

DIQUAT (addendum)

*First draft prepared by
P.V. Shah¹ and Maria Tasheva²*

¹ *Office of Pesticide Programs, Environmental Protection Agency, Washington, DC, United States of America (USA)*

² *Associate Professor Toxicologist, Sofia, Bulgaria*

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Explanation

Diquat is the International Organization for Standardization–approved name for 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service (CAS) number is 85-00-7. The CAS number for diquat ion is 2764-72-9. Diquat is a non-selective, quick-acting contact herbicide and desiccant, causing injury only to the parts of the plant to which it is applied. Diquat interacts with the electron transfer components associated with Photosystem I, which causes inhibition of photosynthesis. Diquat is referred to as a desiccant because it causes a leaf or an entire plant to dry out quickly. It is also used as an aquatic algicide.

Diquat was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1970, 1972, 1977 and 1993. An acceptable daily intake (ADI) of 0–0.008 mg diquat ion/kg body weight (bw) was allocated in 1977. In 1993, JMPR established an ADI of 0–0.002 mg diquat ion/kg bw based on a no-observed-adverse-effect level (NOAEL) of 0.19 mg diquat ion/kg bw per day identified in a 2-year study of toxicity and carcinogenicity in rats, using a safety factor of 100.

Diquat was reviewed at the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues.

Since the last review by JMPR, the following new studies on diquat have been submitted: acute and subchronic neurotoxicity studies, an immunotoxicity study, metabolism studies and a re-evaluation of cataract observations in previous long-term studies. All dose values are expressed as diquat ion. Some of the studies do not comply with good laboratory practice (GLP), as most of the data were generated before the implementation of GLP regulations. Overall, the Meeting considered that the database was adequate for the risk assessment.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption and excretion of diquat were studied by Daniel & Henson (1960) to seek an explanation for the large difference between the acute oral and subcutaneous median lethal dose (LD_{50}) values in the rat. From limited experiments, they found that after single oral administration of [^{14}C]diquat, all of the radioactivity was excreted within 48 hours, about 94% in the faeces and about 6% in the urine. After subcutaneous administration, most of the dose appeared in the urine, which suggested that diquat was poorly absorbed from the gut. Using a chemical method of analysis, they found the excretion in urine to be similar to that measured by radiochemical analysis, but the percentage of the dose appearing in faeces after oral dosing was much lower, ranging between 10% and 40%. This low result in faeces was attributed to the metabolism of diquat by the intestinal flora: when diquat was incubated with washings from rat intestine for 24 hours at 37 °C, only 50% could be recovered by chemical analysis.

The work of Daniel & Henson (1960) was further investigated in a study using radiolabelled diquat dibromide or diquat dichloride in male Wistar rats. In this study, ^{14}C -labelled diquat dibromide (5 or 10 mg diquat ion/kg bw) or dichloride (22 or 24 mg diquat ion/kg bw) was administered by gavage; the dibromide was also administered subcutaneously at a dose of 5 or 6 mg diquat ion/kg bw. Treated rats were housed individually in metabolism cages from which urine and faeces were collected frozen over solid carbon dioxide for up to 96 hours. Urine and faeces were collected daily for the duration of each experiment.

Following oral or subcutaneous administration of diquat, most of the radioactivity appeared in the excreta within 2 days; in a few cases, a small amount was excreted on the 3rd day. Diquat was poorly absorbed from the gut; the bulk of an oral dose appeared in the faeces (84–97%), with limited urinary excretion (4–11%). After a subcutaneous dose, little or none appeared in the faeces (Table 1). The presence of a small amount of metabolites in urine after oral dosing of diquat has been detected. As no such metabolism has been observed after subcutaneous administration, it seems probable that it derives from the absorption of degradation products formed within the gut (Daniel & Gage, 1964, 1966).

The absorption, distribution and excretion of diquat were studied in Wistar rats following a single oral gavage dose of 1 or 100 mg [^{14}C]diquat ion/kg bw in water. Diquat dibromide was radiolabelled in the [2,2',6,6'- ^{14}C] positions. For serial blood collection, three rats of each sex per dose were used, and blood from the tail vein was collected at 1, 2, 4, 8 and 24 hours post-dosing. For the tissue distribution study, three rats of each sex per dose were terminated at 2, 4, 8, 24, 48 and 96 hours post-dosing. The positions of the ^{14}C label of diquat dibromide are shown in Fig. 1.

Following a single oral dose of [^{14}C]diquat at the 1 mg diquat ion/kg bw dose level, the maximum mean blood concentration (0.012 μg equivalents [eq] diquat ion/g) in blood was observed at 2 hours; by 24 hours, all values had declined to near background values (Table 2). Following a 100 mg/kg bw dose, the maximum mean blood concentration (1.428 μg eq diquat ion/g) was observed at 1 hour. By 4 hours, the mean blood concentration had declined to 0.265 μg eq/g, and by 24 hours, to 0.259 μg eq/g. There was no sex difference in blood concentrations.

Table 1. Group mean excretion of radioactivity after single oral or subcutaneous administration of [^{14}C]diquat to male rats

Compound	Dose group	Dose (mg/kg bw)	Dose route	Sample	Daily % excretion					Total % excretion (urine + faeces)
					Day 1	Day 2	Day 3	Day 4	Total	
Diquat dibromide	A	5	Oral	Urine	0	4	0	–	4	101
				Faeces	7	90	0	–	97	
	B	10	Oral	Urine	5	1	0	–	6	96
				Faeces	80	10	0	–	90	
	C	5	Subcutaneous	Urine	94	4	0	–	98	98
				Faeces	0	0	–	–	0	
	D	6	Subcutaneous	Urine	84	3	1	0	88	90
				Faeces	2	0	–	–	2	
	D	6	Subcutaneous	Urine	89	4	0	–	93	93
				Faeces	0	0	–	–	0	
Diquat dichloride	E	22	Oral	Urine	7	2	0	0	9	93
				Faeces	13	57	14	0	84	
	F	24	Oral	Urine	8	2	1	0	11	101
				Faeces	8	44	38	0	90	

Source: Daniel & Gage (1964)

Fig. 1. Radiolabel positions of diquat dibromide

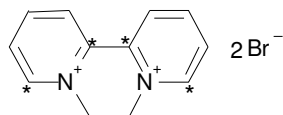


Table 2. Mean blood concentrations of radioactivity over a time course following a single oral dose of 1 or 100 mg [^{14}C]diquat ion/kg bw

Time after dosing (h)	Mean blood concentrations ($\mu\text{g eq diquat ion/g}$) ^a	
	Group A: 1 mg/kg bw	Group B: 100 mg/kg bw
1	0.011	1.428
2	0.012	1.042
4	0.008	0.265
8	0.005	0.240
12	0.003	0.300
24	0.002	0.259

^a Mean of three males and three females.

Source: Johnston et al. (1991)

Following single oral administration of [^{14}C]diquat at a dose level of 1 mg diquat ion/kg bw, the concentration of radioactivity in tissues fell from a maximum at 2–4 hours to near background levels at 24 hours. Excluding the gastrointestinal tract, highest concentrations of radioactivity at the low dose level were found in kidney. In male rats at 2 hours post-dosing, the highest tissue concentration was present in the kidney (0.096 $\mu\text{g eq diquat ion/g}$), which progressively declined to

0.04 µg eq/g by 4 hours and to 0.006 µg eq/g by 24 hours. In female rats, at 2 hours post-dosing, the highest tissue concentration was present in the kidney (0.064 µg eq diquat ion/g), which progressively declined to 0.04 µg eq/g by 4 hours and to 0.006 µg eq/g by 24 hours.

Following single oral administration of [¹⁴C]diquat at a dose level of 100 mg diquat ion/kg bw, the rate of elimination of radioactivity was generally slower than at the low dose level. In male rats at 2 hours post-dosing, the highest tissue concentrations were present in the kidney, lung and liver (5.75, 2.43 and 0.87 µg eq/g, respectively). All other tissue concentrations were below 0.075 µg eq/g. At 4 and 8 hours after dosing, the kidney (3.06 and 1.76 µg eq/g) and lung (2.41 and 0.95 µg eq/g) again showed the highest concentrations. By 8 hours, all other tissue concentrations were lower than 0.47 µg eq/g. In female rats, at 2 hours post-dosing, the highest tissue concentrations were present in the lung, kidney and liver (3.80, 2.27 and 0.83 µg eq/g, respectively). After 4 hours in female rats, concentrations in kidney (2.59 µg eq/g) and liver (1.06 µg eq/g) had risen slightly, but concentrations in lung (1.53 µg eq/g) had declined; by 96 hours, most other tissue concentrations were approaching background values.

There was no apparent sex difference in blood concentrations over a time course following a single oral dose of [¹⁴C]diquat at 1 or 100 mg diquat ion/kg bw, and radioactivity concentrations appeared to be broadly in proportion to dose levels. In general terms, there was no apparent sex difference in the tissue distribution of radioactivity, although concentrations in kidney, liver and lung at 48 hours after a 100 mg/kg bw dose were lower in the female group. In both sexes, ¹⁴C concentrations in lung were disproportionately high at the high dose level (Johnston et al., 1991).

The absorption, distribution, excretion and metabolism of diquat were studied in five male and five female Wistar rats following a single oral gavage dose of 1 mg [¹⁴C]diquat ion/kg bw in water. Diquat dibromide was radiolabelled in the [2,2',6,6'-¹⁴C] positions. Treated animals were housed individually in metabolism cages for collection of urine and faeces. After 7 days, treated animals were killed, and various tissues and blood samples were removed for further analysis.

About 90% of the administered dose was recovered in faeces in 24 hours (Table 3). Approximately 93% of the administered dose was recovered in faeces in 7 days. Approximately 3% of the administered dose was recovered in urine in 7 days. At 168 hours after dosing, the levels of radioactivity in tissues, organs and body fluids were at or close to background values.

Radio-thin-layer chromatographic (TLC) analysis of both pooled urine (0–8 hours) and pooled faecal extract (0–24 hours) samples indicated the presence of a single major radiolabelled component with a retention factor similar to that of [¹⁴C]diquat. Faecal extraction efficiencies were 92.8% for males and 82.5% for females. No urinary or faecal radioactivity co-chromatographed with any of the six reference samples used.

There was apparently no sex difference in the pattern of excretion or the radio-TLC profiles of excreta. An oral dose of diquat was poorly absorbed in the rat. Most of the orally administered dose of diquat was excreted in the first 24 hours, primarily in faeces (about 90%) and to some extent in urine (about 3%). Radio-TLC analysis of pooled urine and faecal samples indicated the presence of a single major radiolabelled component, which co-chromatographed with [¹⁴C]diquat (Johnston, Mutch & Scott, 1994).

The absorption, distribution, excretion and metabolism of diquat were studied in five male and five female Wistar rats following a single oral gavage dose of 100 mg [¹⁴C]diquat ion/kg bw in water. Diquat dibromide was radiolabelled in the [2,2',6,6'-¹⁴C] positions. Treated animals were housed individually in metabolism cages for collection of urine and faeces. After 7 days, treated animals were killed, and various tissues and blood samples were removed for further analysis.

Following a single oral dose of 100 mg diquat ion/kg bw to male and female rats, there was no sex difference in excretion profiles. A mean of 85.5% of the dose was recovered in faeces over

Table 3. Group mean cumulative excretion of radioactivity over 168 hours after single oral administration of [^{14}C]diquat to male and female rats at a dose level of 1 mg/kg bw

	Collection period (h)	Group mean cumulative excretion (%)	
		Males ($n = 5$)	Females ($n = 5$)
Urine	0–8	1.87	2.71
	0–24	2.61	3.36
	0–48	2.68	3.48
	0–72	5.67	3.52
	0–168	2.72	3.55
Faeces	0–24	91.67	88.50
	0–48	94.65	92.41
	0–72	94.74	92.61
	0–168	94.85	92.67
Cage wash	0–24	0.20	0.23
	0–48	0.24	0.26
	0–72	0.25	0.29
	0–168	0.34	0.38
Stomach + contents	168	< LRD	< LRD
Small intestine + contents	168	< LRD	< LRD
Large intestine + contents	168	< LRD	< LRD
Tissues + residual carcass	168	< LRD	< LRD

LRD: limit of reliable determination

Source: Johnston, Mutch & Scott (1994)

168 hours. Excretion via urine accounted for a mean of 5.4% of the administered dose. The mean total radioactivity recovered was 93.2% of the dose (Table 4).

At 168 hours after dosing, a very small proportion of the dose remained in the carcass, accounting for 0.02% of the administered dose in males and 0.01% in females. Seven days after the oral administration, the levels of radioactivity in tissues, organs and body fluids were close to background values. The highest concentrations were detected in the organs of excretion (gastrointestinal tract). There were residues in kidney, lung, liver and lens, with mean concentrations ranging from 0.038 to 0.251 $\mu\text{g eq/g}$. At 96 hours post-dosing, significant residues were seen in the eye lens (Table 5). Radio-TLC analysis of both pooled urine (0–48 hours) and pooled faecal extract (0–72 hours) samples indicated the presence of a single major radiolabelled component with a retention factor similar to that of [^{14}C]diquat (Johnston et al., 1994).

A distribution study was conducted in rats to determine the residue of diquat photodecomposition product. In this study, mature barley plants were sprayed with [^{14}C]diquat at a rate equivalent to 0.77 kg/ha. The plants were then kept in sunlight to allow the diquat to photodegrade. The plants were harvested after 2 weeks, and the straw was fed to rats as 10% of their total diet for up to 20 days. The known photodegradation and oxidation products of diquat are diquat monopyridone, 1,2,3,4-tetrahydro-1-oxopyrido-(1,2-a)-5-pyrazinium salt (TOPPS) and diquat dipyrindone. To analyse the acid-extractable radioactivity on the barley straw, a sample of the powdered straw was extracted with 2 N HCl for 3 hours. The extract was analysed for diquat, diquat monopyridone, TOPPS, diquat dipyrindone and picolinic acid by isotope dilution. All of these compounds have been shown to be stable to the acid extraction conditions used. The picolinic acid analysis also included any picolinamide that was hydrolysed during the acid extraction. The photodegradation products and relative proportions in the acid extraction from the barley plants are shown in Table 6.

