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THE DERMAL TOXICITY OF PARAQUAT

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THE DERMAL TOXICITY OF PARAQUAT

Two samples of paraquat were received for the investigation of percutaneous toxicity in the rabbit.

- A. Paraquat dichloride (28.6% w/v) prepared by the L.T.S. process and labelled Paraquat LTS PP 148 B 650/62/1.
- B. Paraquat dimethosulphate, labelled JF.1824.

The more extensive investigation was carried out on the former preparation; limited tests were performed with JF.1824 to confirm that its toxicity is essentially identical with that of the Paraquat LTS preparation.

To provide a basis for percutaneous toxicity testing the systemic toxicity of Paraquat LTS was first assessed by the intraperitoneal route. In addition, the effect of instilling either preparation into the rabbit's eye was noted.

The following summarises all tests done.

- A. Paraquat LTS PP 148 B 650/62/1
 - (a) Acute systemic (i.-p.) toxicity.
 - (b) Acute percutaneous tests with and without additives.
 - (c) Subacute percutaneous (20 day) toxicity, the lowest dosage applied being 1:100 dilution of the sample supplied.
 - (d) Eye instillation of 1:100 dilution with and without X77 Spreader, an additive prepared by the California Chemical Company and containing a mixture of alkarylpolyoxyethylene glycols, free fatty acids, and isopropanol.
- B. Paraquat dimethosulphate JF.1824
 - (a) Acute percutaneous toxicity.
 - (b) Twenty daily skin applications of 1:100 dilution of the sample supplied.
 - (c) Eye instillation of 1:100 dilution with and without X77 Spreader.

On a previous occasion the dermal toxicity of paraquat dichloride, prepared by recrystallisation, was investigated. The results of this investigation are set out in Appendix II. Detailed histological findings in the present investigation are reported in Appendix I.

A. Paraquat LTS PP 148 B 650/62/1

Intraperitoneal injection

Males: The acute parenteral LD50 of this material was estimated in adult male albino rabbits weighing between 1.82 - 4.5 kg. Each of 20 rabbits was given a single injection and the animals were divided into four equal groups corresponding with the dose given of either 56 mg, 28 mg, 14 mg or 7 mg paraquat ion/kg body weight. Surviving animals were observed for 14 days following injection. Results are summarised in Table 1.

The LD50, calculated from the resulting mortality, was 18.15 mg ion/kg body weight (10.61 - 31.04 mg ion/kg for $P = 0.05$).

Apart from non-specific signs of toxicity, such as testicular tubular degeneration and evidence of thymic lympholysis, the main toxic action appears to involve the proximal convoluted renal tubules and the lungs, which show alveolar-wall thickening with increased cellularity, the cell population consisting of swollen histiocytes and polymorphonuclear leukocytes. No pathological lesions attributable to treatment were seen at the lowest dose level.

Females: A preliminary screening of the toxicity of paraquat LTS was carried out in three rabbits weighing 2.16 - 2.80 kg. A single injection of 56 mg ion/kg body weight caused death of the animal overnight, with effects similar to those seen in male animals. No effect was produced by a dose of 7 mg ion/kg nor, surprisingly, by 28 mg ion/kg. This latter discrepancy from the effect of this dose level in the male was further investigated.

Each of three rabbits, weighing 2.08 - 2.64 kg, was given 28 mg ion/kg by intraperitoneal injection. Over the next week two animals lost weight (up to 31% of the original body weight) and showed some loss of muscle tone. They were then killed and two of them had pale kidneys, emphysematous lungs and mucosal haemorrhages in the stomach.

Although no animal died during the period of observation, 28 mg ion/kg body weight is a severe effect-dose level and it is likely that the LD50 is not significantly different from that found in the males.

Dermal toxicity

Experimental methods: The acute and subacute percutaneous toxicity of this material have been investigated in single-caged albino rabbits weighing

1.59 - 2.89 kg. Tests were done on male animals except where an extra group of females was used.

A standard technique was followed in all tests. The backs of the animals were clipped on the day before testing was begun, avoiding any skin damage. Where possible, the test material was applied in a volume of 1 ml/kg body weight and, when necessary, water was used as a diluent. It was, however, necessary to apply 2 ml/kg body weight of the test material to achieve a skin dose of 560 mg paraquat ion/kg body weight.

Using a soft art brush, the test material was applied to the clipped skins to the limit of spread. This amounted to approximately 10% of the animals' surface area. The applied material was exposed to the air and the treated skin was dry in about one hour. Direct oral contamination was prevented by placing plastic collars (diam 7", 17.7 cm) around the necks of the animals and no scratch marks were noted on the brown-stained skin. After 24 hours the area of application was washed with water and carefully dried.

An acute 24 hour percutaneous test consisted of one such application; a subacute test involved 20 of these complete cycles on 20 consecutive days, unless death intervened. After decontamination the plastic collars were removed.

In some instances, in addition to the standard acute 24-hr test, animals were exposed to skin applications lasting 4, 1, $\frac{1}{4}$ hours in order to assess the effect on toxicity of early decontamination.

The test material was used with or without additives as follows:

1. Paraquat LTS containing 1 in 1,500 X77 spreader
2. " " containing added inhibitors (sod. metaborate 1.42% and sod. benzoate 2.16%)
3. " " no additives

Experiments have revealed no significant difference in toxicity between these three preparations.

1. Paraquat LTS with added spreader

Acute toxicity

(a) Single 24-hr application: 12 male albino rabbits weighing 1.98 - 2.89 kg were divided into four groups of three animals corresponding with an applied

dose of 560 mg, 280 mg, 140 mg, or 70 mg paraquat ion/kg body weight. Animals were observed for 14 days following decontamination, unless death intervened, and were frequently weighed during this period.

Where applications of 560 mg or 280 mg ion/kg were made, animals showed skin erythema followed by hyperkeratosis and desquamation. At all dose levels there was pronounced salivation after an initial latent period of one or two days. This was noted to be associated with glossitis and accompanied by anorexia, weakness, and loss of weight. When salivation decreased in amount, appetite returned and animals began to regain weight.

In addition to non-specific toxic effects, such as thymic and testicular atrophy, post-mortem examination revealed lung congestion, pale kidneys, and gastric haemorrhages. Microscopy showed lung and kidney changes similar to those described following i.-p. injection. Acute ulceration of the lateral margins of the tongue was noted and, in addition, the epithelium of the oesophagus appeared thickened.

Results are summarised in Table 2 and, for the lower three dose levels, body weights, at intervals after the period of exposure, are set out in Figure 1 as a percentage of the initial weights.

The LD50, calculated from the resulting mortality was 236 mg ion/kg body weight, but, because the number of animals in each group was small, this figure is only a rough approximation.

(b) Single application for either 4-hr, 1-hr, or $\frac{1}{2}$ -hr: In order to assess the effect of early decontamination of the skin 280 mg paraquat ion/kg body weight was applied to each of nine rabbits weighing 1.59 - 2.63 kg. One group of three was decontaminated after $\frac{1}{2}$ hr, a further group of three after 1 hr, and the last group after 4 hrs. The animals were observed and weighed as before.

An animal from each of the groups exposed for either 4 hrs or 1 hr died. The animal dying after 1 hr exposure had a severe bronchopneumonia and death was probably unrelated to treatment. Salivation and glossitis was pronounced after an initial delay of a day or two and all animals lost weight. Survivors regained weight. Only in animals exposed for 4 hrs were there lung changes definitely attributable to paraquat and in one of these animals also the oesophageal epithelium appeared necrotic.

These results are summarised in Table 3 - Figure 2.

Subacute toxicity

Fifteen male rabbits, weighing 1.90 - 3.08 kg, were divided into five groups corresponding with daily skin applications of 116 mg, 58 mg, 29 mg, 14.5 mg and 7.25 mg paraquat ion/kg body weight. In addition, the supplied material was diluted 1:100 and applied to two further groups of three rabbits in the same weight range, one male and one female, so as to give a daily skin dose of 2.8 mg ion/kg body weight. Animals surviving 20 applications were observed for a further two weeks. They were then killed, examined post-mortem, and tissues submitted for microscopy. Results are summarised in Table 4 and weight changes are set out in Figs 3 and 4.

From the resulting mortality the subacute LD50 is estimated to lie between 7 - 14 mg ion/kg body weight. Neither clinical nor pathological abnormality was seen at a dose level of 2.8 mg ion/kg body weight (1:100 dilution of the supplied material). At the three highest dose levels a striking feature was excess salivation associated with weight loss and here also kidney damage was seen in addition to lung changes. The latter only were noted at a dose level of 7.25 mg ion/kg. The skin was affected at all dose levels except the lowest but, histopathologically, the appearances were not striking and consisted mainly of hyperkeratosis with occasional intraepidermal pustules. Little if any changes were seen in the subcutaneous tissues.

2. Paraquat LTS with inhibitors

Acute 24-hr toxicity

Six rabbits weighing 1.94 - 2.88 kg each received a single skin application of paraquat. One group of three received 280 mg ion/kg body weight, animals in the other group, 140 mg ion/kg. Effects are summarised in Table 5. They are identical with those seen when the material was used with spreader (Table 2) although, in the absence of spreader, the material was more difficult to apply evenly. Weight changes were similar.

3. Paraquat LTS without additives

Acute 24-hr toxicity

Three groups, each of 3 rabbits, received single skin applications of either 560 mg, 280 mg, or 140 mg paraquat ion/kg body weight. The material, in the absence of spreader, was more difficult to apply evenly. Weight loss was evident at the higher dose levels and, where death occurred, was progressive.

Effects are summarised in Table 6 and are essentially identical with those of paraquat with either spreader or inhibitor (cf. Tables 2 and 5).

Eye instillation

One drop of 1:100 dilution of paraquat LTS was instilled into the eyes of each of three rabbits. There was no immediate effect but on the third day one rabbit showed slight lacrymation and conjunctival congestion, which may have been unrelated to treatment. On the next day no abnormality was to be seen.

A similar experiment was done with the same dilution of paraquat LTS containing 1:1500 X77 spreader. This caused no ill-effect whatever.

B. Paraquat dimethosulphate, JF.1824

Dermal toxicity

The experimental methods used were identical with those used in testing the paraquat LTS preparation. As before, water was invariably used as a diluent.

Acute toxicity

A screening test was done with this material on two groups of three rabbits (1.97 - 2.59 kg) corresponding with an applied skin dose of 240 mg and 120 mg ion/kg body weight. The resulting toxicity was similar to that of the LTS preparation and, in addition to non-specific toxic lesions, the lung, kidney, tongue, and skin were again histologically abnormal. These observations are set out in Table 7 and the effect of the application on the animals' weights are illustrated in Figure 5.

Subacute toxicity

The material was diluted 1:100 with water and 20 daily skin applications made to each of three male (2.21 - 3.09 kg) and three female (2.29 - 2.81 kg) rabbits so that each received a daily skin dose of 2.4 mg/kg body weight.

