The toxicity of diquat

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Clark, D. G. and Hurst, E. W. (1970). Brit. J. Industr. Med., 27, 51-55. The toxicity of diquat. The acute toxicity of diquat has been assessed in several species. The oral LD₅₀ ranged from about 30 mg/kg in cattle to 231 mg/kg in the rat. Large doses of diquat gave rise to symptoms indicative of an action on the central nervous system, but smaller doses did not suggest an obvious mode of action to account for the deaths, which were sometimes delayed for up to 14 days.

The 24-hour percutaneous LD₅₀ in the rabbit was greater than 400 mg/kg. This dose did not irritate the skin. A drop of a 20% aqueous solution of diquat in the conjunctival sac of the rabbit eye caused only slight irritation.

The chronic administration of diquat dichloride in the diet for several months led to bilateral cataract in the rat and the dog. A concentration of 0.05% in the rat diet caused bilateral opacities in all rats within 12 months, but 0.001% diquat did not cause any opacities in two years. Bilateral opacities of the lenses of all dogs occurred within 12 months of administration of 15 mg/kg/day, but 1.7 mg/kg/day was without effect after four years.

Diquat is 1,1′-ethylene,2,2′-dipyridylium; the dibromide has the following structure:

\[
\begin{align*}
\text{CH}_2 & \quad \text{CH}_2 \\
\text{N}^+ & \quad \text{N}^+ \\
[2 \text{ Br}^-] & 
\end{align*}
\]

Diquat is a widely used contact herbicide with a rapid desiccant action similar to that of paraquat (1,1′-dimethyl-4,4′-dipyridylium). The toxicity of paraquat has been described in detail (Clark, McElligott, and Hurst, 1966), but, with the exception of the work of Daniel and Gage (1966) on absorption and excretion of diquat, and the work of Gage (1968) on the toxicity of diquat aerosols, the detailed toxicity of diquat has not been reported previously.

Methods

Young, mature, specific-pathogen-free rats, mice, and guinea-pigs of the Alderley Park (albino) strain were used; their body weights ranged from 180 to 200 g, 20 to 26 g, and 200 to 300 g respectively. The dogs used were of the Alderley Park beagle strain with body weights ranging from 8 to 12 kg. The body weights of the adult Rhode Island hens ranged from 2 to 3 kg and those of the albino rabbits from 1.5 to 2.5 kg. The cattle were adult Friesian cows. All animals, with the exception of the dogs, were allowed free access to as much food as they wanted. Dogs were fed once a day with 'Kennel Kernels' and 'Kennonmeat'.

The diquat was used in the tests as either the dichloride or the dibromide, 99% pure. In the acute toxicity tests it was dissolved in water or physiological saline and administered to the animals by stomach tube or by subcutaneous injection. In long-term experiments it was given in the diet from which it could be recovered quantitatively.

Cutaneous toxicity and skin irritation were determined by applying diquat solution to the shaved, dorsal skin of the rabbit, oral contamination being prevented by means of a plastic collar. After 24 hours the skin was washed with soap and water. The animals were observed for a further three weeks.

The acute LD₅₀s and 95% confidence intervals were calculated from the mortality data by the moving-average interpolation technique of Thompson (1947). When groups of only three animals were used, the LD₅₀s were estimated as being between the dose that killed all the animals in one group and the dose that
killed none of the animals in a group receiving a lower dose.

Blood was examined periodically during the chronic tests for haemoglobin, packed cell volume, and total and differential white cell count. Blood urea was estimated by the method of Skeggs (1957) and serum alkaline phosphatase by the method of Marsh, Fingerhut, and Kirsch (1959). Liver function was assessed by bromsulphthalein retention, the bromsulphthalein being determined by the method of Seligson, Marino, and Dodson (1957). Protein, glucose, and bilirubin in the urine were estimated by the semiquantitative Albustix, Clinistix, and Ictotest respectively (Ames Co., Stoke Poges, Bucks).

Changes in the lenses of the rats and dogs were observed with a torch and the otherwise unaided eye.

