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PARAQUAT: ACUTE ORAL TOXICITY - A SPECIES COMPARISON

by

J R Heylings

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1. INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride) is a very effective broad spectrum contact herbicide widely used throughout the world. Paraquat poisoning resulting from oral ingestion was first reported in 1966(1). Since then, there have been reports of mortality, mainly self-inflicted which have involved this chemical (2). Though paraquat poisoning accounts for a substantial number of suicides throughout the world, proper use of the chemical produces incalculable benefits by improving agricultural productivity in developing countries where the product is used extensively. Ingestion of concentrated liquid formulations of paraquat can lead to death within 1-3 days from shock, multi-organ failure, and upper gastrointestinal tract erosion. Patients who die one week or more after the ingestion usually develop progressive pulmonary fibrosis (3). A common characteristic of both groups of patients is acute renal failure (4,5). Toxicokinetic parameters in man following oral ingestion of paraquat have been largely extrapolated from animal studies (6-9). In addition, important information on the kinetics of paraquat has also been reported from cases of human poisoning (5).

Most studies of acute paraquat toxicity in laboratory animals have been in the rat. In this species only a small proportion of an oral dose (5-10%) of paraquat is absorbed from the gastrointestinal tract but this absorption is rapid (10). It has also been demonstrated that paraquat is selectively absorbed by the jejunum and ileum (11), and by an energy dependent mechanism (12). There is also evidence of energy dependent accumulation in specific organs, particularly the lung (13,14). Virtually the entire absorbed dose is eliminated unchanged into the urine by glomerular filtration and active secretion by the tubular transport mechanism for organic cations (10,15). Therefore, paraquat renal clearance, which can be equated with total body clearance, will decrease as renal failure develops. The dog and rat have been used as models of paraquat toxicity in humans because the toxic effects and kinetics are similar in these species.

In this report a comparison of acute oral paraquat toxicity in several species has been made. Although there have been many papers published on the acute toxicity of paraquat, the main focus of this report is a review of available CTL data where unformulated paraquat dichloride has been given as a single oral dose. Most of the data presented have not been published in the open literature, but are cited in internal CTL reports. The overall objective of this report is to collate the clinical findings following paraquat oral dosing in a single document. This can then be used as a basis for a full comparison of toxic end points across a number of species and to consider how they relate to the known toxicity of paraquat in man.

Species

Paraquat acute toxicity has been studied in a wide range of mammalian species. The majority of studies on paraquat undertaken at CTL as well as in the open literature involve rat or mouse. To these we have included our own in-house and Contract Laboratory data in guinea pig, rabbit, cat, dog and primates. Due to the high incidence of human poisoning with paraquat there are also many individual case reports available. In a few cases of human poisonings sufficient clinical parameters have been documented to allow a comparison to be made with the controlled studies in experimental animals.

Paraquat dosing

Many of the published papers on paraquat involve the commercially formulated product such as GRAMOXONE. The concentration of paraquat in the many formulations which are marketed throughout the world varies. In addition, the formulation adjuvants differ between products which are used for different applications. To remove as much variability as possible only animal data on the active ingredient paraquat dichloride (dissolved in water) has been used in this reported species comparison. Dose levels throughout this report relate to the amount of paraquat ion per kilogram body weight. In man such data on pure paraquat is obviously not available and the information only relates to formulated product.

Parameters measured

Historically, the median lethal dose (MLD) was often used as a relatively simple assessment of the acute toxicity of chemicals. Paraquat studies have been performed for many years and these data have been used here. It is important to point out that under the 1986 Animals (Scientific Procedures) Act such studies and have been replaced by much more objective assessments of likely lethality of paraquat. CTL has more recently designed such studies involving much more objective assessments of paraquat toxicity . This includes the measurement of paraquat in plasma by a sensitive radioimmunoassay technique over a time course of 48 hours. By taking serial blood samples over this time from an animal the full plasma paraquat profile can be obtained. This provides us with the peak blood concentration and the total blood area-under-curve (AUC). Blood paraquat levels indicate the net effect of absorption and excretion of paraquat and provide accurate information on the prognosis following oral dosing. By combining AUC data from animals which have been given different doses of paraquat an assessment of the median lethal AUC for a given species can be derived. This is illustrated in Figure 1. Over the last few years the AUC value has proved to be the best indicator of paraquat acute toxicity.

During the acute phase, and often prior to the development of the characteristic pulmonary changes, renal failure is a common feature of toxicity across all species. Standard clinical tests of renal function which include the measurement of plasma creatinine and blood urea nitrogen (BUN) give additional information on prognosis following paraquat dosing. About 24-48 hours after a lethal amount of paraquat has been absorbed from the GI tract, creatinine and BUN levels are almost invariably elevated. There are also very clear pathological changes in the kidney, lung and GI tract. This report will only focus on the plasma parameters associated with lethality as outlined above.

