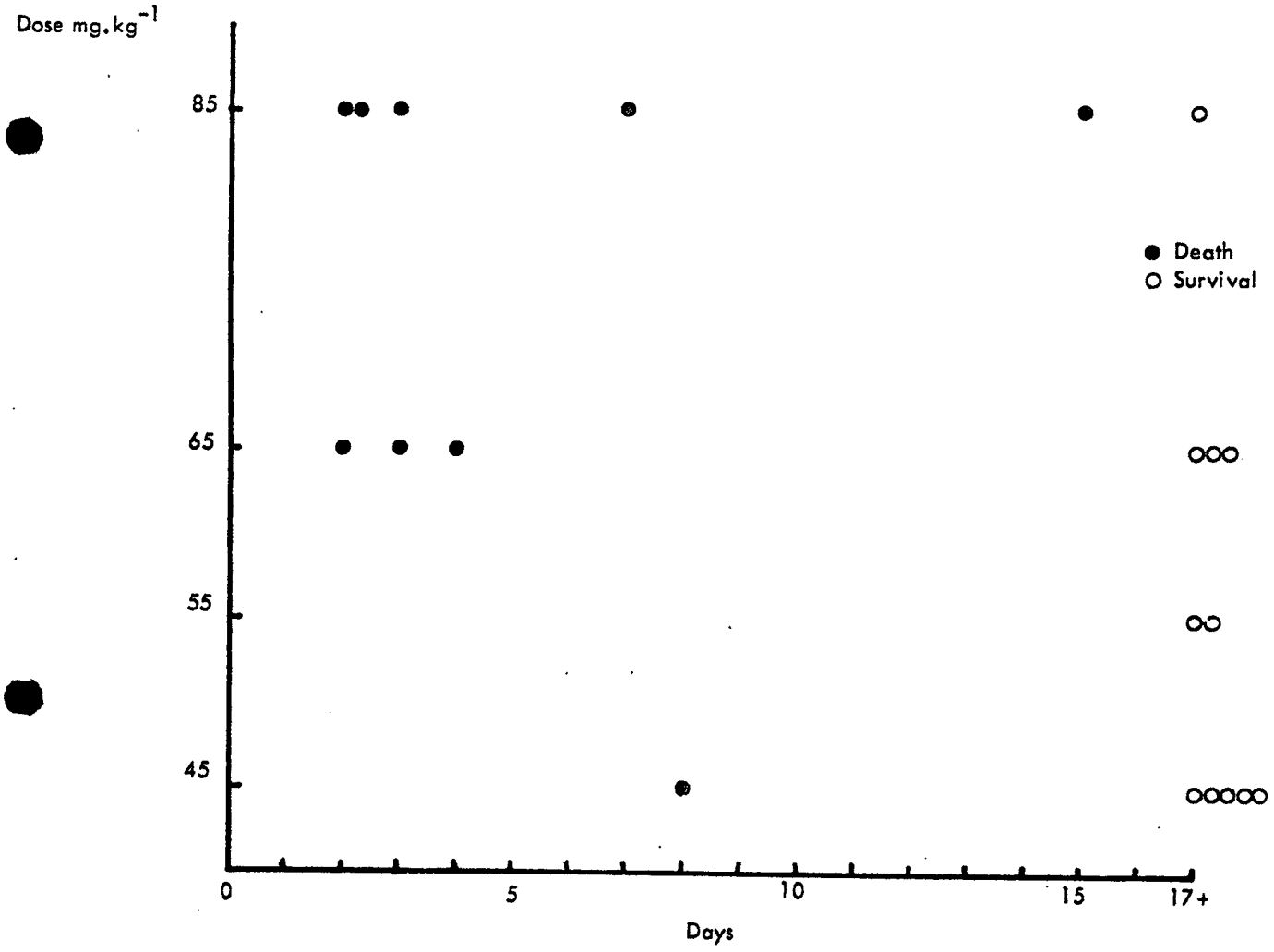


FIGURE 8

A comparison of total urinary paraquat and survival time for orally dosed animals

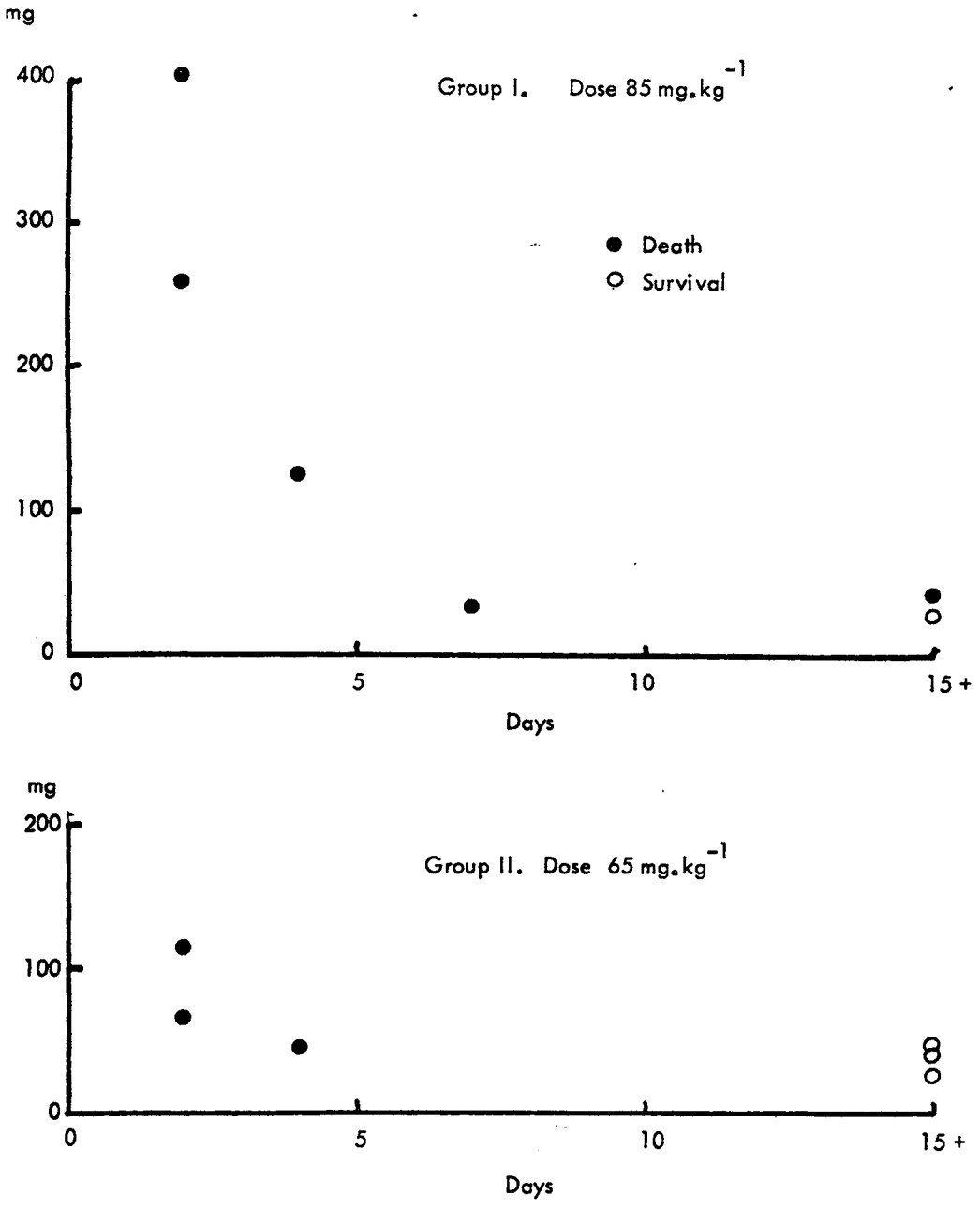


TABLE II

Urine volumes and paraquat levels - orally dosed animals

GROUP I												
Day	Animal No.											
	14		26		22		11		18		15	
	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT
Pre-experiment	156	0	33	0	109	0	110	0	109	0	143	0
(dosed)												
1	420	403	70	25	78	29	220	117	318	260	170	10
2	290	4	20	10	8	tr	52	7	1.4	0.7	132	22
3	Dead		.		60	13	26	0.4	Dead		210	tr .
4			130	0	140	0	Dead				186	0
5			0									
6			48	0								
7			Dead		(Dead day 15)							

GROUP II												
Day	Animal No.											
	4		7		21		13		20		5	
	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT
Pre-experiment	40	0	134	0	89	0	62	0	217	0	60	0
1	70	18	104	35	252	45	178	116	350	42	310	12.5
2	100	2.4	176	6.9	46	1.7	72	?	?		114	53
3	226	3.6	224	tr.	156	0	Dead		?		Dead	
4	55	0	108	tr.	318				84			
5	.		.						Dead			

volumes in ml
paraquat levels in mg
? indicates sample lost
. indicates sample included in next day's volume

TABLE II

(continued)

GROUP III				
Animal No.				
Day	26		29	
	VOL	PQT	VOL	PQT
Pre-experiment	159	0	198	0
1	70	4	240	187
2	144	1	356	tr.
3	78	tr.	280	0
4	42	0	110	

GROUP IV												
Day	Animal No.											
	3		12		17		24		25		27	
	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT	VOL	PQT
Pre-experiment	50	0	84	0	64	0	200	0	76	0	108	0
1	72	6.3	70	147	270	169	192	11	218	69	164	104
2	38	4.5	1	0.4	64	0.6	?		88	1	48	1
3	.		.		84	0	348		38	0	80	0
4	.		.		110		314		102		214	
5	538	0	306 (Dead day 8)	0								

Bacteriology

Bacteriological investigations showed that the lungs of all animals contained bacteria. The results of the tests are shown in Table III.

Lung function tests

Lung Mechanics

No significant changes were observed, but after dosing animals required much longer to recovery from anaesthesia. Considerable difficulty was experienced in getting consistent results using the oesophageal balloon technique on anaesthetized animals.

Lung ventilation (Appendix 9)

The hyperventilation recorded from the i.v. dosed animals was absent from the orally dosed groups, with the exception of No. 26; and respiratory minute volumes were generally lower than the pre-exposure values. However all animals had increased cumulative tidal volumes to 2 % nitrogen at some stage of the test period. This was most marked in the high dose group animals Nos. 14, and 26, which died at 2 and 8 days respectively, and a low dose animal (No. 12) which died at 7 days. For the 3 animals the CVT - 2 % N_2 was increased progressively when tested at 48 hrs. and 4 days. For the other animals, all of which survived the test period, the highest values tended to occur after 7 days. Following a similar pattern, the nitrogen washout times were increased initially, followed by an improvement at 7 days, after which they were again increased in surviving animals. It must be stressed that the results of this test can be very variable under normal conditions, so that with such small groups of animals any interpretation of these figures must be tentative.

Blood gases (Appendix 10)

All animals had low pH, low PCO_2 and high PO_2 when tested pre-exposure, and the 2 animals that died after one week (Nos 12 and 26) exhibited further increases in these parameters at 48 hours. Both animals then developed low PO_2 when tested pre-terminally. No changes considered to be significant were observed in the other animals.