On completion of the chronic toxicity tests the rats and dogs were subjected to a detailed gross and microscopic examination of their tissues. The liver, spleen, kidneys, pancreas, adrenals, lungs, heart, aorta, submaxillary gland, thymus, lymph nodes, five levels of the alimentary canal, gonads, uterus, Fallopian tube, prostate, epididymis, urinary bladder, adrenals, thyroid, parathyroid, pituitary, sciatic nerve, and striated muscle were examined by standard histopathological techniques. Two or more levels of the brain and brain stem and two levels of the spinal cord were examined by neuropathological techniques.

Results

Subcutaneous toxicity in the rat

Diquat salt is completely dissociated in aqueous solution at all pH values and its toxicity should, therefore, not be influenced by the associated anion; diquat dichloride and diquat dibromide should be equitoxic. The results presented in Table 1 are consistent with this view.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Acute Subcutaneous LD₅₀ of Diquat in the Rat</th>
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<tbody>
<tr>
<td><strong>Compound</strong></td>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Diquat dichloride</td>
<td>F</td>
</tr>
<tr>
<td>Diquat dichloride</td>
<td>M</td>
</tr>
<tr>
<td>Diquat dibromide</td>
<td>F</td>
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</table>

Following a single subcutaneous injection of a lethal dose of diquat, there were no symptoms for the first few hours. Pupillary dilatation then occurred and became maximal after about six hours, when the iris was hardly visible and the light reflex was completely abolished. Twenty-four hours after the injection the rats were subdued and lethargic and did not eat much. Over the next few days respiration became laboured, body temperature fell slightly, and body weight was lost. Deaths occurred on days 2 to 13 after injection; in all cases pupillary dilatation persisted until death. Rats dying from day 7 onwards often had greatly distended abdomens due to a grossly swollen caecum. The contents of the caecum and ileum were grey-green or olive-green in colour; spectroscopic examination showed the absorption bands of bile pigments.

A large injection of diquat (four or five times the LD₅₀ dose) gave rise to subdued behaviour within a few minutes and laboured respiration within an hour. Muscular twichings then occurred, leading to generalized convulsions and death within a few hours.

Only slight histological changes were found in the organs of animals dying after an injection of diquat; there was depletion of lymphocytes in the cortex of the thymus and in the spleen. After three or four days these organs were reduced in size; the thymic cortex might contain no thymocytes and the weight of the organ might be as low as 50% of the normal. Using the method of Elton, Zarrow, and Zarrow (1959), it was shown that in the adrenals maximal reduction of the level of ascorbic acid took place within a few hours of the injection.

Acute oral toxicity

The acute oral LD₅₀s of diquat were determined in various animal species. The results presented in Table 2 show that, except in cattle, there was not much species variation in toxicity.

The toxic symptoms following oral administration of diquat were similar in all the species tested. They are described in detail for the rat.

For the first 24 hours after a lethal dose of diquat there were few symptoms. The rats then gradually became lethargic, showed some respiratory difficulty, lost weight, and died between two and 14 days after administration of the diquat. Although slight pupil-
lary dilatation and some swelling of the intestines occurred, the extreme mydriasis and the grossly distended caecum seen after subcutaneous injection were not apparent after oral administration. Post-mortem examination of the rats during the first 24 hours or so showed that the intestines often contained quantities of greenish-yellow or grass-green fluid. This colour, quite different from that following subcutaneous injection, was due to reduction of the diquat during bacterial metabolism and has been shown to occur in vitro with fresh intestinal contents and with actively growing bacterial isolates from these (Hurst, unpublished observations).

Histological changes following oral administration of diquat were minimal. The main change was an inconsistent and patchy loss of keratin from the cardiac end of the stomach. The characteristic lung changes induced by an injection of paraquat (Clark et al., 1966) were never observed. Rats surviving a near lethal dose of diquat were observed for a period of one year following administration, but no further toxic effects were noted.

Dermal toxicity
The 24-hour percutaneous LD₅₀ of diquat in the rabbit was greater than 400 mg cation/kg - the maximum dose that could be applied. At this dose level there were no ill-effects in male or female rabbits, and necropsy and histological examination of the major organs did not reveal any abnormalities. There were no signs of skin irritation.