Acute Oral Toxicity

Rat

The acute oral median lethal dose (MLD) of paraquat in the male and female rat lies in the range of 80-150mg/kg (16, 17, 18, 19). CTL studies indicate that

126-157 as salt (Duerden 1994)

the MLD for paraquat (as dichloride) is 100-120mg/kg in the male AP rat (20). The plasma kinetics following a single oral dose show that PQ is very rapidly absorbed with peak plasma values within one hour of dosing. This declines rapidly over the following 1-2 hours. There is a secondary elevation in plasma PQ (24h after dosing) only at toxic doses (as a consequence of impaired renal excretion). The plasma profiles for three dose levels of PQ are shown in Figure 2. The calculated plasma parameters, creatinine and BUN levels at 100mg/kg (MLD) are shown in Table 1.

Mouse

The acute oral median lethal dose of paraquat (MLD) in the male and female mouse lies in the range 120-250mg/kg (21,22). Recent studies at CTL found the MLD to be 170mg/kg in the female CD1 mouse and 200mg/kg in female AP mouse (23,24). The plasma PQ kinetics following dosing were very similar to rat (Figure 3). Peak plasma PQ occurred within one hour and declined rapidly. Only at a toxic dose in the CD1 strain of mice (200mg/kg) was there a secondary elevation of plasma PQ at 24hrs and this was associated with an elevation in BUN values. Changes in creatinine were less pronounced which may suggest other haemodynamic effects; or even effects of fasting the animals, are occurring in addition to renal impairment. However, in these studies individual animals (23) with high plasma PQ at 24hr had elevated levels of BUN and creatinine. Calculated plasma PQ parameters, creatinine and BUN levels at 200mg/kg (MLD) are shown in Table 1.

Guinea Pig

The acute oral median lethal dose (MLD) of paraquat in male guinea pigs lies in the range 15-30mg/kg (17, 18, 19). There are no CTL studies on paraquat dichloride in guinea pigs. However, the literature is in general agreement that this species is more sensitive to acute paraquat toxicity compared with rodents. There are also differences in the lung toxicity in that unlike the rat, the guinea pig does not develop pulmonary fibrosis. Guinea pigs do, however, develop emphysema, oedema and congestion of the lung tissue.

Cat

The acute oral median lethal dose (MLD) of paraquat in the female cat is 30mg/kg (17). There is no additional information on clinical observations in this citation. There are no CTL studies on paraquat dichloride in this species.

Dog

The acute oral median lethal dose (MLD) of paraquat in the male adult Beagle dog is 10-15mg/kg (25). This value is based studies with several aqueous formulations of paraquat. More recent CTL studies using paraquat dichloride confirm this dose range to be around the MLD (26). The plasma paraquat profile following a single oral dose shows a rapid absorption into blood with peak plasma values occurring at 1-2 hours. As found in other species, plasma values decline rapidly between 2-7 hours and are close to baseline by 24 hours at sub toxic doses (Figure 4). At doses above the MLD the plasma profile is similar with peak values at 1 hour. However, there is a secondary rise in plasma PQ from 12 hours which elevates the AUC and is associated with clinical signs of toxicity which are characteristic of paraquat toxicity in dogs (27). The primary organs affected include the kidney (acute renal failure) GI tract (mucosal erosion) and lung (pulmonary fibrosis). Calculated plasma PQ parameters, creatinine and BUN at a toxic oral dose are shown in Table 1.

Rabbit

The acute oral median lethal dose (MLD) of paraquat in the rabbit is in the range 50-150mg/kg (19). By contrast to rodents, the rabbit displays much less tendancy to involvement of the lungs (28, 29). A much more extensive study of the toxicity and tissue distribution of paraquat has been done at CTL in the female New Zealand White rabbit (30). In this study the MLD was found to be 40-50mg/kg. At toxic doses there was evidence of renal proximal tubular necrosis. As found previously in this species there was no accumulation of PQ in the lung and no pathological changes were observed in this organ. The plasma profile following a 30mg/kg oral dose of PQ is shown in Figure 5. Peak PQ values were found in plasma at 1 hour after dosing. Between 48 and 72 hours the plasma paraquat levels showed a secondary elevation. This was associated with an elevation in plasma creatinine and BUN. In these studies (30) there was decreased urinary output indicating renal functional impairment. Plasma parameters can be compared with other species in Table 1.

