5.6 CHLORMEQUAT (015)

TOXICOLOGY

Chlormequat chloride is the ISO–approved common name for 2-chloroethyltrimethylammonium chloride (IUPAC name), with CAS number 999-81-5. Chlormequat, also called chlorocholine-chloride, belongs to the group of quaternary ammonium compounds that are used as plant growth regulators acting as an inhibitor of the biosynthesis of gibberellins.

Chlormequat chloride was evaluated by the JMPR in 1970, 1972, 1994, 1997 and 1999. In 1972, an ADI of 0–0.05 mg/kg bw was established on the basis of the NOAEL in a study of reproductive toxicity in rats. In 1994, this ADI was withdrawn as the database was considered inadequate by contemporary standards. In 1997, a number of new studies were evaluated, and an ADI of 0–0.05 mg/kg bw was established based on the NOAEL of 4.7 mg/kg bw in a 1-year dog study.

In 1999, the compound was considered again solely to determine an ARfD, which was set at 0.05 mg/kg bw based on a 1-year dog study with a NOAEL of 150 ppm (equal to 4.7 mg/kg/ bw per day), as the clinical signs found were considered to be possibly due to a single dose.

In 2017, the Meeting evaluated new studies/analyses on rodent reproductive and developmental toxicity, acute dog toxicity and case reports on human deaths by suicide using chlormequat chloride.

The studies evaluated by the previous JMPR meetings (1997 and 1999) are included in this assessment and are re-evaluated according to current JMPR policies and procedures.

Most of the unpublished studies evaluated were performed by laboratories that were certified for GLP and that complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the European Union or United States Environmental Protection Agency (USEPA), unless otherwise indicated. Minor deviations from these protocols were not considered to affect the reliability of the studies.

Biochemical aspects

In rats, absorption of chlormequat chloride from the gastrointestinal tract was rapid. Elimination, mainly of nonmetabolized chlormequat, was almost entirely via the urine (approximately 90%) and was essentially complete within 24 hours. Less than 1% of the administered dose remained in the tissues.

In a second metabolism and toxicokinetics study, rats were administered an intravenous dose level of 0.1 mg/kg bw or a high (30 mg/kg bw) or repeated low (0.5 mg/kg bw) oral gavage dose. At 1.5 hours after administration, the highest amounts of radioactivity from the high dose were found in the gastrointestinal tract, followed by the kidneys, the liver and the heart. At termination 168 hours post dosing, very low levels of radioactivity were found in the kidneys, followed by the liver, the heart and the gastrointestinal tract.

For the repeated low-dose study, at 2 hours after the last of the seven daily doses of 0.5 mg/kg bw, the highest amounts of radioactivity were found in the kidneys, followed by the liver, the heart and the gastrointestinal tract, at levels much lower than in the high-dose study. At termination (168 hours post dosing), virtually no radioactivity remained.

Faeces and bile do not play a major role in the elimination.

There were no significant differences in absorption and excretion between the high and low dose levels. Excretion after a 14-day pretreatment was comparable to that after a single oral dose.

The C_{max} increased less than proportionally with dose, whereas the area under the concentration–time curve (AUC) was linear over the tested dose range, indicating that chlormequat chloride has no accumulating potential.

Other than chlormequat chloride, only trace metabolites were found in rat urine; these may have been salts of chlormequat and choline. An unidentified polar metabolite was also found in faeces.

Toxicological data

The LD50 values of chlormequat chloride were 800–1000 mg/kg bw in rats, mice, hamsters, guinea-pigs, sheep and monkeys and 10–80 mg/kg bw in rabbits, cats and dogs.

The dermal LD_{50} was greater than 4000 mg/kg bw in rats and 440 mg/kg bw in rabbits. The rat inhalation LC_{50} was greater than 2.51 mg/L.

Chlormequat chloride is a partial agonist of the nicotinic acetylcholine receptor. The neurological signs of toxicity observed in pharmacological investigative studies may have been due to the pharmacological activity of chlormequat chloride. There were no consistent treatment-related findings at necropsy.

Chlormequat chloride is not irritating to skin and eyes of rabbits and does not induce skin sensitization in guinea-pigs.