Haematology (Appendix 11)

All animals except those in Group IV developed increased platelet concentrations, and Nos. 28, 29 and 30 had increased erythrocyte sedimentation rates. There was also a slight increase in clotting times.

TABLE III

Bacteriology results

Animal No.	Gram stain and morphology	Sensitivity to Antibiotics				
		Penicillin 10 µg	Streptomycin 10 µg	Ampicillin 10 µg	Neomycin 10 µg	Septrim 25 µg
12m TSB	Gram +ve rods plus Gram +ve cocci in chains	S	S	S	S	S
Thio	Gram +ve rods	S	R	R	S	S
3m TSB	Gram +ve cocci in bunches	S	S	S	S	S
	Gram +ve cocci	N.B.G.	N.B.G.	N.B.G.	N.B.G.	N.B.G
4m TSB	Gram +ve cocci	S	R	S	S	S
	Gram +ve rods	S	S	S	S	S
	Gram +ve rods	R	S	R	S	S
Thio	Gram +ve cocci	N.B.G.	N.B.G.	N.B.G.	N.B.G.	N.B.G
7m TSB	Gram +ve cocci chains	S	R	S	R	S
	Gram +ve cocci in bunches	R	S	S	S	S
Thio	Gram +ve cocci in chains	S	R	S	R	S
	Gram -ve rods	S	S	S	S	S
26 m TSB	Gram +ve cocci	S	S	S	S	S
Thio	Gram +ve cocci	S	S	S	S	S
28 m TSB	Gram +ve cocci in bunches	S	S	S	S	S
Thio	Gram +ve cocci	S	S	S	S	S
29 m TSB	Gram +ve cocci in bunches	S	S	S	S	S
Thio	Gram +ve cocci in chains	S	R	S	S	R

S = Sensitive R = Resistant N.B.G. = No Bacterial Growth

Biochemistry (Appendix 12 and Figure 9)

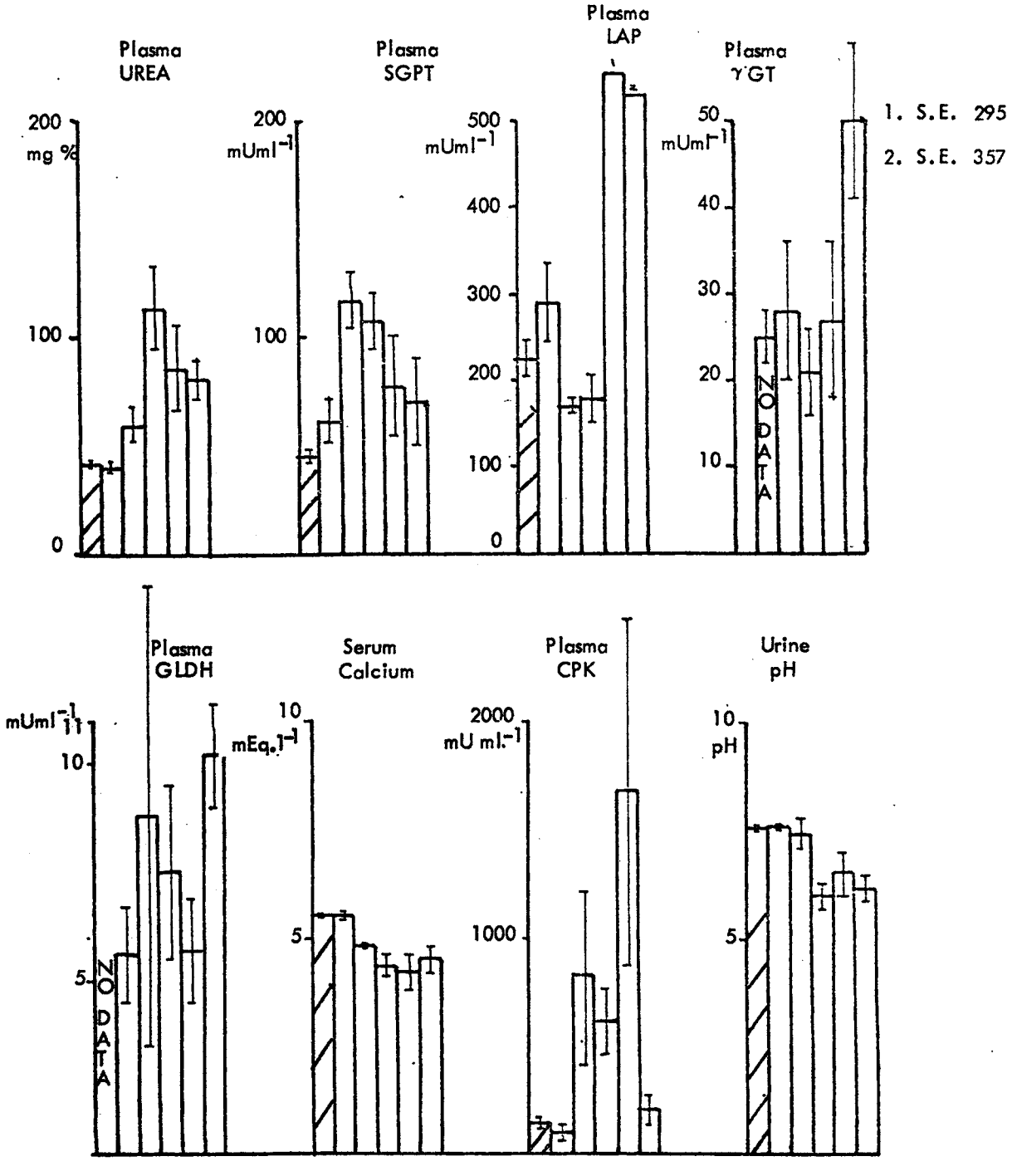
There was a marked increase in plasma urea, SGPT and bilirubin initially in all animals, and a decrease in Ca^{++} and K^{+}. These were followed later in the study by increases in γGT, GLDH and CPK, also LAP in Nos. 12 and 26.

Urinalysis (Appendix 13 and Figure 9)

Forty-eight hours after dosing there was a drop in pH in most animals, and most animals had polyuria at 24 hours followed by oliguria at 48 hours (Urinary volumes and paraquat levels are shown in Table II). The majority of animals also had glycosuria and haematuria, with increased urinary protein levels.

FIGURE 9

Mean changes in blood and urine parameters from all orally dosed animals



The 6 columns shown for each test represent the following:

Column 1: (hatched at base) mean values from 19 untreated cynomolgus monkeys, tested on one occasion.
Column 2: Pre-exposure levels of test animals
Column 3: 24 hours after dosing
Column 4: 48 hours after dosing
Column 5: 7 days after dosing
Column 6: Pre-terminal levels
Standard Error bars are shown

Autopsies

Group	Monkey No.	Observations
Im	14	Died/killed 31.7.74 Stored overnight 4°C. Good bodily condition. Liver: Moderate lobar swelling. Marked generalized pallor. Lung: Generalized congestion. Dorsal aspect of lobes most severely affected (possibly post-mortem effect) Right posterior lobe, posterior border adherent to diaphragm. Stomach: Fundic region, a single area of mucosal haemorrhage (6 x 3 mm).
	26	P.M. 1 hour after death. Lung : Scattered punctate areas of congestion.
IIm	4	Stomach: Glandular region, mucosal surface, a pale raised area (6 mm diam).
	7	Tongue: Occasional dark areas (up to 7 mm diam).
IIIm	28	Intestinal mesentery: Containing 7 parasites (7 m x 2 mm) Portions of small intestine adherent to right abdominal wall. Lung: Right posterior lobe, minimally adherent to parietal pleura. Left posterior lobe, minimally adherent to diaphragm. Right lobes, minimally adherent to each other.
	29	Lung: Left lobes, minimally adherent to parietal pleura. All lobes, multiple dark punctate foci. Portions of small intestine adherent to right abdominal wall. Intestinal mesentery: Containing parasites (7 x 2 mm). Stomach: Gastric/oesophageal junction, a raised pale mass (10 mm diam). Small intestine: A band of haemorrhage over the 30 mm section.

Group	Monkey No.		Observations
IV m	3	Lung:	azygos lobe, an area of consolidation (10 x 15 mm). right anterior lobe, a subpleural fissure (20 x 2 mm).
		Intestinal mesentery:	a haemorrhagic nodule (12 mm diam). Cut surface: white caseous material.
		Kidneys:	minimal bilateral uniform pallor. Left kidney periphery of pelvis, a white band (2 mm diam).
	12	Lung :	generalized congestion. Right posterior lobe, adherent to parietal pleura and diaphragm.

DISCUSSION

Intravenously dosed animals

Paraquat is found to be highly toxic when administered intravenously at the dose levels used in this study. There was some relationship between dose levels and death rate in that the high dose animals tended to become ill the most rapidly, while one low dose animal (No. 9) survived for 9 days, and an animal dosed with 6 $mg.kg^{-1}$ showed no adverse symptoms. However an animal later given 10 $mg.kg^{-1}$ was extremely susceptible, and died within 2 days.

From the results of the tests performed it is clear that in the initial stages the kidney is the most seriously affected target organ, and that the lung and possibly the liver are affected later. Analysis of blood and urine samples indicates that all the animals were suffering from acute renal failure by 48 hours after dosing, and exhibited the following symptoms:

high plasma urea in all animals.
High serum potassium in 6, 8 and 16.
High SGPT levels in all animals.
Increased clotting times in 1, 6, 8 and 10.
low serum calcium in 1,6,8,9 and 10.
Glucose and reducing substances in the urine of all animals.
Oliguria in all animals except no. 1.
Low blood and urine pH in all animals except No. 8.
Blood pigments in the urines of 2, 6, 8 and 16.

These were accompanied by the presence of the following secondary symptoms:

ammoniacal odour due to bacterial breakdown of salivary urea.
Hyperventilation due to acid-base disturbance.
Tall sharply peaked T-waves in the ECG (possibly due to hyperkalemia).
Lethargy, vomiting and muscular twitch.
Pale kidneys.

For the first night after dosing,urine production was increased but the blood sample taken 15 hours after dosing showed already elevated plasma urea levels. It seems likely that this may have been due to excessive production of urea rather than a failure of the kidney to eliminate it. At 48 hours most animals had excreted small amounts of urine (virtual anuria in the case of 3 animals) and this was accompanied by a further considerable increase in plasma urea levels (Figure 6and Appendix 13)

Pulmonary function tests.

The lung function tests and blood gas analyses were difficult to perform because of the poor health of the animals after dosing, and could only be carried out in a few cases. Blood gas data were influenced by 3 factors; stress, renally induced acidosis and direct lung damage.

The stress factor affects the results because when animals are restrained and an arterial blood sample is removed they tend to struggle and hyperventilate.causing low PCO_2 and high PO_2 values, accompanied by transitory metabolic acidosis.