One female animal showed a mild skin erythema but, otherwise, no definite abnormality was noted. A slight weight loss in one animal was reversed even though applications continued and, since such variations in weight occur in untreated rabbits, it is of very doubtful significance. Similarly, one animal

showed minimal lung changes which cannot certainly be attributed to treatment. These results are set out in Table 8 and the animals' weight changes are illustrated in Figure 6.

Eye instillation

One drop of 1:100 dilution of the material supplied was instilled into the eyes of three rabbits. No initial irritation occurred but on the following day, lacrymation and conjunctival congestion was seen in two animals. This persisted for two days in both animals and for a further day in one of them. By this time the eyes were completely normal. At no time was there evidence of corneal damage.

When a similar test was done with this material containing 1:1500 X77 spreader the results were identical.

DISCUSSION

When a single oral dose of paraquat is given to rats metabolic studies give no indication that the nature of the associated anion influences absorption (Daniel and Gage, 1964). Furthermore, both the dichloride and dimethosulphate formulations of paraquat are equitoxic orally when the dose is expressed as paraquat ion (Clark, 1965). The present investigation of percutaneous absorption in rabbits failed to differentiate between the dichloride and dimethosulphate and indicates that here also it is the cation which determines dermal toxicity.

The LD50 calculated from the mortality in rabbits following a single i.-p. injection of paraquat dichloride prepared by the L.T.S. process is 18.15 mg ion/kg body weight (Table 1). In rats the LD50 of the pure dichloride given by the same route is 19 mg ion/kg and the dimethosulphate has a similar toxicity (Clark, 1965) - further evidence that toxic effects are independent of the nature of the anion.

Using ¹⁴C-labelled paraquat, Daniel and Gage (1964) showed that paraquat is poorly absorbed from the gut - less than 20% of orally administered radioactivity appears in the urine. Consequently, it is not surprising that percutaneous toxicity is low compared with that of intraperitoneal injection; whereas the single dose i.-p. LD50 is approximately 18 mg ion/kg body weight, the acute percutaneous LD50 is more than 13 times as great (Table 2).

As expected, repeated skin applications of paraquat proved more toxic than a single skin dose. When paraquat dichloride is applied daily for 20 days to the rabbit skin the subacute LD50 is found to be between 7 - 14 mg ion/kg body weight daily (Table 4). If the site of application is occluded with impermeable material for the duration of exposure to paraquat the subacute LD50 is approximately 4.5 mg ion/kg body weight daily over 20 days (Appendix II). In the present investigation, however, neither clinical nor pathological abnormalities were produced by repeated applications of the supplied sample diluted 1:100 with water (Table 4) although this is more than twice the normal working concentration (0.286% compared with 0.12%).

It is accepted that findings in animal experiments cannot directly be extrapolated to man. Nevertheless, results of toxicity tests in animals may indicate possible toxic effects of chemical substances in man. Where possible, an animal species is chosen for its resemblance to man in the parameters of the test. On these grounds the use of rabbits in testing dermal toxicity may be criticised. There is good reason to assume that results obtained will greatly exaggerate any hazard to man. Tregear (1964) tested a wide spectrum of substances on the skins of common laboratory animals. Contrary to popular belief, the skins of these animals are all more permeable than human skin to the substances he tested. Indeed, he points out that, though the relationship between species is not constant for different substances, the average permeability order is rabbit, rat, guinea-pig, pig, man in descending sequence, rabbit skin being approximately three times more permeable to ions than human skin. It follows, then, that contamination of human skin with paraquat is likely to be much less significant than skin contamination in the rabbit.

The dermal toxicity of paraquat shown by the present investigation, where the site of application was exposed to the air, is less than that shown when the site of application was occluded with polythene sheeting (Appendix II). In the latter instance there is an accumulation of condensed transpired water vapour, which, in addition to the applied aqueous solution, results in hydration of the stratum corneum, which normally contains only 10% of water (Matoltsy, 1958). One would expect the rate of penetration of water soluble chemicals to be faster through hydrated than through normal stratum corneum and, indeed, this has been confirmed by Blank and Scheuplein (1964). Occluded rabbit skin rapidly becomes macerated with separation of large areas of stratum corneum. Now, since transepidermal absorption is

determined by the stratum corneum (Malkinson, 1958) destruction of this layer under an occlusive dressing is followed by rapid penetration of an applied chemical. The results, then, will more closely resemble those of subcutaneous injection rather than percutaneous absorption.

A major difficulty in assessing toxic effects is the accurate appreciation, on the one hand, of coexisting but irrelevant pathological changes and, on the other hand, of effects due to intercurrent disease, which has been precipitated by the intoxication. The latter, although sometimes occurring spontaneously must be regarded as an effect of treatment. This is particularly true of the rabbit, which unlike the rat, is not specific-pathogen-free on admission to these laboratories. Spontaneous respiratory infections, pyelonephritis, and enteritis occur, and such conditions were seen in the present investigation.

The true toxic effects of paraquat are probably best seen in these experiments after intraperitoneal injection. When large doses were given, animals died within a day or two with pronounced muscular weakness and incoordination. The most constant pathological lesions were pulmonary congestion, renal tubular damage, and superficial necrosis of the stomach mucosa. Provided the animals survived this period, they subsequently developed thickening and hypercellularity of the pulmonary alveolar walls (Table 1). This latent period has already been observed by Hurst (1964).

When paraquat is applied to the skin a different picture is seen. Here the most striking effect, after a latent period, is the secretion of copious amounts of brownish saliva. Rabbits so affected refuse to eat and death occurs in a state of cachexia. In some of these animals renal tubular damage and pulmonary changes were seen as before. In others the pulmonary changes were pronounced and alveolar walls were thickened and contained a mixed cell infiltrate, in which plump histiocytes were conspicuous. In a minority the pulmonary changes resembled those described in the rat (Hurst, 1964) but, although greatly thickened alveolar walls obliterated alveoli and caused consolidation in some areas, there was little evidence that the initial change was perivascular or peribronchial as described by Hurst; it appeared to take place diffusely throughout the interstitial tissue of the lung. At low skin doses the lung changes were often of doubtful significance and, in other circumstances, could reasonably be accepted as being within normal limits.

At first the significance of the profuse salivation was not recognised, but it was soon evident that this phenomenon was associated with glossitis

and even ulceration of the tongue and oesophagus. Oral contamination did not appear to be taking place but further investigations, not reported here in full, showed that excess salivation and glossitis did not occur when oral contamination was absolutely excluded by leaving the plastic collars in position after washing the application sites and, in addition, covering the washed and dried skins with dry porous dressings. Conversely, when the rabbits were given drinking water containing 286 p.p.m. of paraquat dichloride (1:1000 dilution of the commercial preparation supplied) they developed glossitis within two days and then rapidly lost weight.

In order to assess the effect of early decontamination of the skin following exposure to paraquat, the rabbits' skins were washed with water after an application of 280 mg ion/kg body weight, so as to give an exposure varying from $\frac{1}{4}$ hr to 4 hr. These rabbits were then returned to their cages without their plastic collars. Lesions definitely attributable to paraquat were not seen either in the lungs or in the kidneys but glossitis and oesophagitis occurred (Table 3) and anorexia and weight loss were pronounced (Fig 2). It was now assumed that, even after decontamination, there was a reservoir of paraquat in the skin and that persistent oral contamination occurred through grooming during the observation period. Consequently it is believed that, following skin application, death, in many cases, is due to wasting following inability to eat due to tongue and oesophageal ulceration or inflammation, the latter condition being caused by continual oral contamination from a skin reservoir. Even during the subacute 20 day test, animals were exposed daily to the possibility of oral contamination because, after washing and drying the skin, they were left without collars until the next application was made.

It is well known that the stratum corneum can act as a skin reservoir for applied substances as the experiments of Vickers (1964) have so elegantly shown. This is particularly probable in the case of paraquat which is readily adsorbed by other materials (Clark, 1965; Clinch and Middleton, 1962). It is thus available for continual application to the tongue during normal grooming with consequent glossitis and failure to eat. It is unlikely to be available for absorption percutaneously since the keratinous layer is shed.

It can be concluded, then, that the present investigation exaggerates the dermal toxicity of paraquat; skin contamination in man is likely to be far less hazardous than suggested by these experiments. In any case, even though rabbit skin is much more permeable to ions than is human skin,

and with the added hazard of oral contamination, no toxic effects were produced in the rabbit by repeated applications of a solution of paraquat more than twice the normal working concentration.

SUMMARY

1. Samples of paraquat dichloride and paraquat dimethosulphate are equitoxic when applied to the skin of rabbits if the dose is expressed as paraquat ion.
2. The acute percutaneous LD50 in rabbits is approximately 240 mg paraquat ion/kg body weight compared with the acute intraperitoneal LD50 of 18.15 mg ion/kg.
3. The subacute LD50 calculated from the mortality caused by up to 20 daily skin applications lies between 7 - 14 mg paraquat ion/kg body weight/daily.
4. There is evidence that much of the mortality caused by the dermal application of paraquat is caused, not by percutaneous absorption but by oral contamination in the stratum corneum.
5. The results of experiments reported here are likely to exaggerate the dermal toxicity of paraquat.

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Table 1.

Paraquat dichloride LTS PP 148 B 650/62/1

Acute Systemic (i.-p.) Toxicity in Male Albino Rabbits

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
56	2.53	1	5/5	-	Early incoordination, becoming complete on day 1 with pronounced muscular weakness.
	2.35	1		1256/64	
	2.80	1		1255/64	<u>P.M.</u> Congestion of lungs and gut. Blotchy kidneys.
	2.16	2		-	<u>Histology:</u> Extensive damage to cells of prox. conv. renal tubules, pulmonary congestion with swollen histiocytes in alveolar walls and interstitial polymorph. Leucocytosis. Superficial necrosis of stomach mucosa with increased mucous production. Swollen centrilobular liver cells.
	1.82	1		-	
28	1.95	-	4/5	1402/64	Increasing incoordination and muscular weakness with respiratory distress progressing to respiratory failure.
	2.31	3		1348/64	
	2.37	1		1328/64	<u>P.M.</u> All animals showed congestion of lungs, some showed renal pallor and 2 animals had a hydrothorax.
	2.07	2		1347/64	<u>Histology:</u> Congestion and interstitial mixed hypercellularity of lungs. Renal tubular damage (P.C.T.). One animal had a fatty liver. Thymic and testicular degenerative changes. The surviving animal (1402/64) showed pronounced thickening of alveolar walls and renal damage was also present.
	2.42	3		-	

Table 1. (continued)

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
14	2.17	-	1/5	-	No immediate effect was noted, but on the 2nd day all showed a slight loss of muscular control but recovery appeared complete. <u>P.M.</u> Lungs appeared emphysematous. <u>Histology:</u> Lungs congested with thickening of alveolar walls. Evidence of thymic regeneration and depression of spermatogenesis. The animal which died had a lung abscess possibly unrelated to treatment.
	2.47	9		1409/64	
	2.16	-		-	
	2.85	-		1449/64	
	2.74	-		1450/64	
7	4.5	-	1/5	1300/64	These animals showed no specific features and the first death was unexpected. <u>P.M.</u> showed congested lungs only. The animal dying on the 6th day had a severe enteritis and death was probably unrelated to treatment. Two animals showed abdominal hair loss. <u>Histology:</u> Congested lungs and kidneys. No specific lesions.
	2.48	3		1261/64	
	2.15	-		-	
	2.5	(6)		1335/64	
	2.47	-		1322/64	