In short-term studies of toxicity of chlormequat chloride in different species, the most important effects were clinical signs related to a generally reduced state of health and reduced feed consumption and body weights. In a 4-week mouse study at dietary concentrations of 0, 500, 1500 or 3000 ppm (equal to 0, 148, 439 and 885 mg/kg bw per day for males and 0, 223, 618 and 1190 mg/kg bw per day for females, respectively), there were no test substance–related effects. The NOAEL was 3000 ppm (equal to 885 mg/kg bw per day), the highest dose tested.

In a 4-week study of acute toxicity of chlormequat chloride in rats at dietary concentrations of 0, 500, 1500, 3000 or 4500 ppm (equal to 0, 47, 137, 258 and 367 mg/kg bw per day for males and 0, 51, 148, 291 and 418 mg/kg bw per day for females, respectively), the NOAEL was 1500 ppm (equal to 137 mg/kg bw per day) based on reduced body weight gain.

In another 4-week oral toxicity study, rats were administered dietary doses of chlormequat chloride of 0, 100, 1000 or 10 000 ppm (equal to 0, 8.5, 95, 1210 and 1110 [recovery group] mg/kg bw per day for males and 0, 9.8, 120, 1240 and 1140 [recovery group] mg/kg bw per day for females, respectively). The NOAEL was 100 ppm (equal to 9.8 mg/kg bw per day) based upon emaciation and clinical signs of weakness observed in females at 1000 ppm (equal to 1240 mg/kg bw per day).

In another 4-week oral toxicity study, rats were administered chlormequat chloride at dietary concentrations of 0, 100, 500 or 2500 ppm (equal to 0, 8.3, 41 and 202 mg/kg bw per day for males and 0, 8.8, 45 and 211 mg/kg bw per day for females, respectively). No treatment-related effects were observed up to 2500 ppm (equal to 202 mg/kg bw per day), the highest dose tested.

In a 90-day oral toxicity study, rats were given chlormequat chloride at dietary levels of 0, 300, 900 or 2700 ppm (equivalent to 0, 21, 61 and 189 mg/kg bw per day for males and 0, 24, 73 and 220 mg/kg bw per day for females, respectively). There were no mortalities or test substance–related clinical signs of toxicity. Reductions in feed consumption, body weight and body weight gain were observed in high-dose males. The NOAEL was 900 ppm (equivalent to 61 mg/kg bw per day) based on reduced body weight gain and feed intake in males at 2700 ppm (equivalent 189 mg/kg bw per day).

In a second 90-day oral toxicity study, rats were given chlormequat chloride at dietary concentrations of 0, 100, 1000 or 5000 ppm (equal to 0, 6.0, 61 and estimated as 305 mg/kg bw per day for males and 0, 7.9, 89 and estimated as 445 mg/kg bw per day for females, respectively). Clinical signs of urinary incontinence were observed in one high-dose female and two high-dose males but not in the recovery group. In addition, three males were found to have prolapsed penis, but this finding was not clearly treatment related and not reported by other study. The NOAEL was 1000 ppm (equal to 61 mg/kg bw per day) based on decreased body weight gain in males at 5000 ppm (approximately 305 mg/kg bw per day).

In a third 90-day oral toxicity study, rats were given dietary doses of chlormequat chloride at 0, 500, 1500 or 4500 ppm (equivalent to 0, 50, 150 and 450 mg/kg bw per day). The 4500 ppm group had their dose raised to 9000 ppm (equivalent to 900 mg/kg bw per day) in the eleventh week and additional groups of 10 males and 10 females were treated at 9000 ppm for 7 weeks, followed by a 6-week recovery period. Body weight gain decreased in the two higher dose groups of 4500/9000 ppm and at 9000 ppm. In the recovery phase, the females recovered completely, but the males did not within the study time frame. Feed consumption also decreased for these high-dose groups. The NOAEL was 1500 ppm (equivalent to 150 mg/kg bw per day) based upon decreased body weight gain and feed consumption at the highest dose.

In a 90-day oral toxicity study, dogs were administered chlormequat chloride in the diet at concentrations of 0, 100, 300 or 600 ppm, with the top dose level raised to 800 ppm in week 8, 1200 ppm in week 10, 2000 ppm in week 11 and 2500 ppm in week 12 (equivalent to 0, 2.5, 7.5 and 15/20/30/50/62.5 mg/kg bw per day). Body weight gain decreased in high-dose females and males from week 9 onwards. Salivation was a consistent treatment-related effect. At the higher doses, increased salivation was observed in two animals in the 300 ppm group 3–6 hours after feeding; this was pronounced in one animal in the 1200 ppm group and in all animals in the 2000 ppm and 2500 ppm groups from week 11. The NOAEL was 100 ppm (equivalent to 2.5 mg/kg bw per day) based on salivation at 300 ppm (equivalent to 7.5 mg/kg bw per day).

