

ACUTE AND SUBACUTE TOXICITY IN SHERMAN STRAIN RATS EXPOSED TO 4,4'- AND 2,2'-DIPYRIDYL

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Acute and subacute toxicity was studied in adult Sherman strain rats exposed to 4,4'- and 2,2'-dipyridyl. The single oral LD50 for 4,4'-dipyridyl was 175 mg/kg in male and 172 mg/kg in female rats; for 2,2'-dipyridyl it was 100 and 107 mg/kg, respectively.

Symptoms of toxicity for 4,4'-dipyridyl included subdued behavior, red stains around mouth and eyes, lacrimation, swelling around the eyes, and occasional convulsions. Rats receiving 2,2'-dipyridyl had subdued behavior, loss of muscle coordination, red urine, tremors, and convulsions. Onset of symptoms was rapid, and most rats died from internal hemorrhage within 2 d after dosing.

Rats given lower single oral doses of 90 mg/kg 2,2'-dipyridyl and 155 mg/kg 4,4'-dipyridyl had no symptoms and no organic pathology 2 wk after dosing. Rats given doses of 5.1 and 25.5 mg/kg 4,4'-dipyridyl and 7.13 and 35.6 mg/kg 2,2'-dipyridyl in their drinking water for 3 mo showed no significant effects that could be related to the consumption of dipyridyl.

INTRODUCTION

Liddle et al. (1980) determined that some of the marijuana brought illegally into the United States from Mexico in 1978 was contaminated with the herbicide paraquat [$C_{12}H_{14}N_2$]2Cl.

Paraquat (Fig. 1) is produced as the dichloride by the reaction of 4,4'-dipyridyl (Fig. 1) with methyl chloride in water (Martin and Worthing, 1974).

When a paraquat-tainted marijuana cigarette is smoked, most of the paraquat is pyrolyzed to 4,4'-dipyridyl, but some enters the lungs unchanged (Trux and Torrey, 1978).

Since the toxic effects of 4,4'-dipyridyl have not been extensively examined, we designed studies to determine the acute toxicity of this

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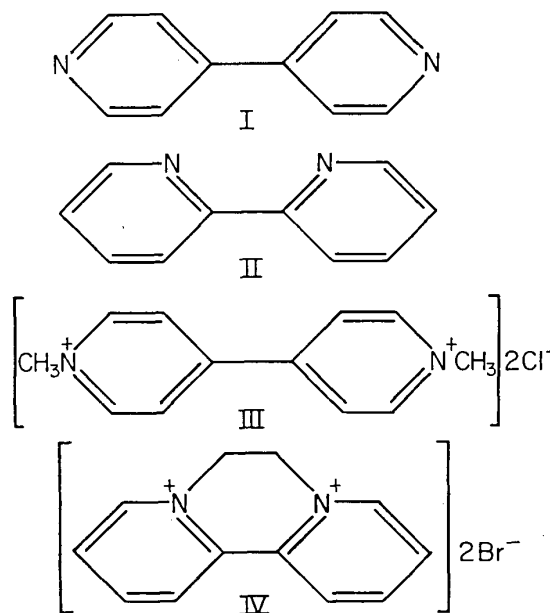


FIGURE 1. Structures of (I) 4,4'-dipyridyl, (II) 2,2'-dipyridyl, (III) paraquat, and (IV) diquat.

chemical, which is paraquat's main combustion product (Martens et al., 1977; Leete, 1978; Pasi, 1978).

Although *N*-methyl-4,4'-dipyridyl has been suggested as a metabolite of paraquat, it is not known whether 4,4'-dipyridyl is a metabolite of paraquat (Pasi, 1978).

For comparison, 2,2'-dipyridyl, an isomer of 4,4'-dipyridyl (Fig. 1) that is found in cigarette smoke (Brown, 1972) and is very similar in structure to the herbicide diquat [$\text{C}_{12}\text{H}_{12}\text{N}_2$] 2Br , was included in the toxicity studies. Diquat (Fig. 1) as the dibromide is made by the reaction of 2,2'-bipyridyl (Fig. 1) and ethylene dibromide in water (Martin and Worthing, 1974). In the scientific literature, 2,2'-dipyridyl has an acute oral LD₅₀ in rats of 256 mg/kg (Windholz, 1976). 2,2'-Dipyridyl induces excretion of excessive amounts of Fe in Fe-overloaded rats by its Fe-chelating properties (Grady et al., 1976). It is toxic when given ip in divided doses of 50 mg/kg·d in 0.25 ml 25% ethanol (Bora et al., 1972). It produces hyperglycemia in rats at 10 mg/kg or higher doses (Kenny and Morey, 1962). It also decreases the output of gastric secretory volumes of pepsin in rats (Bass et al., 1966).