Table 2

Paraquat dichloride LTS PP 148 B 650/62/1 with 1:1500 X77 Spreader

Acute Percutaneous (24-hr.) Toxicity in Male Albino Rabbits

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
560	2.03	-	2/3	-	Initial slight erythema of skin. Beginning on 2nd day there was increasing brown salivation. Reduction in activity. Progressive wt. loss with recovery after a week in the survivor only. Terminal cyanosis.
	2.33	17		1395/64	<u>P.M.</u> Lungs congested and emphysematous, some consolidation. Kidneys pale. Gastric mucosal haemorrhages and increased mucous production. Thymic and testicular atrophy. <u>Histology:</u> Lungs show thickened alveolar walls with a fibroblastic, histiocytic and polymorph reaction. Kidneys: damage to prox. conv. tubules.
	2.10	9		1321/64	
280	2.30	17	2/3	1503/64	Mild initial erythema of skin. All show salivation and weight loss, progressive in lethally affected animals. (Fig. 1) Pronounced desquamation. <u>P.M.</u> Congestion and consolidation of lungs. Kidneys pale. Mucosal haemorrhages in stomach and caecum. <u>Histology:</u> Renal tubular damage in 2/3 animals. Pronounced thickening of lung alveolar walls. Skin: hyperkeratosis with slight ulceration in one area.
	2.33	2		-	
	2.04	-		1484/64	

Table 2 (continued)

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
140	1.98	-	1/3	-	All showed pronounced salivation which lasted 1 week. Glossitis in one animal. <u>P.M.</u> Gastric haemorrhages, kidneys pale, lungs emphysematous. Tongue red. <u>Histology:</u> Ulceration lateral margins of tongue.
	2.58	9		1538/64	
	2.22	-		-	
70	2.46	-	0/3	1655/64	All showed salivation and 2/3 developed glossitis. <u>P.M.</u> Lungs from all animals were either congested or slightly oedematous. <u>Histology:</u> Oesophagus shows mild acanthosis. One lung shows alveolar-wall thickening.
	2.89	-		-	
	2.49	-		1656/64	

Table 3

Paraquat dichloride LTS PP 148 B 650/62/1 with 1:1500 X77 Spreader

Acute Percutaneous Toxicity ($\frac{1}{2}$ hr., 1 hr., and 4 hr.) in Male Albino Rabbits

Dose mg ion/ kg	Duration of expos- ure	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
230	$\frac{1}{2}$ hr	2.19	-	0/3	-	Two animals showed gradual onset of salivation with furring tongue. One became emaciated but all recovered. <u>P.M.</u> Showed congestion of lungs and in one animal thymic atrophy.
		2.63	-		-	
		2.63	-		-	
	1 hr	1.59	9	1/3	1599/64	All showed skin erythema and salivation within 2 days and all developed glossitis. One animal died in a cachectic state. <u>P.M.</u> Consolidation of lung. <u>Histology:</u> Bronchopneumonia and ulceration of tongue in one animal and possible resolving bronchopneumonia in another. Oesophagus of one animal shows submucous oedema.
		2.42	-		1623/64	
		1.78	-		1622/64	
	4 hr	2.80	7	1/3	-	All developed salivation and glossitis which gradually resolved in 2. <u>P.M.</u> One animal's tongue and oesophagus showed white cheesy epithelium and areas of pulmonary consolidation. <u>Histology:</u> Changes in the oesophagi of the survivors were insignificant.
		2.48	-		1624/64	
		2.45	-		1625/64	

Table 4

Paraquat dichloride LTS PP148 B 650/62/1 with 1:1500 X77 Spreader

Subacute Percutaneous (20 day) Toxicity in Albino Rabbits

Dose mg ion/ kg	Sex	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
116	M	1.95	5	3/3	1396/64	Salivation, gradual onset; weakness and incoordination which becomes total. Respiratory difficulty. Skin erythema pronounced. <u>P.M.</u> Lungs congested. Gastric mucosal haemorrhages. Kidney medulla red, testes small. <u>Histology:</u> Lungs: congested, swelling alveolar walls, polymorph infiltrate. Kidneys: damage prox. conv. renal tubule. Thymus and testes atrophic. Skin: hyperkeratosis and microabscesses in Malpighian layer.
		2.41	4		-	
		2.93	6		1407/64	
58	M	2.44	6	3/3	1408/64	Signs as in higher dose. <u>Histology:</u> As before. Liver consistent with fatty change. In animal surviving to 10 days (1448/64) lungs show extensive areas of almost complete consolidation caused by thickening of alveolar septa due to swelling of cells and infiltration with histiocytes and polymorphs.
		2.57	10		1448/64	
		2.38	5		1399/64	
29	M	2.33	16	2/3	1489/64	Gradual onset increased salivation. Some incoordination. Dyspnoea. One animal bloodstained faeces. Papular erythema of skin pronounced. Surviving animal showed lessening of salivation even while application continued. <u>P.M.</u> Lung congestion. Gastric haemorrhage (2/3). <u>Histology:</u> Minimal renal tubular damage. Lung congestion. Atrophy of thymus. Degeneration of testes. Skin hyperkeratosis.
		2.47	9		1446/64	
		2.55	-		1610/64	

Table 4 (continued)

Dose mg ion/ kg	Sex	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
14.5	M	3.08	17	2/3	1530/64	Increased salivation which lessened even before applications ceased. Mild erythema, incoordination and respiratory distress. Prostration in one animal. <u>P.M.</u> Lung congestion. Gastric haemorrhage. Thymic atrophy. <u>Histology:</u> Degenerative changes prox. conv. renal tubules. Lung: thickening and increased cellularity of alveolar walls. Thymus and testes: degenerative changes. Skin: hyperkeratosis, some pustule formation and focal necrosis of epidermis.
		2.50	20		-	
		2.30	-		-	
7.25	M	2.30	-	0/3	1644/64	There was a moderate erythema with minimal papule formation in one animal. No other effects in vivo. <u>P.M.</u> Two of three animals showed lung congestion. <u>Histology:</u> Pronounced thickening of alveolar walls in places amounting to complete consolidation. Kidneys normal.
		2.42	-		1643/64	
		1.90	-		-	
2.8 (1:100 of stock solution)	M	2.11	-	0/3	-	These animals showed no clinical abnormality and nothing abnormal was noted at necropsy.
		2.27	-		-	
		2.24	-		-	
	F	2.15	-	0/3	-	
		2.01	-		-	
		2.33	-		-	

Table 5

Paraquat dichloride LTS PP 148 B 650/62/1 with InhibitorsAcute Percutaneous (24 hr) Toxicity

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
280	2.88	6	2/3	1437/64	Animals developed respiratory difficulty, salivation, and wasting. <u>P.M.</u> Congestion of lungs, mucosal haemorrhages of stomach. Necrosis of oesophageal epithelium. Pale liver. <u>Histology:</u> Renal tubular damage. Centrilobular necrosis and fatty change in liver. Swollen alveolar walls in lungs. Necrosis of oesophageal epithelium, hyperkeratosis and pustule formation in skin. Degenerative changes testes and adrenals.
	2.74	9		1447/64	
	1.94	-		1485/64	
140	2.19	-	1/3	-	Weight loss, pronounced in animal that died. Onset salivation on 2nd day. Gastric mucosal haemorrhages. Lungs congested and slightly emphysematous.
	2.18	11		-	
	2.49	-		-	

Table 6

Paraquat dichloride LTS PP 148 B 650/62/1 (without additives)

Acute Percutaneous (24 hr) Toxicity

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
560	2.10	7	1/3	1299/64	Onset of salivation after 24 hr. Progressive loss of weight for 1 week, then gradual recovery of survivors. Skin dry with papular eruption. Little hair growth in treated areas. <u>P.M.</u> Lungs emphysematous. Kidneys pale, ascites and hydrothorax. <u>Histology:</u> Congested lungs and some inflammation. Kidney tubular damage. Atrophic thymus and testis. Hyperkeratosis and inflammation skin.
	2.85	-		1393/64	
	2.54	-		1394/64	
280	2.72	11	1/3	1451/64	Gradual onset moderate salivation with progressive weight loss in fatally affected animal. <u>P.M.</u> Lung congestion, gastric haemorrhage. Skin desquating. Thymic atrophy. <u>Histology:</u> Mild renal tubular damage. Congestion of lungs. Skin hyperkeratosis and subacute inflammation.
	2.77	-		1487/64	
	2.34	-		1486/64	
140	2.29	-	0/3	1490/64	Gradual onset of a mild degree of excess salivation. No definite effect on weight. Apparent recovery. <u>P.M.</u> Mild congestion of lungs with slight excess fluid in thorax and abdomen. <u>Histology:</u> Congestion of lungs and swelling of alveolar walls. No effect on kidney. Degeneration of testis.
	2.62	-		-	
	2.74	-		-	

Table 7

Paraquat dimethosulphate, JF.1824Acute percutaneous (24 hr) Toxicity in Male Albino Rabbits

Dose mg ion/ kg	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
240	2.1	9	3/3	1546/64	On day following application there was a blotchy erythema of skin. On the 2nd day increased salivation was noted. Animals subdued. <u>P.M.</u> Congested lungs, glossitis, gastric haemorrhage with excess mucin, pale kidneys. <u>Histology:</u> Lungs congested. One animal shows broncho-pneumonia, probably terminal. Kidney: evidence of degeneration and regeneration of prox. conv. tubules. Adrenals, thymus, testis show non-specific signs of toxic stress. Skin: hyperkeratosis with superficial necrosis in areas.
	2.59	7		1536/64	
	2.31	9		1537/64	
120	2.38	-	2/3	-	On day following application salivation noted which became pronounced. Unsteady and weak. After 12 days salivation less obvious. <u>P.M.</u> Glossitis, pale kidneys, enlarged adrenals, gastric haemorrhage and excess gastric mucus. <u>Histology:</u> Ulceration of tongue, necrosis of oesophageal epithelium. Degen. prox. conv. renal tubules. Adrenal necrosis in one animal.
	1.97	20		1621/64	
	2.42	9		1600/64	

Table 8

Paraquat dimethosulphate, JF.1824

Subacute Percutaneous (20 day) Toxicity in Albino Rabbits

Dose mg ion/ kg	Sex	Weight kg	Time of Death (days from start)	Mortality	Histology Ref.	Observations
2.4 (1:100 of stock solu- tion)	M	2.21	-	0/3	1641/64	There was a mild erythema of skin of one female, otherwise no definite clinical effects. A slight weight loss in one animal was reversed even while applications were continued. <u>P.M.</u> Lungs showed some areas of mild congestion. <u>Histology:</u> Kidneys normal. Lungsshow areas of mild thickening of alveolar walls with swollen histiocytes and polymorphonuclear leukocytes.
		2.23	-		1640/64	
		3.09	-		-	
	F	2.29	-	0/3	1653/64	
		2.55	-		1654/64	
		2.81	-		-	