In a 1-year study, dogs were administered chlormequat chloride in the diet at concentrations of 0, 150, 300 or 1000 ppm (equal to 0, 4.7, 9.2 and 31 mg/kg bw per day for males and 0, 5.2, 10 and 32 mg/kg bw per day for females, respectively; corrected for purity), diarrhoea was seen at 300 ppm in two males during the first and second weeks of the study. Salivation and vomiting was also seen at this dose, starting at week 1 and occurring intermittently thereafter. The NOAEL was 150 ppm (equal to 4.7 mg/kg bw per day), based on diarrhoea, vomiting and salivation at the LOAEL of 300 ppm (equal to 9.2 mg/kg bw per day).

The overall NOAEL in the 90-day and 1-year dog studies was 4.7 mg/kg bw per day based on clinical signs, vomiting, salivation and decreased body weight gain at the LOAEL of 300 ppm (equal to 9.2 mg/kg bw per day).

In a 110-week mouse study of chlormequat chloride at dietary concentrations of 0, 150, 600 or 2400 ppm (equal to 0, 21, 84 and 336 mg/kg bw per day for males and 0, 23, 91 and 390 mg/kg bw per day for females, respectively), the NOAEL was 2400 ppm (equal to 336 mg/kg per day), the highest dose tested.

In an 18-month study, mice were administered dietary dose levels of chlormequat chloride at 0, 250, 1000 or 4000 ppm (equal to 0, 20, 79 and 323 mg/kg bw per day for males and 0, 22, 91 and 352 mg/kg bw per day for females, respectively). Although there were no treatment-related increases in the incidence of neoplasms up to the highest dose tested, the limited end-points assessed prevent identification of a NOAEL for non-neoplastic effects.

In a 102-week cancer bioassay study, dietary doses of chlormequat chloride at 0, 500 or 2000 ppm (equivalent to 0, 70 and 290 mg/kg bw per day) were given to mice for 102 weeks. No increases in tumour incidences were found.

In a 78-week toxicity and carcinogenicity study, rats were given chlormequat chloride at dietary concentrations of 0, 281, 937 or 2810 ppm (equal to 0, 12, 43 and 136 mg/kg bw per day for males and 0, 15, 56 and 172 mg/kg bw per day for females, respectively). Tumour incidences were not increased. The NOAEL was 937 ppm (equal to 43 mg/kg bw per day) based on reduced body weight at 2810 ppm (equal to 136 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study, rats were given chlormequat chloride at dietary concentrations of 0, 280, 940 or 2800 ppm (equal to 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, respectively). No treatment-related neoplastic and nonneoplastic histopathological changes were seen. The NOAEL was 940 ppm (equal to 42 mg/kg bw per day), based on reduced weight gain and feed consumption at the highest dose.

In carcinogenicity study conducted by the National Cancer Institute, rats were given chlormequat chloride at dietary concentrations of 0, 1500 or 3000 ppm (equivalent to 0, 75 and 150 mg/kg bw per day). There was no test substance–related increase in mortalities or signs of clinical toxicity in any of the treatment groups. In male rats, dose-related islet cell adenomas of the pancreas were observed. However, given the difficulties in diagnosis of these lesions and considering the available data quality, the Meeting concluded that under the conditions of this bioassay, there was no convincing evidence that chlormequat chloride is carcinogenic in male and female Fischer F344 rats up to the highest dose tested (3000 ppm).

The Meeting concluded that chlormequat chloride is not carcinogenic in mice and rats.

Chlormequat chloride has been adequately tested in a wide range of in vitro and in vivo mutagenicity/genotoxicity assays. The Meeting concluded that the overall weight of evidence indicates that chlormequat is unlikely to be genotoxic.