In this paper we report the results of a study of acute and subacute toxicity in rats exposed to 4,4'- and 2,2'-dipyridyl.

METHODS

4,4'-Dipyridyl and 2,2'-dipyridyl were obtained from Aldrich Chemical Co. (Milwaukee, Wis.).¹ For acute toxicity studies 2% solutions in 20% ethanol were given by gavage. Controls were given the 20% ethanol solvent. In subacute toxicity studies solutions of 0.01 or 0.05% in drinking water were used; controls were given water only. To protect the solutions from degradation caused by ultraviolet light, we used amber containers or Al foil wrapping.

Acute oral LD50 values were obtained in 4 groups of 10 adult male or female Sherman rats² that had been fasted 24 h before they were dosed. In additional studies, we gave smaller single doses of 155 or 90 mg/kg 4,4'- or 2,2'-dipyridyl to 10 rats each by gavage to observe the effect of nonlethal doses. In subacute toxicity studies, 3 groups of 6 and 3 groups of 10 rats received 0, 0.01, or 0.05 2,2'-dipyridyl or 4,4'-dipyridyl, respectively, in drinking water *ad libitum*.

Solutions were administered in conventional clear watering bottles. Water consumption was measured with metric cylinders twice weekly for dose calculations, and rats were weighed biweekly for 3 mo. Rats were kept in individual cages in temperature- and humidity-controlled surroundings on a 12-h dark, 12-h light cycle. Water and Purina Rat Chow (Ralston-Purina Co., St. Louis, Mo.) were given *ad libitum*. Survivors were killed 2 wk after dosing in the acute toxicity studies and 3 mo after the beginning of the subacute toxicity studies. At autopsy, selected tissues were fixed in 4% buffered formaldehyde for study with a light microscope. Tissues were stained with hematoxylin and eosin. LD50 values were calculated by the method of Litchfield and Wilcoxon (1949).

RESULTS

4,4'-Dipyridyl

The acute oral LD50 values of 4,4'-dipyridyl were 175 mg/kg in male and 172 mg/kg in female rats, with 95% confidence limits of 167–184 mg/kg and 159–186 mg/kg, respectively.

Immediate symptoms of toxicity included subdued behavior with labored breathing, lacrimation, and red stains around eyes, nose, and mouth. Fluid discharge from nose and mouth was noted after several hours. Convulsions occurred 1 or 2 d after dosing, usually immediately

¹ Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

² These Cesarean-obtained, barrier-sustained rats are from the colony at the Centers for Disease Control, U.S. Department of Health and Human Services; they have been randomly bred since 1950. They are descendants of the Sherman strain developed at Columbia University from the Osborne-Mendel rat (Kimbrough et al., 1964).

before death. The lowest lethal dose tested was 160 mg/kg for male and 130 mg/kg for female rats. A dose of 150 mg/kg in male and 100 mg/kg in female rats produced no symptoms of poisoning other than subdued behavior.

Autopsies of rats that died from acute doses of 4,4'-dipyridyl revealed massive pulmonary hemorrhage. Microscopic lung changes included pulmonary edema, intra-alveolar hemorrhage, congestion, and atelectasis (Figs. 2 and 3). Kidneys and spleen also were congested.

Rats given 0.01 or 0.05% 4,4'-dipyridyl in water received an average of 5.62 or 25.5 mg/kg·d, respectively (Table 1). Of 10 rats receiving 0.05% 4,4'-dipyridyl in drinking water, 2 died of dehydration early in the experiment and 8 lost a great amount of weight (Fig. 4) initially but regained most of it.

We found no consistent microscopic changes in the lungs.