UNINHIBITED LTS WITH 1 IN 1500 X77 SPREADER

SINGLE 24 HOUR SKIN APPLICATION, 3♂ RABBITS/GROUP

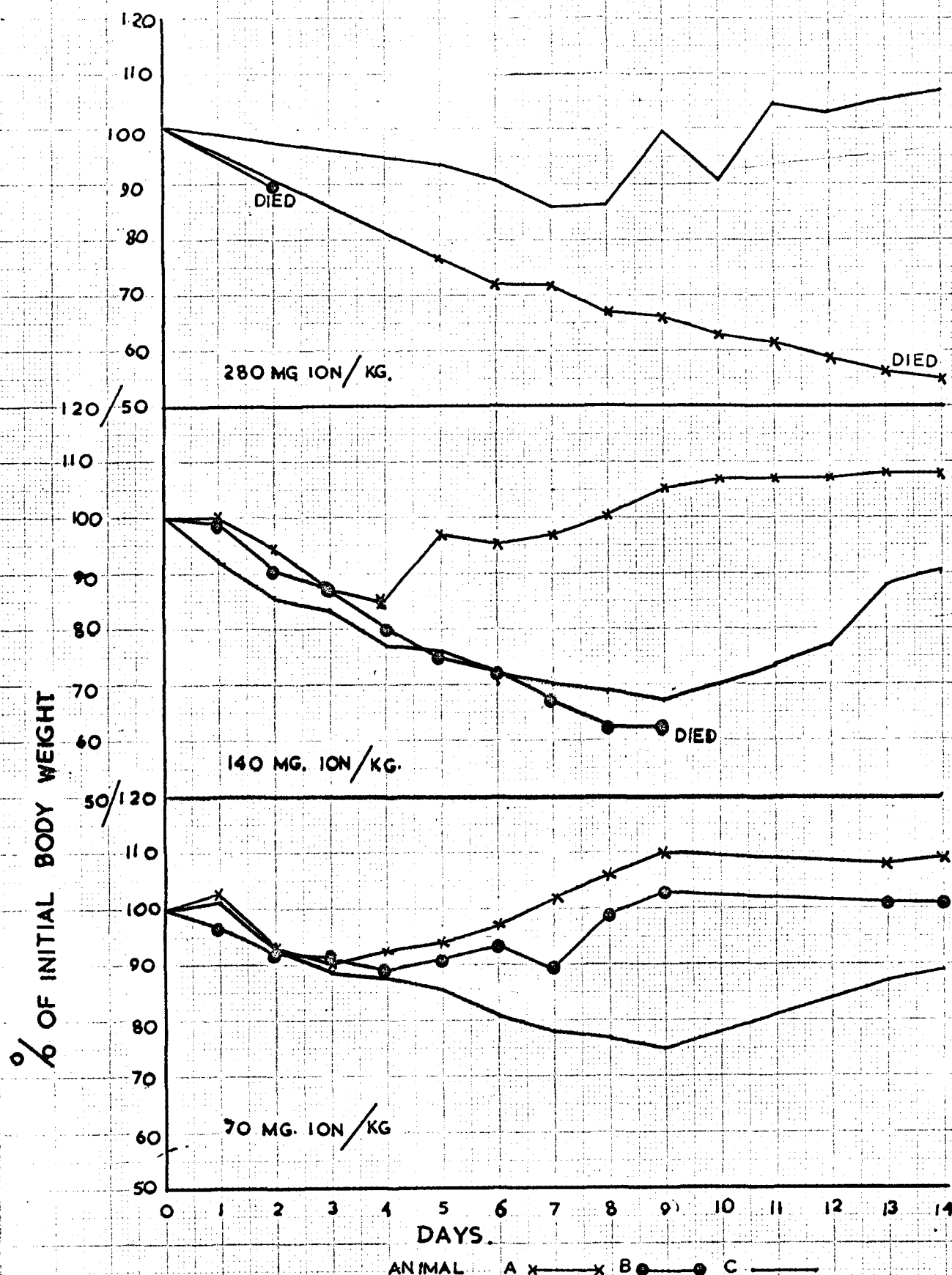
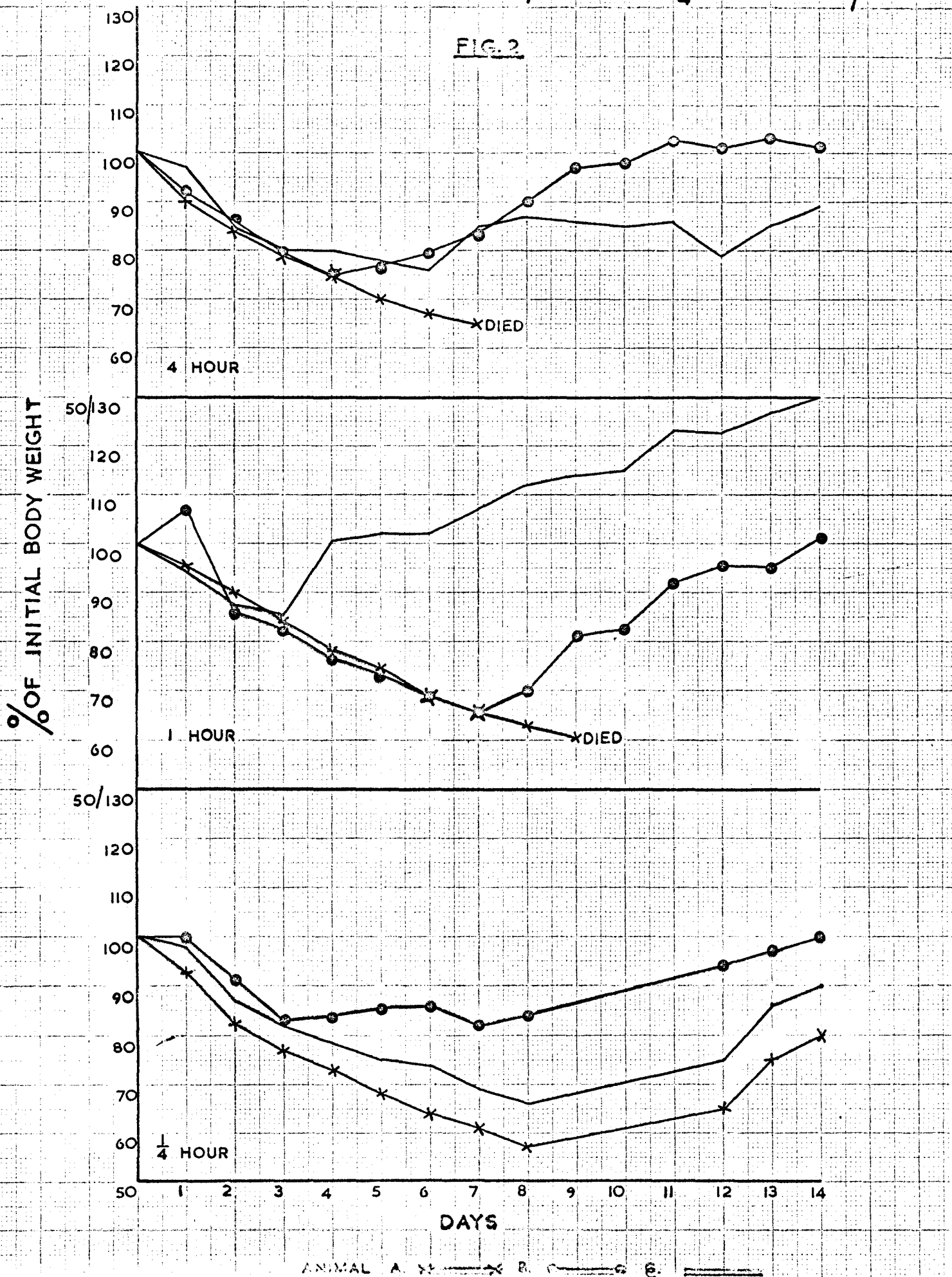


FIG.1

UNINHIBITED LTS WITH 1 IN 1500 X77 SPREADER

SINGLE SKIN APPLICATION OF 280 MG./ON/KG. FOR 4, 1, & $\frac{1}{4}$ HR. 3♂ RABBITS/GROUP

FIG. 2



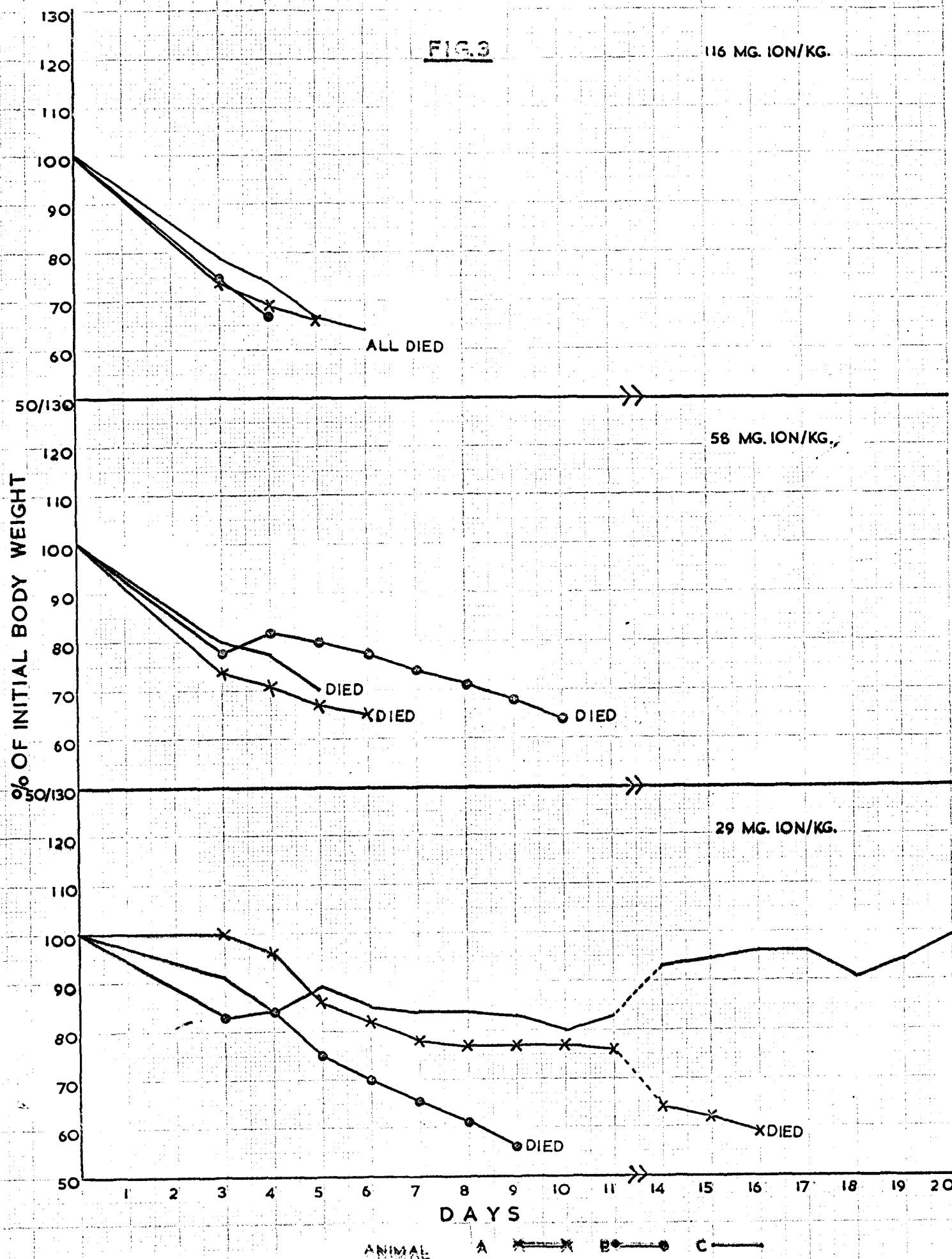
UNINHIBITED LIS WITH TRISCO X77 SPREADER