In view of the lack of genotoxic potential and absence of carcinogenicity in mice and rats, the Meeting concluded that chlormequat is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study of reproductive toxicity in rats at dietary concentrations of chlormequat chloride at 0, 300, 900 or 2700 ppm (equal to 0, 29, 86 and 250 mg/kg bw per day for males and 0, 23, 69 and 230 mg/kg bw per day for females, respectively). The NOAEL for parental toxicity was 900 ppm (equal to 69 mg/kg bw per day) based on reduced feed consumption and decrease in body weight and body weight gain in female and male parental animals of both generations at 2700 ppm (equal to 230 mg/kg bw per day). The NOAEL for offspring toxicity was 900 ppm (equal to 69 mg/kg bw per day) based on reduced pup weight gain and retarded development during lactation at 2700 ppm (equal to 230 mg/kg/ bw per day). The NOAEL for reproductive toxicity was 900 ppm (equal to 69 mg/kg bw per day) based on reduced numbers of pregnancies and delivered pups at 2700 ppm (equal to 230 mg/kg bw per day). Reversible and transient tremor and hypersensitivity were also observed in F_0 and F_1 females at 2700 ppm (equal to 230 mg/kg bw per day), mainly during or after the lactation period. At this dose level there were some indications of an impairment of the reproductive function (a higher number of mating partners did not show signs of fertility within the scheduled mating period).

In another multigenerational study, rats were given dietary dosages of chlormequat chloride at 0, 10, 100 or 1000 ppm (equal to 0, 0.7, 6.8 and 69.3 mg/kg bw per day for males and 0, 1, 10.2 and 105 mg/kg bw for females, respectively). There were no parental mortalities in any group, no signs of clinical toxicity, no treatment-related effects on feed consumption at any time and no significant effects upon body weight. The parental NOAEL was 100 ppm (equal to 6.8 mg/kg bw per day), based on reduced body weight gain, clinical signs seen during lactation (females) and marginal anaemia at 1000 ppm (equal to 69.3 mg/kg bw per day). The NOAEL for offspring toxicity was 1000 ppm (equal to 69.3 mg/kg bw per day), the highest dose tested. The NOAEL for effects on reproductive function was 1000 ppm (equal to 69 mg/kg bw per day).

In a third multigenerational study, rats were given dietary doses of chlormequat chloride at 0, 100, 500 or 2500 ppm (equal to 0, 8.4, 41.4 and 211 mg/kg bw per day for males and 0, 9.1, 46.3 and 241 mg/kg bw per day for females, respectively). The parental NOAEL was 500 ppm (equal to 41.4 mg/kg bw per day for males and 46 mg/kg bw per day for females) based on reduced body weight and body weight gain at 2500 ppm (equal to 211 mg/kg bw per day). The offspring NOAEL was 500 ppm (equal to 41.4 mg/kg bw per day) based on reduced pup weight and pup weight gain and dystrophic changes in the skeletal muscle of the offspring. The reproductive NOAEL was 2500 ppm (equal to 211 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study, rats were administered gavage doses of 0, 25, 75 or 225 mg/kg bw per day. There were no indications of malformations. The NOAEL for maternal toxicity was 75 mg/kg bw per day based on clinical signs and reduced body weight and feed consumption at 225 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 225 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, rabbits were administered gavage doses of chlormequat chloride at 0, 10, 20 or 40 mg/kg bw per day. There was a slight but statistically significant increase in mean post-implantation loss at 40 mg/kg bw per day, but the number of live pups per dam was greater at the high dose than in the controls. The NOAEL for maternal toxicity was 10 mg/kg bw per day based upon salivation seen at 20 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 20 mg/kg bw per day based on increased post-implantation loss and slightly reduced fetal weights.

The Meeting concluded that chlormequat chloride is not teratogenic.

A number of studies retrieved from a search of the scientific literature investigated the potential effects of chlormequat on sperm quality and function and endocrine activity. These studies did not provide any evidence of endocrine activity of chlormequat. Some effects were observed on sperm function in certain in vitro and in vivo non-standard studies that may be consistent with possible minor effects on fertility observed in some standard multigeneration studies. The Meeting considered that these studies did not provide useful information for quantitative risk assessment.

Human data

No adverse effects on the health of workers involved in the normal manufacture of chlormequat were observed.

Lethal poisoning cases with commercial products containing chlormequat have been reported in the literature and in three case studies of oral intake from a manufacturing plant. From these studies the estimated lethal dose in humans appears to be consistent with the LD_{50} in dogs, which are more sensitive than rodents.