Table 4. Group mean cumulative excretion of radioactivity over 168 hours after single oral administration of [^{14}C]diquat to male and female rats at a dose level of 100 mg/kg bw

	Collection period (h)	Group mean cumulative excretion (%)	
		Males ($n = 5$)	Females ($n = 5$)
Urine	0–8	1.09	1.11
	0–24	2.94	2.79
	0–48	5.31	4.78
	0–72	5.67	5.02
	0–168	5.70	5.11
Faeces	0–24	43.44	30.16
	0–48	77.02	69.54
	0–72	82.90	82.40
	0–168	84.17	86.83
Cage wash	0–24	2.55	1.39
	0–48	2.70	1.52
	0–72	2.78	1.58
	0–168	2.90	1.71
Stomach + contents	168	< LRD	< LRD
Small intestine + contents	168	< LRD	< LRD
Large intestine + contents	168	< LRD	< LRD
Tissues + residual carcass	168	0.02	0.01
Total recovery	0–168	92.78	93.86

LRD: limit of reliable determination

Source: Johnston et al. (1994)

Table 5. Distribution of radioactivity in tissues/organs over 96 hours following a single oral dose of 100 mg [^{14}C]diquat ion/kg bw to female rats (Group F)

	Distribution ($\mu\text{g eq diquat ion/g}$) ^a					
	2 h	4 h	8 h	24 h	48 h	96 h
Bone	0.285	0.441	0.157	0.154	0.140	0.085
Brain	0.131	0.123	0.058	0.056	0.490	0.119
Fat (renal)	0.216	0.127	0.115	0.209	0.322	0.094
Heart	0.440	0.347	0.240	0.249	0.154	0.015
Kidneys	2.272	2.590	1.389	2.054	0.739	0.251
Lens	0.225	0.344	0.112	0.046	0.094	0.097
Liver	0.833	1.060	0.339	0.476	0.164	0.059
Lungs	3.802	1.526	1.854	0.355	0.105	0.038
Muscle	0.247	0.231	0.151	0.111	0.156	0.032
Ovaries	0.570	0.400	0.196	0.272	0.069	0.011
Spleen	0.279	0.435	0.220	0.217	0.100	0.082
Residual carcass	0.630	0.634	0.498	2.265	1.745	0.007
Stomach	134.0	198.2	137.9	154.4	7.823	0.227
Stomach contents	9 259	9 977	9 648	5 756	274.5	9.578
Small intestine	78.97	32.17	26.89	84.24	12.03	0.833
Small intestine contents	1 947	672.8	656.3	1 388	192.8	17.48

	Distribution ($\mu\text{g eq diquat ion/g}$) ^a					
	2 h	4 h	8 h	24 h	48 h	96 h
Large intestine	49.67	112.5	89.40	84.37	38.86	2.544
Large intestine contents	1 992	2 849	2 050	1 721	1 219	79.25
Whole blood	0.235	0.232	0.108	0.244	< LRD	< LRD
Plasma	0.420	0.352	0.163	0.386	0.055	0.017

LRD: limit of reliable determination

^a Each value represents the mean of three animals.

Source: Johnston et al. (1994)

Table 6. Percentages of [¹⁴C]diquat and its photoproducts acid-extracted from [¹⁴C]diquat-treated barley straw

	% of total barley straw radioactivity extracted
Total radioactivity extracted	81
Diquat	21
Diquat monopyridone	2
TOPPS	12
Diquat dipyrindone	1
Picolinic acid	2
Unidentified photoproducts	62

TOPPS: 1,2,3,4-tetrahydro-1-oxopyrido-(1,2-a)-5-pyrazinium salt

Source: Leahey, Burgess & Mills (1974)

Analysis of the tissues of these rats showed that the maximum radioactive residues in the tissues analysed were as follows: muscle, 0.007 $\mu\text{g eq/g}$; fat, 0.008 $\mu\text{g eq/g}$; kidney, 0.03 $\mu\text{g eq/g}$; liver, 0.02 $\mu\text{g eq/g}$; heart, 0.01 $\mu\text{g eq/g}$; and lungs, 0.01 $\mu\text{g eq/g}$. Necropsy of the rats revealed no gross abnormalities in any of the tissues, and no behavioural abnormalities occurred during the experiment (Leahey, Burgess & Mills, 1974).

In a study investigating the extent of absorption of [¹⁴C]diquat residues on wheat chaff, a sample of [¹⁴C]diquat-treated wheat chaff was produced at Zeneca Agrochemicals, Jealott's Hill Research Station (Heath & Leahey, 1989). The bioavailability of the radioactivity associated with a nominal dose of 100 mg (84 kBq) of this wheat chaff was investigated in the AlpK:APfSD rat. Three male and three female rats were given three consecutive daily doses of wheat chaff, by gavage, as an aqueous suspension. An additional three male and three female bile duct-cannulated rats were given a similar single oral dose. Urine, faeces and, where applicable, bile were collected over 48 hours from the bile duct-cannulated rats and over 120 hours from the non-cannulated rats. At the end of these periods, the animals were killed, and selected tissues and the residual carcass were sampled. Excreta, tissues and carcasses were analysed for radioactivity.

The radioactivity in bile represented less than 0.7% of the dose for either sex. The urine from bile duct-cannulated rats contained 4.2% and 2.9% of the administered dose for males and females, respectively. The urine from non-cannulated rats contained 2.2% and 2.4% of the cumulative dose to males and females, respectively. The remainder of the administered radioactivity was excreted in the faeces. Very low levels of radioactivity were detected in all tissues for both bile duct-cannulated and non-cannulated rats. All mean measurements in the residual carcass were below the limit of detection. The radioactivity associated with the [¹⁴C]diquat-treated wheat chaff was poorly absorbed following either single or repeated oral dosing. The bioavailability of oral doses of radiolabelled diquat residues on wheat chaff is therefore very low in the rat (Lappin, Platt & Davies, 1993).

1.2 Biotransformation

A comparative metabolism study was conducted in Wistar rats via single oral gavage or single subcutaneous administration of diquat dibromide. In this study, a single oral dose of 45 mg [^{14}C]diquat ion/kg bw in water (radiolabelled in 1,1'-[U- ^{14}C]ethylene-2,2'-bipyridylium ion) was administered to each of five male rats by gavage. A single subcutaneous dose of 10 mg [^{14}C]diquat ion/kg bw in water was administered to each of five male rats. Urine and faeces were collected at 24-hour intervals for 96 hours after dosing. Diluted urine samples and acid extracts of faecal samples were further subjected to metabolic identification. A separate *in vitro* study was conducted to evaluate the nature of the radioactivity present in the rat caecal content.

Rats given a single oral dose of 45 mg diquat ion/kg bw excreted 6.3% and 89.3% of the administered dose in urine and faeces, respectively, within 4 days, mainly in the first 48 hours. Diquat was the major radioactive component in the urine (5.1% of the dose). Diquat monohydrate represented 0.2% of the dose in the urine. Diquat dipyrindone and unknown degradation products represented 0.1% and 0.3% of the dose, respectively. The chromatographic analysis of the faecal extract showed that diquat represented 57.1% and diquat monopyrindone 4.3% of the dose. The unidentified radioactivity (4.1% of the dose) included one discrete unidentified metabolite. Diquat dipyrindone was not detected.

Following subcutaneous injection of 10 mg diquat ion/kg bw, rats excreted 87.1% of the dose in the urine and 4.6% in the faeces within 4 days. The urine contained mainly diquat (75% of the dose), together with diquat monopyrindone (about 3% of the dose) and diquat dipyrindone (at least 6% of the dose). Diluted sulfuric acid extracted 4.3% of the radioactivity from faeces, 0.6% of which was diquat. No further analysis of radiolabelled faecal metabolites was done.

Methanol extracted 98% of the radioactivity present in caecal contents following incubation with [^{14}C]diquat. Chromatographic analysis showed that two compounds were present: 88% was identified as parent diquat, and 7.8% as diquat monopyrindone. This finding confirmed that the gut microflora possesses the capacity to metabolize diquat at least to diquat monopyrindone.

The study author concluded that the extent of diquat metabolism in rats is considerably lower than predicted previously (Daniel & Gage, 1966), those predictions being based on erroneous comparisons of radioactivity measurements and chemical analyses (Mills, 1976).

Urine and faecal samples from the previous study (Johnston et al., 1994) in which male and female Wistar rats were administered a single oral gavage dose of 100 mg [^{14}C]diquat ion/kg bw in water were subjected to metabolic identification. Urine samples used for metabolite identification represented separate 0- to 48-hour pools for male and female rats administered a single oral gavage dose of 100 mg diquat ion/kg bw. Faecal samples used for metabolite identification represented separate 0- to 72-hour pools for male and female rats administered a single oral gavage dose of 100 mg diquat ion/kg bw.

The mean proportion of administered radioactivity recovered in urine following a single oral dose of 100 mg diquat ion/kg bw was 5.4% (5.7% for males and 5.1% for females). Radio-high-performance liquid chromatographic (HPLC) analyses of 0- to 48-hour pooled urine samples exhibited one major peak with a retention time consistent with that of diquat. This peak accounted for 76.2% and 79.8% of the total excreted radioactivity in 48 hours for males and females, respectively. In addition, three approximately equivalent minor peaks were observed by radio-HPLC analysis, with retention times consistent with picolinic acid, diquat dipyrindone and diquat monopyrindone (Table 7). The mean proportion of administered radioactivity recovered in faeces following a single oral dose of 100 mg diquat ion/kg bw was 85.5% (84.2% for males and 86.8% for females). Radio-HPLC analyses of 0- to 72-hour pooled faecal samples exhibited one major peak with a retention time consistent with that of diquat. The peak accounted for 86.7% and 75.4% of the total excreted radioactivity within 72 hours for male and female rats, respectively. One minor peak was observed in the female faecal sample with a retention time between 9 and 10 minutes, which did not correspond to any of the analysed reference standards. This accounted for 6.4% of total excreted radioactivity in faeces within

72 hours in females. A broad band of radioactivity was also observed between 2 and 6 minutes, which accounted for 6.2% of total excreted radioactivity within 72 hours for male rats and 9.3% for female rats (Williams, Cameron & McGuire, 1991).

Table 7. Metabolite identification of excreta derived following oral administration of diquat in rats following a single dose of 100 mg diquat ion/kg bw

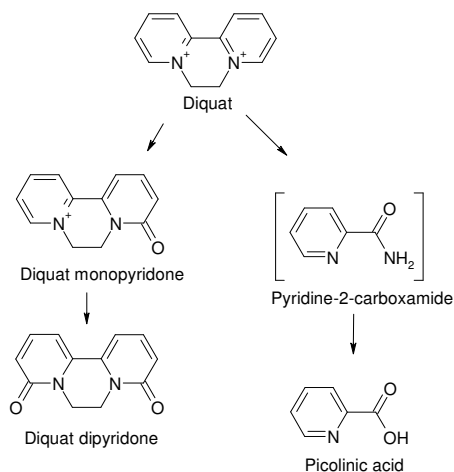
	% of administered dose					
	Males			Females		
	Urine (0–48 h)	Faeces (0–72 h)	Total excreta	Urine (0–48 h)	Faeces (0–72 h)	Total excreta
Total urinary excretion (0–168 h)	5.70	N/A	N/A	5.11	N/A	N/A
Excretion in 0–48 h urine	5.31	N/A	N/A	4.78	N/A	N/A
Total faecal excretion (0–168 h)	N/A	84.17	N/A	N/A	86.83	N/A
Excretion in 0–72 h faeces	N/A	82.90	N/A	N/A	82.40	N/A
Diquat	4.05	71.87	75.92	3.81	62.13	65.94
Diquat dipyridone		ND			ND	
Diquat monopyridone	0.92	ND	0.92	0.75	ND	0.75
Picolinic acid		ND			ND	
TOPPS	ND	ND	ND	ND	ND	ND
Pyridine-2-carboxamide	ND	ND	ND	ND	ND	ND
Total identified	4.97	71.87	76.84	4.56	62.13	66.69
Unidentified at RT 9–10 min	–	–	–	–	5.27	5.27
Unidentified at RT 2–6 min	–	5.14	5.14	–	7.66	7.66
Total unidentified	–	5.14	5.14	–	12.93	12.93
Total accounted for	4.97	77.01	81.98	4.56	75.06	79.62

N/A: not applicable; ND: not detected; RT: retention time

Source: Williams, Cameron & McGuire (1991)

The biotransformation pathway proposed for diquat is presented in Fig. 2.

Fig.2. Proposed biotransformation pathway for diquat



The biotransformation of diquat is postulated to proceed either by progressive oxidation of the pyridine rings to form diquat monopyridone and diquat dipyridone or by the cleavage of one of the pyridine rings to form picolinic acid, possibly via pyridine-2-carboxamide as an intermediary metabolite, although this was not identified. However, the total proportion of the dose metabolized to diquat monopyridone, diquat dipyridone and picolinic acid amounted to less than 1%. Furthermore, it is possible that these various metabolites were produced by the intestinal flora and absorbed as such into the systemic circulation and were then subject, like the parent diquat, to rapid urinary excretion. Similarly, the unidentified minor metabolites in faeces are also likely to be formed by gut flora metabolism, and the available evidence suggests that these were not absorbed into systemic circulation and were therefore not bioavailable to the rat, but were excreted directly in the faeces (Williams, Cameron & McGuire 1991).