THE EFFECT OF 20 DAILY SKIN APPLICATIONS ON THE BODY WEIGHT OF RABBITS

3 ♂ ANIMALS/GROUP

FIG. 3

116 MG. ION/KG.



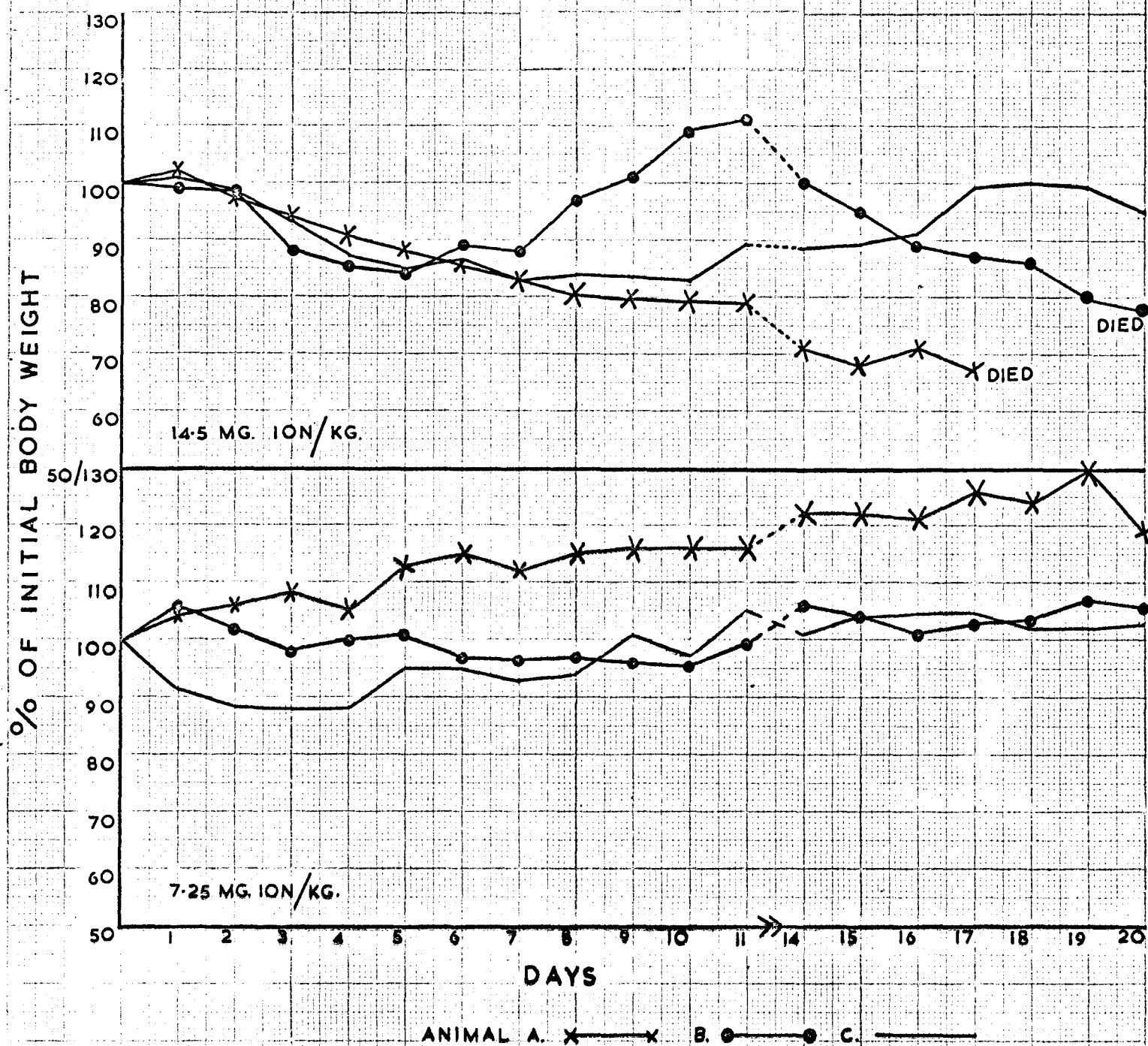


FIG. 3 CONTINUED

UNINHIBITED LTS WITH 1 IN 1500 X77 SPREADER

20 DAILY SKIN APPLICATIONS OF A 1 IN 100 DILUTION. 3 RABBITS/GROUP

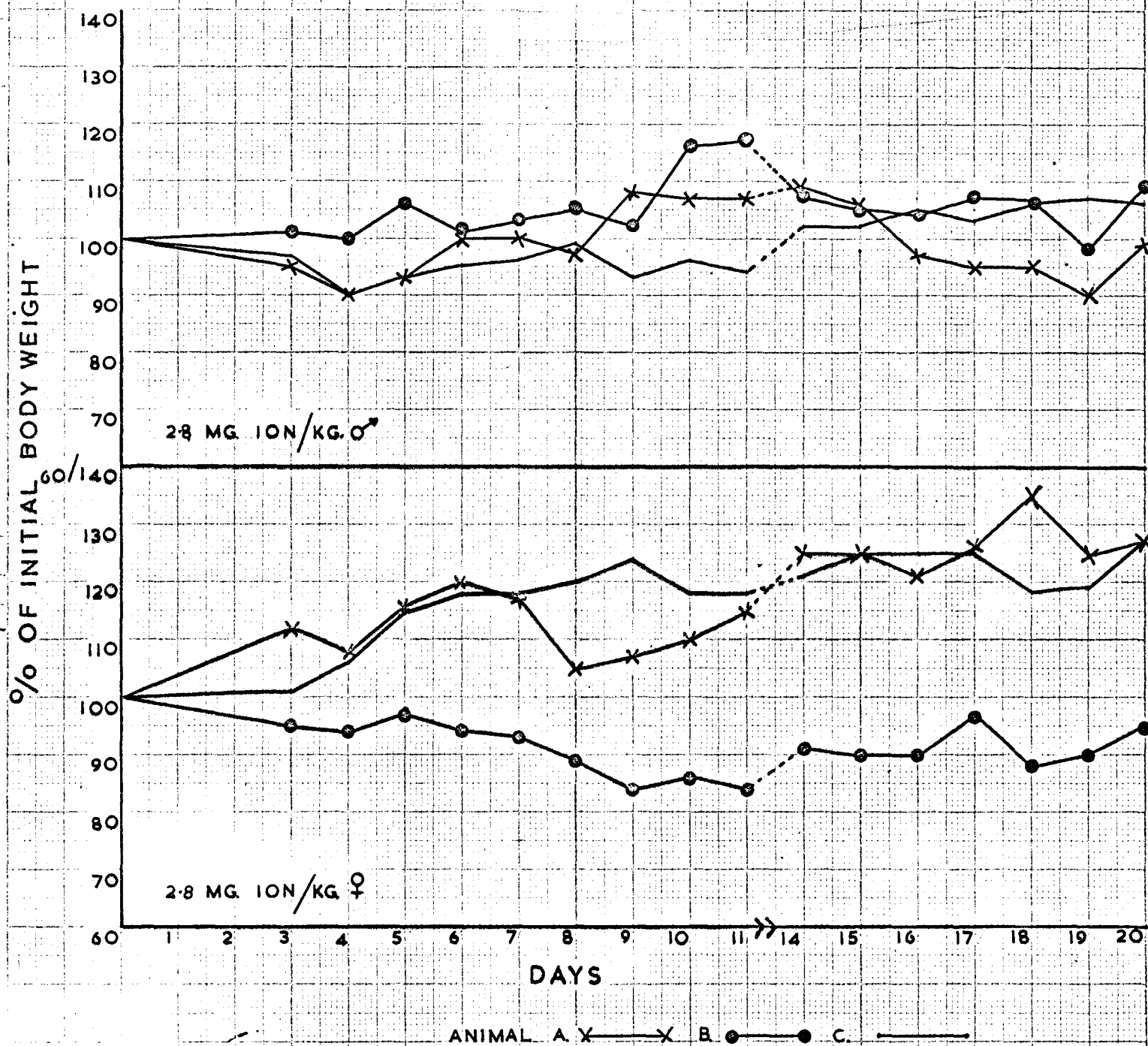


FIG. 4

JF.1824.