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

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.05 mg/kg bw established on the basis of the overall NOAEL of 4.7 mg/kg bw per day for diarrhoea, vomiting and salivation in the 1-year and 90-day studies of toxicity in dogs, and using a safety factor of 100.

The Meeting reaffirmed the ARfD of 0.05 mg/kg bw on the basis of a NOAEL of 4.7 mg/kg bw for clinical signs (diarrhoea, vomiting and salivation) observed in the 1-year study in dogs. These effects were observed multiple times during the first week of treatment and were likely to be elicited after a single dose.

A toxicological monograph was prepared.

Levels relevant to risk assessment of chlormequat chloride

Species	Study	Effect	NOAEL	LOAEL
Mouse	One hundred and ten week study of toxicity and carcinogenicity[a]	Toxicity	2 400 ppm, equal to 336 mg/kg bw per day[b]	–
		Carcinogenicity	2 400 ppm, equal to 336 mg/kg bw per day[b]	–
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	940 ppm, equal to 42 mg/kg bw per day	2 800 ppm, equal to 120 mg/kg bw per day

		Carcinogenicity	2 800 ppm, equal to 120 mg/kg bw per day[b]	–
	Two-generation reproductive toxicity study[a,d]	Reproductive toxicity	900 ppm, equal to 69 mg/kg bw per day	2 700 ppm, equal to 230 mg/kg bw per day
		Parental toxicity	100 ppm, equal to 6.8 mg/kg bw per day	1 000 ppm, equal to 69.3 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 41.4 mg/kg bw/day	2 500 ppm, equal to 211 mg/kg bw per day
	Prenatal/developmental toxicity study[c]	Maternal toxicity	75 mg/kg bw per day	225 mg/kg bw per day
		Embryo/fetal toxicity	225 mg/kg bw per day[b]	–
Rabbit	Prenatal developmental toxicity study[c]	Maternal toxicity	10 mg/kg bw per day	20 mg/kg bw per day
		Embryo/fetal toxicity	20 mg/kg bw per day	40 mg/kg bw per day
Dog	Ninety-day and 12-month toxicity studies[a,d]	Toxicity	150 ppm, equal to 4.7 mg/kg bw per day	300 ppm, equal to 9.2 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

[d] Two or more studies combined.

Estimate of acceptable daily intake (ADI)

0–0.05 mg/kg bw

Estimate of acute reference dose (ARfD)

0.05 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 chlormequat

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly and almost completely absorbed
Dermal absorption	Less than 10%
Distribution	Widely distributed

Potential for accumulation	Low potential for bioaccumulation
Rate and extent of excretion	Rapid excretion mainly into urine (90%)
Metabolism in animals	Mostly excreted unchanged, little metabolism to choline or N-choline, salts of chlormequat
Toxicologically significant compounds in animals and plants	Chlormequat chloride
Acute toxicity	
Mouse, LD_{50}, oral	405 mg/kg bw
Rat, LD_{50}, oral	433 mg/kg bw
Rabbit, LD_{50}, oral	~75 mg/kg bw
Dog LD_{50}, oral	37 mg/kg bw
Rat, LD_{50}, dermal	>4 000 mg/kg bw
Rabbit, LD_{50}, dermal	>440 mg/kg bw
Rat, LC_{50}, inhalation	>2.51 mg/L
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Non-irritating
Guinea-pig, dermal sensitization	Non-sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight gain, neurological effects (salivation, tremors)
Lowest relevant oral NOAEL	4.7 mg/kg bw (dog)
Lowest relevant dermal NOAEL	150 mg/kg bw
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight gain, neurological effects (salivation, tremors)
Lowest relevant NOAEL	
Carcinogenicity	Not carcinogenic in mice and rats
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Target/critical effect	Reduced fertility
Lowest relevant parental NOAEL	6.8 mg/kg bw per day
Lowest relevant offspring NOAEL	41.4 mg/kg bw per day
Lowest relevant reproductive NOAEL	69 mg/kg per bw day
Developmental toxicity	
Target/Critical effect	Reduced body weight gain during lactation and focal dystrophy of muscles
Lowest relevant maternal NOAEL	10 mg/kg bw day (rabbit)
Lowest relevant embryo/fetal NOAEL	20 mg/kg bw day (rabbit)