The second cause of metabolic acidosis is the failure of the kidney to maintain a normal acid-base balance. This leads to an excessive production of CO_2, as the acid is buffered by the blood bicarbonate, giving a high blood PCO_2 which in turn causes hyperventilation resulting in a high PO_2. This occurred in all animals at 48 hours, the symptoms produced depending upon the severity of the acidosis, and there was some relationship between the dose levels of paraquat and the degree of response. For the high dose animal (No. 1), this led to a very low pH and bicarbonate concentration, but a low PCO_2 and little evidence of hyperventilation. Both of the Group II animals showed the classical response in that the PCO_2 was raised due to the buffering action of bicarbonate, and the PO_2 was very high, indicating a considerable degree of hyperventilation. The Group III animals showed signs of mild acidosis, in that the PCO_2 was elevated slightly, and the pH slightly acidic, but normal PO_2 values gave little evidence of hyperventilation. The Group IV animal (No. 16) appeared to be highly susceptible to paraquat, and although it received a low dose of 10 $mg.kg^{-1}$ it behaved like a high dose animal, with a very low pH and a very high PO_2 (Appendix 10).

The nitrogen washout and lung mechanics tests results indicate that apart from the metabolic/renal effects on respiration there was also some direct lung damage. The increased CVT - 2 % N_2 from the nitrogen washout tests of 6,9 and 10 at 44 hours shows that ventilation was impaired at this stage. No. 9 which was tested on 3 occasions (Figure 5) shows some improvement in ventilation after 44 hours, but the further impairment of lung function can be deduced by comparing the PO_2 of the pre-exposure and pre-terminal blood samples with RMVs from the washout tests done on the same days. When the pre-exposure RMV was 2000 ml, the PO_2 was 108.7 mm Hg, while pre-terminally when the RMV was 2800 ml, the PO_2 was only 90.4 mm Hg.

This lung damage may be partly due to oedema caused by the renal failure, and partly due to the direct effect on the lung of paraquat. It seems likely that the poor condition of No. 9 after 8 days, when it had apparently overcome the renal symptoms, and the congestion and consolidation of the lung in most animals when examined after death, must be due to paraquat. In No. 9, where the paraquat had longer to work, the lungs were more severely damaged than in the other animals, and the fall in dynamic lung compliance suggests that they had become mechanically 'stiffened'.

T-wave abnormalities were detected in several cases, and notched P waves were recorded from No. 1. The very high creatine phosphokinase level in No. 16 may be indicative of myocardial or general muscular damage.

Orally dosed animals

In general the symptoms shown by the orally dosed animals were similar to those of the intravenously dosed groups, but less severe. The majority of animals had increased urine production and evidence of kidney damage within 24 hours, followed by oliguria or anuria at 48 hours. By this time they had the same symptoms of renal failure as the intravenously dosed animals, namely: high blood urea, high SGPT, low urine pH, glycosuria, low Ca^{++} and increased clotting times. However the urea was not as high as in the i.v. animals and severe metabolic acidosis was detected in only one animal (No. 26), which had the low pH and high PO_2 characteristic of the i.v. groups at 48 hours.

Of the 8 animals on which lung function tests were performed, early changes were detected in 3 animals, the high dose group Nos 14 and 26 and a low dose animal No. 12. These then died at 2, 8, and 7 days respectively, 12 and 26 having low PO_2 pre-terminally. For the other animals, all of which survived the test period, the most severe lung symptoms tended to occur after 7 days. It seems likely that all the animals went through a renal crisis at 48 hours, when No. 14 died, while paraquat-induced lung damage increased progressively, leading to the deaths of 12 and 26 and lung function impairment in the other animals at 7 - 15 days.

There was evidence of liver damage from the raised levels of Gamma GT and GLDH in all animals, especially in Nos.12 and 26, where LAP values were also increased. The very high CPK levels and T-wave abnormalities may indicate heart damage (Figure 9).

Considering the results from all the orally dosed animals, the relationship between the dose of paraquat and survival time does not appear to be a simple one, and it is not possible to make definitive statements on the basis of the small number of animals used in this study. However, two kinds of positive correlation do seem to be emerging. Firstly there is a relationship between the dose administered and the death rate (Figure 7), in that from groups of 6 animals 5 died at 85 $mg.kg^{-1}$, 3 at 65 $mg.kg^{-1}$, and 1 at 45 $mg.kg^{-1}$. Secondly there appears to be some correlation between the amount of paraquat excreted and the death rate for any particular dose level. It is therefore possible that animals which fail to excrete large amounts of paraquat have not absorbed all of the administered dose, and that this may account for the survival of some of the animals in the high and medium dose groups. This is supported by the fact that only one of the animals which vomited overnight after dosing (No. 22) subsequently died, and this was after 15 days. However, some paraquat must be absorbed fairly quickly, since No. 28, although it was sick 10 minutes after dosing, and other animals which were sick and had diarrhoea 3 - 4 hours after dosing all showed some symptoms of poisoning, in terms of abnormal values in their blood biochemistry.

A further complication is that the substance appears to cause both an acute and a sub-acute phase of toxicity. The acute phase reaches a peak at between 2 and 4 days when 6 of the 16 animals died, and when all the animals on ICI/50 showed blood and lung function changes. This is also the period when most of the i.v. dosed animals died, and is probably due to acute renal failure and general toxaemia. Animals surviving this period usually exhibit less severe symptoms for the next few days and then either go on to recover following a bout of increased lung dysfunction or die between 7 and 15 days, as did 3 orally dosed animals and 1 i.v. dosed animal. Unfortunately this later phase is the one where we expect to see the development of pulmonary fibrosis, and the one we are trying to induce. It is possible that it will be difficult to find a dose which will reliably induce the second phase in a large proportion of animals without killing most of them during the primary phase.

Whether or not animals die from acute renal failure, or survive long enough to succumb to the later lung damage, may well depend upon the dosing regime employed. The animals in this study were dosed 23 hours after being fed in the case of ICI/50, and 18 hours after being fed for ICI/52; they have been observed to eat as soon as they are fed, and during observations at night and during the early part of the day, they have rarely been seen to feed at other times. Their stomachs were therefore probably empty when they were dosed, and since the dose was contained in 40 ml of water a large amount of paraquat is probably absorbed quickly. This would have provided a high blood level of paraquat for a relatively short time, sufficient to cause the severe renal damage.

Since it is now known that paraquat is taken up actively by the lung from low blood concentrations, it is likely that if food was included with the dose to slow down absorption, it would be possible to induce lung lesions without first causing fatal renal damage. This is supported by the fact that of the 3 monkeys surviving 65 $mg.kg^{-1}$ of paraquat, 2 received a small amount of 'Complan' with the dose.

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APPENDIX 1

Clinical signs - i.v. dosed animal (ICI/40)

Group I Animal No. 1 Dose 32 $mg.kg^{-1}$

Group	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
11/6/74		34 ml	Good, wt. 4150 Average food consumption 250 $g.day^{-1}$.	* Slightly high total protein, K^+, Na^+,	Normal
Test period - Dosed 17/6/74					
17/6	0		2 hours after dosing, no symptoms.		
18/6	1	355 ml 103 mg.pqt. Glucose and reducing subs.	Active, vomited overnight, ammoniacal smell, Food consumption 140 g.	High blood urea.	
19/6	2	80 ml. low pH glucose	in extremis, hyperventilation, hypothermia food consumption 145 g, killed 13.00 hrs. ECG-high, peaked T-waves, notched P-waves,	Slightly raised prothrombin index, and SGPT; high urea, low Ca^{++}, pH, PCO_2, and HCO_3.	

Autopsy: moderate lung congestion, pallid liver, pallid kidneys, large discoloured spleen, gaseous distension of gut.
(N.B. Food consumption figures refer to previous night's intake). * all animals showed these symptoms pre-expsoure.

APPENDIX 1

(continued)

Group I Animal No. 2 Dose 32 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
11/6/74		25 ml	Good, wt. 4600 g. Average food consumption 223 g.day^{-1}	Slightly raised urea, total protein, Na^+, K^+.	Normal
Test period - Dosed 17/6/74					
17/6	0		2 hours after dosing, lethargic, lying on bottom of cage, eating little, excessive salivation. Food consumed, 140 g.		
18/6	1	105 ml. 95 mg.pqt. Glucose, blood pigments	Lethargic, ammoniacal odour, oily secretions on body fur. Food consumed 35 g.	High urea, SGPT, bilirubin. (No gas data)	(No data)
19/6	2	2 ml Glucose, Reducing subs. Blood pigments, low pH	Died during previous night. Food consumed 40 g.		

Autopsy: Moderate lung congestion, pallid liver and kidneys.

APPENDIX 1

(continued)

Group II Animal No.6 Dose 24 mg.kg^{-1}

Date	Day	Urine	General conditions	Blood	Lung function
Pre-exposure					
11/6/74		19 ml	Good, wt 3400g. Average daily food consumption 228 g.	Slightly raised total protein, SGPT.	Normal
Test period - Dosed 17/6/74					
17/6	0		2 hours after dosing, lethargic, lying on bottom of cage, not eating, food consumed previous night 195 g.		
18/6	1	165 ml. 74 mg, pqt. Blood pigments	Food consumed, 80 g; lethargic.	High urea, SGPT.	
19/6	2	1 ml reducing subs, glucose, blood pigments low pH and α cells	Food consumed 35 g., in extremis hyperventilation, hypothermia, sporadic pulse, killed 11.30 hrs. ECG - T-waves abnormal and inverted abnormal-shaped P-waves.	High prothrombin index, urea, SGPT, LAP, K^+, PO_2 low pH, and Ca^{++}	Long N_2 washout time

Autopsy: Lung congestion/consolidation, pallid liver and kidneys, moderate gaseous distension of gut.

APPENDIX 1

(continued)

Group II Animal No. 8 Dose 24 $mg.kg^{-1}$

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
11/6/74		60 ml	Good, wt 3850 g. Average daily food consumption 223 g.	Slightly raised urea, total protein, Na^+ K^+.	Normal
Test period - Dosed 17/6/74					
17/6	0		Food consumed 220 g. 2 hours after dosing, lethargic, lying on bottom of cage, not eating.		
18/6	1	55 ml. 41 mg, pqt. Reducing subs., glucose blood pigments	Food consumed, 210 g, vomited overnight, lethargic. 26 hrs after dosing, in extremis, hyperventilation, hypothermia, prolapsed anus, pupil constriction, reflex intact but random eye movements, no blink reflex, cornea drying out and becoming opaque, killed 18.00 hrs. ECG - T-wave abnormalities.	Slightly raised prothrombin time, high urea, SGPT, LAP, bilirubin K^+, PO_2 low pH, Ca^{++}	(No data)

Autopsy: Lungs slightly oedematous, frothy exudate in trachea, pallid liver and kidneys, gaseous distension of gut.