Monkey

The acute oral median lethal dose (MLD) of paraquat in male and female monkeys is 50mg/kg (18). The toxic effects of PQ in this species are important since the primate has respiratory and thoracic similarities to man which may be determinants of pathological distribution within the lung. Like humans, animals displayed gross pathological lesions in the lung, liver and kidney at

lethal doses. Animals had emphysema, congestion and haemorrhage of the lung, centrolobular necrosis of the liver and tubular necrosis of the kidney. There are no CTL studies on this species on unformulated product.

Man

The acute oral median lethal dose (MLD) in man is about 45mg/kg. This value is based on a subset of a study from ICI Japan (31), where the amount of paraquat ingested could be reasonably accurately ascertained in 69 cases. The MLD is equivalent to 3 grammes of paraquat and this figure is widely accepted in the literature (5, 32). A plot of paraquat ingested versus mortality has been constructed and is shown in Figure 6. There are many publications on the detailed clinical and pathological effects of paraquat in man and it is widely accepted that pulmonary failure and renal function impairment are primary toxic end points (2-6, 32, 33). Information on the plasma paraquat profile following poisoning is obviously very sparse. There are only a few case studies where early blood samples have been taken together with serial samples over the first 24 hours. However, there are good prognostic indicators of survival based on plasma paraquat and time after ingestion which have been successfully used in the management of poisoning cases (34). By combining kinetic information from experimental animals and available human plasma data a prediction of the plasma profile in man can be obtained. This is presented in Figure 7 for the medial lethal dose of 45mg/kg. For comparison, toxic oral doses in other species shown on the same scale. This demonstrates the general similarity in plasma profile and AUC at doses which are approaching or at the median lethal dose.

Conclusion

Acute oral median lethal doses for paraquat differ quite widely between the 8 species reviewed here. The dog has the lowest MLD at 15mg/kg and mouse the highest MLD at 200mg/kg. Generally, the rodent species can tolerate larger doses of paraquat than higher mammals. Man and primates are quite similar in response to the chemical with MLD values around 50mg/kg. Interestingly, despite the wide range in MLD values across species, the peak plasma paraquat

concentrations, AUC and markers of renal function are very comparable at a near lethal dose (Figure 7). This suggests that the species differences in toxicity are probably due to differences in gastrointestinal absorption of paraquat rather than differences in systemic tolerance to the chemical.

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TABLE 1

SPECIES	MLD mg/kg paraquat ion	$LPEAK_{50}$ µg/ml paraquat ion	$LAUC_{50}$ µg/ml.h paraquat ion	Plasma Creatinine at MLD mg/dl (normal range)	Plasma BUN at MLD mg/dl
RAT	100	5 - 10	50 - 100	0.4 - 0.5 (0.3 - 0.4)	30 - 50 (20 - 30)
MOUSE	200	6 - 8	30 - 50	0.5 - 1.5 (0.5 - 1.5)	100 - 200 (50 - 70)
GUINEA PIG	20	---	---	---	---
RABBIT	50	4 - 6	20 - 40	2 - 5 (0.5 - 1.5)	100 - 150 (30 - 50)
CAT	30	---	---	---	---
DOG	15	7 - 10	40 - 50	1.5 - 2.0 (0.5 - 1.5)	30 - 50 (20 - 30)
MONKEY	50	---	---	---	---
MAN	45	5 - 10	20 - 30	1.8 - 2.0 (0.8 - 1.0)	15 - 20 (10 - 15)

Plasma AUC represents (0-24h). Plasma Creatinine and BUN are values at 48-72hr. $LPEAK_{50}$ is the peak plasma PQ concentration at MLD. $LAUC_{50}$ is the AUC at MLD. MLD is Median Lethal Dose.

FIGURE 1

Relationship between AUC of Plasma Paraquat and Incidence of Mortality in the Dog