Neurotoxicity	
Acute neurotoxicity NOAEL	No specific studies were conducted
Subchronic neurotoxicity NOAEL	
Developmental neurotoxicity NOAEL	

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

Summary

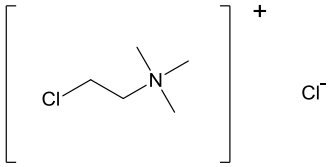
	Value	Study	Safety Factor
ADI	0–0.05 mg/kg bw	90-day and 1-year studies in dogs	100
ARfD	0.05 mg/kg bw	1-year study in dogs	100

RESIDUE AND ANALYTICAL ASPECTS

Chlormequat chloride is a plant growth regulator which acts primarily by reducing cell elongation, but also by lowering the rate of cell division. It inhibits the synthesis of gibberellins. It was scheduled for periodic review evaluation by the 2017 JMPR at the 48th Session of the CCPR (2016). Chlormequat was previously evaluated by the JMPR in 1970, 1972, 1994 (periodic review), 1997, 1999 and 2000. It was evaluated for toxicology in 1997 and 1999 at which time an acute reference dose was established.

The manufacturer supplied information on identity, physicochemical properties, plant, animal and confined crop metabolism, environmental fate, methods of residue analysis, freezer storage stability, registered use patterns, supervised residue trials, fate of residues in processing, and animal transfer studies.

The IUPAC name is 2-chloroethyl-trimethylammonium chloride.



Chlormequat-chloride

Plant Metabolism

The Meeting received plant metabolism studies conducted on wheat and grapes, together with a considerable amount of supporting metabolism information previously provided to the 1994 JMPR.

Chlormequat chloride, radiolabelled in both carbons of the chloroethyl group, was applied to grapevines as three consecutive foliar applications at growth stages BBCH 13–15, 15–17 and 57. The application rates were 180, 360 and 90 g ai/ha (total 630 g ai/ha).

Leaves were sampled from the immature plants immediately before and 22 days after the last application. The mature grapes were harvested at BBCH 89 (90 DALA) and the remaining plant material was separated into leaves, branches and stalks. With methanol (×3) and water (×2) extractions, 99% of the radioactive residues in grapes and 95% of the radioactive residues of leaves were extracted.

In total, 0.18 mg eq/kg (98% TRR) in grapes and 1.65 mg eq/kg (84% TRR) in leaves was identified as the active substance chlormequat chloride. Minor unidentified components totalled 0.004 mg eq/kg in grapes and 0.10 mg eq/kg in leaves. In total 100% and 89% TRR was identified or characterised in grapes and leaves respectively. Unextracted residues in grapes after solvent extraction were <1% TRR and in leaves 5% TRR.

In a study in wheat, [1,2-14C-ethyl]-chlormequat chloride was applied once at 1380 g ai/ha by foliar application to wheat plants grown in a phytotron. Forage was collected at 0, 28 and 84 days after application, while grain and straw were collected at harvest maturity 118 days after application.

Residues were readily extractable from forage and straw using methanol (79–90% TRR extracted from forage and 81% TRR from straw). Extractability from grain was lower, with 37% TRR extracted using methanol, together with a further 17% released using a methanol/water reflux. A significant proportion of the radioactivity in grain had been incorporated into biomolecules, with 16% TRR present as starch, and 36% TRR present as lignin. A smaller proportion of the residue in straw (5.1% TRR) had been incorporated into lignin. Incorporation into protein or cellulose was not significant in either straw or grain.

Parent was the largest individual identified component in wheat matrices, at 9.7–42 mg eq/kg (67–86% TRR) in forage, 36–37 mg eq/kg (78–81% TRR) in straw, and 0.37–0.41 mg eq/kg (28–30% TRR) in grain. Small amounts of betaine were identified in grain (up to 0.054 mg eq/kg, 4.7% TRR),

and straw (0.06 mg eq/kg, 0.1% TRR), with unidentified components at up to 2.4 mg eq/kg (6.2% TRR) in forage, up to 1.8 mg eq/kg (3.8% TRR) in straw, and up to 0.026 mg eq/kg (1.5% TRR) in grain.