APPENDIX 1

(continued)

Group III Animal No. 9 Dose 16 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
11/6/74		17 ml	Good, wt 3400 g. Average daily food consumption 210 g.	Slightly raised total protein, K^+, Na^+, pH.	Normal
Test period - Dosed 17/6/74					
17/6/74	0		Food consumed 245 g. 2 hours after dosing, no symptoms.		
18/9	1	605 ml. 17 mg pqt. trace of reducing subs.	Active, food eaten 170 g, but vomited overnight.	Slightly raised urea, Na^+.	
19/9	2	33 ml. tr. pqt. trace of reducing subs., glucose, low pH	Food consumed, 105 g, active.	High WBC, urea PCO_2 low Ca^{++}, pH.	Long N_2 washout time high RR, low VT. Low compliance.
20/9	3	46 ml trace pqt.	Less active, hyperventilation, food consumed 85 g. 300 g wt. loss.	pH, normal: PCO_2 lower.	
21/9	4	98 ml no pqt.	Slight recovery. Food consumed, fruit only but double measure - 140 g.		Long N_2 washout time, high RR, low VT, but symptoms less severe than previously.
22/9	5	50 ml approx.	Active, but occasional coughing, hypothermic and shivering, hyperventilation.		

APPENDIX 1

(continued)

Group III Animal No. 9(cont)Dose 16 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
23/9	6	50 ml approx.	From this day onward lethargic, but condition appears slightly improved, however exhausted by stress of lung tests on day 8, and died after being bled on day 9.		
24/6	7	50 ml approx.			
25/6	8	150 ml		Low PO_2.	Long N_2 washout time, high RR, low VT. compliance further reduced.
26/6	9	113 ml. glucose, reducing substances	wt. loss 550 g.	Raised ESR, retics, platelets, high urea, glucose, SGPT, K^+ CPK. low Ca^{++}, PCV Hb, RBC, WBC.	

Autopsy: Lungs; gross consolidation and congestion, kidneys and liver pallid, heart enlarged with bubbles of gas in pericardium stomach distended with gas.

APPENDIX 1

(continued)

Group III Animal No. 10 Dose 16 $mg.kg^{-1}$

Date	Day	Urine	General condition	Blood	Lung ventilation
Pre-exposure					
11/6/74		136 ml	Good, wt. 3850 g, Average daily food consumption 215 g.	Slightly raised total protein, K^+.	Normal
Test period - Dosed 17/6/74					
17/6	0		Food consumed 190 g. No symptoms, eating		
18/6	1	85 ml 44 mg pqt. Reducing subs. glucose lowered pH	Food consumed 190 g. Lethargic, vomited overnight.	Raised urea.	
19/6	2	57 ml trace pqt. Reducing subs. glucose low pH	Lethargic, ammoniacal odour, food consumed 30 g.	Slightly raised PTI, high urea, SGPT, LAP, PCO_2, low Ca^{++} pH.	Long N2 washout time, low VT, raised RR, CVT.
20/6	3	43 ml no pqt.	Lethargic, eyes 'glazed', sporadic nystagmus, hyperventilation, hypothermia, died during lung function test at 18.00 hours. Food consumed 30 g. Wt. loss 350 g.	No data.	

Autopsy: Lungs, consolidation/congestion, kidneys and liver pallid, stomach distended with gas.

APPENDIX 1

(continued)

Clinical signs - i.v. dosed animals (ICI/50)

Group IV Animal No. 16 Dose 10 $mg.kg^{-1}$

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
		229 ml Glucose, Reducing subs	Good, wt 4500 g.	Normal	Normal
Test period - Dosed 22/7/74					
22/7	0		No symptoms.		
23/7	1	68 ml. 15 mg, pqt. glucose, blood pigments	Slightly lethargic.	High urea, SGPT, K^+.	
24/7	2	61 ml trace pqt. low pH. glucose, trace of reducing subs	09.00 hrs., Slightly lethargic. 14.00 hrs., Very ill, dyspnoea, hypothermia, low blood pressure - killed in extremis.	High urea, SGPT, K^+ CPK, PO_2, low glucose, pH, PCO_2.	

Autopsy: Lung: consolidation and congestion
Liver: pallid
Kidney: pallid

APPENDIX 2

Lung mechanics - i.v. dosed animals (ICI/40)

Group	Animal No.	Time	VT	RR	RMV	DEDC	VTP	CDYNL	RLI	RLE	RLI/RLE	RL
I	1	Pre-exposure Too ill to test	55.6	33	1805	54	4.3	14.06	.011	.012	118	.011
	2	Pre-exposure Died day 1	49.2	26	1242	53	4.1	12.39	0.23	.040	62	.029
II	6	Pre-exposure	48.0	28	1351	55	5.3	9.3	.035	.044	87	.042
		44 hours	34.6	44	1529	59	3.5	10.7	.005	.005	132	.006
		(Anaes. Intubated)	20.5	38	781	56	1.4	15.17	.011	.012	100	.012
	8	Pre-exposure Too ill to test	52.9	19	977	53	3.7	14.60	.014	.021	68	.019
III	9	Pre-exposure	38.3	33	1249	52	3.5	11.15	.013	.025	69	.016
		44 hours	25.1	95	2397	52	5.1	4.93	.010	.027	35	.017
		8 days	32.4	92	2976	55	10.3	3.20	.012	.019	68	.016
	10	Pre-exposure	45.0	25	1122	56	4.0	11.06	.010	.010	120	.009
IV	16	Pre-exposure (Anaes. Intubated) died day 2.	39.6	18	725	57	1.1	36.52	.017	.025	70	.023

APPENDIX 3

Lung ventilation - i.v. dosed animals (ICI/40)

Group	Animal No.	Time	VT (ml)	RR (min^{-1})	RMV ($ml.min^{-1}$)	T-2 % (min)	N-2 %	CVT-2 % (ml)
I	1	Pre-exposure	88.9	33	2932	1.07	35	3108
		Died day 2						
	2	Pre-exposure	91.6	29	2632	1.84	53	4820
		Died day 1						
II	6	Pre-exposure	53.2	35	1855	1.75	61	3238
		44 hours after dosing	48.6	41	1995	2.41	99	4815
	8	Pre-exposure	59.1	19	1134	2.43	47	2760
		Died day 1						
III	9	Pre-exposure	68.8	30	2021	1.73	51	3505
		44 hours after dosing	27.2	82	2222	2.63	216	5863
		4 days after dosing	29.7	77	2287	1.90	146	4350
		8 days after dosing	28.4	99	2808	1.68	167	4715
	10	Pre-exposure	78.8	22	1749	2.25	50	3940
		49 hours after dosing	35.8	38	1371	3.57	137	4875
IV	16	Pre-exposure	126	38	4766	0.48	18	2304
		Died day 2 - too ill to test						

APPENDIX 4

Blood gas analysis - i.v. dosed animals (ICI/40)

Group	Animal No.	Time	pH	PCO_2 (mm Hg)	PO_2 (mm Hg)	Base excess ($mEq.l^{-1}$)	Bicarb.conc. ($mEq.l^{-1}$)
I	1	Pre-exposure	7.281	26.6	108.2	-12.9	12.3
		44 hours	7.137	23.4	107.3	-20	7.6
	2	Pre-exposure	7.335	28.7	110.8	- 9	15
		Died day 1					
II	6	Pre-exposure	7.353	28.3	112.8	- 8.7	15.6
		44 hours	7.235	31.6	125.4	-13.0	13
	8	Pre-exposure	7.324	31.1	109.0	- 9	15.8
		26 hours	7.293	30.9	125.1	-10	12.8
III	9	Pre-exposure	7.270	25.9	108.7	-13.7	11.5
		44 hours	7.297	39.3	100	- 6.3	17
		3 days	7.398	34.6	101.8	- 2.3	21.2
		8 days	7.477	32.6	90.4	+ 1.3	24
	10	Pre-exposure	7.343	30.3	103.5	- 8.2	16
		44 hours	7.297	39.3	100.0	- 6.3	17
		3 days	7.371	23.8	105.5	- 9.8	13.5
IV	16	Pre-exposure	7.238	28.4	102.2	-14	11.7
		48 hours	7.130	22.0	132.7	-21	6.8

APPENDIX 5

Haematology - i.v. dosed animals (ICI/40)

Pre-exposure

Animal No.	ESR mm/hr.	PCV %	Hb g %	RBC 10^6/cmm	Retics %	WBC 10^3/cmm	%					Platelets 10^3/cmm	PTI secs
							N	L	E	B	M		
GP I 1	0	43	11.0	5.3	<2	9.0	34	62	4	0	0	435	11.4
2	0	45	11.9	5.3	<2	11.0	45	52	3	0	0	250	10.9
GP II 6	0	49	13.6	5.4	<2	6.9	41	58	1	0	0	290	11.1
8	0	42	11.6	5.4	<2	10.8	75	22	3	0	0	290	11.3
GP III 9	0	46	13.4	5.6	<2	10.4	65	32	3	0	0	385	10.6
10	0	44	12.6	5.3	<2	11.4	29	64	7	0	0	430	11.6
GP IV 16	0	46	13.4	5.2	<2	22.0	57	38	5	0	0	255	10.1
Controls													
3	0	44	11.6	5.4	<2	10.0	39	60	1	0	0	440	11.9
4	0	45	11.4	5.0	<2	12.2	72	27	1	0	0	345	11.4
5	13	38	9.4	4.7	<2	10.8	61	38	1	0	0	395	11.1
7	6	42	11.4	5.0	<2	11.6	62	35	2	0	1	540	11.4

24 hours after dosing

Animal No.	ESR mm/hr.	PCV %	Hb g %	RBC 10^6/cmm	Retics %	WBC 10^3/cmm	%					Platelets 10^3/cmm	PTI secs
							N	L	E	B	M		
GP I 1	0	46	14.2	5.4	<2	8.2	70	30	0	0	0	325	12.6
2	1	39	11.0	4.6	<2	10.8	83	17	0	0	0	280	12.8
GP II 6	0	42	11.2	5.0	<2	6.9	60	39	1	0	0	330	12.6
8	2	44	11.8	5.0	<2	9.4	77	19	1	0	3	490	13.8
GP III 9	0	40	10.9	4.9	<2	12.8	78	21	0	0	1	335	11.3
10	2	45	10.8	5.4	<2	11.0	87	13	0	0	0	415	11.8
GP IV 16	0	45	13.0	6.2	<2	8.2	77	23	0	0	0	490	12.3
Controls													
3	0	43	12.2	5.3	<2	9.0	54	42	3	0	1	260	12.3
4	0	42	11.2	5.0	<2	8.6	65	35	0	0	0	680	11.6
5	0	39	10.6	4.4	<2	16.0	72	25	2	0	1	290	11.4
7	3	40	10.4	4.6	<2	13.0	54	44	2	0	0	380	12.1

APPENDIX 5

(continued)

48 hours after dosing

Animal No.	ESR mm/hr.	PCV %	Hb g %	RBC 10^6/cmm	Retics %	WBC 10^3/cmm	% N	% L	% E	% B	% M	Platelets 10^3/cmm	PTI secs
GPI 1	0	48	14.0	5.6	<2	9.8	71	28	1	0	0	355	16.8
2	DEAD												
GPII 6	Clotted												17.6
8	2	43	11.3	5.3	<2	9.4	38	61	0	0	1	480	15.1
GPIII 9	1	44	11.4	5.4	<2	28.6	89	9	2	0	0	385	11.3
10	2	48	13.0	5.6	<2	24.8	89	11	0	0	0	430	13.8
GPIV 16	Clotted												16.6
Controls													
3	0	42	11.0	5.0	<2	11.0	41	54	1	0	4	335	11.8
4	2	43	11.0	4.9	<2	12.8	31	66	0	0	2	750	11.1
5	2	40	9.8	5.0	<2	19.4	77	20	3	0	1	305	10.5
7	3	41	11.0	5.1	<2	14.6	40	57	3	0	0	405	11.1