1.3 Dermal absorption

(a) *In vivo*

¹⁴C-ring-labelled diquat dibromide was dissolved in deionized water and applied topically at a single dose of 0.05, 0.5 or 5.0 mg onto an unabraded 10 cm² application site on the shaved dorsal trunk of each of 12 male Sprague-Dawley® Crl:CD® (SD)BR rats per dose group. Treated animals were kept in metabolism cages for collection of urine, faeces and volatiles. Four animals per dose were sacrificed at 2, 10 and 24 hours post-exposure. Urine, faeces, blood, carcass and skin from application sites were analysed for radioactivity.

The mean recovery of radioactivity ranged from 93.5% to 99.6% of the administered dose. The absorbed dose included the radioactivity in urine, faeces, cage wash, residual carcass and blood. The radioactivity remaining at the application site after skin washing was considered the absorbable dose. The mean systemic absorption following dermal application of 0.05 mg diquat dibromide per rat was 1.3%, 2% and 2.5% at 2, 10 and 24 hours, respectively. At these times, 2.6%, 3.1% and 3.3% of the dose were recovered from the skin application site after skin washing. The mean systemic absorption following dermal application of 0.5 mg diquat dibromide per rat was 1.2%, 3.6% and 2.1% at 2, 10 and 24 hours, respectively. At these times, 1.2%, 1.3% and 1.0% of the dose were recovered from the application site of the skin. The mean systemic absorption following dermal application of 5.0 mg diquat dibromide per rat was 1.8%, 3.4% and 3.4% at 2, 10 and 24 hours, respectively. At these times, 0.6%, 0.8% and 0.7% of the dose were recovered from the application site of the skin.

The dermal absorption of diquat dibromide through the skin of male rats is low. Approximately 6% of the administered dose was absorbed through rat skin in 24 hours when skin-bound residue after skin washing was included (Brorby & Griffis, 1987).

(b) *In vitro*

In vitro dermal absorption of ¹⁴C-ring-labelled diquat dibromide from a soluble concentrate formulation through human epidermis was studied. The doses were applied as the concentrate formulation (200 g diquat ion/L) and as 1 : 100 volume per volume (v/v) (2 g/L) and 1 : 200 v/v (1 g/L) spray strength dilutions of the formulation in water. The doses were applied to the epidermal membranes at a rate of 10 µL/cm² and left unoccluded for an exposure period of 24 hours. The absorption rate of diquat from the concentrate formulation through human epidermis was 0.082 µg/cm² per hour during the 24-hour exposure period. The absorption rate of diquat from the 1 : 100 v/v dilution through human epidermis was 0.001 µg/cm² per hour during the 24-hour exposure period. The absorption rate of diquat from the 1 : 200 v/v dilution through human epidermis was 0.001 µg/cm² per hour during the 24-hour exposure period (Johnson, 2009).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with diquat (including skin and eye irritation and dermal sensitization studies; see below) are summarized in Table 8.

Table 8. Acute toxicity of diquat

Species	Strain	Sex	Route	Purity; vehicle	LD ₅₀ (mg ion/kg bw) or LC ₅₀ (mg ion/L)	Reference
Rat	Wistar derived (Alpk:APfSD)	M + F	Oral	21.2% w/w diquat ion; deionized water	LD ₅₀ M = 214 (180–271) F = 222 (203–241)	McCall & Robinson (1990a)
Rat	Wistar derived (Alpk:APfSD)	M + F	Dermal	21.2% w/w diquat ion; undiluted	LD ₅₀ > 424	McCall & Robinson (1990b)
Rat	Sprague-Dawley	M + F	Inhalation (whole body)	19.5% w/w diquat ion; distilled water	LC ₅₀ (4 h) M = 0.121 F = 0.132 Combined = 0.125	Bruce, Griffis & Wong (1985)
Rabbit	New Zealand White	F	Skin irritation	20.7% diquat ion; undiluted	Moderate to severely irritating	Robinson (1998a)
Rabbit	New Zealand White	F	Eye irritation	20.7% diquat ion	Mildly irritating	Robinson (1998b)
Guinea-pig	Dunkin Hartley	F	Skin sensitization (Magnusson and Kligman method)	267 g/L; deionized water	Sensitizer	Rattray & Robinson (1990)

F: female; LC₅₀: median lethal concentration; LD₅₀: median lethal dose; M: male; w/w: weight per weight

(a) *Lethal doses*

In an acute oral toxicity study, groups of five male and five female fasted young adult Alpk:APfSD (Wistar-derived) rats were given a single oral dose of diquat dibromide (purity 21.2% weight per weight [w/w] diquat ion) in deionized water at a dose of 100, 150, 200, 225 or 250 mg diquat ion/kg bw and observed up to 15 days.

Following a dose of 100 mg/kg bw, there were no mortalities and no signs of toxicity. At 150 mg/kg bw, one male rat died, and at 200 mg/kg bw, there were two mortalities (one male and one female). Surviving animals at these two dose levels showed signs of slight to moderate toxicity, which persisted until day 7. Signs of extreme toxicity were seen in all animals at 225 and 250 mg/kg bw. There were four mortalities (two males and two females) at 225 mg/kg bw by day 8, but surviving animals recovered by day 12. All animals dosed at 250 mg/kg bw were either found dead or killed in extremis by day 5. At 150 and 250 mg/kg bw, clinical signs of toxicity seen included decreased activity, hypothermia, piloerection, reduced splay reflex, distended abdomen, sides pinched in, ungroomed, upward curvature of the spine, increased breathing depth, decreased breathing depth, increased breathing rate, breathing irregular, stains around mouth and/or nose, dehydrated, urinary incontinence, diarrhoea, chromodacryorrhoea and splayed gait.

The acute oral LD₅₀ for males was 214 mg diquat ion/kg bw (95% confidence interval [CI] 180–271). The acute oral LD₅₀ for females was 222 mg diquat ion/kg bw (95% CI 203–241) (McCall & Robinson, 1990a).

In an acute dermal toxicity study, five male and five female young adult Alpk:APfSD rats were each given a single 24-hour dermal application of diquat dibromide (purity 21.2% w/w diquat ion) at 2000 mg/kg bw. The test substance was applied undiluted to the shorn backs of the rats and held in place under an occlusive dressing. The animals were observed daily for 15 days.

There were no mortalities, and no significant signs of toxicity were seen. Signs of moderate skin irritation were seen. No treatment-related effects were observed on body weight or upon macroscopic examination.

The acute dermal LD₅₀ of diquat dibromide to males and females was greater than 2000 mg/kg bw (equivalent to > 424 mg diquat ion/kg bw) (McCall & Robinson, 1990b).

In an acute inhalation toxicity study, groups of young adult Sprague-Dawley CD rats (five of each sex) were exposed by inhalation (whole body) for 4 hours to aerosols containing 19.5% w/w diquat ion in distilled water at target concentrations of 0 (controls), 0.15, 1.0 and 4.0 mg/L. Approximately 97–99% of each aerosol was smaller than 10 µm (mass median aerodynamic diameter 2.2–2.8 µm). The average total measured concentrations were 0, 0.16, 1.1 and 3.9 mg/L. Specified tissues were submitted for histological examination.

All animals exposed to 3.9 mg/L (211 µg diquat ion/L) died 2–10 days after exposure. Three males and three females exposed to 1.1 mg/L (118 µg diquat ion/L) died 3–5 days after exposure. There were no deaths in animals exposed to 0.16 mg/L (86.1 µg diquat ion/L).

Signs of toxicity included squinted eyes and salivation (seen during exposure at all concentrations); laboured breathing, increased respiration rate, abnormal respiratory sounds; nasal, oral and/or ocular discharge; slight hindquarter ataxia/weakness; sores/alopecia on the throat; and weight loss. At necropsy, treatment-related lung changes were present in animals exposed to 1.1 and 3.9 mg/L. Histopathological examination revealed exposure-related, subchronic inflammation, congestion, oedema and alveolar/septal thickening in the lungs of all rats exposed to 1.1 and 3.9 mg/L. Three males and two females in the 0.16 mg/L group had subchronic inflammation and alveolar/septal thickening of the lungs.

It was concluded that the acute inhalation median lethal concentration (LC₅₀) of diquat ion was 121 µg/L for males, 132 µg/L for females and 125 µg/L for both sexes combined (Bruce, Griffis & Wong, 1985).

(b) Dermal and ocular irritation

In a primary dermal irritation study, three young adult female New Zealand White albino rabbits were dermally exposed to 0.5 mL of diquat dibromide technical concentrate (20.7% w/w diquat ion) on an area (approximately 2.5 cm × 2.5 cm) of the shorn flank for 4 hours. The animals were assessed for up to 23 days for any signs of skin irritation. Irritation was scored by the method of Draize.

Very slight or well defined erythema was seen in two animals for up to 7 days and in one animal for up to 17 days. Very slight or slight oedema was seen in all animals for up to 2 or 7 days. There were no additional signs of irritation in one animal. Additional signs seen in the remaining two animals included desquamation, skin thickening and development of new skin with associated sparse hair growth. All signs of irritation had completely regressed by day 23 in both animals.

Diquat dibromide technical concentrate is a moderate to severe irritant following a single 4-hour application to rabbit skin (Robinson, 1998a).

In a primary eye irritation study, 0.1 mL of diquat dibromide technical concentrate (20.7% w/w diquat ion) was instilled into the conjunctival sac of the left eye of each of three young adult female New Zealand White rabbits. The eyes were examined for up to 8 days to assess the grade of ocular reaction. Irritation was scored by the method of Draize.

No corneal or iridial effects were observed. Conjunctival effects included slight to moderate redness, slight chemosis and slight to moderate discharge. All effects had completely regressed by day 8.

Diquat dibromide technical concentrate is a mild irritant (class 4 on a 1–8 scale) to the rabbit eye (Robinson, 1998b).

(c) *Dermal sensitization*

Groups of 20 test and 20 control young adult female Alpk:Dunkin Hartley guinea-pigs were used to assess the sensitization potential of diquat using a method based on the maximization test of Magnusson and Kligman. For the main study, the concentrations used were 0.1% weight per volume (w/v) in deionized water for the induction intradermal injections, 100% for the topical induction applications, and 10% w/v in deionized water and 100% for the challenge applications. A positive control study was conducted using essentially the same methodology and using formaldehyde as the test substance.

Following challenge with the undiluted test sample, scattered mild redness was seen in 5/16 test and 0/17 control animals. The net response was calculated to be 31%. Following challenge with a 10% w/v preparation of the test sample in deionized water, scattered mild redness was seen in 1/16 test and 0/17 control animals. The net response was calculated to be 6%. No erythematous reactions were seen in any animal, test or control, following challenge with deionized water. The net response was zero. The positive control study provided a response as expected. Challenge of previously induced guinea-pigs with undiluted diquat elicited a moderate sensitization response, and challenge with a 10% w/v preparation elicited a weak sensitization response.

Diquat is considered to be a skin sensitizer in the guinea-pig (Ratray & Robinson, 1990).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

No data are available.

Rats

In a 90-day toxicity study, diquat technical, as the dibromide (26.9% diquat ion), was administered to 12 Alpk:APfSD rats of each sex per dose in the diet at a dose level of 0, 20, 100 or 500 ppm diquat ion (equal to 0, 1.7, 8.5 and 39.5 mg diquat ion/kg bw per day for males and 0, 1.9, 9.2 and 41.5 mg diquat ion/kg bw per day for females, respectively). The clinical condition of the animals, including body weight gain and feed consumption, was monitored during the study, and the eyes were examined ophthalmoscopically. Urine and blood were examined during the study. At necropsy, blood was taken for clinical chemistry and haematology, and selected organs were weighed and examined histologically.

There were no mortalities. All rats survived the experimental period until scheduled termination. Males and females fed 500 ppm diquat ion showed a statistically significant decrease in growth throughout the study, resulting in a mean body weight at termination that was 26% below that of the control group for males and 15% lower for females. Feed consumption was reduced (approximately 25% below control values) throughout the study for males and females receiving 500 ppm diquat ion. During the later stages of the study, the majority of male and female rats fed 500 ppm diquat ion showed clinical changes in the eyes, which were opaque and pale. Cataract formation was also observed at this dose at 8 weeks, and at necropsy, corneal opacity was observed in 7/12 males and 4/12 females. At histological examination, cataract was seen in 12/12 males and 11/12 females. Additionally, a low incidence of focal inflammation of the tongue and epithelium of the palate was observed.

Reduced plasma total protein and albumin levels were seen at 500 ppm, possibly caused by low feed intake. In the high-dose males (Table 9), cholesterol, triglycerides and alanine transaminase (ALT) were statistically significantly reduced compared with control values at termination. In the high-dose females, ALT and aspartate transaminase (AST) were statistically significantly reduced compared with control values at termination. A marked reduction in urinary protein was seen in males

in weeks 3 and 13 (2 and 12 weeks of treatment, respectively). At week 3, urinary pH was statistically significantly increased and specific gravity was decreased in both males and females dosed with 500 ppm diquat ion. All organ weights (absolute, but not relative) were reduced, almost certainly a reflection of poor feed intake. Treatment-related effects were not seen at 100 ppm.

Table 9. Intergroup comparison of selected blood clinical chemistry parameters (week 13)

Parameter	Males ^a				Females ^a			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Albumin (g/100 mL)	4.62	4.70	4.57	4.47*	4.65	4.61	4.57	4.52
Total protein (g/100 mL)	6.32	6.35	6.27	5.98**	6.19	6.16	6.16	6.04
ALT (mU/mL)	57.8	54.1	55.1	45.8**	46.9 (11)	43.8	45.8	24.7**
AST (mU/mL)	59.6	61.1	56.0	58.5	64.3 (11)	64.0	57.6	50.9**
Cholesterol (mg/100 mL)	60.9	62.1	60.4	49.6*	61.6	64.0	60.0	60.1
Triglycerides (mg/100 mL)	143	143	134	77**	86 (11)	75	73	71

ALT: alanine transaminase; AST: aspartate transaminase; U: units; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided; compared with control group mean)

^a Number of animals, when less than 12, shown in parentheses.