SINGLE 24 HOUR SKIN APPLICATION. 30³ RABBITS/GROUP

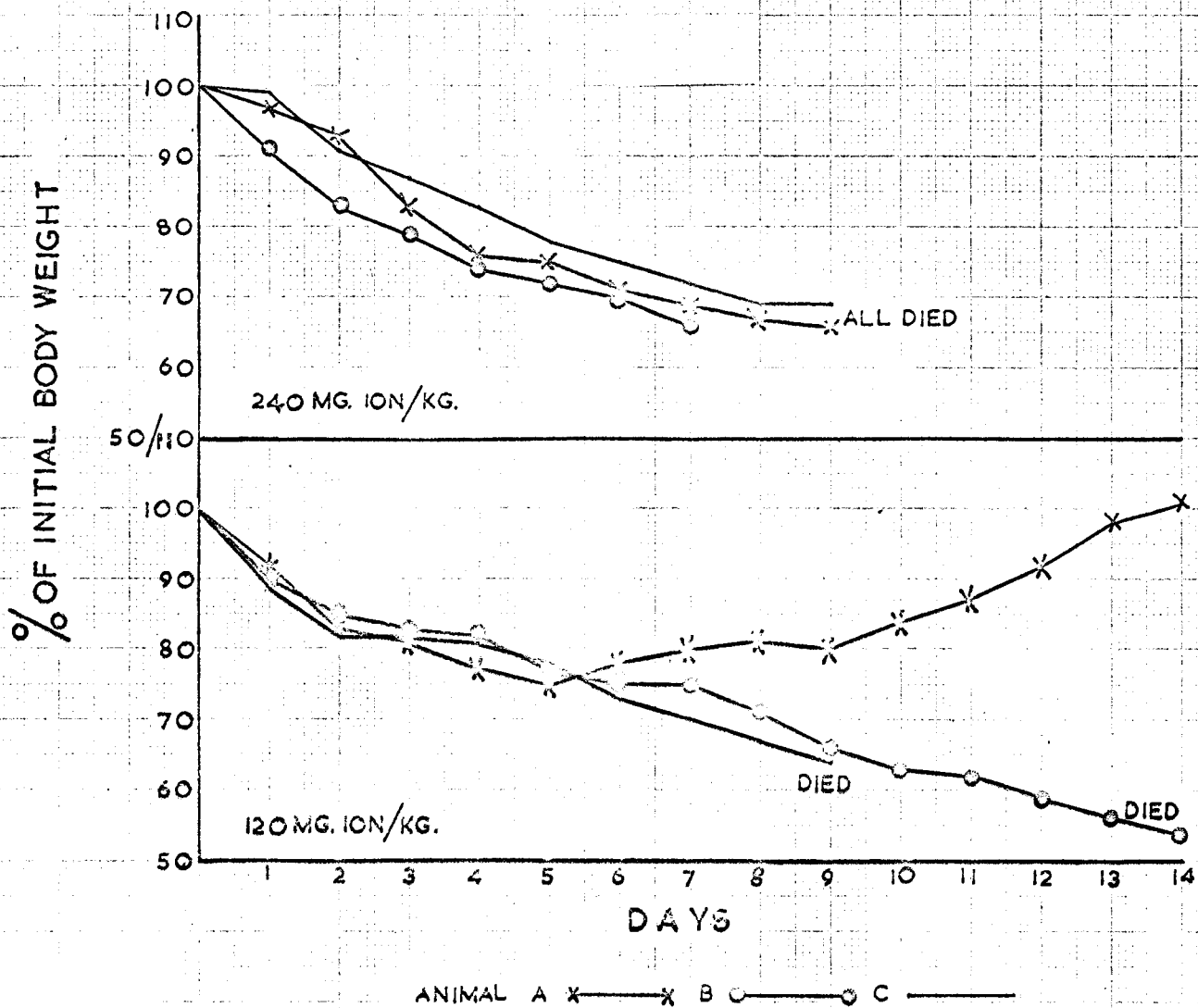


FIG.5

JF. 1824.

20 DAILY SKIN APPLICATIONS OF A 1 IN 100 DILUTION. 3 RABBITS/ GROUP

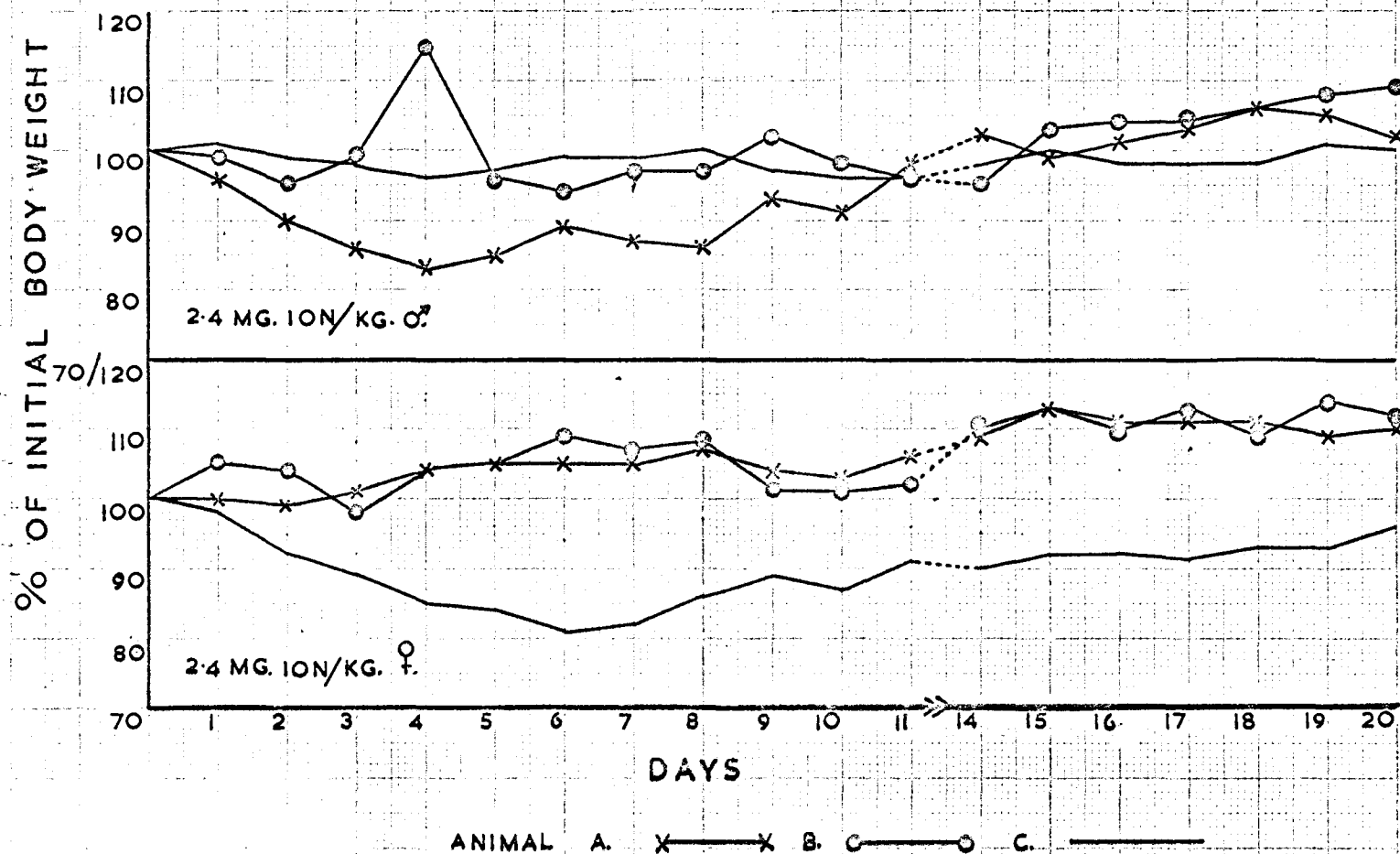


FIG. 6

APPENDIX I

Detailed Histological Findings in Individual Animals

1255/64

Kidney: Swelling, vacuolation, degeneration and shedding of cells of p.c. tubules.

Lung: Congested. Thickening of alveolar walls. Swelling of histiocytes and interstitial polymorphonuclear leucocytosis.

Stomach: Superficial acute necrosis of gastric mucosa.

1256/64

Kidney: Extensive degeneration and necrosis of cells of p.c. tubules with some areas in which cells are characteristically vacuolated. Some tubules are dilated and lined by flattened basophilic cells and surrounding these there is a lymphocytic infiltrate.

Stomach: Conspicuous mucus production.

Lungs: Severely affected. In addition to features seen in 1255, there are small discrete collections of swollen histiocytes.

Liver: Swelling and pallor of centrilobular cells, but vacuolation is only rarely seen.

1261/64

Kidney: A few foci of interstitial lymphocytes.

Lungs: Intense congestion and some haemorrhage.

1299/64

Lungs: Acute bronchiolitis and some large vessels contain fibrinous thrombi. One lung is congested and shows some alveolar-wall thickening.

Kidney: Damage to P.C.T. characterised by swelling, proteinaceous casts, and degeneration of individual cells.

Liver: Congestion. Insignificant vacuolation of cells. Kupffer cells are prominent and lymphocytes are increased.

1300/64

Kidney: Intense congestion.

Liver: Cells appear foamy with some small vacuoles. These appearances are probably mainly due to glycogen.

Lungs: Intensely congested. There is probably some swelling of histiocytes, but changes are not pronounced.

1321/64

Kidney: Some post-mortem change but nothing specific seen.

2.

Adrenals: Nil specific.
Stomach: Autolysis of pyloric mucosa.
Liver: Post-mortem change and intense lympho-histiocytic infiltration of portal tracts.
Lungs: Congested and interstitial polymorphonuclear leucocytosis.

1322/64

Adrenal: Swelling and degeneration of cells, probably post-mortem.
Kidney: Cloudy swelling consistent with P.M. change.
Lung: Normal apart from one area of congestion and haemorrhage, possibly artefactual.
Liver)
Heart) Nil of note.

1328/64

Kidney: Occasional degeneration, necrosis and desquamation of cells of P.C.T.
Liver: Pronounced hepatocellular vacuolation, presumably fatty change.
Lungs: Intense congestion and one superficial area of collapse, interstitial thickening, swelling of cells and polymorph infiltration. In addition, apparently at the apex, there is thickening of alveolar walls, cell swelling and polymorph infiltration with accompanying emphysema.

1335/64

Lungs: Patchy alveolar-wall thickening with swelling of cells and polymorph infiltration.
Adrenal: Very occasional degenerate cells. General appearances are normal.
Thymus: Nil of note.
Liver: N.A.D.
Kidney: No active damage is seen but there are many areas with altered basophilic tubules, lymphocytic infiltration, casts and some scar tissue. The appearances are consistent with a pyelonephritis and may not be due to treatment.

1347/64

Liver: The appearances are those of the fasting state.
Kidney: Degeneration, necrosis, desquamation, and vacuolation of cells most pronounced in p.c. tubules. Occasional degenerative cells are also seen at other levels of the renal tubules. Dilated tubules lined by basophilic low epithelium are frequently seen in the cortex.
Lung: Intense congestion with some areas of thickening of alveolar walls.

1348/64

Lung: Congestion, oedema, alveolar-wall thickening, and hypercellularity, including many polymorphs.

Testis: Tubular degeneration and inhibition of spermatogenesis.

Adrenal: Cells swollen. Possibly P.M. change.

Liver: Changes consistent with post-mortem effect.

Kidney: Changes are partly post-mortem but there is evidence of individual cell necrosis and desquamation in prox. conv. tubules.

Thymus: Some atrophy.

1393/64

Kidney: Cloudy swelling of proximal convoluted tubules.

Liver: The appearances are those of the fasting animal.

Testis: Although the germinal epithelium appears active, sperm formation is depressed.

Thymus: Atrophic.

Adrenal: Nil of note.

Stomach: Nil of note.

Lung: One area of septal thickening with swelling of cells and polymorphonuclear increase. There are also several foci of mononuclear cells with prominent histiocytes and an occasional larger focus with necrotic centre and a prominent polymorphonuclear content.

Skin: One biopsy shows hyperkeratosis.

1394/64

Kidney: Nil of note.

Liver: Badly fixed. No relevant features.

Stomach: N.A.D.

Testis: Spermatogenesis is almost completely inhibited and some tubules appear necrotic. Giant cells are present occasionally in the tubules.