The daily application of 20 mg/kg diquat to the rabbit skin for 20 days did not cause any systemic effects but did lead to some mild erythema, thickening of the skin, and some scabbing. The daily application of 40 mg/kg caused loss of weight, unsteadiness, and muscular weakness. Four of the six rabbits in the group died after eight to 20 applications. The subacute LD₅₀ is therefore estimated to be 20 to 40 mg/kg/day.

Chronic toxicity in the rat
Groups of 25 male and 25 female rats were fed diets containing 0·1, 0·05, 0·025, 0·01, 0·005, and 0·001% diquat dichloride for two years.

No deaths resulted from feeding these diets. Food consumption was reduced and some reduction in growth rate occurred in animals receiving 0·1% diquat in the diet, but diets containing less than 0·1% diquat were without effect on bodyweight or food consumption. Haematological examination, analysis of the urine, and gross and microscopic pathological examination did not reveal any changes, other than in the eye, at any dietary level of diquat. Rats receiving diets containing 0·005% or more diquat developed cataracts during the course of the experiment. In the group fed a diet of 0·1% diquat, partial or complete opacities were present in one or both lenses within six months. At 0·05% opacities were seen in the lenses of some animals by four months and all animals had bilateral cataracts by 12 months. At 0·025% some degree of opacity was noted in all animals by 18 months. A diet of 0·01% and 0·005% led to slight opacities in some animals after 12 months, but approximately three-quarters of the rats were unaffected at the end of the experimental period. Feeding for two years with a diet containing 0·001% diquat did not cause any opacities of the lens in any animals.

At an early stage of cataract development the eyes seemed slightly paler and more transparent than normal and the blood vessels of the iris stood out with greater clarity. When the first signs of cataract were evident macroscopically, no conclusive microscopic abnormality of the eye could be detected. At a later stage the lens became quite opaque. Still later, a variety of secondary changes occurred in the lens and in the other structures of the eye. Anterior or posterior synechiae were observed, together with haemorrhage into the vitreous humour and detachment of the retina.

In a study of the development of cataract, rats were fed a diet known to produce cataracts within a few months (0·05% diquat). After feeding on this diet continuously for periods ranging from a few days to eight weeks, they were given a normal diquat-free diet for the remainder of one year. Cataracts did not develop in any of these rats. Thus, continuous and prolonged exposure to diquat is necessary for the formation of cataract in the rat; temporary exposure to a known cataract-producing diet does not lead to cataract.

Since it is known that some forms of cataract are influenced by light (Siddall, 1965; Cameron, 1967; McDonald, Snell, and Lerner, 1967) an experiment was undertaken to study the effect of light on diquat cataract. Rats were fed 0·05% diquat diet under conditions of darkness, the darkness being total except for very dim light for a few minutes a day during cage cleaning, etc. A further group of rats was given a similar diet under conditions of continuous illumination. After three months feeding on this diet, both groups of rats showed an equal number of cataracts. Thus, light does not influence the development of diquat cataract.

The concentration of ascorbic acid in the aqueous humour of an eye showing cataract is often lower than that of a normal eye (Pirie and Van Heyningen, 1956), but ascorbic acid (200 mg/ml) in the drinking water of rats receiving diquat in the diet did not influence the development of cataract.

Chronic toxicity in the dog
Groups of three male and three female dogs were given diquat dichloride, mixed with their food, for periods of two to four years. Doses of 15 mg/kg
and 5 mg/kg were given daily for two years, 1-7 mg/kg for four years, and 0-8 and 0-4 mg/kg for three years.

Administration of these doses of diquat did not cause any deaths. Growth was comparable to that of control animals in all experimental groups, and there were no changes in the blood picture, urine analysis, blood urea, serum alkaline phosphatase or liver function attributable to the diquat. Histopathological examination of the tissues at the end of the experimental periods did not reveal any changes due to treatment.

