

## CHLORMEQUAT (addendum)

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### Explanation

Chlormequat was evaluated by the JMPR in 1970, 1972, 1994, and 1997 (Annex 1, references 14, 18, 71, and 80). In 1972, an ADI of 0–0.05 mg/kg bw was established on the basis of a study of reproductive toxicity in rats, but in 1994 the Meeting withdrew this ADI on the grounds that the data package was inadequate. In 1997, an ADI of 0–0.05 mg/kg bw was allocated. The compound was considered by the present Meeting to determine an acute reference dose, as requested at the Thirty-first Session of the Codex Committee on Pesticide Residues (Codex Alimentarius Commission, 1999), and short-term studies of toxicity in dogs, long-term studies of toxicity in rats and mice, a study of developmental toxicity in rabbits, and a two-generation study in rats were reviewed.

### Evaluation for Acute Reference Dose

#### 1. Short-term studies of toxicity

##### *Dogs*

A 1-year study of toxicity was carried out in groups of five male and five female beagles fed diets containing chlormequat chloride (purity, 67.4%) at doses of 0, 150, 300, or 1000 ppm, equal to 0, 4.7, 9.2, and 31 mg/kg bw per day in males and 0, 5.2, 10, and 32 mg/kg bw per day in females. The animals were initially observed daily and specifically for diarrhoea, salivation, lachrymation, and incoordination before and 2, 4 and 6 h after feeding. After study day 91, the animals were examined weekly. Food consumption was determined daily, while the animals were weighed weekly. Clinical chemical and haematological examinations were carried out before the start of administration of the test material and three times during the study. Ophthalmological examinations were performed before the start of the study and near its end. At autopsy, the organs were examined grossly and histopathologically. Of the animals at the highest dose, one male died at 42 days and one female at 20 days; diarrhoea was seen in all males and in three females at this dose, starting in the first week. During the fourth week, the dog that died showed vomiting, spasm, staggering, emaciation, and apathy, and pulmonary oedema and atrophy of the thymus were found *post mortem*. This animal also showed some abnormalities in clinical chemistry and haematology (increased prothrombin time, alanine aminotransferase and alkaline phosphatase activity, and urea, creatinine, total protein, and cholesterol concentrations and decreased polymorph and lymphocyte counts). The bitch that died at 20 days also had pulmonary oedema and thymic changes (multiple haemorrhages). Diarrhoea was also seen in two males at 300 ppm in the first and second weeks of the study, and salivation was seen at this dose, starting at week 1 and intermittently thereafter. No treatment-related effects were seen at 150 ppm, the NOAEL, equal

to 4.7 mg/kg bw per day, on the basis of diarrhoea and salivation at the next highest dose. Because these effects occurred early in the study, they were considered relevant to setting an acute reference dose. No abnormality was observed on ophthalmological examination and, in the animals that survived to term, no gross or histopathological abnormality was seen that was related to the treatment (Mellert et al., 1993).

## 2. Long-term studies of toxicity

### *Mice*

Groups of 50 male and 50 female B6C3F1/CrIbR (VAF) mice were given diets containing chlormequat chloride (67.4% pure) at doses of 0, 150, 600, or 2400 ppm for 100 weeks, equal to intakes of chlormequat of 0, 21, 84, and 340 mg/kg bw per day in males and 0, 23, 91, and 390 mg/kg bw per day in females. Satellite groups comprising a further 10 mice of each sex received chlormequat at the same dietary concentrations for 52 weeks, with measured intakes of chlormequat of 0, 23, 89, and 355 mg/kg bw per day for males and 0, 28, 109, and 445 mg/kg bw per day for females. The animals were inspected daily and more thoroughly weekly for clinical status. Body weight and food consumption were recorded weekly during the first 14 weeks of the study and thereafter every 4 weeks. The animals in the satellite group were killed at 52 weeks and those in the main group at 110 weeks. Blood was taken from all sacrificed animals and differential blood counts carried out. The mice were examined *post mortem* both grossly and histopathologically. No clinical signs were observed that appeared to be related to treatment. Survival was not affected by the test material at any dose. Animals of each sex killed at 52 weeks showed a reduction in weight gain at the highest dose at some times, but the significance of this finding is hard to assess because of the small group sizes. No such reduction was seen in the main groups, killed at 110 weeks. No significant differences in feed consumption were seen between groups. Minor inter-group differences in leukocyte counts lacked consistency and are unlikely to be of biological significance. No treatment-related differences in organ weights were seen between groups. The only organ-specific findings that appeared to be related to treatment were found in the female reproductive tract at autopsy, which were increased incidences of ovarian tubular downgrowth and of cystic endometrial hyperplasia at the two higher doses. There was no treatment-related increase in tumour incidence. The NOAEL was 150 ppm, equal to 23 mg/kg bw per day, on the basis of histopathological effects in the uterus and ovaries at the next highest dose (Mellert et al., 1994).