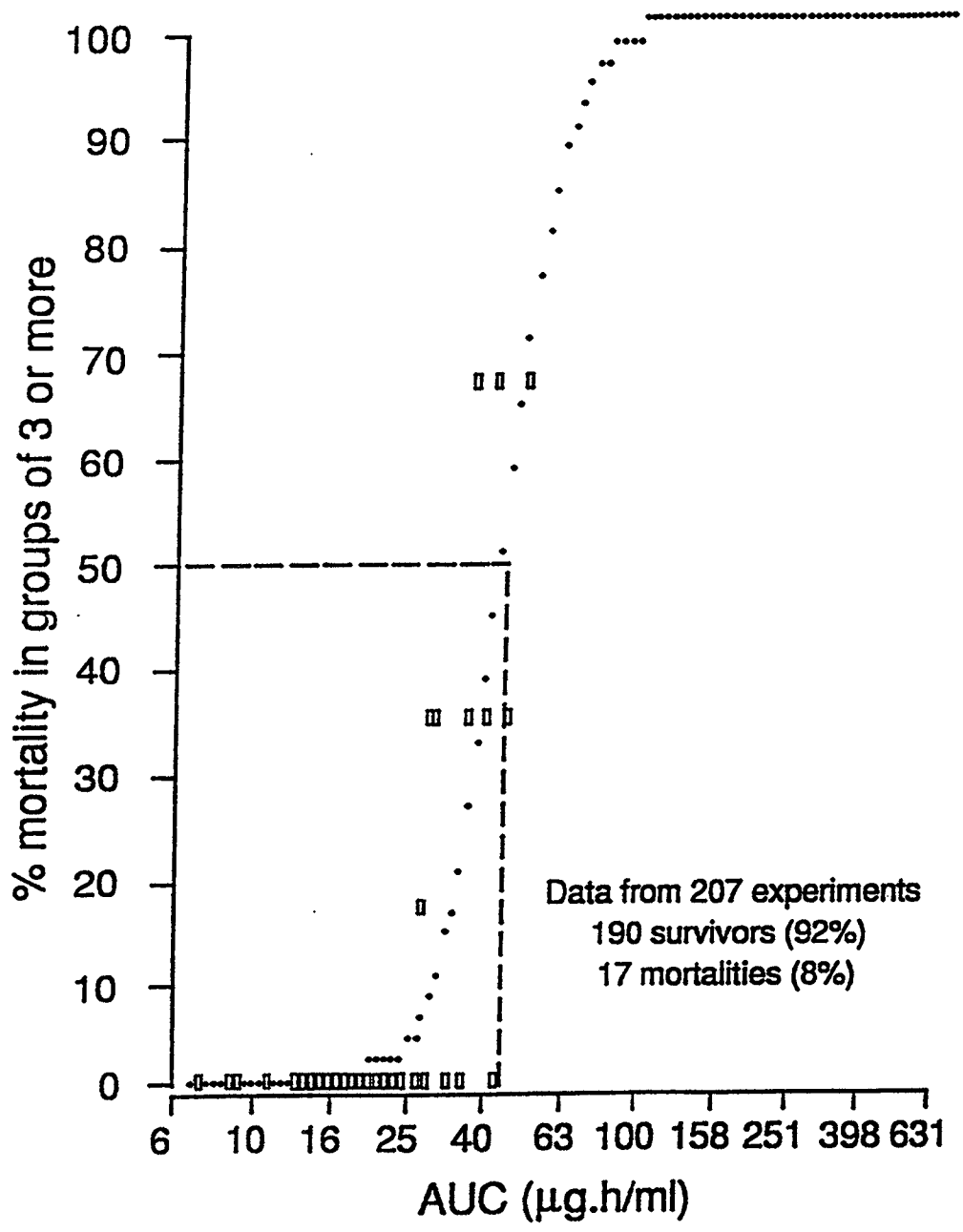

FIGURE 2

Rat plasma paraquat following a single oral dose

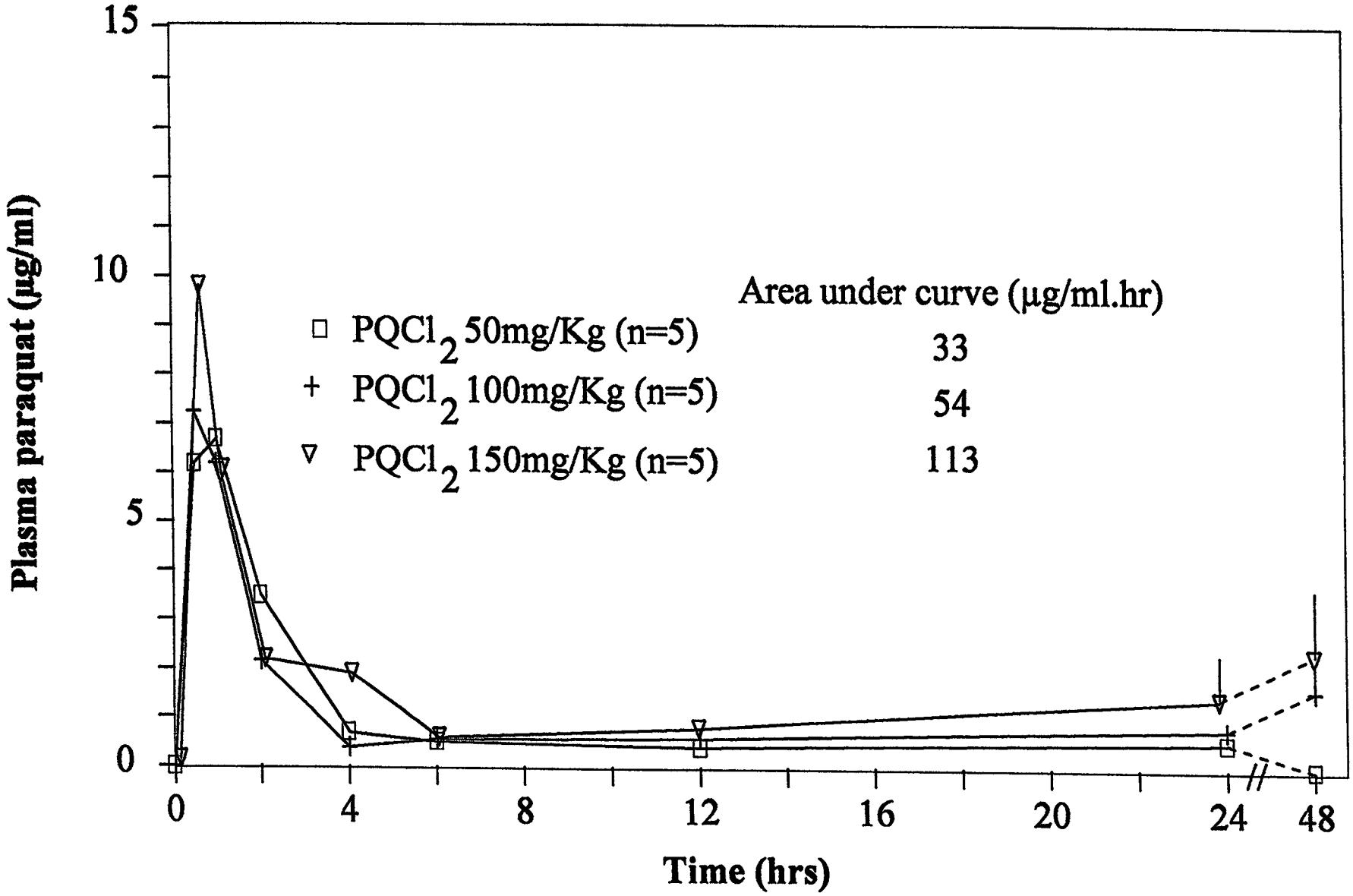

FIGURE 3