Terminal

Animal No.	ESR mm/hr	PCV %	Hb g%	RBC 10^6/cmm	Retics %	WBC 10^3/cmm	% N	% L	% E	% B	% M	Platelets 10^3/cmm	PTI secs
GP III 9	10	37	8.0	4.4	4.8	5.0	57	43	0	0	0	550	

APPENDIX 6

Clinical chemistry - i.v. dosed animals (ICI/40)

Pre-exposure

Animal	No.	Urea mg %	Glucose mg %	Total protein g %	Serum Proteins %					SGPT $mU.ml^{-1}$ units	LAP GR units	Bilirubin mg %	γGT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^{+} $mEq.l^{-1}$	K^{+} $mEq.l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mU.ml^{-1}$
					Alb	α1	α2	β	γ									
GPI	1	41	80	10.6	36	6	8	26	24	30	202	0.2			159	6.1	5.6	30
	2	52	92	10.2	43	6	10	34	7	48	100	0.3			164	5.7	5.4	31
GP II	6	44	86	9.6	37	5	10	36	12	66	224	0.3			154	4.4	5.3	88
	8	53	80	10.0	43	2	7	32	10	34	281	0.2			157	5.7	5.4	28
GP III	9	38	96	10.0	40	6	10	34	10	23	279	0.3			160	5.7	5.8	30
	10	30	90	10.6	34	8	14	33	11	46	256	0.2			150	5.8	5.4	25
GP IV	16	44	84	8.6	45	4	14	20	17	47	226	0.3	22.2	4.5	147	5.1	5.7	346
Controls	3	63	86	10.0	43	7	8	27	5	78	551	0.3			161	6.0	5.5	53
	4	40	94	11.0	34	7	14	33	12	50	289	0.3			158	5.7	5.5	15
	5	50	84	10.0	36	10	12	30	12	83	295	0.3			155	5.9	5.2	26
	7	48	84	10.8	37	6	10	30	17	49	218	0.2			158	5.8	5.8	41

APPENDIX 6

(continued)

24 hours after dosing

Animal No.		Urea mg %	Glucose mg %	Total protein g %	Serum Proteins %					SGPT $mU.ml^{-1}$ units	LAP GR units	Bilirubin mg %	γGT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^+ $mEq.l^{-1}$	K^+ $mEq.l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mEq.l^{-1}$
					Alb	α1	α2	β	γ									
GPI	1	106	90	10.0	55	3	8	23	11	35	191	0.3			151	5.0	5.2	36
	2	110	64	9.2	48	3	12	25	12	181	x	0.7			161	5.0	4.2	x
GPII	6	106	98	10.0	50	3	7	27	13	85	172	0.4			153	5.1	5.2	41
	8	56	72	9.6	46	3	10	26	13	33	151	0.4			154	5.2	4.2	x
GPIII	9	64	98	9.0	40	4	11	28	17	20	x	0.2			160	5.0	6.0	x
	10	66	94	7.6	45	6	10	27	12	x	x	0.2			154	4.7	5.0	x
GPIV	16	128	52	9.8	44	6	13	14	23	83	279	x			149	5.6	x	x
Controls	3	54	116	8.4	48	7	15	19	11	59	224	0.1			141	4.2	5.3	21
	4	33	94	9.6	36	3	11	32	18	46	175	0.1			152	5.1	5.7	x
	5	34	98	7.8	46	3	7	26	18	45	178	0.1			153	6.8	5.5	50
	7	40	100	9.4	52	4	9	22	13	34	143	0.1			152	5.6	5.6	11

APPENDIX 6

(continued)

48 hours after dosing

Animal No.		Urea mg %	Glucose mg %	Total protein g %	Serum Proteins %					SGPT $mU.ml^{-1}$ units	LAP GR units	Bilirubin mg %	γGT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^{+} $mEq l^{-1}$	K^{+} $mEq\ l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mEq.l^{-1}$
					Alb	α1	α2	β	γ									
GPI*	1	170	180	7.7	56	3	5	22	14	62	236	0.3			143	4.7	3.6	88
	2	DEAD																
GPII*	6	200	98	8.2	55	3	6	22	14	394	320	0.4			150	5.9	3.6	59
*	8	110	56	8.5	47	3	6	30	14	239	303	0.6			150	7.4	3.7	54
GPIII	9	102	78	7.5	48	4	10	30	8	36	260	0.1			152	4.5	3.8	55
	10	188	100	8.2	47	3	10	30	10	229	333	0.1			148	4.3	2.9	40
GPIV	16	196	32	8.5	x					x	217							3680
Control	3	44	100	7.3	56	2	4	25	13	55	398	0.1			155	4.9	5.3	41
	4	32	86	7.0	42	4	10	34	10	37	287	0.1			154	5.5	5.8	26
	5	42	90	6.1	51	3	10	25	11	41	298	0.1			150	3.9	5.0	19
	7	42	76	7.5	52	4	8	22	14	41	290	0.1			154	6.1	5.6	27

x Terminal

APPENDIX 6

(continued)

Pre-terminal

Animal No.		Urea mg %	Glucose mg %	Total protein mg %	Serum Proteins %					SGPT units units	LAP GR units	Bilirubin mg %	γ GT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^{+} $mEq.l^{-1}$	K^{+} $mEq.l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mEq.l^{-1}$
					Alb	1	2											
GPIII	9	89	111	6.8	40	4	8	35	13	60	170	0.3			144	6.4	3.1	1651
GPIV	16	224		8.5	40	6	11	30	13	687	242	0.4	5.1	1.0	178	9.8	5.7	1434

APPENDIX 7

Urinalysis - i.v. animals (ICI/40)

Pre-exposure

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
GP I	1	7.6	34	1030	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	2	6.6	25	1035	0	0	0	0	0	0	0	1	0	0	0	1	0	0
GP II	6	8.1	19	1042	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	8	6.9	60	1033	0	0	0	0	0	0	0	0	0	0	0	1	0	0
GPIII	9	6.0	17	1036	0	0	0	0	0	0	0	1	0	0	0	1	0	0
	10	7.5	136	1015	0	0	0	0	0	0	0	0	0	0	0	2	0	0
GP IV	16	7.5	229	1020	0	+++	+	0	0	0	0	1	0	0	0	1	0	0

APPENDIX 7

(continued)

24 hours after dosing

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
GP I	1	6.1	340	1020	0	+++	+	0	0	0	0	0	0	0	0	3	0	0
	2	7.9	100	1020	0	0	+	0	0	0	+	0	0	0	0	1	0	0
GP II	6	8.0	180	1015	0	0	0	0	0	0	+	0	0	0	0	2	0	0
	8	7.8	520	1031	0	+++	+	0	0	0	+	0	0	0	0	1	0	0
GP III	9	7.6	580	1005	0	tr	0	0	0	0	0	1	0	0	0	1	0	0
	10	6.6	80	1040	30	+++	+	0	0	0	0	2	0	0	0	3	0	0
GP IV	16	8.3	47	1025	10	0	+	0	0	0	+	2	2	1	0	2	0	0

tr = trace

APPENDIX 7

(continued)

48 hours after dosing

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
GP I	1	5.4	7.4	1020	10	tr	+	0	0	0	0	0	1	0	0	3	0	0
	2	5.7	3.6	1020	20	tr.	+	0	0	0	+	0	0	0	0	2	0	0
GP II	6	4.4	1.6	1025	10	+++	+	0	0	0	+	0	0	0	1	3	0	0
	8	6.8	0.2	1019	10	++	+	0	0	0	+	0	0	0	0	2	0	0
GP III	9	5.4	2.5	1026	20	tr	+	0	0	0	0	1	0	0	0	2	0	0
	10	5.0	4.9	1040	20	+++	+	0	0	0	0	0	2	0	0	2	0	0
GP IV	16	6.7	4.8	1025	10	tr	+	0	0	0	0	0	0	0	0	1	0	0

tr = trace

APPENDIX 7

(continued)

Pre-terminal (8 days)

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
GP III	9	68	110	1025	0	0	++	++	0	0	0	0	0	0	0	2	0	0

APPENDIX 8

Clinical signs - orally dosed animal (ICI/50)

Group 1. Animal No. 14 Dose 85 $mg.kg^{-1}$

Date	Day	Urine (ml)	General condition	Blood	Lung function
Pre-exposure 18/7/74		156	Good, wt. 5300 g. Average food consumption 230 day g.	Low pH, high gamma GT.	Good.
Test Period. Dosed 29/7/74 15.00 hrs.					
30/7	1	420. 403 mg. pqt. glucose, blood pigments.	Healthy.	High urea, SGPT, gamma GT at pre-exposure value.	
31/7	2	290. 4.mg pqt,	Weak, diarrhoea. Collapsed and was killed at 17.00 hrs.	High urea, SGPT, CPK, K^+, low gammaGT, pH.	Hyperventilation, long washout time.

Autopsy: Lung: Generalized congestion, Liver: Swollen, generalized pallor, Kidney: Normal

APPENDIX 8

(continued)

Group 1 . Animal No. 26 Dose 85 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure 30/7/74		44	Good, wt. 5000 g . Average daily food consumption 213 g	Slightly high SGPT, K^+;	Normal
Test Period. Dosed 31/7/74					
1/8	1	70 25mg pqt. glucose	Diarrhoea.	High glucose, SGPT, CPK .	
2/8	2	20 10 mg pqt. glucose, protein low pH	Diarrhoea.	High platelets, urea, SGPT Na^+, K^+, CPK, very low pH, PCO_2.	Long washout time high CVt.
3/8	3	65	Ibid.		
4/8	4	65	Ibid.	Low PO_2.	Very long washout time high CVt.
5/8	5	0	Diarrhoea, lethargic ,		
6/8	6	48	Lethargic, mucus secretion from nose,		
7/8	7	Low pH blood pigments	Oily secretions on body fur. Dyspnoea, lethargic.	High urea, glucose, SGPT, LAP, low Ca^{++},	
8/8	8		Died 14.00 hrs. wt 4800 g	High urea , glucose, SGPT LAP, gamma GT.	Improved washout time .

Autopsy: Lung: Scattered areas of congestion.