Thymus: Almost complete atrophy.

Lung: Congested with small consolidated areas containing lymphocytes and foamy histiocytes, some of which contain necrotic centres and here the polymorphonuclear content is conspicuous. One area contains a prominent giant cell.

Skin: Hyperkeratosis with one area showing a mixed inflammatory infiltrate of the upper dermis and a micro abscess in the stratum spinosum.

1395/64

Kidney: Tubular dilatation, individual cell necrosis and desquamation. Many dilated tubules are lined by basophilic epithelium and contain proteinaceous casts.

4.

Lungs: Show large areas with oedema and apparent destruction of alveoli. There is great thickening of the surviving alveolar walls with a fibroblastic, histiocytic and polymorphonuclear reaction. Many of the histiocytes are greatly swollen with large hyperchromatic nuclei.

Testis: Degenerative changes are seen but spermatogenesis is not completely suppressed.

Salivary gland: Nil of note.

Liver: Pronounced centrilobular fatty change.

Adrenal: Lipid depletion and degeneration of occasional cell in zona fasciculata.

Heart: Nil of note.

Thymus: Atrophic.

Stomach: Nil of note.

1396/64

Lung: Congested with areas of swelling of alveolar walls, swelling of cells, and interstitial polymorphonuclear leucocytosis.

Adrenal: Moderately lipid depleted with condensation of cytoplasm of individual cells in zona fasciculata.

Kidney: Congested with swelling of p.c. tubules and degeneration of occasional cells. Some tubules are lined by a basophilic epithelium and, in some areas, there is an interstitial cortical infiltrate of lymphocytes with an occasional polymorph.

Liver: Fasting appearance with minimal centrilobular vacuolation of cells in some areas. There is an increase in lymphocytes in the portal tracts.

Thymus: Mild lympholysis.

Testis: Tubular degeneration and suppression of spermatogenesis.

Heart: Nil definite.

Caecum: Congested with mucosal oedema, congestion and haemorrhage. The normal glandular pattern is largely obliterated by oedema and subacute inflammation.

Skin: Hyperkeratosis, focal parakeratosis, and occasional micro-abscesses in the stratum spinosum. There is little underlying reaction.

1399/64

Kidney: Swelling of proximal convoluted tubules with individual cell necrosis and desquamation. Dilated tubules lined by a flattened basophilic epithelium and containing eosinophilic casts are conspicuous.

Liver: Extensive vacuolation of cells.

Adrenals: Swelling of cells of zona fasciculata with polymorphonuclear infiltration.

Lungs: Congested.
Testis: Tubular degeneration and inhibition of spermatogenesis.
Thymus: Atrophic.
Skin: Very slight hyperkeratosis.

1402/64

Kidney: Individual cell necrosis and degeneration with foci of vacuolated cells in the p.c. tubules.
Lung: Area of pronounced swelling of alveolar walls with swelling and probable increase of histiocytes and polymorphonuclear infiltration.

1407/64

Kidney: Congested with swollen p.c. tubules and degeneration and desquamation of individual cells. Some dilated tubules lined by a basophilic epithelium and containing proteinaceous casts are present in the cortex.
Lung: Intense congestion, some oedema, and a moderate interstitial polymorphonuclear leucocytosis.
Liver: Congested with a fasting appearance.
Adrenal: Appears lipid depleted with condensation of cytoplasm of individual cells. Fasciculata appears in disarray.
Thymus: Atrophic.
Testis: Degenerative changes in tubules and suppression of spermatogenesis.
Skin: Hyperkeratosis with, in places, almost complete degeneration of Malpighian layer. Minimal round cell reaction in the upper dermis.

1408/64

Kidney: Swelling of P.C.T. with individual cell necrosis. Many dilated tubules, containing proteinaceous casts, are lined by flattened basophilic epithelium. Congestion is extreme.
Liver: Widespread vacuolation of cells, most pronounced centrilobularly.
Adrenals: Congestion. Degeneration of individual cells and swelling in zona fasciculata.
Lungs: Congested.
Testis: Tubular degeneration and depression of spermatogenesis.
Skin: Hyperkeratosis.

1409/64

Lung: Oedematous and congested with a small area of consolidation showing central suppuration.

1437/64

Kidney: Occasional necrosis, degeneration and desquamation of individual liver cells in the prox. conv. tubules with proteinaceous casts. Many tubules are dilated and lined by a low basophilic epithelium.

Liver: Centrilobular necrosis and fatty change.

Adrenal: Cells appear swollen and lipid depleted.

Lungs: Alveolar-septal walls are swollen and hypercellular with prominent large hyperchromatic macrophages and numerous polymorphs.

Testis: Degenerative tubular changes with inhibition of spermatogenesis.

Heart: Nil of note.

Oesophagus: Complete necrosis of the oesophageal epithelium with an acute inflammatory reaction.

Stomach: Superficial oedema of the mucosa.

Skin: Hyperkeratosis and superficial pustule formation.

1447/64

Kidney: Congested. Swelling of p.c. tubules with individual cell necrosis and desquamation. Dilated tubules are lined by a low basophilic epithelium.

Lungs: Congested with mild thickening of alveolar walls and moderate increase in interstitial polymorphs and histiocytes.

Adrenals: Cortical cells are swollen and an occasional one is degenerate.

Liver: Cells appear swollen and there is a considerable lympho-histiocytic infiltrate of portal tracts.

Thymus: Atrophic.

Testis: Diminished spermatogenesis.

Skin: Moderate hyperkeratosis.

1449/64

Kidney: Fixation appears unsatisfactory but no specific lesions are noted.

Thymus: Lymphocytes appear decreased and mitotic activity increased.

Liver: Centrilobular cells appear pale but no specific features.

Lung: Congested. A very few areas of thickening of alveolar walls and two small areas with a mixed inflammatory infiltrate with large histiocytes containing pigment, presumably haemosiderin.

Adrenals: Nil of note.

Testis: Although the germinal epithelium is actively dividing, there is little sperm formation and the epididymis is devoid of sperm.

1450/64

Kidney: An occasional tubule is lined by low basophilic epithelium and a few contain slightly basophilic casts. A very few glomeruli show periglomerular fibrosis. No necrotic cells are seen.

Lung: Alveolar septa are thickened by cellular swelling and proliferation.
 Testis: Spermatogenesis appears depressed, epididymis normal.
 Thymus: Considerable mitotic activity in the reticulum cells.
 Adrenal: A small cortical adenoma. Nil of note.

1451/64

Kidney: Swelling, degeneration, necrosis and desquamation of cells in P.C.T. Dilated tubules containing casts and lined by flattened basophilic epithelium are prominent.
 Lung: Thickening of alveolar septa, congestion, and two small lung abscesses.
 Testis: Tubular degeneration and inhibition of spermatogenesis.
 Adrenal: Appears lipid depleted with some cellular degeneration.
 Liver: Fasting appearance with minimal vacuolation of cells in a few areas.
 Stomach: Congested with some superficial necrosis of the mucosa showing brown-yellow pigmentation.
 Skin: Hyperkeratosis and a mild diffuse lympho-histiocytic infiltrate in the upper dermis.

1484/64

Kidney: No definite abnormality detected.
 Adrenals: N.A.D.
 Lungs: Congested with large areas of alveolar wall thickening, swelling of cells and infiltration of histiocytes. In places this amounts to consolidation and here the mixed inflammatory infiltrate is pronounced.
 Testis: Degenerative changes in the tubules and suppression of spermatogenesis.
 Skin: Mild hyperkeratosis with one area showing a mixed chronic inflammatory infiltrate in the upper dermis.
 Liver: Nil specific.
 Thymus: N.A.D.

1485/64

Kidney)
 Adrenal) N.A.D.
 Lung: One area shows thickening of alveolar walls, due to swelling of cells, and increased polymorphonuclear infiltration.
 Liver: Congested.
 Testis: Inhibition of spermatogenesis and giant cell formation.
 Skin: There is pronounced hyperkeratosis and moderate acanthosis. In the dermis and upper subcutis there is haemorrhage and a mixed inflammatory reaction.

1486/64

Kidney: A very occasional degenerate cell is present in the proximal convoluted tubule. Throughout the cortex tubules lined by a basophilic low epithelium are seen, indicating, presumably, earlier tubular damage.

Adrenals: Cells appear compact, unlike the more usual lipid-containing appearance.

Lungs: Congested with areas showing some thickening of alveolar walls. This latter appears due to oedema, swelling of cells, and mixed inflammatory infiltrate.

Liver: Nil of note.

Testis: Sperm formation appears depressed.

Skin: Mild hyperkeratosis only.

1487/64

Kidney: A few dilated tubules lined by a low basophilic epithelium are present, but no active necrosis is seen.

Lungs: Congested. There is a small area of subacute inflammation probably unrelated to treatment.

Liver: One focal granulomatous reaction is present.

Thymus: N.A.D.

Testis: Suppression of spermatogenesis.

Adrenals: N.A.D.

Skin: Hyperkeratosis with mild focal chronic inflammation in the upper dermis.

1490/64

Testis: Tubular degeneration and suppression of spermatogenesis.

Kidney: Nil of note.

Lungs: Thickening of alveolar walls, swelling of cells, and interstitial polymorphonuclear infiltration. Congestion.

Liver: Round cell infiltration of portal tracts.

Adrenals: Normal.

1503/64

Skin: Slight hyperkeratosis and one very tiny area of ulceration.

Stomach: Submucous oedema.

Lung: Congested. There is an area of swollen alveolar septa with polymorph infiltration.

Liver: Congested. A few areas of vacuolation of cells. There is increased fibrosis and round cell infiltration of portal tracts with one area of apparent bile duct proliferation. The liver cells have a fasting appearance.

Thymus: Atrophic.

Testis: Sperm formation appears depressed and there is evidence of tubular damage characterised by occasional giant cell formation.

Kidney: Considerable damage to P.C.T. characterised by degenerate cells, many of which are shed into the lumen. Many tubules are lined by a flattened epithelium.

1530/64

Kidney: Degeneration and desquamation of individual cells of prox. conv. tubules. Some tubules are dilated and lined by a low basophilic epithelium.

Lung: Congested with thickening and increased cellularity of alveolar walls. The cell population consists of swollen histiocytes, many with hyperchromatic nuclei and numerous polymorphs.

Liver: Pallor and vacuolation of centrilobular cells, but little sudanophilic material is seen.

Thymus: Atrophic.

Testis: Tubular degenerative changes and diminished spermatogenesis.

Adrenals: Swelling of cells.

Skin: Hyperkeratosis and pustule formation with focal necrosis of epidermis.

1536/64

Kidney: Shows post-mortem changes but also evidence of ante-mortem effect consisting of vacuolation of occasional cells in p.c. tubules and some eosinophilia of epithelium.

Testis: Tubular degenerative changes and diminished spermatogenesis.

Liver: Centrilobular vacuolation.

Adrenal: Degeneration of individual cells.

Thymus: Atrophy.

Lungs: Patchy pneumonia, probably terminal.