Kidney changes (Table 1) included Ca concretions in tubules of two rats (Fig. 5) and nephrosclerosis in one of six rats given 0.01% 4,4'-dipyridyl. The tubular epithelium contained brown pigment in three rats, nephrosclerosis was present in one rat, and occasional thickening of Bowman's glomerular capsule was noted in two rats receiving 0.05% 4,4'-dipyridyl.

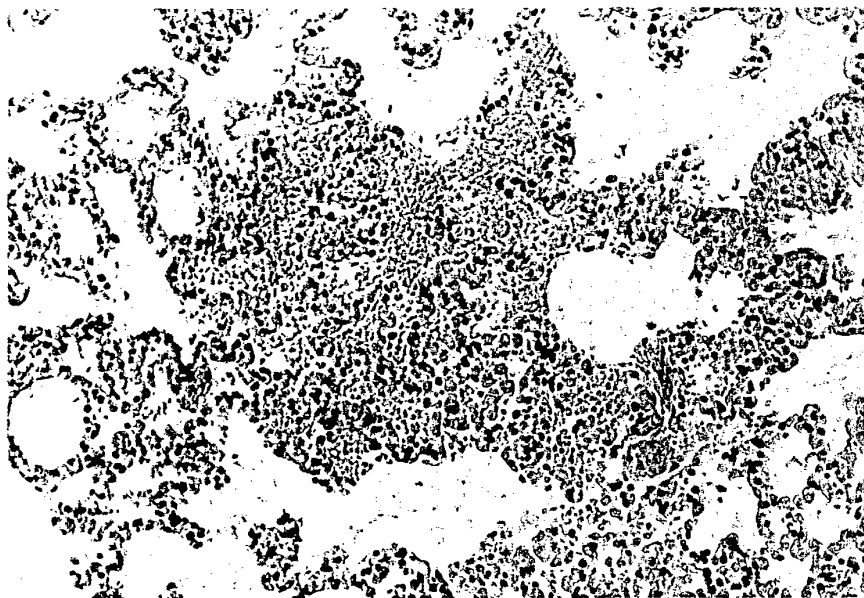


FIGURE 2. Section of lung from rat given a single toxic dose of 4,4'-dipyridyl. Note areas of hemorrhage and strands of fibrin in the alveoli. H&E, X76.



FIGURE 3. Section of lung from rat given a single toxic dose of 4,4'-dipyridyl. Note atelectasis. H&E, X76.

TABLE 1. Mean Water Consumption, Daily Dose, and Kidney Pathology for Subacute Toxicity Studies

Observation	Control ^a	4,4'-Dipyridyl concentration (%)		2,2'-Dipyridyl concentration (%)	
		0.01	0.05	0.01	0.05
Mean water consumption, ml/d	26.2	17.2	10.7	21.4	18.0
Mean daily dose, mg/kg	0.00	5.62	25.5	7.13	35.6
Hydronephrosis	1/15				
Tubules occasionally containing Ca concretions		2/6		3/6	
Nephrosclerosis		1/6	1/8	1/6	
Bowman's capsule of glomeruli occasionally thickened			2/8		1/10
Tubular epithelium containing brown pigment			3/8		1/10
Tubular epithelium containing enlarged atypical nuclei					2/10
Tubular epithelium containing degenerative changes			1/8		
Normal	13/15	4/6	2/8	2/6	7/10

^aNumbers are for two control groups. For one rat no kidney tissue was submitted.