Source: Hodge (1989a)

The NOAEL was 100 ppm (equal to 8.5 mg diquat ion/kg bw per day), based on decreased body weight gain and feed consumption, changes in clinical chemistry parameters, increased urine volume, decreased urinary specific gravity, minor changes in haematological values, erosion of the tongue and oral cavity and ocular changes at 500 ppm (equal to 39.5 mg/kg bw per day) (Hodge, 1989a).

The purpose of a second 90-day toxicity study in Sprague-Dawley rats (described below) was to determine the NOAEL for cataract formation in rats. In a previous 90-day study in the Alpk:APfSD strain of rat (Hodge, 1989a), the no-effect level for ocular toxicity of diquat ion was 100 ppm. In a 2-year chronic toxicity/carcinogenicity study conducted in the Sprague-Dawley strain of rat, the no-effect level for ocular toxicity of diquat ion at 90 days, based on ophthalmoscopy, was 15 ppm. At the next higher dose of 75 ppm, 1/50 males and 3/50 females showed evidence of treatment-related cataracts (Colley et al. 1985).

In this second 90-day toxicity study, groups of 12 male and 12 female Sprague-Dawley rats were fed diets containing 0, 30, 60 or 300 ppm diquat ion (equal to 0, 2.4, 4.7 and 23.2 mg diquat ion/kg bw per day for males and 0, 2.7, 5.0 and 25.3 mg diquat ion/kg bw per day for females, respectively) for 90 consecutive days. Clinical observations, body weight and feed consumption were measured. An ophthalmoscopic examination was performed on all rats prior to commencement and prior to termination of treatment. At the end of the study, all animals were killed and examined postmortem, and the eyes were removed and stored.

There were no toxicologically significant clinical observations. At 300 ppm, body weight and feed consumption were generally low compared with control values throughout the study for males and low compared with control values during the 1st week of treatment for females. Posterior lens opacities were present in week 13 for 10/12 males and 9/12 females at 300 ppm. A lens opacity

plaque was also seen for one female at this dose level. There were no ophthalmoscopic abnormalities in animals in the control, 30 and 60 ppm diquat ion groups.

The NOAEL was 60 ppm (equal to 4.7 mg diquat ion/kg bw per day) for ocular lesions and lens opacities evident at 300 ppm (equal to 23.2 mg diquat ion/kg bw per day) (Noakes, 2003).

When the results of the two 90-day toxicity studies in rats were combined, the overall NOAEL was 100 ppm (equal to 8.5 mg diquat ion/kg bw per day), with an overall lowest-observed-adverse-effect level (LOAEL) of 300 ppm (equal to 23.2 mg diquat ion/kg bw per day).

Dogs

A 1-year toxicity study in dogs was carried out using diquat dibromide technical (26.7% w/v diquat ion) added to the feed. Groups of four male and four female Beagles received diquat at a dose of 0, 0.5, 2.5 or 12.5 mg diquat ion/kg bw per day (achieved intakes 0, 0.46, 2.42 and 11.48 mg diquat ion/kg bw per day for males and 0, 0.53, 2.53 and 13.21 mg diquat ion/kg bw per day for females, respectively) for 52 weeks. Clinical condition, body weight and feed consumption were monitored throughout. Ophthalmoscopy, haematology and clinical chemistry were carried out. The animals were killed and necropsied at 52 weeks, and a range of organs was examined and processed for histological examination.

No treatment-related effects on survival, clinical signs, haematology, clinical chemistry, urine analysis or gross pathology (except eye) were observed at any dose level. Decreased body weight gains were observed only during the first 2 weeks of dosing in both sexes at the high dose level (males 46% and females 32% of control; Table 10), although there was no decrease in feed consumption at any time point. There was a statistically significant decrease in white blood cells and neutrophil count in males of all treatment groups at a single time point (week 4), which was ascribed to raised counts in one control. There were decreased platelet counts in top-dose females at 4, 26 and 52 weeks. Raised plasma chloride levels observed in the top-dose animals were attributed to bromide ion interference. Plasma triglyceride levels in the males given 12.5 mg diquat ion/kg bw per day were higher than in the controls throughout the study; at 4 and 26 weeks, these increases were statistically significant. Statistically significant increases in relative and absolute kidney weights were observed in both sexes at 12.5 mg diquat ion/kg bw per day. There were decreases in absolute and relative adrenal weights in all treatment groups in the males, which were statistically significant only in the case of relative weights. Additionally, there was a decrease in the absolute and relative weights of the epididymides in all test groups compared with the controls; this finding was statistically significant only for absolute weights in the 2.5 mg diquat ion/kg bw per day group and for relative weights in the top-dose group. Changes in organ weights did not correspond to any histopathological changes.

Table 10. Intergroup comparison of body weight gain from start of study: selected time points

Week	Body weight gain (kg)							
	Males				Females			
	0 mg diquat ion/kg bw per day	0.5 mg diquat ion/kg bw per day	2.5 mg diquat ion/kg bw per day	12.5 mg diquat ion/kg bw per day	0 mg diquat ion/kg bw per day	0.5 mg diquat ion/kg bw per day	2.5 mg diquat ion/kg bw per day	12.5 mg diquat ion/kg bw per day
1	0.27	0.32	0.30	0.17	0.25	0.15	0.07	0.00*
2	0.70	0.88	0.75	0.32*	0.47	0.38	0.30	0.15**
3	0.92	1.13	1.00	0.67	0.60	0.55	0.50	0.35
12	2.67	3.30	2.75	2.60	1.82	1.65	1.90	1.47

*: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided; compared with control group mean)

Source: Hopkins (1990)

At necropsy, bilateral lens opacity was observed in all high-dose males and 3/4 high-dose females. Unilateral lens opacity was observed in two females that received 2.5 mg diquat ion/kg bw per day at necropsy. The first occurrences of lens opacity were at week 8 and week 40 in one female each in the 2.5 mg diquat ion/kg bw per day dose group. The first occurrences of lens opacity at the high dose level were at week 16 in males and week 24 in females. Inflammatory changes were seen at the top dose in the large intestine, consisting of reduction in mucosal thickness, loss and abnormality of mucosal glands, epithelial hyperplasia in crypts and increased goblet cell activity.

The NOAEL was 0.53 mg diquat ion/kg bw per day, based on lens opacity (cataracts) in females at 2.53 mg diquat ion/kg bw per day (Hopkins, 1990).

(b) *Dermal application*

No data are available.

(c) *Exposure by inhalation*

No data are available.

2.3 *Long-term studies of toxicity and carcinogenicity*

Mice

In a 2-year toxicity and carcinogenicity study, groups of 60 male and 60 female mice (C57BL/10JfCD-1/Alpk) were fed a diet containing 0, 30, 100 or 300 ppm diquat (equal to 0, 3.56, 11.96 and 37.83 mg diquat ion/kg bw per day for males and 0, 4.78, 16.03 and 48.27 mg diquat ion/kg bw per day for females, respectively). The test material was technical-grade diquat dibromide containing 26.7% w/v diquat ion. Haematological parameters were assessed at weeks 53 and 79 and at study termination. Various tissues were taken at necropsy and processed for histological examination. At 12 months, 10 mice of each sex per dose were sacrificed.

There were no treatment-related adverse effects on survival of either sex, although a greater number of treated females (37%, 47% and 40% with increasing dose) were killed in extremis compared with the control females (25%). Treatment-related clinical signs were observed in both sexes and included an increased incidence of eye discharge (males in the 100 ppm dose group and mice of both sexes in the 300 ppm dose group) and an increased incidence of thin appearance (males) and subdued behaviour (females in the 100 ppm group and males and females at 300 ppm). Decreased body weight was observed throughout the study in both sexes (males 3.5–8.7% and females 2.9–9.4% below control) at 300 ppm and in males at 100 ppm (3.8–4.7% below control) from week 95 until termination, compared with the controls. Feed consumption was decreased for both sexes at the high dose level. Changes in certain haematological parameters at 300 ppm were also seen – namely, statistically significantly decreased neutrophil count and increased lymphocyte count in both sexes at weeks 53 and 79. There was also a significant increase in total white blood cells at 2 years in males. At termination, platelet count, white blood cell count and lymphocyte count were all slightly higher than control values in males fed 300 ppm diquat, with marginal increases in females receiving 300 ppm diquat. White blood cell counts in males only showed a statistically significant difference. Relative kidney weights were increased significantly in male mice fed 100 or 300 ppm diquat by approximately 5% and 7%, respectively. There was a slight treatment-related increase in microscopic lesions in the kidney (tubular dilatation in both sexes and tubular hyaline droplet formation in females), liver (extramedullary haematopoiesis in both sexes) and mesenteric lymph nodes (lymphoid proliferation in females) at the high dose level, mainly at the terminal kill.

Diquat was not carcinogenic in this study. The number of tumour-bearing mice was the same in the control and diquat-treated male groups. In the females, there was a reduction in the number of tumour-bearing mice in the mid-dose (100 ppm) and high-dose (300 ppm) groups, relative to the controls. Most of the tumour-bearing mice in each group, including the controls, had single, malignant and metastatic tumours.

The NOAEL was 30 ppm (equal to 3.56 mg diquat ion/kg bw per day), based on reduction in body weight gain, increased relative kidney weights and ocular discharges at 100 ppm (equal to 11.96 mg diquat ion/kg bw per day) and above. There was no evidence of carcinogenicity in mice at doses up to and including 300 ppm (equal to 37.83 mg diquat ion/kg bw per day), the highest dose tested (Hodge, 1992).

Rats

In a combined chronic toxicity and carcinogenicity study, male and female Sprague-Dawley rats (50 of each sex per dose) were fed diets containing 0, 5, 15, 75 or 375 ppm diquat dibromide (purity 19.54% w/w diquat ion; equal to 0, 0.19, 0.58, 2.91 and 14.88 mg diquat ion/kg bw per day for males and 0, 0.24, 0.72, 3.64 and 19.44 mg diquat ion/kg bw per day for females, respectively) for 104 weeks. In addition, 10 rats of each sex per dose were treated similarly and killed at 12 months. Ophthalmoscopy was carried out before dosing and periodically during the study. Haematological and biochemical variables were measured on 10 animals from each main group before dosing and at 26, 52, 78 and 104 weeks and in five satellite animals at week 52.

Treatment had no adverse effect on survival. With the exception of effects on the eye, there were no treatment-related clinical signs of toxicity. Changes in the eyes were observed at the two highest dose levels and consisted of an initial appearance of the eye being clearer and more transparent than usual. This was followed temporally by opacity, which first occurred in week 10 at 75 ppm (one male) and in week 11 at 375 ppm (two females). From week 45, a number of rats with total opacity showed a dark red discolouration of the eyes, which became darker with time. This was due to extensive haemorrhage in the anterior chamber of the eye, revealed by ophthalmoscopic examination. Body weight gains were decreased at the high dose level in both sexes throughout the study (weeks 0–13: males 84% and females 80% of control; overall: males 86% and females 75% of control), and a corresponding depression in average feed consumption was observed at the high dose level for both sexes for the first 26 weeks and for females throughout week 78 of the study. No treatment-related effects on body weight or feed consumption were observed at 5, 15 or 75 ppm. After 26 weeks, there was a reduction in mean corpuscular volume and in haemoglobin in females and males, respectively. Minimal reductions in red blood cell parameters were observed in males at 15 ppm and above at 52 and 78 weeks. Other changes were prolonged activated partial thromboplastin times at 75 and 375 ppm in females at 52 and 104 weeks and at 15 ppm in females at 52 weeks. Blood urea nitrogen was elevated at 52 weeks at 75 and 375 ppm and at 78 weeks in the 375 ppm females. At 52 weeks, the 75 and 375 ppm groups exhibited lower total protein and albumin levels. On ophthalmoscopic examination (Table 11), lenticular opacities were seen in the 75 and 375 ppm groups at 13 weeks, and the findings progressed at subsequent examinations. Ophthalmoscopy at 104 weeks showed severe lens opacities at 375 ppm in all surviving rats, whereas less severe ones were seen at 75 ppm. At 15 ppm, a single instance of cataract was seen in each sex. Histological examination of the eyes postmortem showed advanced cataractogenesis in all animals at 375 ppm and in about 80% of the 75 ppm group. There was a low prevalence of cataract at 15 ppm: cataract-type changes were seen in 0/22 control and 3/22 treated males and 0/22 control and 2/20 treated females.

There were no differences in urine clinical chemistry parameters that were considered to be related to treatment. There were no compound-related effects on organ weights. Males at the two highest dose levels displayed an increased incidence of nephropathy in the kidneys, and the high-dose males displayed increased incidences of arteritis/periarteritis in blood vessels and paracortical cell hyperplasia in the lymph nodes. There was no treatment-related increase in any tumour type in either sex.