Skin: Hyperkeratosis. No inflammation.

1537/64

Kidneys: P.C. tubules appear swollen and some are lined by a basophilic epithelium and show occasional mitoses. Occasional degenerate cells are desquamated.

Lungs: Congested but not otherwise severely affected, although septal walls appear slightly thickened in some areas.

1538/64

Tongue: Active ulceration of lateral margin.

10.

1546/64

Adrenals: Cortical cells swollen and an occasional cell appears degenerate.
Kidney: Numerous degenerate individual cells in p.c. tubules.
Liver: Centrilobular vacuolation and disarray. Occasional degenerate liver cells.
Lung: Two small areas of increased interstitial cellularity with a mixed cell population.
Testis: Tubular degeneration. In the region of the rete testis there is a small mass of adrenal-like tissue, probably heterotopic adrenal tissue.
Thymus: Atrophy.
Skin: Hyperkeratosis with superficial slough. The appearances suggest an earlier partial skin necrosis with underlying regeneration.

1599/64

Lung: Bronchopneumonia.
Tongue: Ulceration.
Oesophagus: Submucous oedema and pronounced superficial desquamation of epithelium.

1600/64

Kidney: Dilatation and pronounced vacuolation of some areas of prox. conv. tubules and occasional desquamated degenerate cells.
Salivary gland: N.A.D.
Oesophagus: Pronounced desquamation of superficial layers of epithelium, and acanthosis.
Adrenal: Area of necrosis and haemorrhage. Surviving cells are swollen and mitotic activity is increased.
Tongue: Acute ulceration and oedema.
Heart: Extensive focal necrosis of heart muscle with calcification. There is also interstitial myocarditis.

1610/64

Lung: One area shows thickening of alveolar walls with swelling of histiocytes. Throughout the lungs there are focal aggregates of a subacute inflammatory type.

1621/64

Tongue: Superficial necrosis of epithelium with areas of acute inflammation. The underlying muscle is very oedematous and there are foci of muscle necrosis with an acute inflammatory reaction.
Oesophagus: There appears to be superficial dissolution of the epithelium without a vital reaction.

Kidney: This organ contains virtually no normal proximal convoluted tubules: they are dilated, contain desquamated degenerate cells, and are lined by low basophilic epithelium.

Adrenal: Necrosis.

Liver: P.P. fibrosis.

1622/64

Oesophagus: There is some acanthosis of the epithelium and a mild chronic inflammatory infiltrate in the submucosa.

1623/64

Oesophagus: N.A.D.

Lungs: Thickening of alveolar walls, emphysema, and areas of active subacute inflammation. The appearances suggest a broncho-pneumonia and may be unrelated to paraquat.

1624/64

Oesophagus: A sparse submucous infiltrate of chronic inflammatory cells of doubtful significance.

1625/64

Oesophagus: A sparse submucous chronic inflammatory infiltrate of doubtful significance.

1640/64

Kidney)
Adrenal)
Testis) N.A.D.
Thymus)
Liver)
Oesophagus)

Lungs: Areas of congestion, alveolar wall thickening and increased cellularity with swollen histiocytes.

1641/64

Kidney)
Adrenal) N.A.D.
Oesophagus)

Lungs: Congested with areas showing thickening of alveolar walls and some oedema together with increased cellularity with swollen histiocytes and many polymorphs.

Testis)
Liver) N.A.D.
Thymus)

12.

1643/64

Oesophagus: N.A.D.

Liver: Periportal fibrosis and lympho-histiocytic infiltration.

Thymus)

Testis)

Kidneys) N.A.D.

Adrenals)

Lungs: Interstitial thickening and swelling of histiocytes occurring both perivascularly and in areas apparently unconnected with large vessels.

1644/64

Kidney)

Adrenal)

Thymus)

Liver)

N.A.D.

Oesophagus: Epithelium appears slightly thickened.

Testis: N.A.D.

Lungs: Congestion. Thickening of alveolar walls with increased cellularity, mainly histiocytic. In places this amounts almost to complete consolidation.

1653/64

Oesophagus: N.A.D.

Lung: Area of interstitial thickening with histiocytic reaction.

Liver)

Thymus)

Adrenal)

Kidney)

N.A.D.

1654/64

Kidney: Pyelonephritis.

Oesophagus)

Adrenals) N.A.D.

Liver: Moderate periportal fibrosis and lymphohistiocytic infiltration.

Thymus: N.A.D.

Lung: Congestion and interstitial thickening with swelling of histiocytes and polymorph infiltration.

1655/64

Kidney: N.A.D.

Liver: N.A.D.

Testis: No definite abnormality although one giant cell is present in one tubule.

Adrenal: One small cortical adenoma present.

Oesophagus: Moderate acanthosis.

Thymus: N.A.D.

Lungs: There is an area of alveolar-wall thickening with swelling of histiocytes and some increase in polymorphonuclear leukocytes.

1656/64

Kidney: No abnormality which can definitely be attributed to treatment.

Testis)

Liver)

Adrenals) N.A.D.

Lungs)

Thymus: Regenerative activity.

Oesophagus: Moderate acanthosis.

APPENDIX II

Paraquat Dichloride Purified By Recrystallisation

Dermal toxicity

The acute toxicity and subacute percutaneous toxicity of paraquat have been investigated in albino rabbits of both sexes in the same way as described for diquat. Rabbits with initial body weights between 1.5 and 3.0 kg were used. The area of application was kept covered by polythene sheet held in place by a jacket of stiff canvas.

(a) Acute toxicity

Single applications in aqueous solution were made to dehaired dorsal skin with the concentration adjusted to give an approximately constant volume of 1 ml/kg for all dose levels.

In two male and two female rabbits to which single doses of 1 g/kg were applied, one female died after 24 hr and one male after 48 hr. The two surviving animals were somewhat inactive but survived until killed 7 days later.

In a similar group to which a dose of 0.5 g/kg was applied, one male died after 2 days and one female after 4 days. The other female was severely affected and was killed after 5 days. The remaining male survived for a week but was inert and in poor condition.

Nothing abnormal was found at post-mortem examination or histopathological examination of the animals in the 1 g/kg group apart from some vascular congestion of the lungs. In two of the animals in the 0.5 g/kg group early fatty degeneration of the liver was found and some degeneration of the tubules in the renal cortex.

(b) Subacute toxicity

The results of repeated daily application of a number of dose levels to groups of 5 male and 5 female rabbits are recorded in the table below. The area of application was clipped of hair a day or so before the start of the experiment and was examined carefully for injury. It was kept covered by polythene sheet etc. as previously described to prevent oral contamination.

Dose (mg/kg)	Sex	Weight Loss (% initial wt.)	Time of Death (days from start)	Mortality	
50	M		4 4 6 6 7	5/5	Local effects. Skin became very moist after 1 application. Some reddening followed by sloughing of superficial epidermal layers. Systemic effects. Reduction in activity after third application. P.M. and histology. 1 animal. Vascular congestion lungs - areas of collapse. Some dilatation and degeneration of renal tubules.
25	M		4 6 7 7 8	5/5	Findings similar to 50 mg/kg above.
12.5	M	22) 31) 21) 20.4 18) 10)	7 8 13 14 21	5/5	Local effects. Reddening and sloughing after 3 to 5 days. Ulceration and scab-formation 2 animals after 7 and 13 days. Systemic effects. 1 animal nervous after 5 days - some incoordination, died after 7 applications. 2 animals - increasingly inert - respiratory difficulty 12-14 days, incoordination and weakness in 1 surviving animal died in observation period. P.M. and histology. Pulmonary congestion - 3, gastric ulceration - 2, renal tubular degeneration - 1.
12.5	F	16 32	4 4 4 6 10	5/5	Local effects. Only early signs of reddening and some subcutaneous oedema in animal dying after 4 applications. Systemic effects. 1 animal developed tremors and increased salivation, died next day. 3 animals - increasingly inert - died overnight. 1 animal - incoordinate. P.M. and histology. Pulmonary congestion - 3, haemorrhage into gastric mucosa - 2, renal tubular degeneration - 3.

Dose (mg/kg)	Sex	Weight Loss (% initial wt.)	Time of Death (days from start)	Mortality	
6.25	M	24.5)	12	3/5	Local effects. Reddening and sloughing began 5-9 days - 4 animals. Ulceration and splitting of skin in 3 animals. 1 animal unaffected. Systemic effects. 2 animals died after 2-3 days of increasing activity, weakness and incoordination after 19-20 days in 2 animals. P.M. and histology. Pulmonary congestion - 2, areas of necrosis in liver - 1, areas of necrosis in pancreas - 1.
		13)	12		
		11) 9	21		
		-) + 3)			
6.25	F	22)		0/5	Local effects. Earliest changes between 8-10 days. Scab-formation after 13 days in 4 animals. Systemic effects. All animals showed reduced activity and 3/5 had some incoordination and weakness after 14 days. Recovery of wt. and activity occurred in all during observation period.
		24)			
		38) 22			
		10) 17)			
3.13	M	-)	-	0/5	Local effects. Mild reddening in 3 animals between 7 and 9 days. Scab-formation in 3 after 12-14 days. Systemic effects. None apart from wt. loss in 3/5. Animals gained wt. in observation period.
		4.5)			
		12.5) 7			
		21) + 3)			
3.13	F	29.5)	21	2/5	Local effects. Early changes in all between 5th-7th day. Scab-formation and ulceration in all between 8th-13th day. Systemic effects. 2 animals became weak and unsteady towards end of period: died in observation period. P.M. and histology. Ulceration and necrosis of gastric mucosa - 1. Some renal tubular degeneration - 2.
		+ 35)	25		
		23.5) 9			
		27) -)			

Dose (mg/kg)	Sex	Weight Loss (% initial wt.)	Time of Death (days from start)	Mortality	
1.56	M	17) 18) -) 4.6 + 6) + 6)	-	0/5	Local effects. Early changes in 3 animals between 7-15 days. Scab-formation 2nd, 10th, 12th days. Systemic effects. Reduced activity and wt. loss in 1 animal in third week.
1.56	F	3.5) -) 12.5) 7.6 8.5) 6)	4	1/5	Local effects. Early changes 5-7th day in 4. Scab-formation 11th-15th day. Systemic effects. One animal died after 4 applications. No effect in 4/5. P.M. and histology. No signs apart from vascular congestion of lungs, liver and spleen.

The results of single applications suggest that paraquat is a little more readily absorbed from the skin than diquat. This is in keeping with the minor differences in their oral toxicity which can be related to differences in absorption from the gut.

Calculation of a subacute (20 day) percutaneous LD50 from the combined mortality result for both sexes gave a figure of 6.24 mg/kg/day (4.5 mg/kg as paraquat ion) (confidence interval 4.6-8.5 mg/kg for $P = 0.5$). This is little different from the subacute percutaneous LD50 for diquat (5.7 mg/kg/day diquat ion) and as the parenteral toxicities of diquat and paraquat are of the same order, it is a further indication that the subacute percutaneous LD50 of these substances is largely a measure of their penetration through damaged skin.