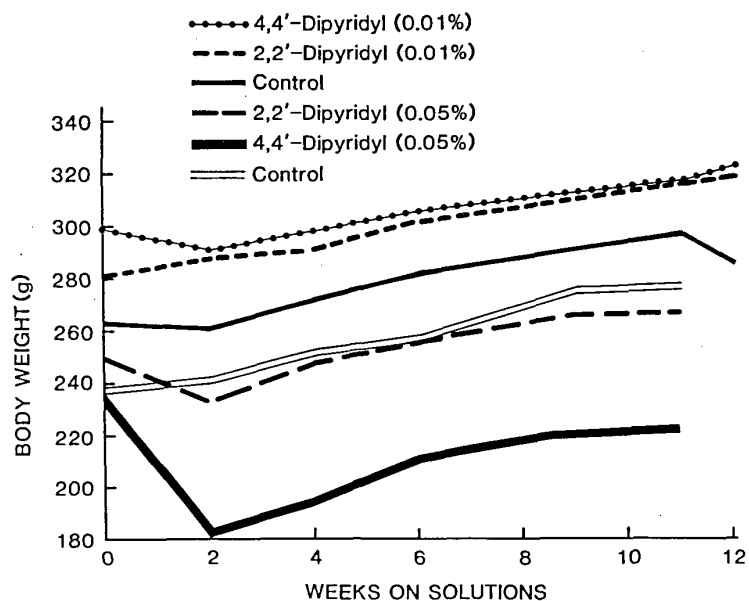


FIGURE 4. Body weights of rats dosed daily with 2,2'- or 4,4'-dipyrityl in water.



FIGURE 5. Section of kidney from rat given 4,4'-dipyrityl in drinking water. Note Ca concretions within tubules. H&E, X76.

2,2'-Dipyridyl

The acute oral LD50 values of 2,2'-dipyridyl were 100 mg/kg in male and 107 mg/kg in female rats, with 95% confidence limits of 89–112 mg/kg and 101–113 mg/kg, respectively.

Symptoms of toxicity included immediate subdued behavior, loss of muscle coordination, and red urine. Tremors were observed several hours later, and convulsions immediately before death occurred 1 or 2 d after dosing. The lowest lethal dose given was 80 mg/kg for male rats and 90 mg/kg for females.

Microscopic changes in the organs of rats that died after receiving 2,2'-dipyridyl were the same as those seen in rats that died after receiving 4,4'-dipyridyl.

Microscopically, there were no significantly different changes in the organs of 2-wk survivors receiving 4,4'- or 2,2'-dipyridyl and controls receiving 20% ethanol for either sex tested in acute toxicity studies.

In subacute toxicity studies rats given 0.01 or 0.05% 2,2'-dipyridyl in drinking water had no symptoms of toxicity such as central nervous system (CNS) effects or red urine, but they did gain less weight and drink less water than controls. Microscopically, no lung changes were present. Kidney changes (Table 1) included Ca concretions occasionally found in tubules of three rats and nephrosclerosis in one of six receiving 0.01% 2,2'-dipyridyl. Bowman's glomerular capsule occasionally was thickened, and the tubular epithelium contained a brown pigment in one rat each. The tubular epithelium occasionally had enlarged, atypical nuclei in 2 of 10 rats receiving 0.05% 2,2'-dipyridyl.

DISCUSSION

The acute oral LD50 value of paraquat is 100 mg/kg in male and 110 mg/kg in female Sherman rats (Kimbrough and Gaines, 1970). LD50 values obtained in this study were higher for 4,4'-dipyridyl and essentially equal for 2,2'-dipyridyl.

The steepness of the lethality curve is remarkable for both compounds tested. Doses of 150 and 100 mg/kg 4,4'-dipyridyl in male and female rats, respectively, produced no deaths, while the LD50s were 175 and 172 mg/kg. Lethalities of 90 and 80% were observed at 200 mg/kg for male and female rats, respectively.

Similarly, 75 mg/kg 2,2'-dipyridyl had no effect in male and female rats, while the LD50 values were 100 and 107 mg/kg, respectively. At 150 mg/kg there was 100% mortality in males, and 120 mg/kg caused 80% mortality in female rats.

There is a very small difference between the no-effect and 100% lethality doses for each compound.

Red drainage around the mouth, eyes, and nose in rats given an LD50

dose of paraquat has been reported (Sharp et al., 1972). The same symptom was observed in rats dosed with 4,4'-dipyridyl in our study. It is not known whether both unchanged compounds produce this drainage, a common metabolite exists, or 4,4'-dipyridyl is a metabolite of paraquat that produces this symptom.

Microscopic lung changes associated with paraquat poisoning in rats have often been reported and include vascular congestion, intra-alveolar hemorrhage, edema, extensive fibrosis, and extensive epithelial proliferation (Pasi, 1978; Kimbrough and Gaines, 1970). Rats given one toxic dose of 4,4'-dipyridyl also showed vascular congestion, intra-alveolar hemorrhage, and edema, but no extensive fibrosis or epithelial proliferation.