Benign pheochromocytoma of the adrenal gland was seen in males receiving 75 or 375 ppm diquat dibromide, but this was not dose related and achieved statistical significance only at the 75 ppm level. There were two instances in males of statistically significant positive trends with dose: osteosarcoma and thyroid follicular cell adenoma. In both cases, these findings reflected elevated incidences at the 375 ppm level only, which were not statistically significant by Fisher's exact test, and there was no evidence of any increasing incidence below this dose level. There was a significant

Table 11. Intergroup comparison of ophthalmology: selected lens lesions

Finding	Week	Incidence of lens lesions									
		Males					Females				
		0 ppm	5 ppm	15 ppm	75 ppm	375 ppm	0 ppm	5 ppm	15 ppm	75 ppm	375 ppm
Point posterior subcapsular polar opacities	13	0/50	0/50	0/50	8/50	6/49	0/50	2/50	1/50	5/50	0/50
	26	0/50	0/50	0/50	0/49	0/49	0/50	0/50	0/50	0/50	2/49
	52	0/50	0/49	0/50	1/49	0/48	0/49	0/50	0/47	2/50	0/48
	104	0/22	0/16	0/22	0/21	0/24	0/20	0/22	1/20	0/20	0/27
Posterior subcapsular polar opacities	13	0/50	0/50	1/50	0/50	0/49	0/50	0/50	0/50	0/50	0/50
	26	1/50	2/50	3/50	3/49	0/49	0/50	1/50	0/50	5/50	0/49
	78	0/46	1/44	0/44	0/40	0/40	0/39	0/41	0/42	0/41	0/46
Triangular posterior subcapsular polar opacities	13	0/50	0/50	0/50	1/50	32/49	0/50	0/50	0/50	3/50	40/50
	26	0/50	0/50	0/50	2/49	9/49	0/50	0/50	0/50	5/50	10/49
	52	0/50	0/49	0/50	1/49	3/48	0/49	0/50	0/47	5/50	3/48
	78	0/46	0/44	1/44	3/40	0/40	0/39	0/41	0/42	4/41	1/46
	104	0/22	0/16	1/22	9/21	0/24	0/20	0/22	0/20	1/20	0/27
Total cataract	13	0/50	0/50	0/50	1/50	11/49	0/50	0/50	1/50	0/50	2/50
	26	0/50	0/50	0/50	2/49	38/49	0/50	0/50	0/50	0/50	37/49
	52	0/50	0/49	0/50	2/49	45/48	0/49	0/50	0/47	0/50	43/48
	78	1/46	0/44	0/44	2/40	40/40	0/39	0/41	0/42	2/41	45/46
	104	0/22	0/16	1/22	3/21	24/24	0/20	0/22	1/20	3/20	27/27
Plaque opacities	52	1/50	0/49	3/50	1/49	0/48	0/49	1/50	0/47	0/50	0/48
	78	3/46	2/44	3/44	2/40	0/40	0/39	2/41	0/42	6/41	0/46
	104	5/22	4/16	6/22	3/21	0/24	5/20	3/22	0/20	3/20	0/27

Source: Colley et al. (1985)

increase in the number of females with multiple neoplasia and with malignant neoplasms at 5 and 75 ppm. No other change attributable to the test material was seen.

The NOAEL was 5 ppm (equal to 0.19 mg diquat ion/kg bw per day), based on cataractous changes seen at 15 ppm (equal to 0.58 mg diquat ion/kg bw per day). There was no evidence of carcinogenic potential. The NOAEL for carcinogenicity was 375 ppm (equal to 14.88 mg diquat ion/kg bw per day), the highest dose tested (Colley et al., 1985). This was the study on which the ADI was based at the 1993 JMPR.

Subsequent to the 1993 JMPR, the ophthalmoscopy and ocular histopathology data from the 2-year chronic toxicity and carcinogenicity study in rats by Colley et al. (1985) were re-evaluated by the same laboratory that had conducted the original study (specifically by the principal pathologist); in particular, histopathology data were correlated with ophthalmoscopy findings. The evaluation demonstrated a consistent pattern of diquat-induced lesion (bilateral triangular posterior subcapsular polar opacities that progress to more extensive opacities and total cataract) in animals at 75 and 375 ppm. However, none of the animals at 15 ppm demonstrated the consistent pattern; when considered with the histopathology, there was no treatment-related increase in overall cataractous change. It was concluded that there was no evidence of diquat cataractogenic response at 15 ppm (Harling, Buist & Gopinath, 1997a,b).

Based on the above re-evaluation of the data, the present Meeting identified the NOAEL as 15 ppm (equal to 0.58 mg diquat ion/kg bw per day), as the incidence and severity of cataracts were comparable with those of the controls.

2.4 Genotoxicity

The results of studies of genotoxicity with diquat technical are summarized in Table 12. All the studies, either in vitro or in vivo, were negative except for the mammalian cell cytogenetic assay (human lymphocytes), which gave an equivocal response in the absence of metabolic activation and a positive response in the presence of metabolic activation at cytotoxic doses. Diquat is not considered to possess any mutagenic or genotoxic potential.

Table 12. Results of studies of genotoxicity with diquat

Type of study	Organism/cell line	Dose range tested	Purity (%) ^a	Result	Reference
In vitro					
Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538; <i>Escherichia coli</i> (WP2 uvrA PMK101)	0.05–100 µg/plate (+S9 mix) 0.01–50 µg/plate (–S9 mix) (in water)	25.8	Negative ±S9 mix	Callander (1986)
Mammalian cell gene mutation assay	L5178Y mouse lymphoma cells	3.13–50 µg/mL (+S9 mix) 6.25–100 µg/mL (–S9 mix) (in 1% DMSO)	25.8	Negative ±S9 mix	Cross (1986)
Mammalian cell cytogenetic assay	Human lymphocytes (chromosomal aberrations)	26.7–534.8 µg/mL (+S9 mix) 13.4–534.8 µg/mL (–S9 mix) (in 0.85% saline)	100	Equivocal –S9 mix Positive +S9 mix	Wildgoose et al. (1986)
In vivo					
Mouse micronucleus	Male and female C57BL/6J/Alpk mice	0, 62.5 and 100 mg/kg bw (gavage in deionized water)	25.8	Negative	Sheldon, Richardson & Shaw (1986)
DNA repair (unscheduled DNA synthesis) assay	Rat liver Alpk:AP rats	0, 225, 450 or 900 mg/kg bw (gavage in deionized water)	25.8	Negative	Trueman (1987)
Dominant lethal assay	CD-1 mice	0, 0.1, 1.0 or 10 mg/kg bw for 5 consecutive days (gavage in 0.5% Tween 80 in water)	28.6	Negative	McGregor (1974)

DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; S9: 9000 × g supernatant fraction from rat liver homogenate; w/w: weight per weight

^a Purity expressed as diquat ion (w/w).

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

A multigeneration reproduction study was conducted using technical-grade diquat dibromide, containing 26.7% w/v diquat ion. Groups of Alpk:APfSD Wistar-derived rats (30 of each sex) were fed diets containing 0, 16, 80 or 400/240 ppm diquat ion (equal to 0, 1.6, 7.9 and 38.7 mg/kg bw per day for males and 0, 1.7, 8.4 and 40.4 mg/kg bw per day for females, respectively, during the premating period). After 12 weeks, the animals were mated and allowed to rear the litters that resulted (F_{1a}). The process was repeated with 30 male and 30 female parents per group selected from the F_{1a} litters, these F_1 parents being mated 11 weeks after selection. The dose received by the top-dose F_1 rats was reduced after 4 weeks to 240 ppm. Ophthalmoscopic examination of the eyes was carried out on the controls and top-dose group at 12 weeks and on all the F_0 animals at termination (24 weeks). A similar examination was carried out on the F_1 rats 4 weeks after selection, at 11 weeks before mating and at termination (21 weeks).

No treatment-related mortality was observed. Parental toxicity was observed mostly in the high-dose group in both generations as increased incidences of clinical signs (red/brown urine, piloerection and ulcers on palate and tongue) and increased incidences of ophthalmoscopic signs (eye opacity, partial or total lenticular cataracts and iritis). At a dose level of 80 ppm, there was a low incidence of mouth lesions in both generations and a slightly increased incidence of partial cataract in F_1 females. At the high dose in both generations, a statistically significant ($P < 0.01$) decreased body weight gain during the premating period (males, 15–40%; and females, 10–37%), gestation (20–23%) and lactation (32–80%) and statistically significant ($P < 0.05$ or 0.01) decreased feed consumption during the premating period (males, 7–40%; and females, 5–33%), gestation (8–14%) and lactation (16–28%) were observed. The nature of the diquat-induced cataracts varied from animal to animal and also varied according to the time period of examination. The less severe cataracts (partial) were characterized by the following features: increased transparency or clearing of the lens (believed to result from a change in optical density or loss of lines of discontinuity rather than opacity); focal areas of opacity (usually posterior) with or without increased demarcation of the posterior suture lines; and vacuoles (usually posterior). The more severe cataracts (total) were characterized either by opacity of the whole or greater part of the lens or by shrinkage of the lens with opacification. The cataracts tended to be bilateral, although the degree of change in each eye was not necessarily the same. In both F_0 and F_1 parents, the severity and incidence of cataracts increased with time, and females were more severely affected than males. Cataracts, first seen at week 12 in F_0 females and at week 13 in males and 2 weeks earlier in each sex in the F_{1a} and F_{1b} generations, were also observed in the F_1 adults. Although cataract formation was mostly confined to the 240 ppm group, a low incidence was seen at 80 ppm in the F_1 female parents (3/30 at premating ophthalmoscopic examination and 4/30 at examination before termination of the study). Diquat had no effect on fertility in either sex. At the terminal histopathological examination, cataract was seen in the highest-dose group only of F_0 and F_1 parents. There was an increase in pathological changes in the renal tract in the F_1 and F_2 pups. Pup weight gain was lower in the top dose in both generations compared with controls. A statistically significant decrease in total litter weight was apparent for most time points for the F_{1a} litters. The slightly lower values for pup weight gain in the F_{1a} litters at 80 ppm were considered not to be related to treatment, as no similar effects were seen in the F_{2a} litters, and total litter weight was not affected.

The NOAEL for parental systemic toxicity was 16 ppm (equal to 1.6 mg diquat ion/kg bw per day), based on a low incidence of mouth lesions in both generations and a slightly increased incidence of cataracts at 80 ppm (equal to 7.9 mg diquat ion/kg bw per day). The NOAEL for reproductive toxicity was 400/240 ppm (equal to 38.7 mg diquat ion/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 80 ppm (equal to 7.9 mg diquat ion/kg bw per day), based on decreased pup body weights seen in both generations at 400/240 ppm (equal to 38.7 mg diquat ion/kg bw per day) (Hodge, 1990).

(b) *Developmental toxicity**Rats*

In a developmental toxicity study, diquat dibromide (purity 26.2% w/v diquat ion) was administered by gavage, in deionized water, to pregnant Alpk:APfSD Wistar-derived rats (23–24 per group) from gestation days 7 through 16. The dose levels used were 0, 4, 12 and 40 mg/kg bw per day, expressed as diquat ion. Animals were sacrificed on gestation day 22, and uteri were examined for live fetuses and intrauterine deaths.

A dose-related, statistically significant decrease in body weight gain compared with control values was seen across all dose groups during the first 3 days of dosing. There was a clear statistically significant decrease in body weight gain (about 22% compared with controls) in the 40 mg diquat ion/kg bw per day dose group throughout the dosing period, with the majority of the animals losing weight initially. The effect seen at 4 and 12 mg diquat ion/kg bw per day was evident only during the first few days of dosing and was very mild at 4 mg diquat ion/kg bw per day. A dose-related, statistically significant reduction in feed consumption compared with controls was seen across all dose groups during the first 3 days of dosing. This effect persisted throughout the dosing period for animals in the 12 and 40 mg diquat ion/kg bw per day groups and continued at the top dose for the first 3 days post-dosing. It did not persist in the 4 mg diquat ion/kg bw per day group. Significant reductions in fetal weight, litter weight and gravid uterine weight as well as fetal defects in ossification were seen at 40 mg diquat ion/kg bw per day. At the high dose, increased incidences of fetuses with haemorrhagic kidney (five in four litters; $P < 0.05$), compared with none in the control group, were also seen (Table 13). Minor evidence of reduced ossification was seen at the lower doses, but these were considered not to be of biological significance.

Table 13. Intergroup comparison of selected fetal defects and variants in a developmental toxicity study in rats

Observation	0 mg/kg bw per day	4 mg/kg bw per day	12 mg/kg bw per day	40 mg/kg bw per day
No. of litters examined	24	24	23	20
No. of fetuses examined	297	291	269	239
Total incidence of major defects	1	2	1	4
No. of fetuses showing major skeletal defects	1	1	1	1
No. of fetuses showing minor skeletal defects only	106	102	97	132**
Incidence of haemorrhagic kidneys (fetus/litter)	0	1/1	0	5*/4*
Incidence of calcaneum not ossified (fetus/litter)	133/22	139/21	157**/22	149**/19
Incidence of odontoid not ossified (fetus/litter)	107/22	114/19	110/17	102/19
Incidence of partially ossified 4th lumbar transverse process (fetus/litter)	232/24	210/24	182/22	128/19
Mean <i>manus</i> score	2.20	2.25	2.18	2.52**
Mean <i>pes</i> score	2.92	2.93	2.92	2.98

*: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided; compared with control group mean)

Source: Wickramaratne (1989)

The NOAEL for maternal toxicity was 12 mg diquat ion/kg bw per day, based on reduced body weights and feed consumption seen at 40 mg diquat ion/kg bw per day. The NOAEL for embryo and fetal toxicity was 12 mg diquat ion/kg bw per day, based on reduced fetal weight, haemorrhagic kidney and increased incidence of soft tissue and minor skeletal anomalies at 40 mg diquat ion/kg bw per day (Wickramaratne, 1989).

Rabbits

In a developmental toxicity study, groups of 20 New Zealand White rabbits were administered diquat dibromide technical (26.2% w/v diquat ion) in deionized water by gavage at a dose of 0, 1, 3 or 10 mg diquat ion/kg bw per day from days 7 to 19 (inclusive) of gestation. On day 30 of gestation, the females were killed and the uteri examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined externally and for visceral abnormalities, eviscerated and stained for skeletal abnormalities.