APPENDIX 8

(continued)

Group II Animal No. 4 Dose 65 $mg.kg^{-1}$

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
12/7/74		40 ml	Good, wt. 3750 g. Average daily food consumption 173 g.	High platelet count, low pH, low PO_2.	Normal
Test period. Dosed 15.00 hours 15/7/74			Dose mixed with approximately 5 g 'Complan' during administration. Regurgitated approximately 1 ml of fluid during dosing.		
16/7/74	1	70 18 mg. pqt. glucose, blood pigments.	Healthy, active, diarrhoea and some vomiting during night.	High SGPT, low K^+	
17/7	2	100 2.4 mg. pqt.	Ammoniacal odour of breath.	High urea, high SGPT, low K^+.	Long N2 washout time
18/7	3	226 3.6 mg pqt.	Ammoniacal odour, oily secretions on body fur.		
19/7	4	55 1.3 mg pqt.	Ammoniacal odour.		
20/7	5	61 0 mg pqt.	Ibid. Diarrhoea, wt loss 250 g.		
21/7	6	61	Ibid.		
22/7	7	61	Ibid.		N2 washout normal
29/7			Wt. loss 50 g - 2/8/74 wt. gain 100 g.		30/7/74 N2 washout time long, high CVt.
The animal had diarrhoea until 2/8/74, and increased apocrine and sebaceous secretions, after which it recovered. Slight drop in food consumption during first week. Terminal wt. 3650 g.					
7/8			Terminal test Autopsy: Lung, liver and kidney normal.	High urea	Long washout time, high CVt.

APPENDIX 8

(continued)

Group II (cont) Animal No. 7 Dose 65 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure 12/7/74		134	Good, wt.3950g Average daily food consumption 248 g	Normal, low pH, low PCO	Normal
Test period. Dosed 15.00 hrs 15/7/74			Dose mixed with 5 g 'Complan'.		
16/7	1	104 35 mg.pqt.	Healthy, active, slight shivering, diarrhoea.	High SGPT.	
17/7	2	176 6.9 mg.pqt. Blood pigments in urine, day 1 and 2,	Ammoniacal odour.	High urea, SGPT, gamma GT, low K^+, low pH, PCO_2	Normal
18/7	3	224 trace pqt.	Diarrhoea, low food consumption.		
19/7	4	108 no pqt.	Diarrhoea, normal food consumption, ammoniacal odour.		
20/7	5	71	Oily secretions on body fur. Diarrhoea no wt.loss.		
21/7	6	71			
22/7	7	71	Ibid.	High urea, gamma GT.	Longer washout time
30/7	15	56	Diarrhoea still, wt. loss 200 g		Washout time normal
5/8	24		Solid stools.		Longer washout time

Sacrificed on 8/8/74 terminal wt.3850 g

Autopsy: Tongue: Occasional dark areas.

APPENDIX 8

(continued)

Group III Animal No. 28 Dose 55 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
18/7/74		159	Good. wt. 4700 g . Average daily food consumption 246 g	Slightly high SGPT, low pH, PCO_2.	Normal, hyperventilating
Test period. Dosed 15.00 hrs 22/7/74			Vomited after 10 minutes, while still anaesthetised		
23/7	1	70 4 mg pqt. glucose, blood pigments	No symptoms.	High urea, SGPT, bilirubin, gamma GT.	
24/7	2	144 1 mg. pqt.		High urea, SGPT, platelets, low pH, PO_2	Long N2 washout time
25/7	3	78 trace pqt.			
26/7	4	42 no pqt.		Normal pH, PCO_2	Slightly improved N2 washout
27/7	5	145			
28/7	6	145			
29/7	7	145	(30/7/74) wt. loss 350 g Food consumption halved.	High urea, SGPT, gamma GT, platelets.	Washout further improved
30/7	8	88		High CPK, low pH, PCO_2	
31/7	9	200		(2/8/74) Low pH, PCO_2	Washout further improved.
2/8	11	96	Wt. loss further 250 g		
7/8	16			High urea, glucose, SGPT, gamma GT, low pH.	Longer washout time

Sacrificed 8/8/74 terminal wt. 4150 g

Autopsy: Slight lung adhesions

APPENDIX 8

(continued)

Group III Animal No. 29 Dose 55 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure 18/7/74		198	Poor, old animal. wt.4000 g .Average daily food consumption 242 g	High glucose, SGPT, low pH.	Long washout time
Test period. Dosed 15.00 hrs 22/7/74					
23/7	1	240 187 mg pqt. glucose, blood pigments	Lethargic, possibly slightly sick overnight.	High urea, SGPT, bilirubin, gamma GT.	
24/7	2	356 trace pqt. low pH		High SGPT, gamma GT.	Very long washout time
25/7	3	280 no pqt.	Lethargic.		
26/7	4	110	Lethargic.	Low pH, slightly high PO2.	Washout time to pre-exposure value
27/7	5	170			
28/7	6	170			
29/7	7	170 blood pigments	Low food consumption, wt. loss 300 g	High SGPT, CPK, platelets, ESR.	
30/7	8	38			Long washout time
31/7	9	136			
1/8	10	240			
2/8	11	304			Longer washout time
7/8	16	Blood pigments	Wt. loss further 100 g , low food consumption.	High urea, gamma GT, low pH,	Long washout time
8/8	Sacrificed wt.3500g				

Autopsy: Multiple dark punctate foci, slight adhesions. Small intestine, a band of Haemorrhage (30 mm).

Sacrificed 8/8/74 terminal wt. 3500g at which time animal still losing wt. very poor food consumption.

APPENDIX 8

(continued)

Group IV Animal No. 3 Dose 45 mg.kg^{-1}

Pre-exposure

Date	Day	Urine	General condition	Blood	Lung function
18/7/74		50	Good, wt.4750g Average daily food consumption 195 g.	Slightly high SGPT, low pH, PCO_2	Good
Test period. Dosed 24/7/74 17.00 hours					
25/7	1	72 6.3 mg.pqt. Glucose, Ketones.	Diarrhoea.	High urea, glucose.	
26/7	2	38 4.5 mg pqt. low pH, glucose.	Slight diarrhoea.	High urea, SGPT, CPK, low K^+.	Normal
27/7	3	179			
28/7	4	179			
29/7	5	179	Wt. loss 400 g. Food consumption slightly reduced.		
30/7	6	112	Ammoniacal odour.		
31/7	7	132 Glucose		High urea, glucose, CPK.	Increased N2 washout time
1/8	8	150			
5/8	12	86			Washout time greatly increased
8/8	15		Wt. loss further 300 g	High urea, glucose, gamma GT, slightly raised ESR.	Washout time normal
9/8	16	Sacrificed wt. 4050g			

Autopsy: Consolidation, subpleural fissure. Kidney: pallid.

APPENDIX 8

(continued)

Group IV Animal No. 12 Dose 45 mg.kg^{-1}

Date	Day	Urine	General condition	Blood	Lung function
Pre-exposure					
18/7/74		84	Good, wt. 4950g. Average food consumption 247 g.	High SGPT, LAP.	Good
Test period. Dosed 24/7/74 17.00 hrs					
25/7	1	70 147 mg.pqt. glucose, blood pigments	Green faeces.	High urea, SGPT.	
26/7	2	1 trace pqt.		Very high urea, high SGPT, CPK, gamma GT.	Long washout time
27/7	3	102			
28/7	4	102			
29/7	5	102	Cough, wt. loss 350 g . food consumption halved.		
30/7	6	86	Cough.	Blood gas normal.	Improved washout time
31/7	7	86 Ketones, glucose	Killed in extremis wt. 4600 g	High urea, SGPT, LAP, Na^+, K^+ CPK. Low glucose.	

Autopsy: Lung: congestion, adhesions.

APPENDIX 8

Clinical signs - orally dosed animals (ICI/52)

Group I Dose 85 $mg.kg^{-1}$

Date	Day	No. 22 Condition	No. 11 Condition	No. 18 Condition	No. 15 Condition
Test period.	Dosed 5/8/74	at 10.00 hrs.			
6/8/74	1	Vomited overnight.	Slight regurgitation of fruit, diarrhoea. Polyuria.	Blood in faeces, lethargic, diarrhoea at 20.00 hours	Diarrhoea.
7/8	2	Diarrhoea, oliguria.	Diarrhoea, lethargic, dyspnoea, slight oliguria.	Polyuria. In extremis, killed 10.00hours	Diarrhoea.
8/8	3	Slight vomiting overnight.	In extremis, dyspnoea, oliguria, killed 10.00 hours.		Diarrhoea.
9/8 - 13/7		No further symptoms.			
20/8	15	In extremis, dyspnoea, died.			Recovered.
Autopsy.		Lungs: consolidation.		Lung: no abnormalities. Kidney: normal. Liver: pallid.	

Synopsis: 1 death after 2 days (No. 18)
1 death after 3 days (No. 11)
1 death after 15 days (No. 22)

APPENDIX 8

Clinical signs - orally dosed animals (ICI/52)

Group II Dose 65 mg.kg^{-1}

Date	Day	No. 21 Condition	No. 13 Condition	No. 20 Condition	No. 5 Condition
Test period.	Dosed 2/8/74	at 10.00 hrs.			
2/8	0	Good.	Diarrhoea, 17.00 hours.	Good.	Good.
3/8	1	Polyuria, vomited overnight.	Diarrhoea, possibly vomited. polyuria.	Good, polyuria.	Good, polyuria.
4/8	2	Good, slight oliguria.	Lethargic.	Good.	Moribund - killed.
5/8	3	Diarrhoea.	Died overnight.	Good.	
6/8	4	Diarrhoea, polyuria.		Moribund killed.	
7/8 - 13/8		No further symptoms.			
Autopsy:			Lung: congestion/consolidation. Gut: mesenteries swollen and fatty grey-black colouration	Lung: consolidation/congestion. Multiple pleural adhesions to chest wall and diaphragm. Kidneys: pale and enlarged. Liver: pale and dark patches.	Lung: Congestion/ consolidation.

Synopsis: 1 death after 2 days (No. 5)
1 death after 3 days (No. 13)
1 death after 4 days (No. 20)

APPENDIX 8
(continued)
Clinical signs - orally dosed animals (ICI/52)

Group IV Dose 45 mg.kg^{-1}

Date	Day	No. 17 Condition	No. 24 Condition	No. 25 Condition	No. 27 Condition
Test period.	Dosed 2/8/74	at 10.00 hours			
2/8	0	Vomited.	Good.	Vomited.	Good.
3/8	1	Vomited overnight, polyuria.	Good.	Good, polyuria.	Slight vomiting overnight
4/8	2	No further symptoms.	Good.	Good.	Good.
5/8	3	No further symptoms.	Polyuria.	Oliguria.	Good.
6/8	4	No further symptoms.	Polyuria.	Good.	Good.
7/8	5	Increased apocrine and sebaceous secretions, lethargic.	Diarrhoea.	Good.	Diarrhoea.
8/8	6	Lethargic.	Diarrhoea.	Good.	Diarrhoea.
9/8 - 12/8	Condition of No. 17, worsening, reached a crisis on 12/8/74 with rapid shallow breathing, requiring great effort.				
13/8	11	Slight recovery, sitting on perch.			
14/8		Gradual recovery.			

Synopsis: No deaths, one animal very ill at 11 days, then recovered.