Kimbrough and Gaines (1970) found that rats died from paraquat-induced lung hemorrhage and edema 5-6 d after dosing. Death from 4,4'-dipyridyl-induced lung hemorrhage and edema occurred in female rats 1-2 d and in males 1-3 d after dosing. Thus 4,4'-dipyridyl seems to act more rapidly than paraquat.

Administration of $\geq 0.03\%$ paraquat in the diet (about 12.0 mg/kg·d) led to pulmonary fibrosis in experimental animals after 15 d (Conning et al., 1969). A dose of 25.5 mg/kg·d 4,4'-dipyridyl did not produce any consistent lung pathology in our study.

Although we observed no lung fibrosis after the oral administration of paraquat's major combustion product, acute toxic effects of pulmonary hemorrhage and edema seem to occur after exposure to dipyridyls as well as to paraquat. Toxic effects of inhaled dipyridyl isomers on the lungs should also be determined, because these compounds are not well absorbed from the gastrointestinal tract, primarily since the systemic dosage concentrations at the alveoli are much lower.

Our LD50 value for 2,2'-dipyridyl was much lower than the value reported in the literature. Such factors as age, strain, sex, time fasted before dosing, solvent used, and whether the compound degraded in solution could account for the differences.

Clark and Hurst (1970) found the acute oral LD50 of diquat to be 231 mg/kg in the rat; symptoms included lethargy after 24 h and respiratory difficulty, weight loss, and death 2-14 d after administration. The onset of symptoms from 2,2'-dipyridyl toxicity was much the same, with full recovery of body weight within 2 wk in survivors and death within 2 d in fatalities.

Clark and Hurst (1970) also found that a large dose of diquat rapidly affected the CNS, but smaller doses (although lethal) did not produce symptoms that indicated an obvious mode of action to account for the deaths. In our experiments, 2,2'-dipyridyl produced rapid CNS effects but also caused convulsions and death from internal hemorrhage.

Clark and Hurst (1970) also found bilateral cataracts in rats and dogs resulting from the chronic administration of diquat dichloride. Eyes were not examined with a slit lamp, but gross inspection of the eyes showed no abnormalities during the 3 mo of 2,2'-dipyridyl administration.

Although Daniel and Gage (1966) showed that diquat is not metabolized after it is absorbed into the bloodstream in experimental animals, the toxic effects are very similar to those of 2,2'-dipyridyl. This suggests a common mode of action of the two compounds.

Many compounds color the urine; for example, the drug phenazopyridine, which has a single pyridine ring, colors the urine orange to red. The urine was colored red to violet in rats given 2,2'-dipyridyl.

Red urine has not been reported in studies of diquat or 2,2'-dipyridyl toxicity. It is not known whether the coloration is due to an Fe-2,2'-dipyridyl complex. The coloration was not due to hemolysis, since tests with reagent strips did not indicate blood in the urine.

Red urine was not observed in the studies of subacute toxicity caused by exposure to 2,2'-dipyridyl.

Both paraquat and diquat, given to rats orally or sc as an acute LD50 dose, caused mild hydropic degeneration of proximal convoluted tubules and reduced renal excretory flow (Lock, 1979; Lock and Ishmael, 1979). Gage (1968) observed pale and discolored areas in the kidneys after exposing rats to paraquat in aerosols.

Degenerative changes or enlarged atypical nuclei were seen occasionally in the tubular epithelium of rats receiving 0.05% 4,4'- or 2,2'-dipyridyl. It is therefore possible that at least some of the effects observed in kidneys of animals exposed to paraquat or diquat were caused by metabolites of these compounds. This should be further investigated.

The results of the subacute toxicity studies do not indicate an additive effect on the lungs when rats are fed small concentrations per day of either 4,4'- or 2,2'-dipyridyl; however, renal damage was occasionally observed in rats given 0.05% 4,4'- and 2,2'-dipyridyl in drinking water. The conventional artificial ultraviolet lighting in our facilities may have caused some breakdown of solutions in the clear watering bottles.

Although 2,2'-dipyridyl has been determined qualitatively in cigarette smoke, quantitative measurements have not been reported, and no information is available on the toxicity of chronically inhaled trace amounts of 2,2'-dipyridyl in cigarette smoke.

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