Nothing remarkable was observed in the low-dose group (1 mg diquat ion/kg bw per day). The mid-dose group (3 mg diquat ion/kg bw per day) was associated with decreased maternal body weight gain (229% less than controls; $P < 0.01$) and feed consumption (45% less than controls; $P < 0.01$), which were observed only during the first 3 days of dosing. The high-dose group (10 mg diquat ion/kg bw per day) showed greater decreases in body weight gain (343%; $P < 0.01$) and feed consumption (74%; $P < 0.01$) compared with the mid-dose group during the first 3 dosing days, and the decreases continued throughout the dosing period. Two 10 mg diquat ion/kg bw per day females lost weight throughout dosing and then rapidly gained weight during the post-dosing period. Analyses were done both with and without these two females (Table 14).

Table 14. Intergroup comparison of body weight gain (all females) in a developmental toxicity study in rabbits

Period	Body weight gain (g)				
	0 mg/kg bw per day	1 mg/kg bw per day	3 mg/kg bw per day	10 mg/kg bw per day (all females)	10 mg/kg bw per day (excluding two females)
Initial weight (day 1)	3 418.1	3 401.3	3 344.5	3 368.2	3 357.1
Predosing (days 1–7)	130.2	131.0	158.4	171.9	165.0
During dosing (days 7–19)	237.4	309.9	236.4	47.5**	175.1
Days 7–10	42.6	61.4	–54.8**	–103.0**	–
Days 10–13	57.3	66.3	90.8	26.3	–
Days 13–16	109.3	140.3	131.8	94.1	–
Post-dosing (days 19–30)	312.4	387.1	293.3	424.8*	352.4
Overall (days 1–30)	680.1	828.0*	688.2	644.3	692.5

*: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided; compared with control group mean)

Source: Hodge (1989b)

There were no treatment-related effects on numbers of corpora lutea, preimplantation or postimplantation loss, litter size, sex distribution, gravid uterus weight or litter weight. Fetal weight was marginally lower at 10 mg diquat ion/kg bw per day. Some evidence of fetotoxicity was observed at 10 mg/kg bw per day (mottled and friable livers and small increase in minor skeletal defects at 3 and 10 mg diquat ion/kg bw per day, in the form of partially ossified sternebrae). The elevation in the proportion of fetuses with minor skeletal defects was significant at 3 and 10 mg diquat ion/kg bw per day; there was a non-significant increase in the lowest-dose group. The incidences were not statistically significant using a litter-based method of analysis (Table 15).

The NOAEL for maternal toxicity was 1 mg diquat ion/kg bw per day, based on reduced weight gain and feed consumption at 3 mg diquat ion/kg bw per day. The NOAEL for embryo and fetal toxicity was 3 mg diquat ion/kg bw per day, based on skeletal anomalies at 10 mg diquat ion/kg bw per day (Hodge, 1989b).

Table 15. Intergroup comparison of selected fetal defects and variants in a developmental toxicity study in rabbits

	0 mg/kg bw per day	1 mg/kg bw per day	3 mg/kg bw per day	10 mg/kg bw per day
No. of litters examined	18	15	20	13
No. of fetuses examined	147	134	129	96
Total incidence of major defects	2	8	2	4
No. of fetuses showing major external/visceral defects	2	8*	2	3
No. of fetuses showing major skeletal defects	1	3	1	1
No. of fetuses showing minor external/visceral defects only	60	56	64	39
No. of fetuses showing minor skeletal defects only	64	65	71*	53*
Liver – friable (fetus/litter)	8/5	7/2	5/2	14*/3
Gall bladder – small blood clot(s) attached (fetus/litter)	18/8	21/9	33**/13	14/8
Vertebral column – 27 presacral vertebrae (fetus/litter)	46/14	54/12	49/15	49**/12
Sternebrae – not ossified 6th (fetus/litter)	0/0	6*/2	3/3	6**/5**
Sternebrae – partially ossified 6th (fetus/litter)	9/6	14/8	19*/8	16**/8
Extra ribs – 13th normal length (fetus/litter)	76/15	61/13	89**/18	71**/12
Mean <i>manus</i> score	1.61	1.60	1.62	1.80
Mean <i>pes</i> score	1.43	1.45	1.40	1.65*

*: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided; compared with control group mean)

Source: Hodge (1989b)

2.6 Special studies

(a) Acute neurotoxicity

Rats

In an acute neurotoxicity study, diquat dibromide (technical grade) was administered in a single gavage dose to 10 male and 10 female Sprague-Dawley rats at a dose of 0, 25, 75 or 150 mg/kg bw, expressed as diquat ion. These rats were assessed for reactions in functional observational battery and motor activity measurements at 6 hours post-dosing and on days 8 and 15.

Evidence for neurotoxicity was observed only during the daily clinical observations. In the 75 mg/kg bw group, females had an increased incidence of diarrhoea (2/10) and staining of the nose (3/10), compared with the controls (0/10). Females in the 150 mg/kg bw group also had piloerection (7/10), urinary incontinence (3/10), mouth staining (3/10), upward curvature of the spine (3/10), tiptoe gait (3/10), hunched posture (2/10), subdued behaviour (2/10) and sides pinched in (1/10). These signs were observed up to day 9 of the study. At 150 mg/kg bw, slightly decreased body weights (4–5%) were observed by day 8 in both sexes, and decreased body weight gains (males 17%; females 23%) were observed during the 1st week only at this dose level in both sexes (Table 16). Feed consumption was decreased in the mid-dose (males 10%) and high-dose (males 22% and females 35%) groups during the 1st week following dosing. One female in the 150 mg/kg bw group, with all of the above signs, was sacrificed in extremis on study day 6. Males were less affected than females. The clinical signs observed in this study may not be due to direct neurotoxicity.

The NOAEL for general toxicity was 75 mg diquat ion/kg bw, based on a range of findings, including clinical signs, at 150 mg diquat ion/kg bw. The NOAEL for neurotoxicity was 150 mg diquat ion/kg bw, the highest dose tested (Horner, 1992a).

Table 16. Intergroup comparison of body weight in an acute neurotoxicity study in rats

	Body weight (g)							
	Males				Females			
	0 mg/kg bw ^a	25 mg/kg bw	75 mg/kg bw	150 mg/kg bw	0 mg/kg bw	25 mg/kg bw	75 mg/kg bw	150 mg/kg bw
Day 1 (post-dosing)	170.6	173.0	169.2	173.2	144.2	144.1	143.5	143.9
Day 8	246.9	251.5	240.1	236.5**	188.4	183.9	185.6	178.3**
Day 15	288.1	295.5	282.5	286.4	207.7	203.6	204.6	203.3

***P* < 0.01 (Student's *t*-test, two-sided; compared with control group mean)

^a Doses expressed as diquat ion.

Source: Horner (1992a)

(b) *Subchronic neurotoxicity*

Rats

In a subchronic neurotoxicity study, diquat dibromide (purity 20.8%) was administered to 12 Alpk:APfSD rats of each sex per group at a dietary level of 0, 20, 100 or 400 ppm (equal to 0, 1.6, 7.9 and 32.4 mg/kg bw per day for males and 0, 1.9, 9.5 and 38.5 mg/kg bw per day for females, respectively, expressed as the diquat ion) for 13 weeks. Five rats of each sex per dose were used for neuropathological examination at study termination.

There were no treatment-related deaths. Treatment-related clinical signs were observed at the high dose level in both sexes at the end of the study: opaque eyes: 7/12 males and 8/12 females (weeks 13 and 14); eye pallor: 3/12 males and 1/12 females (weeks 12 and 13); and decreased visual placement response: 3/12 males and 1/12 females (week 14). Decreased body weights (9%) were observed throughout the study in both sexes at the high dose level (Table 17); decreased body weight gains (males 20% and females 18%) and decreased feed utilization were also observed at this dose level in both sexes. There was an increased incidence of cataracts and/or posterior lens opacities at the high dose level in both sexes at study termination. Total cataracts were observed in 5/12 high-dose males and 7/12 high-dose females. Posterior opacity of the lens was observed in 8/12 high-dose males and 6/12 high-dose females.

Table 17. Intergroup comparison of body weights (selected time points) in a subchronic neurotoxicity study in rats

Week	Body weight (g)							
	Males				Females			
	0 ppm	20 ppm	100 ppm	400 ppm	0 ppm	20 ppm	100 ppm	400 ppm
1	196.8	201.7	200.9	199.6	159.4	154.8	156.9	152.9
4	318.5	319.9	313.3*	294.5**	218.8	202.8*	210.2	198.7**
8	402.1	398.4	397.7	359.8**	254.4	238.9*	247.1	227.2**
14	477.8	478.3	475.7	420.4**	276.7	271.6	267.3	249.8**
Body weight gain, 0–14 weeks	278.1	274.8	268.5	221.2	116.3	114.8	111.8	95.5
% of controls	–	99	97	80	–	99	96	82

P* < 0.05; *P* < 0.01 (Student's *t*-test, two-sided; compared with control group mean)

Source: Horner (1992b)

There was no evidence of neurotoxicity. No treatment-related effects were reported in the functional observational battery (weeks 5, 9 and 14 post-dosing), motor activity assessment (weeks 5, 9 and 14 post-dosing) and gross pathological/histopathological examination at study termination.

The NOAEL for neurotoxicity was 400 ppm (equal to 32.4 mg diquat ion/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 100 ppm (equal to 7.9 mg diquat ion/kg bw per day), based on evidence of eye lesions and reductions in body weight gain and feed consumption at 400 ppm (equal to 32.4 mg diquat ion/kg bw per day) (Horner, 1992b).

Karuppagounder et al. (2012) published a high-dose study investigating the chronic neurotoxic effects of diquat. The study involved experiments conducted in young adult male C57Bl6 mice given 10 mg diquat/kg bw via intraperitoneal injection twice a week for 6 weeks (i.e. a total dose of 120 mg/kg bw). The authors reported a “mild degeneration of substantia nigra dopaminergic neurones” and stated that it correlated well with the behavioural and neurochemical results. However, these findings were not observed in the guideline subchronic neurotoxicity study in rats in which doses of up to 38.5 mg diquat ion/kg bw per day were administered in the diet.

(c) *Immunotoxicity*

In an immunotoxicity study, diquat dibromide (purity 22.0% w/w diquat ion) was administered to female CD-1 mice (10 per dose) in the diet at a concentration of 0, 100, 200 or 350 ppm of diquat ion (equal to 0, 23, 44 and 81 mg diquat ion/kg bw per day, respectively) for 28 days. Animals in the positive control group (10 females) received an oral gavage dose of cyclophosphamide at 10 mg/kg bw per day (dosing volume 5 mL/kg bw) for 28 days. On day 25, animals in all groups were immunized with a suspension of sheep red blood cells (sRBC) by intravenous injection (2×10^8 sRBC/mL, 0.25 mL/animal). Animals were sacrificed on day 29 by carbon dioxide inhalation, and blood samples were collected for analysis of anti-sRBC immunoglobulin M (IgM) titre with an enzyme-linked immunosorbent assay. The natural killer cell activity was not evaluated in this study. All animals were evaluated for mortality, clinical signs, body weight changes, feed consumption and water consumption. Terminal body weight and liver, spleen and thymus weights were measured for all animals at necropsy.

All mice survived the scheduled treatment period, and there were no treatment-related clinical signs. At day 28, animals receiving 200 or 350 ppm of diquat ion had a lower body weight compared with controls receiving only untreated diet. There was also a lower body weight change over the study period in these groups, compared with controls receiving only untreated diet. There were no differences in feed consumed or organ weights that were considered to be related to treatment with diquat dibromide. There were no abnormal findings noted at necropsy. There was no immunosuppressant effect with diquat dibromide.

The systemic toxicity NOAEL was 100 ppm diquat ion (equal to 23 mg diquat ion/kg bw per day), based on decreases in body weights and body weight gains seen at 200 ppm (equal to 44 mg diquat ion/kg bw per day). Under the conditions of the study, after 28 consecutive days of dietary treatment of female CD-1 mice with up to 350 ppm diquat ion (equal to 81 mg diquat ion/kg bw per day), there was no evidence of an immunosuppressant effect on the humoral immune system (Donald & Marr, 2011).

2.7 *Studies on metabolites*

Diquat monopyridone (6,7-dihydro-4-oxodipyrido(1,2-a:2',1'-c) pyrazinium) was the major metabolite following oral dosing and could therefore be the toxicologically active species following administration by this route. An acute (single dose) toxicity study and a 2-week repeated-dose toxicity study were conducted in rats to evaluate its toxicity. In the acute toxicity study in Alderley Park rats, six female rats were given a single gavage dose of diquat monopyridone dissolved in deionized water at 0 and 4000 mg/kg bw. No mortality occurred. The oral LD₅₀ of this compound in rats was in excess of 4000 mg/kg bw. In a separate study, groups of male and female Alderley Park rats (10 of each sex

per group) were given diquat monopyridone dissolved in deionized water and administered by stomach tube at 0 or 1000 mg/kg bw per day, 5 days/week, for 2 weeks. No clinical, haematological, biochemical or histopathological abnormalities were observed, except for a decreased number of lymphocytes in both males and females (Parkinson, 1974).

The acute toxicity of diquat monopyridone and diquat dipyrindone (6,7-dihydro-4,9-dioxodipyrido(1,2-a:2',1'-c) pyrazine) was evaluated in rats. Preliminary data suggest that diquat monopyridone is less toxic than diquat when administered orally. Diquat monopyridone and diquat were administered at equivalent doses of 90 $\mu\text{mol/kg}$ bw (i.e. 16 mg active ingredient/kg bw) to groups of 10 male Alderley Park rats. The animals in the monopyridone group showed no adverse effects for the entire 14-day period studied, with no deaths occurring. In the diquat group, however, all animals appeared subdued by day 3, and many animals developed abdominal distension. Nine animals of the original group of 10 were dead by the 14th day after dosing, with the remaining animal exhibiting abdominal distension. When diquat dipyrindone was administered at a dose of 90 $\mu\text{mol/kg}$ bw (i.e. 16 mg active ingredient/kg bw) to 10 rats, the animals showed no adverse effects for the entire 14-day period studied, with no deaths occurring. Both diquat dipyrindone and diquat monopyridone (major metabolites of diquat) are less toxic than diquat dichloride when administered by subcutaneous injection to male rats (Crabtree, 1976).