APPENDIX 9

Lung ventilation - orally dosed animals (ICI/50)

Group	Animal No.	Time	VT (ml)	RR (min^{-1})	RMV ($ml.min^{-1}$)	T-2 % (min)	N-2 %	CVT- 2 % (ml)
		Pre-exposure						
I	14		141	46	6560	.44	20	2875
	26		88.2	43	3796	.77	33	2910
II	4		53.5	54	2875	.81	44	2335
	7		64.6	52	3374	1.01	53	3397
III	28		107	43	4657	.84	36	3840
	29		60.9	28	1690	1.67	46	2777
IV	3		99.8	50	5033	.69	35	3495
	12		93.0	59	5531	.49	29	2732
		48 hours after dosing						
I	14		31.1	81	2515	2.05	166	5165
	26		80.4	49	3929	1.15	56	4505
II	4		42.6	46	1975	1.75	81	3450
	7		72.8	49	3593	.95	47	3395
III	28		49.7	31	1520	2.49	76	3780
	29		47.1	36	1689	3.04	109	5135
IV	3		62.2	50	3108	1.06	53	3295
	12		47.7	59	2831	1.18	70	3340

APPENDIX 9

(continued)

Group	Animal No.	Time	VT (ml)	RR (min^{-1})	RMV ($ml.min^{-1}$)	T-2 % (min)	N-2 %	CVT-2 % (ml)
		4 days after dosing						
I	14		DEAD					
	26		28.8	91	2615	2.40	218	6275
II	4		-					
	7		-					
III	28		49.7	29	1435	2.15	62	3080
	29		82.4	22	1852	1.65	37	2050
IV	3							
	12 (6 days)		40.7	102	4153	.98	100	4070
		7 days after dosing						
I	14		DEAD					
	26 (8 days)		38.9	64	2483	1.39	89	3460
II	4		40.0	59	2351	1.48	87	3480
	7		54.5	45	2424	1.64	73	3975
III	28		84.4	40	3382	1.04	42	3503
	29		53.7	37	1959	2.17	80	4250
IV	3		91.0	44	3991	1.14	50	4550
	12 (6 days)		40.7	102	4153	.98	100	4070

APPENDIX 9

(continued)

Group	Animal No.	Time	VT (ml)	RR (min^{-1})	RMV ($ml.min^{-1}$)	T-2 % (min)	N-2%	CVT-2% (ml)
		12 days after dosing						
I	14		DEAD					
	26		DEAD					
II	4		53.6	55	2929	1.49	82	4373
	7		56.5	44	2492	1.13	50	2805
III	28		70.0	31	2146	1.42	44	3040
	29		57.8	32	1862	2.89	93	5375
IV	3		77.1	44	3424	1.73	77	5935
	12		DEAD					
		Pre-terminal						
I	14		DEAD see 48 hours					
	26		DEAD see 7 days					
II	4		61.0	45	2776	1.41	64	3905
	7		61.7	38	2322	2.45	92	5680
III	28		90.4	32	2905	1.68	54	4880
	29		78.1	32	2526	2.23	72	5625
IV	3		60.2	46	2793	1.01	47	2830
	12		DEAD See 7 days					

APPENDIX 10

Blood gas analyses - orally dosed animals (ICI/50)

Treatment- Pre-exposure

Group	No.	Weight	pH	PCO_2 (mmHg)	PO_2 (mmHg)	Base excess (mEq litre)
I	14	5300	7.202	27.2	106.3	-16.0
	26	5000	7.270	27.1	111.0	-13.0
II	4	3750	7.212	32.8	96.5	-13.6
	7	3950	7.062	28.0	105.5	-21.8
III	28	4700	7.247	25.2	102.8	-14.8
	29	4000	7.185	33.2	101.3	-14.6
IV	3	4750	7.265	24.5	101.5	-14.0
	12	4950	7.332	26.3	106.2	-10.9

APPENDIX 10

(continued)

Treatment – 48 hours after dosing

Group	No.	Weight	pH	PC_2 (mmHg)	PO_2 (mm Hg)	Base excess (mEq litre)
I	14	5300	7.257	25.7	107.7	-14.1
	26	5000	7.116	21.3	113.6	-21.8
II	4	3500	7.439	27.5	96.0	- 4.9
	7	3950	7.259	26.9	101.1	-13.7
III	28	4700	7.265	27.9	91.9	-13.0
	29	4000	7.412	26.6	106.0	- 6.2
IV	3	4750	7.391	26.9	102.4	- 7.1
	12	4950	7.364	25.2	111.1	- 9.3

APPENDIX 10

(continued)

Treatment - 4 days after dosing

Group	No.	Weight	pH	PCO_2 (mm Hg)	PO_2 (mm Hg)	Base excess (mEq litre)
I (5 days)	26	5000	7.372	28.6	91.9	- 7.2
II	4					
	7					
III	28	4350	7.341	26.9	98.6	- 9.9
(4 days)	29	3700	7.273	29.8	107.0	-12.0
IV	12	4600	7.440	36.2	103.0	+ 1.1

APPENDIX 10

(continued)

Treatment-7 days after dosing

Group	No.	Weight	pH	PCO_2 (mm Hg)	PO_2 (mm Hg)	Base excess (mEq litre)
II	4	3500	7.399	32.9	90.1	- 3.2
	7	3950	7.344	30.1	105.9	- 8.2
III	28	4350	7.243	23.1	110.4	-16.0
	29	3700	7.338	35.2	108.3	- 5.8
IV	3	4600	7.332	29.6	99.5	- 9.0
	12	4350	7.050	23.2	69.7	- 2.4

APPENDIX 10

(continued)

Treatment -12 days after dosing

Group	No.	Weight	pH	PCO_2 (mm Hg)	PO_2 (mm Hg)	Base excess (mEq litre)
I	14	DEAD				
	26					
II	4	3450	7.353	28.2	107.8	- 8.8
(15 days)	7	3750	7.375	30.1	107.6	- 6.0
III	28	4100	7.202	25.5	112.6	-16.7
(11 days)	29	3600	7.248	35.6	100.8	-11.0
IV	3	4050	7.404	32.8	107.8	- 3.1
(12 days)	12	DEAD				

APPENDIX 10

(continued)

Treatment - Pre-terminal

Group	No.	Weight	pH	PCO_2 (mmHg)	PO_2 (mm Hg)	Base excess (mEq litre)
I	14	DEAD (see 48 hrs)				
	26	DEAD (see 5 days)				
II	4	3650	7.398	29.8	106.6	- 4.8
(21 days)	7	3850	7.329	29.3	110.2	- 9.3
III	28	4150	7.289	31.3	109.7	-10.2
(14 days)	29	3500	7.242	36.3	101.6	-11.1
IV	3	4050	7.441	37.5	107.0	+ 2.0
	12	DEAD (see 7 days)				

APPENDIX 11

Haematology - orally dosed animals (ICI/50)

Pre-exposure

Group	Animal No.	ESR mm. $hr.^{-1}$	PCV %	Hb g.100 ml. $blood^{-1}$	RBC millions. ul^{-1}	Retics %	WBC $x10^3$ cells ul^{-1}	%					Plate-lets $x10^3$ ul^{-1}	PTI secs
								N	L	E	B	M		
I	14	0	45	13.8	6.2	<2	10.6	53	47	0	0	0	390	10.8
	26	1	45	11.4	5.4	<2	6.8	32	60	5	0	3	310	11.6
II	4	2	43	11.0	4.9	<2	12.8	31	66	0	0	2	750	11.1
	7	3	41	11.0	5.1	<2	14.6	40	57	3	0	0	405	11.1
III	28	1	45	11.9	5.3	<2	8.6	22	77	0	0	1	300	10.6
	29	4	41	10.8	4.9	<2	9.6	20	67	11	0	2	385	10.9
IV	3	0	42	11.0	5.0	<2	11.0	41	54	1	0	4	335	11.8
	12	0	47	13.6	6.0	<2	12.0	27	72	1	0	0	300	10.5

APPENDIX 11

(continued)

24 hours after dosing

Group	Animal No.	ESR mm. $hr.^{-1}$	PCV %	Hb g.100 ml. $blood^{-1}$	RBC millions ul^{-1}	Retics %	WBC $x10^3$ cells ul^{-1}	%					Platelets $x10^3$ ul^{-1}	PTI secs
								N	L	E	B	M		
I	14	0	49	14.6	5.8	<2	14.0	98	2	0	0	0	530	10.9
	26	0	46	12.7	5.6	<2	8.4	71	27	1	0	1	490	12.1
II	4	0	42	11.8	5.8	<2	16.0	76	23	0	0	1	350	11.4
	7	2	38	11.9	5.0	<2	14.0	89	31	0	0	0	400	11.6
III	28	Clotted												11.1
	29	Clotted												
IV	3													
	12		48	10.5	5.0	<2	7.6	56	44	0	0	0	300	10.9

APPENDIX 11

(continued)

48 hours after dosing

Group	Animal No.	ESR mm. $hr.^{-1}$	PCV %	Hb g.100 ml blood $^{-1}$	RBC millions ul^{-1}	Retics %	WBC $x10^3$ cells ul^{-1}	%					Platelets 10^3 ul^{-1}	PTI secs
								N	L	E	B	M		
I	14	0	41	12.5	5.9	<2	23.0	96	4	0	0	0	570	13.6
	26	1	42	12.8	5.8	<2	15.6	54	43	2	0	1	460	10.4
II	4	1	40	10.4	5.8	<2	16.1	70	30	0	0	0	280	11.0
	7													10.8
III	28	12	43	11.2	4.5	<2	11.2	58	36	5	0	1	590	11.0
	29	38	36	9.0	4.6	<2	11.8	61	38	0	0	1	525	11.6
IV	3	4	38	11.0	4.7	<2	11.0	75	23	1	0	1	440	11.4
	12	2	41	11.6	5.0	<2	10.6	53	47	0	0	0	365	12.4

APPENDIX 11

(continued)

1 week after dosing

Group	Animal No.	ESR mm. hr^{-1}	PCV %	Hb g.100 ml blood $^{-1}$	RBC millions ul^{-1}	Retics %	WBC $x10^3$ cells ul^{-1}	%					Platelets 10^3 ul^{-1}	PTI secs
								N	L	E	B	M		
I	26	2	41	11.6	5.3	<2	15.6	46	54	0	0	0	520	12.9
II	4	4	38	10.2	5.4	<2	12.0	48	51	1	0	0	620	12.5
	7	5	38	10.8	5.5	<2	9.8	60	40	0	0	0	530	12.2
III	28	4	40	11.6	4.8	<2	8.6	49	45	6	0	0	565	10.4
	29	27	33	8.6	4.0	<2	9.8	59	38	1	0	2	535	11.7
IV	3	2	35	10.0	4.8	<2	10.8	60	35	2	0	3	395	11.2
	12	1	39	11.8	6.0	<2	13.1	51	47	1	0	1	330	12.5

APPENDIX 11

(continued)

Terminal

Group	Animal No.	ESR mm hr^{-1}	PCV %	Hb g. 100 ml blood $^{-1}$	RBC millions ul $^{-1}$	Retics %	WBC $x10^3$ cells ul^{-1}	%					Plate lets 10^3 ul^{-1}	PTI secs
								N	L	E	B	M		
I	26	3	43	12.6	4.8	<2	10.4	51	49	0	0	0	335	10.9
	4	2	38	9.8	4.6	<2	18.6	64	36	0	0	0	485	11.2
II	7	3	43	12.2	4.9	<2	12.6	45	55	0	0	0	325	10.9
III	28	16	41	10.6	4.6	<2	12.4	50	50	0	0	0	330	10.4
	29	12	33	8.6	3.8	<2	7.4	46	54	0	0	0	265	10.7
IV	3	5	40	11.0	4.6	<2	11.8	62	38	0	0	0	445	