### *Rats*

Groups of 20 male and 20 female Wistar rats received diets containing chlormequat (purity, 67.4%) as the chloride for 18 months at nominal concentrations of 0, 280, 940, or 2800 ppm (calculated erroneously on the basis of 72% purity), equal to 0, 13, 43, and 140 mg/kg bw per day in males and 0, 17, 56, and 170 mg/kg bw per day in females. The rats were examined daily and more thoroughly once a week. Ophthalmological examinations were carried out at the beginning and end of the study. Food consumption and body weight were determined weekly for the first 14 weeks and thereafter every 4 weeks. Clinical chemistry, haematology, and urinalysis were performed 3, 6, 12, and 18 months after the start of the study. The animals were killed after 18 months and examined grossly and histopathologically. No treatment-related clinical signs were observed in any group, and ophthalmological examination showed no adverse effects. The mortality rate was unaffected by treatment. The body-weight gain of males was reduced throughout the study and that of females at the highest dose from the 58th week, so that by the end of the study the weight of the males was 18% less than that of concurrent controls and that of the females was 10% less. The feed consumption of males was reduced from week 54 and that of females from time to time. The lower doses had no effect on weight gain or feed consumption. Although some differences in clinical chemical and haematological parameters were seen between groups, they were marginal, sporadic, and showed no relation to dose; they were therefore considered not to be of toxicological significance. In particular, no changes were seen in plasma, erythrocyte, or brain cholinesterase activity. At autopsy, the only finding attributable

to treatment was decreased body-weight gain in animals at the highest dose by comparison with controls and consequent differences in the relative weights of the kidney, brain, and liver. No treatment-related histopathological changes were found. The NOAEL was 940 ppm, equal to 43 mg/kg bw per day (Schilling et al., 1992).

Chlormequat chloride (technical grade; purity, 67.4%) was administered to groups of 50 male and 50 female Wistar rats at a nominal dietary concentration of 0, 280, 940, or 2800 ppm for 2 years. The mean daily intakes were 0, 13, 42, and 120 mg/kg bw per day for males and 0, 16, 55, and 170 mg/kg bw per day for females. The animals were observed daily and inspected thoroughly weekly. Body weight and intake of food were determined weekly for the first 14 weeks and every 4 weeks thereafter. At termination, the survivors were examined grossly, and selected tissues were taken for histopathological examination. Clinical chemistry, haematology, and urinalysis were undertaken on 20 animals of each sex per group, and the brains of 20 animals of each sex per group were taken for measurement of acetylcholinesterase activity. No treatment-related clinical signs were seen, and the mortality rate was not affected. Reduced body-weight gain and food consumption were seen in animals of each sex at the highest dose. Weight gain was decreased throughout the study, by 14% in males and 22% in females by comparison with concurrent controls. The food consumption of males was reduced throughout the study and that of females during the latter part. Some inter-group differences were seen in clinical chemical and haematological findings, but they were inconsistent and probably not related to treatment. Chlormequat did not affect plasma, erythrocyte, or brain cholinesterase activity. The material was not carcinogenic, and no treatment-related histopathological changes were seen. The NOAEL was 940 ppm, equal to 42 mg/kg bw per day, on the basis of reduced weight gain and feed consumption at the highest dose (Mellert et al., 1992).

### 3. Reproductive toxicity

#### *Rats*

In a two-generation study, groups of 24 male and 24 female rats were given diets containing chlormequat (purity, 67.4%) at concentrations of 0, 300, 900, or 2700 ppm, equal to 0, 29, 86, and 250 mg/kg bw in males and 0, 23, 69, and 230 in females. At least 70 days after the beginning of treatment, the F<sub>0</sub> parents were mated to produce the F<sub>1a</sub> litters and, subsequently, the F<sub>1b</sub> litters, only the latter being retained until weaning. Groups of 24 males and 24 females from the F<sub>1a</sub> litters were used as the F<sub>1</sub> parents and were given chlormequat as above to produce the F<sub>2</sub> litters. The F<sub>1</sub> adults and the F<sub>2</sub> weanlings were killed at the end of the study. All animals, including pups, were examined daily. The feed consumption of the F<sub>0</sub> and F<sub>1</sub> parents was determined weekly before mating and during gestation and lactation. Body weights were determined weekly, but during gestation and lactation the body weights of the females was determined on days 0, 7, 14, and 20 of gestation, on the day of parturition, and on days 4, 7, 14, and 21 after delivery. Clinical chemical and haematological variables were measured in 12 F<sub>1</sub> animals in each group. All parental animals were examined after death both grossly and histopathologically. The clinical signs seen at the highest dose included tremor. The body-weight gain of F<sub>0</sub> females at 900 ppm was slightly reduced while they were suckling the F<sub>1a</sub> pups, and reduced body-weight gain was seen in animals of each sex and of both parental generations at the highest dose. At this dose, feed consumption and creatinine concentration were also reduced in F<sub>1</sub> females, which had decreased mean numbers of pups per dam and of total delivered pups. The NOAEL for reproductive toxicity was 900 ppm, equal to 69 mg/kg bw per day, and that for systemic toxicity was 300 ppm, equal to 23 mg/kg bw per day, on the basis of reduced body-weight gain (Hellwig & Hildebrand, 1993).