Mouse plasma paraquat profile following a single oral dose

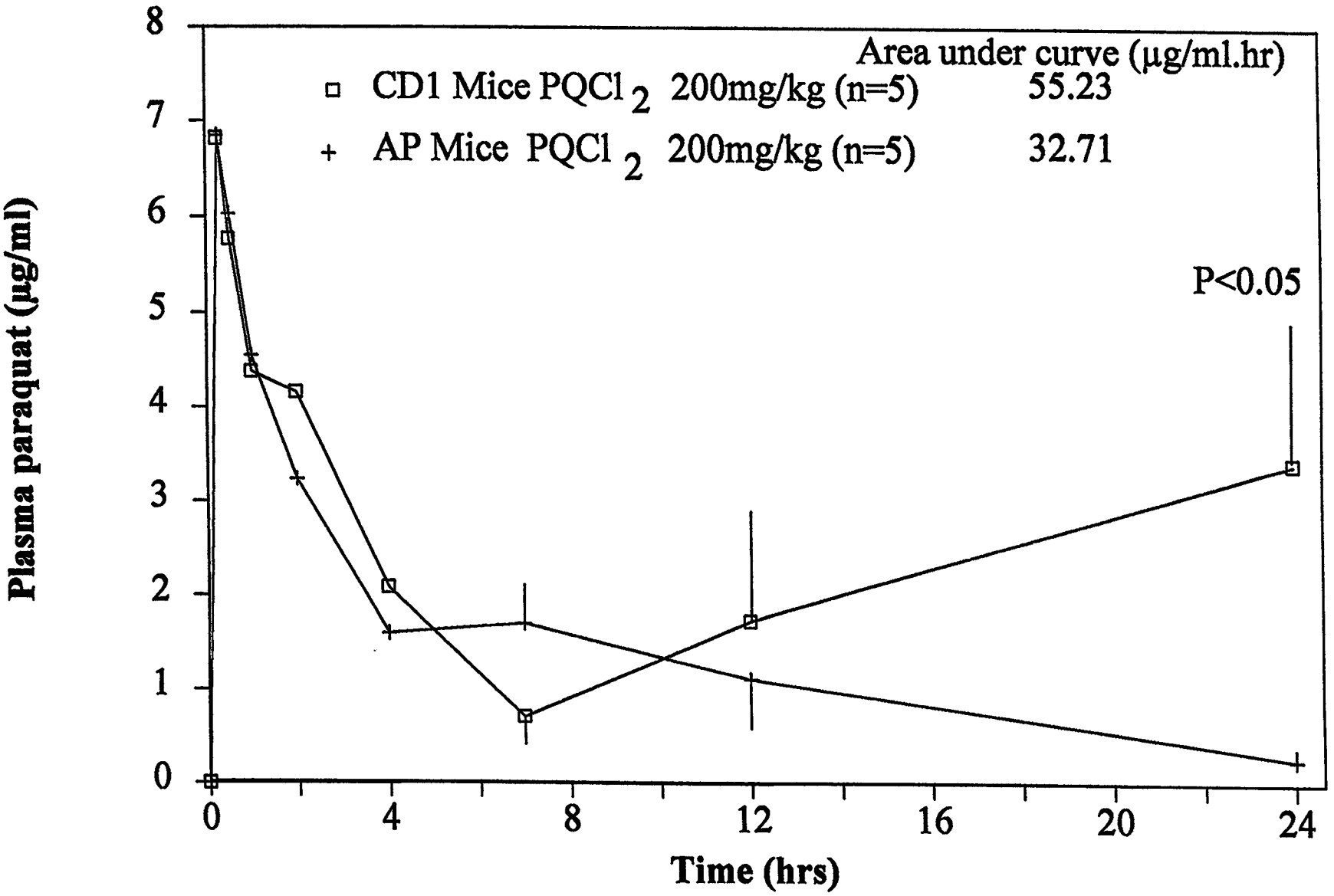

FIGURE 4

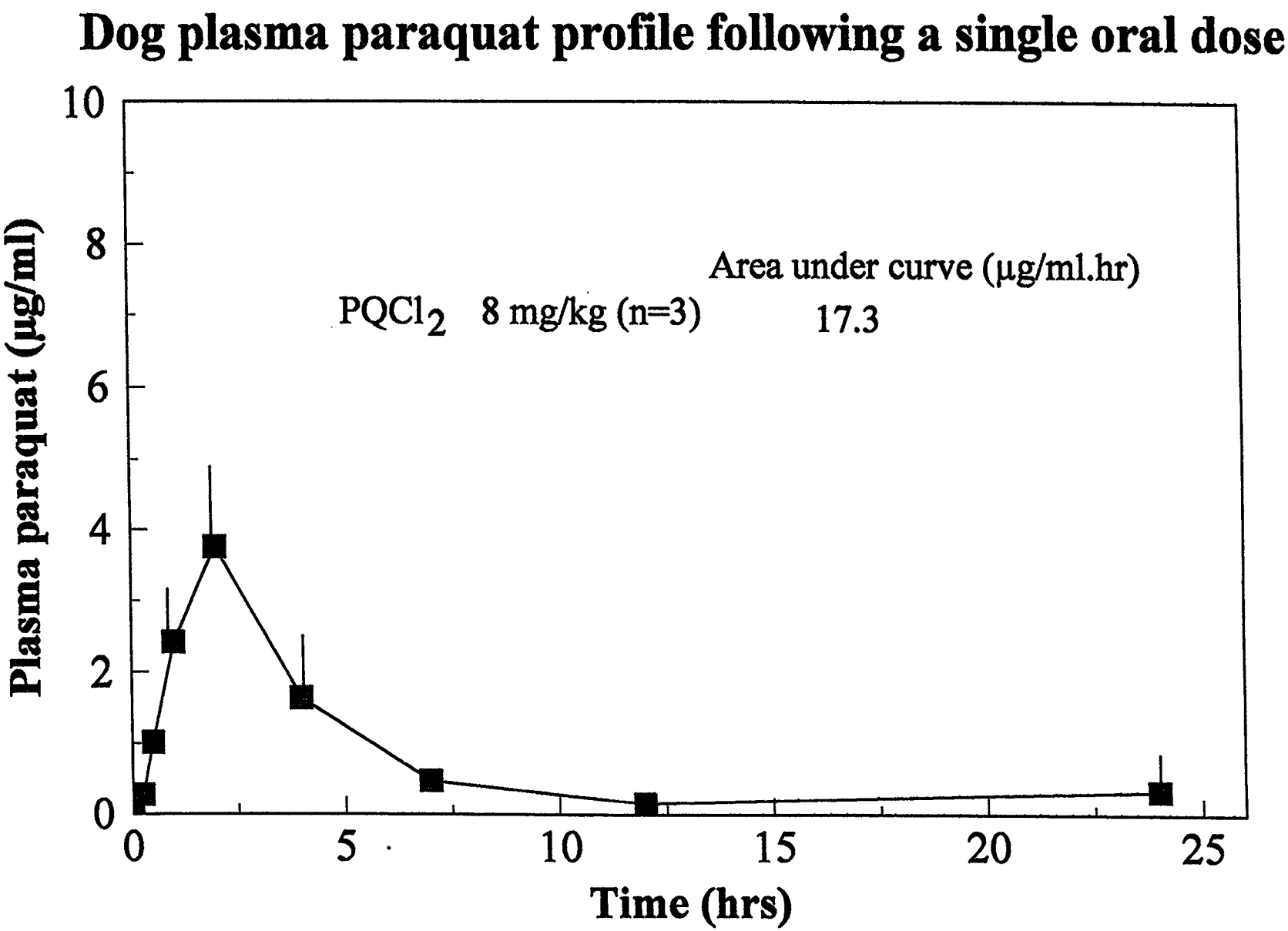
Dog plasma paraquat profile following a single oral dose
Plasma paraquat (µg/ml)
Time (hrs)
PQCl2 8 mg/kg (n=3)
Area under curve (µg/ml.hr)
17.3
0
2
4
6
8
10
0
5
10
15
20
25