Cataracts again developed in some of the animals during the course of the experiment. Bilateral opacities of the lens of all dogs occurred after 10 to 11 months of administration of 15 mg/kg/day. At 5 mg/kg/day one lens was affected by the 11th month and all eyes were involved by 15 to 17 months. Animals tolerated 1-7 mg/kg/day for four years without developing cataracts, and doses of 0-8 and 0-4 mg/kg/day for approximately three years.

Eye irritation
One drop of a 20% solution of diquat in the conjunctival sac of the rabbit eye gave rise to slight injection of the palpebral and bulbar conjunctivae which persisted for two days. Pupillary dilatation did not occur.

Effects in man
No cases of fatal poisoning in man have been reported, but inflammation and bleeding of the nasal mucosa have been observed in people handling crystalline diquat powder in the laboratory and following field use. Nasal bleeding in the field has invariably been the result of inhaling the droplets arising from splashing due to careless mixing of the concentrate, or to exceptional field conditions when an operator has spent a considerable time walking through spray drift that has not dispersed. Under conditions such as these a filter respirator will prevent any nasal effects (Gage, 1968).

A concentrated (20%) solution of diquat in contact with the base of the nail for a few minutes can cause a disturbance in nail growth. A white opaque area appears and, with the continuing growth of the nail, this gradually moves distally until it is lost in the normal course of growth and wear. On histological examination the opaque patch is seen to consist of a collection of cells of the nail-bed which have retained their nuclei instead of maturing in the normal way into acellular horny keratin. More prolonged exposure to concentrated solutions results in cracking across the base and subsequent shedding of the nail, which is then replaced by the growth of a normal nail. Exposure to the dilute solutions used for spraying does not lead to any effects on the nail.

Concentrated solutions of diquat have also been reported to cause a delay in the healing of superficial cuts on the hands.

Discussion
The results presented here show that diquat is a compound of moderate oral toxicity which does not readily penetrate the skin and is without marked local irritant properties.

The acute toxicity of diquat by oral administration is approximately 10 times less than by subcutaneous injection, suggesting that its oral toxicity is moderate because of poor absorption from the gastrointestinal tract. This has been shown to be so by Daniel and Gage (1966), who found that less than 10% of an oral dose of diquat is excreted in the urine of the rat, the remainder being excreted in the faeces. Such absorption is consistent with the observation that cations of strong bases are very poorly absorbed from the rat small intestine (Schanker, Tocco, Brodie, and Hogben, 1958).

Administration of a single, lethal dose of diquat by stomach tube or by subcutaneous injection rarely gives rise to any toxic symptoms during the first 24 hours following injection, but deaths occur several days later. Since it has been shown that over 90% of a subcutaneous injection is excreted in the urine within 24 hours (Daniel and Gage, 1966), the delay in the appearance of toxic symptoms and death is probably not due to a slow penetration and a slow build-up to a lethal concentration at the site of action. The delay may, however, be due to the retention of a small amount of diquat which slowly exerts its effects over several days, culminating in the death of the animal. Alternatively, during the short period before it is excreted, diquat may initiate an irreversible change in some critical system which then progresses slowly until death ensues.

Although a large dose of diquat in the rat gives rise to symptoms indicative of a rapid action in the central nervous system, the symptoms following a smaller, though lethal, dose do not suggest an obvious mode of action to account for the deaths. There are certainly none of the proliferative changes in the lungs which are so conspicuous following oral administration of the closely related compound, paraquat (Clark et al., 1966). The persistent mydriasis following the subcutaneous injection of diquat in the rat suggests that blockade of the parasympathetic nerves may play some part but it is difficult to see how this could account for the deaths several days later. Whatever changes do occur in the body, they must be due to the diquat molecule per se as Daniel and Gage (1966) have shown that diquat is not metabolized once it has been absorbed into the blood stream of experimental animals.
The chronic administration of diquat in the diet for several months leads to bilateral cataract in the rat and the dog. Development of these cataracts is not influenced by the presence or absence of light, or the presence of excess ascorbic acid in the diet. Continuous exposure to diquat is necessary to produce the cataracts since it has been shown that neither a single oral dose nor feeding for a short period of time leads to the formation of cataract.

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References

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