APPENDIX 12

Clinical chemistry - orally dosed animals (ICI/50)

Pre-exposure

Group	Animal No.	Urea mg %	Glucose mg %	Total Protein g %	Serum Proteins %					SGPT $mU.ml^{-1}$ units	LAP GR units	Bilirubin mg %	γGT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^{+} $mEq.l^{-1}$	K^{+} $mEq.l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mU.ml^{-1}$
					Alb	α1	α2	β	γ									
I	14	44	84	8.3	53	5	8	18	16	31	155	0.1	31.9	4.2	156	5.7	5.4	80
	26	33	50	7.8	52	4	10	20	14	60	195	0.3	5.8	2.2	154	5.3	5.4	80
II	4	32	86	7.0	42	4	10	34	10	37	287	0.1	27.8	6.5	154	5.5	5.8	26
	7	42	76	7.5	52	4	8	22	14	41	290	0.1	30.0	6.9	154	6.1	5.6	27
III	28	42	101	7.8	56	4	9	20	11	53	253	0.1	15.1	5.1	152	5.1	5.4	361
	29	37	134	7.6	55	5	9	21	10	99	199	0.1	24.2	1.1	151	5.2	6.1	74
IV	3	44	100	7.3	56	2	4	25	13	59	398	0.1	29.7	8.0	155	4.9	5.3	41
	12	39	92	8.0	51	5	14	16	14	11	534	0.1	31.8	10.9	107	5.4	5.2	107

APPENDIX 12

(continued)

24 hours after dosing

Group	Animal No.	Urea mg %	Glucose mg %	Total Protein g %	Serum Proteins %					SGPT mU.ml^{-1} units	LAP GR units	Bilirubin mg %	γ GT mU.ml^{-1}	GLDH mU.ml^{-1}	Na^{+} mEq.l^{-1}	K^{+} mEq.l^{-1}	Ca^{++} mEq.l^{-1}	CPK mEq.l^{-1}
					Alb	α1	α2	β	γ									
I	14	75	100	8.8	50	2	6	30	12	169	167	0.2	32.2	24.3	153	4.9	4.8	626
	26	42	146	7.8	48	2	6	29	15	162	141	x	x	x	155	4.6		198
II	4	50	83	11.0	48	7	14	19	12	112	190	0.3	23.9		145	3.6	4.8	218
	7	50	88	11.4	50	8	8	22	14	91	183	0.3			148	4.2	5.0	495
III	28	74	80	8.8	48	6	14	18	14	64	167	0.6	54.0	x	144	5.3	x	x,
	29	72	70	8.2	45	9	11	20	15	97	180	0.7	42.8	4.4	147	5.5	x	x
IV	3	74	128	8.7	44	5	9	30	12	101	x	0.2	9.8	6.0	143	4.3	4.7	x
	12	112	65	9.0	51	7	9	24	9	139	x	0.2	3.4	0.6	146	3.8	4.7	x

APPENDIX 12

(continued)

48 hours after dosing

Group	Animal No.	Urea mg %	Glucose mg %	Total Protein g %	Serum Proteins %					SGPT $mU.ml^{-1}$ units	LAP GR units	Bilirubin mg %	γGT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^+ $mEq.l^{-1}$	K^+ $mEq.l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mEq.l^{-1}$
					Alb	α1	α2	β	γ									
I	14	130	62	8.5	57	3	6	21	13	163	154	0.3	6.5	9.9	141	5.9	x	1446
	26	110	107	7.4	48	2	5	31	14	157	74	0.2	x		158	5.6	5.3	866
II	4	60	80	8.0	48	6	12	19	15	96	193	0.2	23.0		139	3.4	5.0	136
	7	54	99	8.2	54	5	10	20	11	92	191	0.1	43.2		134	3.4	4.5	310
III	28	82	100	7.7	50	2	10	21	17	60	120	0.1	8.8	4.6	147	5.5	4.3	312
	29	94	55	7.8	50	5	16	25	14	82	124	0.1	18.3	4.1	154	4.9	4.0	395
IV	3	156	95	8.4	56	3	7	19	15	90	276	0.2	18.1	14.3	142	3.6	3.8	916
	12	216	80	8.6	55	3	6	20	16	115	299	0.3	27.9	4.7	141	4.3	3.5	578

APPENDIX 12

(continued)

1 week after dosing

Group	Animal No.	Urea mg %	Glucose mg %	Total Protein g %	Serum proteins %					SGPT mU.ml^{-1} units	LAP GR units	Bilirubin mg %	γGT mU.ml^{-1}	GLDH mU.ml^{-1}	Na^+ mEq.l^{-1}	K^+ mEq.l^{-1}	Ca^{++} mEq.l^{-1}	CPK mEq.l^{-1}
					Alb	α1	α2	β	γ									
	26	140	124	9.2	48	2	6	27	17	214	2284	0.3	*	*	148	5.6	3.5	271
	4	50	67	10.0	41	11	16	10	22	45	200	0.2	22.7	6.8	147	4.8	*	*
	7	65	62	9.6	43	8	13	16	20	49	199	0.3	49.4	*	146	4.0	*	*
	28	60	68	7.6	48	2	5	31	14	68	187	0.3	45.6	3.6	147	4.9	4.1	1068
	29	45	109	7.2	47	3	8	29	13	63	194	0.2	12.5	3.0	150	5.5	5.0	1524
	3	58	120	7.5	49	2	6	28	15	33	187		*	9.8	153	4.1		630
	12	180	56	8.6	50	3	7	28	12	67	626		4.3	5.2	158	6.4		4915

APPENDIX 12

(continued)

Terminal

Group	Animal No.	Urea mg %	Glucose mg %	Total Protein g %	Serum proteins %					SGPT $mU.ml^{-1}$ units	LAP GR units	Bilirubin mg %	γGT $mU.ml^{-1}$	GLDH $mU.ml^{-1}$	Na^{+} $mEq.l^{-1}$	K^{+} $mEq.l^{-1}$	Ca^{++} $mEq.l^{-1}$	CPK $mU.ml^{-1}$
					Alb	α1	α2	β	γ									
I	26	114	132	7.6	48	2	4	29	17	160	2310	0.2	90.6	4.9	147	5.4	3.3	169
II	4	56	80	9.0	45	3	4	30	18	46	137	0.1	31.8	10.7	146	5.3	4.6	176
	7	80	78	10.0	47	3	6	27	17	53	138	0.2	36.3	10.1	150	5.1	5.4	380
III	28	76	114	8.0	49	2	6	27	16	53	249	0.2	58.8	10.8	144	4.8	4.3	391
	29	60	90	7.8	49	2	5	27	17	50	168	0.1	49.9	11.0	146	4.9	4.7	65
IV	3	96	112	9.0	45	2	7	30	16	25	179	0.1	30.9	13.6	149	3.7	4.9	74

APPENDIX 13

Urinalysis - orally dosed animals (ICI/50)

Pre-exposure

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
I	14	7.6	156	1025	0	tr	0	0	0	0	0	0	0	0	0	1	0	0
	26	7.7	44	1025	0	0	0	0	0	0	0	0	0	0	0	2	0	0
II	4	7.5	40	1039	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	7	7.5	134	1024	0	0	0	0	0	0	0	0	0	0	0	2	0	0
III	28	7.5	159	1020	20	tr	0	0	0	0	0	0	0	0	0	2	0	0
	29	7.5	198	1015	0	0	0	0	0	0	0	0	0	0	0	2	0	0
IV	3	7.7	50	1028	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	12	7.7	84	1034	10	0	0	0	0	0	0	1	0	0	0	1	0	0

tr = trace

APPENDIX 13

(continued)

24 hours after dosing

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
I	14	7.6	44	1025	0	0	+	0	0	0	+	0	0	0	0	2	0	0
	26	7.1	37	1035	10	tr	+	0	0	0	0	1	2	0	0	2	0	0
II	4	7.3	56	1035	0	0	+	0	0	0	+	1	0	0	1	3	0	0
	7	8.1	90	1024	0	0	0	0	0	0	+	0	0	0	1	3	0	0
III	28	8.1	48	1019	10	0	+	0	0	0	+	0	1	0	0	2	0	0
	29	5.5	216	1018	0	0	+	0	0	0	+	1	1	0	1	2	0	1
IV	3	7.8	50	1025	20	tr	+	0	0	0	0	2	1	0	0	2	0	1
	12	7.8	45	1025	20	0	+	0	0	0	+	2	1	0	1	3	0	0

tr = trace

APPENDIX 13

(continued)

48 hours after dosing

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
I	14	6.1	212	1005	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	26	5.3	18	1025	100	0	+	0	0	0	0	0	0	0	0	3	0	0
II	4	5.7	88	1010	20	0	0	0	0	0	0	0	0	0	0	2	0	0
	7	7.7	164	1010	20	0	0	0	0	0	+	0	0	0	0	2	0	0
III	28	5.9	126	1010	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	29	5.1	220	1013	0	0	0	0	0	0	0	0	0	0	0	1	0	1sp
IV	3	5.2	17	1020	0	0	+	0	0	0	0	0	0	0	0	3	0	0
	12	7.0	68	1030	0	0	0	0	0	0	0	0	0	0	0	3	0	0

tr = trace

APPENDIX 13

(continued)

1 week after dosing

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
I	26	5.3	88	1020	20	0	0	0	0	0	+	1	1	0	1	1	0	0
II	4	8.4	74	1024	0	0	0	0	0	0	0	0	0	0	0	3	0	0
	7	7.6	48	1035	0	0	0	0	0	0	0	0	1	1	1	2	0	0
III	28	7.1	16	1026	10	0	0	0	0	0	0	0	0	0	0	3	0	0
	29	7.1	64	1020	0	0	0	0	0	0	+	0	0	0	0	3	0	0
IV	3	5.2	104	1018	0	0	+	0	0	0	0	0	0	0	0	1	0	0
	12	5.1	65	1030	0	++	+	0	0	0	0	1	0	0	0	1	0	0

APPENDIX 13

(continued)

Terminal

Group	Animal No.	pH	Volume mls	SG	Protein mg %	Total red subs	Glucose	Ketones	Bile pigments	Urobilinogen	Blood pigments	Microscopy						
												E	P	M	R	O	C	A
II	4	6.4	58	1031	0	0	0	0	0	0	0	0	0	0	0	3	0	0
	7	7.1	100	1027	0	0	0	0	0	0	0	0	0	0	0	3	0	0
III	28	6.6	248	1018	0	0	0	0	0	0	0	0	0	0	0	3	0	0
	29	5.8	192	1020	0	0	0	0	0	0	+	0	0	0	0	3	0	0
IV	3	4.9	122	1020	0	0	+	0	0	0	0	0	0	0	0	2	0	0