FIGURE 5

Rabbit plasma paraquat profile following a single oral dose

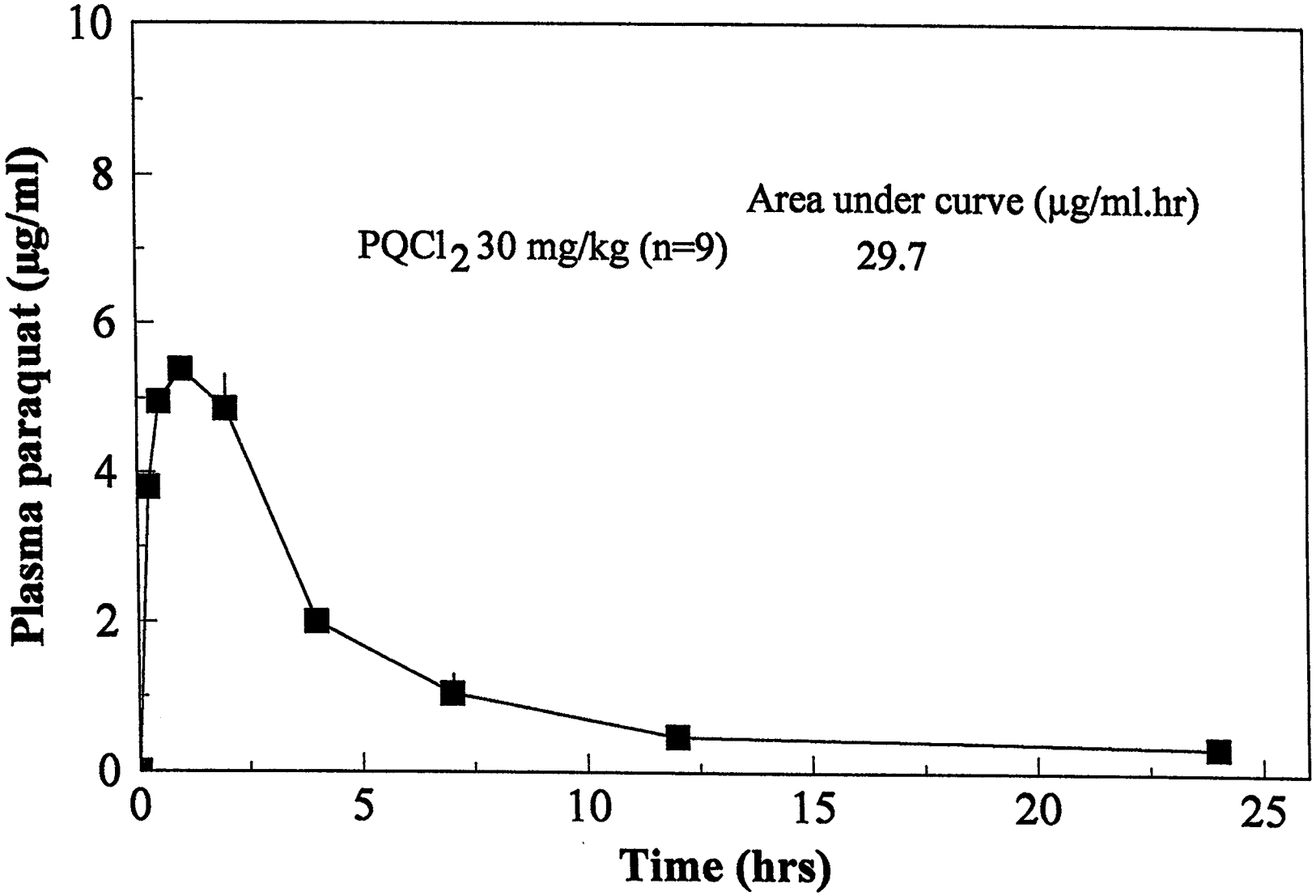

FIGURE 6

Human mortality following