Summary of plant metabolism

Metabolism data in grapes and wheat were provided, together with a considerable amount of supporting literature. Parent was observed to be the major component of the radioactive residues in grape berries and leaves, and in wheat grain, straw and forage. Betaine was observed as a very minor component (<5% TRR) in grain and straw. A number of older metabolism studies (in pot grown wheat and barley, brassicas, and tomatoes) first considered by the 1994 JMPR showed similar behaviour, with metabolism of chlormequat chloride only occurring to a limited extent, with minor amounts of choline also being observed. Greater degrees of metabolism were noted in other non-contemporary studies, including in wheat treated via a root application, in which significant metabolism to choline, then betaine, glycine and serine, with ultimate incorporation into biomolecules and evolution of radiolabelled CO_2 being observed.

Confined Rotational Crops

A study was undertaken to investigate the metabolism of chlormequat chloride in the representative crops spring wheat, lettuce and white radish after three plant back intervals using ^{14}C-chlormequat chloride (radiolabelled in both carbons of the chloroethyl group) sprayed onto bare soil in plastic containers at 2 kg ai/ha. The crops were each sown at 30, 120 and 365 days after the soil application, representing the first, second and third rotation.

In lettuce leaf parent was not observed at any of the three plant back intervals. In radish root and leaf, parent was observed at 0.008–0.009 mg eq/kg (19–20% TRR) at the 30 day plant back interval (PBI) while at the 120 day PBI it was no longer present. Parent was the major component in wheat straw at the 30 day PBI (0.072 mg eq/kg, 22% TRR) and at the 120 day PBI parent was no longer detected. Parent was observed in wheat grain at the 30 and 120 day PBIs only (0.015 and 0.009 mg eq/kg, 9 and 4% TRR respectively). Polar degradation products (not identified) were found in most samples at low levels (except for 120 day PBI wheat straw and chaff, containing ≤ 0.011 mg eq/kg (≤ 8.3% TRR) and 0.022 mg eq/kg (13% TRR) respectively, the totals in each matrix were ≤ 0.01 mg eq /kg (≤ 47% TRR)).

At the 365 day PBI, only lettuce and wheat grain residues were characterised, and no parent was detected, with only minor polar degradates found at ≤ 0.003 mg eq/kg (≤ 31% TRR).

In general, chlormequat chloride was converted to mainly polar degradation products and at longer plant back intervals parent was no longer detected or only found at low levels.

In another study, the metabolism of chlormequat chloride was investigated in the representative crops spring wheat, green beans, carrots and head lettuce from three consecutive rotations using 14C-chlormequat chloride added to loamy sand soil giving an application rate of 1.5 kg ai/ha, then stored in a drum for 30 days. After 30 days the soil was diluted using untreated soil to simulate ploughing. The crops were planted/sown at 30 days after the soil application. Beans, carrots and lettuce were cultivated in a greenhouse while spring wheat was grown in a phytotron with fluorescent lamps. Only total residues were reported: concentrations of total residue in the edible parts of the four crops ranged from 0.003 mg eq/kg in beans at harvest to 0.052 mg eq/kg in wheat grain at harvest, but were < 0.01 mg eq/kg for lettuce heads and carrot roots. In wheat forage and straw, bean forage, and carrot leaves, the total residues ranged from 0.016–0.066 mg eq/kg.

In summary, chlormequat chloride is metabolised in rotational crops to unidentified polar components, with only relatively low levels of parent found (≤ 0.072 mg eq/kg, ≤ 32% TRR at the 30-day PBI; ≤ 0.009 mg eq/kg, ≤ 9.2% TRR at the 120-day PBI; not detected at the 365-day PBI).

Environmental fate in soil

The Meeting received information on aerobic soil metabolism, hydrolysis, aqueous photolysis, and a field dissipation study. Only the aerobic soil metabolism study and the field dissipation study, which are relevant to the current evaluation were considered.

The route and rate of degradation of ^{14}C-chlormequat chloride was studied in an aerobic laboratory study in three European soils, at 20 ± 2 °C and a period of 120 days. Parent compound ^{14}C-chlormequat chloride was the only major radioactive fraction detected in the soil extracts. Mineralisation to CO_2 was the major route of degradation besides formation of bound residues. Four minor metabolites (<3% AR) were detected in the soil extracts. DT_{50} values for chlormequat chloride ranged from 10.2–36.5 days, while DT_{90} values ranged from 33.8–121 days.