An acute oral toxicity study was conducted for a plant metabolite, TOPPS (R32245), to determine its toxicity relative to that of diquat. TOPPS was not found in the rat metabolism study, but it has been detected in a livestock metabolism study. In the acute oral toxicity study, groups of five male and five female fasted adult Alpk:AP (Wistar-derived) rats were given a single dose of TOPPS (purity 100%) in deionized water at 2000, 3000 or 5000 mg/kg bw and observed for up to 15 days.

No signs of toxicity or mortality were observed in the animals dosed with 2000 mg/kg bw. At 3000 and 5000 mg/kg bw, the signs of toxicity seen were sedation, tremors, piloerection, upward curvature of the spine, decreased breathing rate and increased breathing depth in surviving animals. All the males were found dead and two females survived following a dose of 3000 mg/kg bw. All animals in the 5000 mg/kg bw group died. All deaths occurred between 1 and 5 hours after dosing.

The acute oral LD_{50} was 2449 mg/kg bw for male rats and 2942 mg/kg bw for female rats (Southwood, 1987).

The mutagenic potential of TOPPS was determined using a deoxyribonucleic acid (DNA) repair test (rec-assay) and reverse mutation assay. TOPPS was found to be negative for mutagenicity in the DNA repair assay using *Bacillus subtilis* strains and in the reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2 uvrA, in the presence and absence of metabolic activation (Ohta, Watanabe & Tsukamoto, 1987).

Plasma corticosteroid concentrations were significantly elevated 15 minutes after intraperitoneal injection of 90 μmol diquat/kg bw into phenobarbitone-anaesthetized rats. Similar effects were noted after subcutaneous dosing. After oral administration, there was a delay of approximately 1 hour before significantly elevated concentrations were observed. The magnitude of the increase after intraperitoneal administration of diquat was dose related up to 26 $\mu\text{mol/kg}$ bw, as was the duration of the response. The increase in plasma corticosteroid concentration in diquat-treated rats could be prevented by pretreating animals with dexamethasone, which also reduced adrenocorticotrophic hormone (ACTH) concentrations in these animals. Experiments in vivo and in vitro indicated that diquat did not increase the steroidogenic response of adrenals to ACTH. It was concluded that the increased adrenal steroid synthesis observed at early times after diquat administration is caused by release of ACTH from the pituitary (Crabtree & Rose, 1976).

Administration of diquat prevents the normal depletion of liver glycogen in starved rats. There is an increase in blood glucose, which returns to normal after approximately 7 hours. Diquat administration increases plasma corticosteroids for at least 24 hours, but plasma ACTH is elevated only for 4 hours. Adrenal cyclic adenosine monophosphate is increased after diquat administration for at least 24 hours. It is suggested that diquat increases the response of the adrenal cortex to ACTH (Crabtree et al., 1973).

Following oral administration of 900 μmol diquat ion/kg bw to male rats, there was a rapid accumulation of water in the lumen of the gastrointestinal tract of all animals, which reached a maximum at 24 hours (about 14 mL water per rat at 24 hours). Blood, liver and muscle showed significant dehydration. Deaths occurred over 9 days, with about 50% occurring within the first 3 days. Animals that did not gain weight 24 hours after the oral dose died within the first 3 days and had significantly greater gastrointestinal water content and more pronounced haemoconcentration than animals that did gain weight. After subcutaneous injection of 90 μmol diquat ion/kg bw, there was only a minimal increase in water content of the gastrointestinal tract at 24 hours. Both mean water content and dry weight of the gastrointestinal tract increased with time. At 14 days, surviving animals could be divided into two distinct groups: those that showed severe abdominal distension and those that appeared normal. Only the abdominally distended animals showed increased water content and dry weight of the gastrointestinal tract. Severe changes were limited to animals that died between 2 and 8 weeks following dosing and had significantly increased tissue water.

It was concluded that diquat has an effect on water distribution within the body following oral dosing, and early death is associated with rapid fluid loss into the gastrointestinal tract. Gastrointestinal distension following subcutaneous administration appeared to be an unrelated phenomenon (Crabtree, Lock & Rose, 1976).

Following the administration to rats of approximately equitoxic doses of paraquat and diquat by intraperitoneal injection, the pattern of mortality was different. After dosing with paraquat, there were no deaths in the first 24 hours, whereas 22% of the rats given diquat died in this period. About 34% of the paraquat-treated rats died on the 3rd day after dosing. The death of paraquat-poisoned rats has been shown to be related to lung damage, as measured by a greatly increased water content. There was no similar increase in the water content of lungs taken from rats poisoned with diquat. Thymidine incorporation into lung DNA was significantly decreased 1 day after dosing with both paraquat and diquat, but became greatly elevated in a proportion of those rats given paraquat, which survived 3 days after dosing. In diquat-poisoned rats, thymidine incorporation into lung DNA remained depressed for at least 8 days.

It was concluded that (1) most rats given paraquat by intraperitoneal injection die from acute lung damage characterized by a dramatic increase in the lung water content; (2) only a small proportion die many days later from lung fibrosis characterized by an increase in thymidine incorporation into DNA; and (3) diquat does not cause lung damage as characterized by increases in either the water content of lung or thymidine incorporation into DNA (Smith & Rose, 1977).

3. Observations in humans

No data were provided by the company, other than the publication by Sechi et al. (1992).

In cases of acute diquat poisoning following oral ingestion, management of the massive fluid losses into the gastrointestinal tract and their potential circulatory and renal consequences is critical, with special attention given to maintaining adequate hydration of the patient. Intestinal paralysis and fluid loss are prominent features of diquat intoxication and may lead to abdominal distension, tissue dehydration, hypotensive shock and severe cerebrovascular complications due to brain stem infarction and/or intracranial haemorrhage (Oreopoulos & McEvoy, 1969; Vanholder et al., 1981; Jones & Vale, 2000; Saeed, Wilks & Coupe, 2001).

Despite the lack of findings indicating neurotoxicity in animals, diquat has aroused interest because its analogue, paraquat, is perceived as being structurally similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is known to elicit a condition similar to Parkinson disease in humans. MPTP and its metabolite 1-methyl-4-phenylpyridine (MPP⁺) accumulate in melanin and cause degeneration of the melanin-containing neurons in the substantia nigra. Diquat has therefore also come under scrutiny as a possible environmental factor in the development of Parkinson disease (Sechi et al., 1992).

There is a report in the published literature of a 72-year-old farmer with a history of diabetes and transient right-sided hemiparesis who developed erythema of the skin with hyperkeratosis and conjunctivitis after exposure of the hands to a 10% diquat solution for about 10 minutes. About 10 days later, he developed akathisia with moderate hyperexcitability and insomnia. Over a period of 5 days, he became dysphonic, bradykinetic and rigid. Treatment with carbidopa/levodopa and bromocriptine significantly improved his symptoms. A magnetic resonance imaging scan 4 months after the onset of the illness showed small, multiple, bilateral, symmetric areas of high signal intensity in the caudate nuclei and putamen and in the white matter near the ventricular wall. The authors suggested a causal relationship to diquat exposure. However, there were no clinical signs suggestive of systemic diquat poisoning, nor has there been any other description of Parkinson-like illness following diquat exposures (Sechi et al., 1992).

The results of large epidemiological studies specifically investigating exposure to diquat and the development of Parkinson disease have not shown any evidence of an increased risk (Tanner et al., 2009).

Most cases of diquat poisoning reported to date have resulted from the intentional, usually suicidal, ingestion of concentrated solutions; rarely, accidental ingestion has occurred as a consequence of decanting diquat concentrates into soft drink bottles. Over the period 1968–1999, only 30 cases of diquat poisoning were reported in detail in the literature, of which 13 (43%) were fatal. Local and systemic effects have been reported following diquat exposure, with systemic features being invariably associated with ingestion. In severe and usually fatal cases, gastrointestinal mucosal ulceration, paralytic ileus, hypovolaemic shock, acute renal failure and coma have been reported (Jones & Vale, 2000).

In a report in the published literature, a 52-year-old Caucasian man with a history of alcohol abuse who ingested about 160 mL of “weed killer” (confirmed as diquat ion) with suicidal intent after his wife left him developed aggressive behaviour, oliguric renal failure and intracerebral bleeding. The patient was successfully managed and made a complete recovery. In this paper, special attention was given to the major clinical differences between diquat and paraquat intoxication (Saeed, Wilks & Coupe, 2001).

In a report in the published literature, two cases of diquat poisoning were described. In the first case, a 35-year-old, previously healthy woman ingested a 14% diquat solution. The poisoning had a fulminant course, consisting of severe stomach ache, vomiting, cardiocirculatory shock, respiratory failure and cardiac arrest 20 hours post-ingestion. Autopsy revealed myocardial infarction, bronchopneumonia and incipient renal damage. In the second case, a 64-year-old man developed severe gastroenteritis, corrosive lesions of mucosal surfaces, acute renal injury, arrhythmias, brain stem infarction and bronchopneumonia. The patient died 18 days post-exposure. The most prominent findings on autopsy were pontine haemorrhage and infarction, bronchopneumonia, left ventricle papillary muscle infarction and renal tubular damage. Cardiocirculatory disturbances led to fatal complications, the heart and brain infarction. The study authors pointed out the heart as one of the most severely affected organs in diquat poisoning (Jović-Stošić, Babić & Todorović, 2009).

In a report in the published literature, an 18-year-old man accidentally took a mouthful of undiluted diquat. Although he spat most of it out, he is quite certain that he swallowed a small quantity. Over the next 10 hours, he felt worse and developed diarrhoea (5–6 episodes). The patient reported difficulty in swallowing. There was a small ulcer on the upper surface of the tongue and another on the right side of his pharynx. He was not cyanosed, and there were no abnormal physical signs in the chest. The diquat excretion lasted for about 11 days (Oreopoulos & McEvoy, 1969).

Comments

Biochemical aspects

When administered orally, [^{14}C]diquat is poorly absorbed (< 10%) from the gastrointestinal tract of rats and eliminated mainly via the faeces (about 94%) during the first 24 hours. The small amount absorbed is rapidly eliminated via the urine (about 6%). Biliary excretion represented less than 0.7% of the administered dose. Peak tissue and blood levels were seen at approximately 2 hours, followed by a rapid decline. Liver, kidney, gastrointestinal tract and lung had the highest residues immediately following dosing; at 96 hours, significant residues were seen primarily in the eye lens.

After oral administration of [^{14}C]diquat to rats (45 mg diquat ion/kg bw), the major excreted product was diquat in both urine (about 6% of the dose) and faeces (about 89% of the dose); diquat monopyridone was the main metabolite in the faeces (5% of dose), but a minor one in the urine. In another oral study in rats (100 mg diquat ion/kg bw), small amounts of diquat dipyrindone and picolinic acid were found, in addition to the monopyridone, in urine. The biotransformation of diquat is postulated to proceed either by progressive oxidation of the pyridine rings to form diquat monopyridone and diquat dipyrindone or by the cleavage of one of the pyridine rings to form picolinic acid, possibly via pyridine-2-carboxamide as an intermediary metabolite, although this was not identified.

Toxicological data

The acute oral LD_{50} in rats was 214 mg diquat ion/kg bw. There were no mortalities or clinical signs of toxicity at 100 mg/kg bw. The acute dermal LD_{50} in rats was greater than 424 mg diquat ion/kg bw. The 4-hour acute inhalation LC_{50} in rats was 0.121 mg/L. Diquat was moderately to severely irritating to rabbit skin and mildly irritating to rabbit eyes. Diquat was a skin sensitizer in the Magnusson and Kligman test.

Unlike paraquat, diquat is not actively taken up by lung slices, and lung toxicity is not characteristic of diquat poisoning. The eye was the main target organ following short-term repeated exposure in rats and dogs. In addition, effects on kidney, liver and haematological parameters were also observed.

In a 90-day toxicity study in rats using dietary concentrations of 0, 20, 100 and 500 ppm (equal to 0, 1.7, 8.5 and 39.5 mg diquat ion/kg bw per day for males and 0, 1.9, 9.2 and 41.5 mg diquat ion/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 8.5 mg diquat ion/kg bw per day), based on decreased body weight gain and feed consumption, changes in clinical chemistry parameters, increased urine volume, decreased urinary specific gravity, minor changes in haematological values, erosion of the tongue and oral cavity and ocular changes at 500 ppm (equal to 39.5 mg/kg bw per day). An investigative 90-day toxicity study in rats was conducted at dietary concentrations of 0, 30, 60 and 300 ppm diquat ion (equal to 0, 2.4, 4.7 and 23.2 mg diquat ion/kg bw per day for males and 0, 2.7, 5.0 and 25.3 mg diquat ion/kg bw per day for females, respectively) to determine the NOAEL for cataract formation in rats. The NOAEL was 60 ppm (equal to 4.7 mg diquat ion/kg bw per day) for ocular lesions and lens opacities evident at 300 ppm (equal to 23.2 mg diquat ion/kg bw per day). The overall NOAEL in the 90-day toxicity studies in rats was 100 ppm (equal to 8.5 mg diquat ion/kg bw per day), with an overall LOAEL of 300 ppm (equal to 23.2 mg diquat ion/kg bw per day).

In a 1-year feeding study in dogs, with achieved dietary intakes of 0, 0.46, 2.42 and 11.48 mg diquat ion/kg bw per day for males and 0, 0.53, 2.53 and 13.21 mg diquat ion/kg bw per day for females, the NOAEL was 0.53 mg diquat ion/kg bw per day, based on lens opacity (cataracts) in females at 2.53 mg diquat ion/kg bw per day.