#### *Rabbits*

In a study of developmental toxicity stated to have been conducted in accordance with 1966 US Food and Drug Administration guidelines and the 1975 Association of the British Pharmaceutical Industry guidelines, inseminated Himalayan ChBB:HM rabbits were given chlormequat chloride (purity, 99%) at doses of 0, 1.5, 3, 6, or 12 mg/kg bw per day by gavage on days 6–18 after insemination. The group sizes were not the same at each dose, there being 15 controls, 15 animals

at 1.5 mg/kg bw per day, 21 at 3 and 6 mg/kg bw per day, and 14 at the highest dose. The animals were killed 28 days after insemination; the fetuses were removed, sexed, and weighed, and the placentas were weighed. The fetuses were eviscerated and their organs examined macroscopically. Additionally, their skeletons were X-rayed, and the heads were fixed and sectioned for examination. The two higher doses caused rapid respiration, salivation, and apathy in one animal in each group. These effects were observed on day 13 after insemination in animals at 6 mg/kg bw per day and on day 14 after insemination in those at 12 mg/kg bw per day. One animal at 3 mg/kg bw per day and two at 6 mg/kg bw per day died. The body-weight gain of rabbits at the highest dose was decreased, and feed consumption was affected in all treated groups. Two animals at 6 mg/kg bw per day and one at 12 mg/kg bw per day aborted. No treatment-related effects were seen on the numbers of corpora lutea or implants or the weight or sex of the fetuses. No teratogenic effects were seen. It might be concluded that there was no NOAEL for maternal toxicity, as feed consumption was reduced at all doses; however, this effect may have been due to reduced palatability. The NOAEL for maternal toxicity was 6 mg/kg bw per day on the basis of decreased weight gain at 12 mg/kg bw per day, provided that the single instance of rapid respiration, salivation, and apathy at 6 mg/kg bw per day can be ignored. This study was difficult to interpret: the clinical signs observed at 6 and 12 mg/kg bw per day were consistent with a cholinergic agonist effect, but comparison of the findings in these two groups showed little evidence of a dose-response relationship (BASF, 1979).

### Comments

The oral LD<sub>50</sub> of chlormequat was 200–1000 mg/kg bw in rodents and > 800 mg/kg bw in monkeys but was much lower, approximately 50 mg/kg bw, in cats and dogs.

In a 1-year study in dogs given chlormequat chloride (purity, 67.4%) in the diet at concentrations of 0, 150, 300, or 1000 ppm, diarrhoea was seen at 300 ppm in two males during the first and second weeks of the study, and salivation was also seen at this dose, starting at week 1 and intermittently thereafter. Consequently, the NOAEL was 150 ppm, equal to 4.7 mg/kg bw per day, on the basis of diarrhoea and salivation at the next highest dose. Because these findings were seen early in the study, they were considered relevant to setting an acute reference dose.

Three long-term studies—two in rats and one in mice—showed that chlormequat was not carcinogenic. None of the studies showed acute effects.

In a two-generation study of reproductive toxicity in rats given chlormequat in the diet, clinical signs such as tremor were seen at the highest dose (250 mg/kg bw per day). Reproductive toxicity was also seen at this dose and systemic toxicity at the intermediate dose (69 mg/kg bw per day). The NOAEL for systemic toxicity was 23 mg/kg bw per day, and the NOAEL for reproductive toxicity was 69 mg/kg bw per day.

In a study of developmental toxicity in rabbits given chlormequat chloride at doses of 0, 1.5, 3, 6, or 12 mg/kg bw per day by gavage on days 6–18 after insemination, the body-weight gain of animals at the highest dose was decreased and the feed consumption of all treated animals was affected, possibly because of reduced palatability. The NOAEL for maternal toxicity was 6 mg/kg bw per day and that for developmental toxicity was 12 mg/kg bw per day, the highest dose tested.

An acute reference dose of 0.05 mg/kg bw was established on the basis of the NOAEL of 4.7 mg/kg bw per day in the 1-year study in dogs, as the clinical signs that were found were considered to be acute. A 100-fold safety factor was used.

### Toxicological evaluation

#### *Levels that cause no toxic effects*

Mouse: 150 ppm, equal to 23 mg/kg bw per day (2-year study of toxicity and carcinogenicity)

- Rat: 940 ppm, equal to 42 mg/kg bw per day (2-year study of toxicity and carcinogenicity)  
 900 ppm, equal to 69 mg/kg bw per day (reproductive toxicity in a two-generation study of reproductive toxicity)  
 300 ppm, equal to 23 mg/kg bw per day (systemic toxicity in a two-generation study of reproductive toxicity)
- Rabbit: 6 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)  
 12 mg/kg bw per day (developmental toxicity in a study of developmental toxicity)
- Dog: 150 ppm, equal to 4.7 mg/kg bw per day (1-year study of toxicity)

*Estimate of acute reference dose*

0.05 mg/kg bw

### References

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