oral ingestion of paraquat

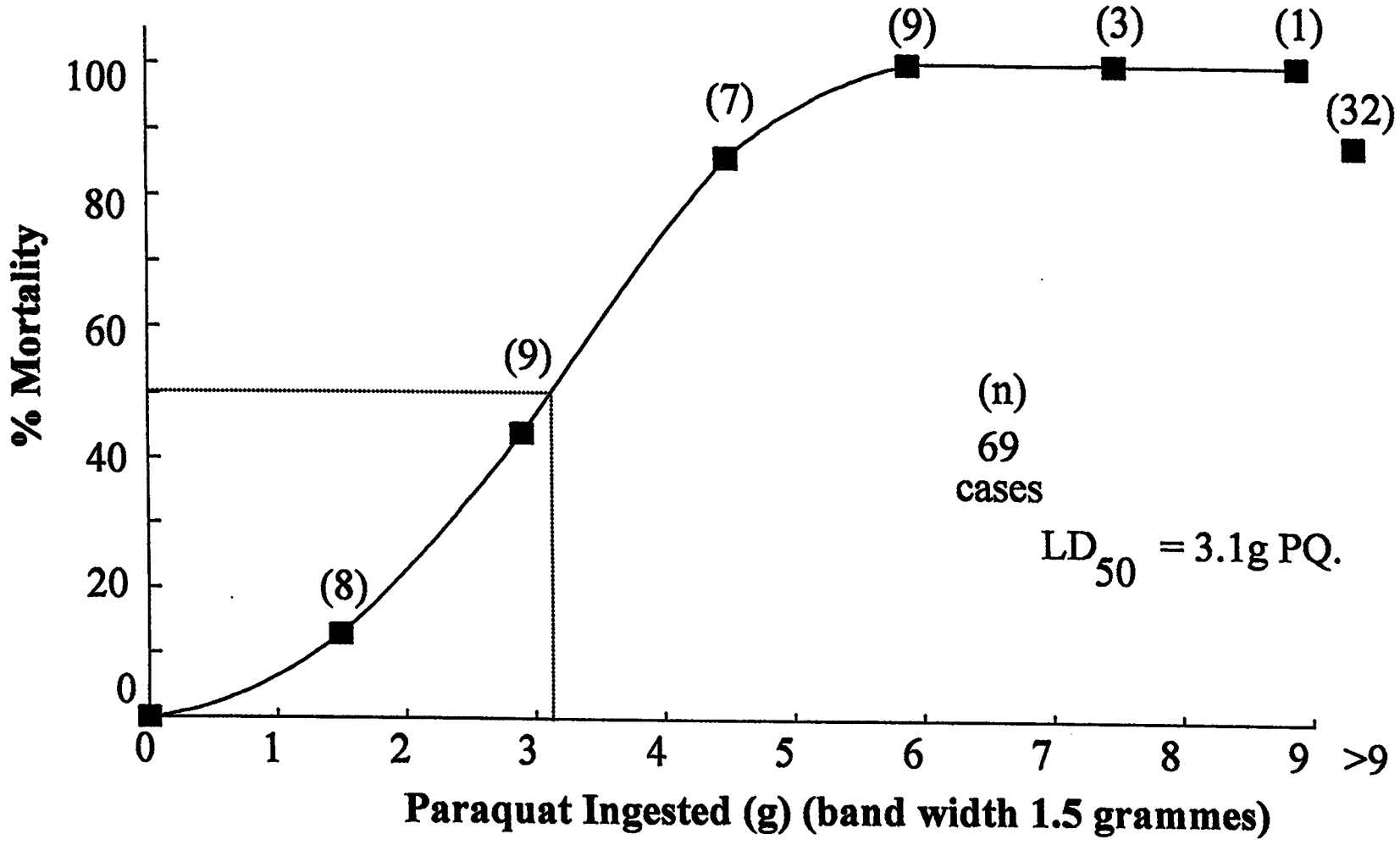

FIGURE 7

Paraquat plasma profiles in 6 species following a single oral dose