In a 2-year toxicity and carcinogenicity study in mice using dietary concentrations of 0, 30, 100 and 300 ppm (equal to 0, 3.56, 11.96 and 37.83 mg diquat ion/kg bw per day for males and 0, 4.78, 16.03 and 48.27 mg diquat ion/kg bw per day for females, respectively), the NOAEL was 30 ppm (equal to 3.56 mg diquat ion/kg bw per day), based on reduction in body weight gain, increased

relative kidney weights and ocular discharges at 100 ppm (equal to 11.96 mg diquat ion/kg bw per day) and above. There was no evidence of carcinogenicity in mice at doses up to and including 300 ppm (equal to 37.83 mg diquat ion/kg bw per day), the highest dose tested.

A 2-year study of toxicity and carcinogenicity in rats was conducted using dietary concentrations of 0, 5, 15, 75 and 375 ppm (equal to 0, 0.19, 0.58, 2.91 and 14.88 mg diquat ion/kg bw per day for males and 0, 0.24, 0.72, 3.64 and 19.44 mg diquat ion/kg bw per day for females, respectively). There were no effects on survival, changes in haematology, clinical chemistry or urine analysis parameters or neoplastic changes that were considered to be of toxicological significance at any dose level. A treatment-related incidence of opacities of the lens was found in male and female animals receiving 75 and 375 ppm. Among rats receiving 375 ppm, these lesions progressed to total opacification of the lens, affecting all surviving rats receiving 375 ppm when examined at week 104. A low, but slightly higher than in controls, incidence of rats with opacities was seen in the 15 ppm group at 104 weeks only. Cataractous change was observed in the lenses of rats at 75 and 375 ppm. The NOAEL was concluded to be 5 ppm (equal to 0.19 mg diquat ion/kg bw per day) in the 1993 JMPR evaluation. Since that evaluation, the cataract data at 15 ppm have been re-evaluated by the original pathologists. Cataracts were seen in controls and all dose groups, and the incidence and severity were dose related. The present Meeting noted the conclusion of the re-evaluation of the cataract data: that, in contrast to the observations at 75 and 375 ppm, there was no evidence of progression of the disease at 15 ppm that differed from the controls. The present Meeting identified the NOAEL as 15 ppm (equal to 0.58 mg diquat ion/kg bw per day), as the incidence and severity of cataracts were comparable with those of the controls. There was no evidence of carcinogenicity in this study.

The Meeting concluded that diquat is not carcinogenic in mice or rats.

Diquat was tested for genotoxicity in vitro and in vivo in an adequate range of assays. It gave an equivocal response in the absence of metabolic activation and a positive response in the presence of metabolic activation in the mammalian cell cytogenetic assay; however, it was negative in the in vivo mouse micronucleus assay and dominant lethal assay.

The Meeting concluded that diquat is unlikely to be genotoxic in vivo.

On the basis of the lack of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that diquat is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study of reproductive toxicity in rats using dietary concentrations of 0, 16, 80 and 400/240 ppm (equal to 0, 1.6, 7.9 and 38.7 mg diquat ion/kg bw per day for males and 0, 1.7, 8.4 and 40.4 mg diquat ion/kg bw per day for females, respectively), the NOAEL for parental systemic toxicity was 16 ppm (equal to 1.6 mg diquat ion/kg bw per day), based on a low incidence of mouth lesions in both generations and a slightly increased incidence of cataracts at 80 ppm (equal to 7.9 mg diquat ion/kg bw per day). The NOAEL for reproductive toxicity was 400/240 ppm (equal to 38.7 mg diquat ion/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 80 ppm (equal to 7.9 mg diquat ion/kg bw per day), based on decreased pup body weights seen in both generations at 400/240 ppm (equal to 38.7 mg diquat ion/kg bw per day).

In a developmental toxicity study in rats that tested gavage doses of 0, 4, 12 and 40 mg diquat ion/kg bw per day, the NOAEL for maternal toxicity was 12 mg diquat ion/kg bw per day, based on reduced body weights and feed consumption seen at 40 mg diquat ion/kg bw per day. The NOAEL for embryo and fetal toxicity was 12 mg diquat ion/kg bw per day, based on reduced fetal weight, haemorrhagic kidney and increased incidence of soft tissue and minor skeletal anomalies at 40 mg diquat ion/kg bw per day.

In a developmental toxicity study in rabbits that tested gavage doses of 0, 1, 3 and 10 mg diquat ion/kg bw per day, the NOAEL for maternal toxicity was 1 mg diquat ion/kg bw per day, based on reduced weight gain and feed consumption at 3 mg diquat ion/kg bw per day. The NOAEL for embryo and fetal toxicity was 3 mg diquat ion/kg bw per day, based on skeletal anomalies at 10 mg diquat ion/kg bw per day.

The Meeting concluded that diquat is not teratogenic in rats or rabbits.

Diquat has been tested for neurotoxicity in acute and repeated-dose studies in rats. In neither study was there any indication of neurotoxicity. In an acute neurotoxicity study in rats that tested gavage doses of 0, 25, 75 and 150 mg diquat ion/kg bw per day, the NOAEL for general toxicity was 75 mg diquat ion/kg bw, based on a range of findings, including clinical signs, at 150 mg diquat ion/kg bw. In the 90-day study of neurotoxicity in rats using dietary concentrations of 0, 20, 100 and 400 ppm (equal to 0, 1.6, 7.9 and 32.4 mg/kg bw per day for males and 0, 1.9, 9.5 and 38.5 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 100 ppm (equal to 7.9 mg diquat ion/kg bw per day), based on evidence of eye lesions and reductions in body weight gain and feed consumption at 400 ppm (equal to 32.4 mg diquat ion/kg bw per day).

The Meeting concluded that diquat is not neurotoxic.

In a 28-day study of immunotoxicity in female mice using dietary concentrations of 0, 100, 200 and 350 ppm (equal to 0, 23, 44 and 81 mg diquat ion/kg bw per day), there was no evidence of immunotoxicity at any dose tested.

Toxicological data on metabolites and/or degradates

Diquat monopyridone, the major metabolite following oral dosing, was less toxic than the parent compound. The oral LD₅₀ for diquat monopyridone was greater than 4000 mg/kg bw. Male and female rats orally administered diquat monopyridone at a dose of 1000 mg/kg bw per day, 5 days/week, for 2 weeks showed no clinical, haematological, biochemical or histopathological abnormalities, except for a decreased number of lymphocytes in both males and females.

TOPPS, a metabolite in livestock but not in rats, was less toxic than the parent compound. The acute oral LD₅₀ of TOPPS in rats was 2449 mg/kg bw. There was no evidence of genotoxicity in the DNA repair test.

Human data

No reports of adverse health effects in manufacturing plant personnel were provided. In humans, intestinal paralysis and fluid loss are prominent features of diquat intoxication and may lead to abdominal distension, tissue dehydration, hypotensive shock and severe cerebrovascular complications due to brain stem infarction and/or intracranial haemorrhage.

The Meeting concluded that the existing database on diquat was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.006 mg diquat ion/kg bw on the basis of a NOAEL of 0.58 mg diquat ion/kg bw per day in the 2-year carcinogenicity study in rats on the basis of cataracts seen at 2.9 mg diquat ion/kg bw per day. A safety factor of 100 was applied. This ADI is supported by the NOAEL of 0.46 mg diquat ion/kg bw per day observed in a 1-year toxicity study in dogs on the basis of cataracts seen at the LOAEL of 2.53 mg diquat ion/kg bw per day. The present ADI is based on the same study and end-point selected by the 1993 JMPR, but using a different NOAEL value. The current Meeting identified the NOAEL as 0.58 mg diquat ion/kg bw per day, as the incidence, progression with time and severity of cataracts at this dose were comparable with those in the controls.

An ARfD of 0.8 mg/kg bw was established on the basis of a NOAEL of 75 mg diquat ion/kg bw in an acute neurotoxicity study in rats, based on clinical signs and decreased body weight gains in the 1st week and decreased feed consumption seen at the LOAEL of 150 mg diquat ion/kg bw. A safety factor of 100 was applied. This ARfD is further supported by the acute oral toxicity study in

rats (LD₅₀ = 214 mg/kg bw) in which no mortality or clinical signs of toxicity were observed at 100 mg/kg bw. The Meeting considered reductions in body weight gain seen at the start of gavage dosing in several studies (e.g. developmental toxicity studies) to be secondary to gastrointestinal irritation and not relevant to establishing an ARfD. The Meeting concluded that the critical effects in the longer-term studies of eye lesions were not likely to be produced following a single dose, as the eye lesions were normally evident only after several weeks of continuous dosing.

Levels relevant to risk assessment of diquat

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity ^a	Toxicity	30 ppm, equal to 3.6 mg diquat ion/kg bw per day	100 ppm, equal to 12.0 mg diquat ion/kg bw per day
		Carcinogenicity	300 ppm, equal to 37.8 mg diquat ion/kg bw per day ^b	–
Rat	Acute neurotoxicity study ^c	Toxicity	75 mg diquat ion/kg bw	150 mg diquat ion/kg bw
	Ninety-day studies of toxicity ^{a,d}	Toxicity	100 ppm, equal to 8.5 mg diquat ion/kg bw per day	300 ppm, equal to 23.2 mg diquat ion/kg bw per day
	Two-year study of toxicity and carcinogenicity ^a	Toxicity	15 ppm, equal to 0.58 mg diquat ion/kg bw per day	75 ppm, equal to 2.9 mg diquat ion/kg bw per day
		Carcinogenicity	375 ppm, equal to 14.9 mg diquat ion/kg bw per day ^b	–
	Two-generation study of reproductive toxicity ^a	Reproductive toxicity	400/240 ppm, equal to 38.7 mg diquat ion/kg bw per day ^b	–
		Parental toxicity	16 ppm, equal to 1.6 mg diquat ion/kg bw per day	80 ppm, equal to 7.9 mg diquat ion/kg bw per day
		Offspring toxicity	80 ppm, equal to 7.9 mg diquat ion/kg bw per day	400/240 ppm, equal to 38.7 mg diquat ion/kg bw per day
Rabbit	Developmental toxicity study ^c	Maternal toxicity	12 mg diquat ion/kg bw per day	40 mg diquat ion/kg bw per day
		Embryo and fetal toxicity	12 mg diquat ion/kg bw per day	40 mg diquat ion/kg bw per day
	Developmental toxicity study ^c	Maternal toxicity	1 mg diquat ion/kg bw per day	3 mg diquat/kg bw per day
		Embryo and fetal toxicity	3 mg diquat ion/kg bw per day	10 mg diquat ion/kg bw per day
Dog	One-year study of toxicity ^a	Toxicity	0.53 mg diquat ion/kg bw per day	2.53 mg diquat ion/kg bw per day

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

^a Dietary administration.

^b Highest dose tested.

^c Gavage administration.

^d Two or more studies combined.

Estimate of acceptable daily intake

0–0.006 mg/kg bw

Estimate of acute reference dose

0.8 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to diquat

<i>Absorption, distribution, excretion and metabolism in mammals</i>	
Rate and extent of oral absorption	Poor, ~10% in rat
Dermal absorption	About 6% in 24 h, rat skin
Distribution	Widely distributed, highest levels in eye lens
Potential for accumulation	Potential accumulation only in eye lens
Rate and extent of excretion	Rapid, absorbed dose extensively excreted (> 90%) in urine and bile within 96 h
Metabolism in animals	Metabolism was limited, with < 20% of the urinary residues (< 1% of the administered dose) consisting of metabolites
Toxicologically significant compounds in animals, plants and the environment	Diquat ion
<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	214 mg diquat ion/kg bw
Rat, LD ₅₀ , dermal	> 424 mg diquat ion/kg bw
Rat, LC ₅₀ , inhalation	0.121 mg diquat ion/L
Rabbit, dermal irritation	Moderately to severely irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Sensitizing (Magnusson and Kligman)
<i>Short-term studies of toxicity</i>	
Target/critical effect	Cataract, body weight (rat, dog)
Lowest relevant oral NOAEL	0.53 mg diquat ion/kg bw per day (dog)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Cataract (rat), kidney lesions (mouse)
Lowest relevant NOAEL	0.58 mg diquat ion/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans from the diet
<i>Genotoxicity</i>	
	Not genotoxic in vivo
<i>Reproductive toxicity</i>	
Target/critical effect	No reproductive toxicity
Lowest relevant parental NOAEL	1.6 mg diquat ion/kg bw per day

Lowest relevant offspring NOAEL	7.9 mg diquat ion/kg bw per day
Lowest relevant reproductive NOAEL	38.7 mg diquat ion/kg bw per day, the highest dose tested
<i>Developmental toxicity</i>	
Target/critical effect	Reduced body weight, delayed ossification, skeletal anomalies at maternally toxic doses
Lowest relevant maternal NOAEL	1 mg diquat ion/kg bw per day (rabbit)
Lowest relevant embryo and fetal NOAEL	3 mg diquat ion/kg bw per day (rabbit)
<i>Neurotoxicity</i>	
Acute and subchronic neurotoxicity	Not neurotoxic
<i>Other toxicological studies</i>	
Immunotoxicity	Not immunotoxic
Studies on metabolites	Diquat monopyridone, diquat dipyrindone and TOPPS were less toxic than parent diquat
<i>Medical data</i>	
	No reports submitted; characteristics of intoxication obtained from published literature

LC₅₀: median lethal concentration; LD₅₀: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level; TOPPS: 1,2,3,4-tetrahydro-1-oxopyrido-(1,2-a)-5-pyrazinium salt

Summary

	Value	Study	Safety factor
ADI	0–0.006 mg/kg bw	Two-year study of carcinogenicity in rats	100
ARfD	0.8 mg/kg bw	Acute study of neurotoxicity in rats	100

ADI: acceptable daily intake; ARfD: acute reference dose

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