Field dissipation data conducted with a sandy loam soil, together with data for a clay soil in a greenhouse, showed rapid microbiological degradation in both cases. The observed field behaviour was consistent with the results of the laboratory study. Chlormequat was extensively mineralised and CO_2 was the ultimate product of degradation. Other degradation products could not be identified. DT_{50} values ranged from <1–28 days, while DT_{90} values were less than 100 days.

Chlormequat is not considered to be persistent in soil.

Animal metabolism

The Meeting received animal metabolism studies with chlormequat in rats, hens and goats.

Rats

Evaluation of the metabolism studies in rats was carried out by the WHO Core Assessment Group.

Goats

A study on the metabolism of chlormequat chloride was conducted with the test compound labelled in both positions of the chloroethyl group. Two lactating goats were dosed orally twice daily for seven consecutive days, at 25 ppm in the diet. Milk was sampled twice daily, prior to dosing in the morning and afternoon. Animals were sacrificed approximately 23 hours after the last dose.

A total of 49 and 30% of the total administered dose was eliminated in the urine and faeces respectively (cumulative over 7 days). TRRs were 1.5 mg eq/kg in kidney, 0.36 mg eq/kg in liver, 0.23 mg eq/kg in muscle, 0.022 and 0.008 mg eq/kg in renal and omental fat respectively, and 0.24 and 0.20 mg eq/kg in 56 hour and 144 hour milk respectively.

Initial methanol extraction of kidney, liver, muscle and renal fat resulted in extraction efficiencies of 92, 77, 90 and 67% respectively. Pepsin hydrolysis of the post-extracted solid (PES) released 7.3, 15 and 7.7% TRR for kidney, liver, and muscle respectively. Protease released a further 1.5% for liver.

Milk was initially extracted with acetonitrile recovering 17 and 20% from the 56 hour and 144 hour samples respectively. Pepsin hydrolysis of the solvent-precipitated solids released 63 and 80% TRR from the 56 hour and 144 hour samples, respectively, while protease released a further 16% TRR from the 56 hour sample.

Metabolism in ruminants only occurred to a very limited extent. Parent was the only compound identified in kidney, liver and muscle (83, 42 and 76% TRR respectively). It was also the only identified compound in milk accounting for <5% TRR in the 56 hour and 144 hour milk samples (0.011 and 0.002 mg eq/kg respectively). Release of significant proportions of the radioactivity by pepsin and protease hydrolysis indicated that a substantial part of the radioactivity was present as macromolecules, formed by incorporation of chlormequat chloride by biosynthetic pathways.

Hens

A study on the metabolism of chlormequat chloride was conducted with the test compound labelled in both positions of the chloroethyl group. Ten laying hens were dosed orally once daily for 14 consecutive days, at 12 ppm. The hens were sacrificed approximately 23 hours after the last dose.

A total of 93% of the total administered dose was eliminated in the excreta (cumulative after 14 days). Egg yolk and egg white accounted for 0.34 and 0.05% of the administered dose respectively (cumulative after 14 days). A plateau level of approximately 0.97 mg eq/kg in composite egg yolk samples was reached at 264 hours (11 days) after the first administration, while concentrations in egg white were much lower and did not reach a plateau level. TRRs in tissues were 0.36 mg eq/kg in liver, 0.35 mg eq/kg in kidney, 0.12 mg eq/kg in muscle and 0.062 mg eq/kg in abdominal fat.

Methanol extraction of liver, kidney, muscle and egg yolk extracted 66, 65, 75 and 62% TRR respectively, while proteolytic enzyme hydrolysis after solvent extraction released further substantial amounts of the radioactivity (26%, 28%, 15%, and up to 13% respectively of the TRR).

In egg white, the solvent extraction only extracted ~5% TRR, however pepsin enzymatic hydrolysis released a significant proportion of the unextracted fraction (85 and 87% TRR for the 96 hour and 264 hour fractions respectively). In fat most of the radioactive residues remained unextracted after solvent extraction, enzyme hydrolysis and acid reflux (65% of TRR still unextracted, however absolute levels were low, with fat only containing a total of 0.062 mg eq/kg).

Parent chlormequat chloride was the only identified compound. It was found in kidney and liver (0.023 and 0.007 mg eq/kg, or 7 and 2% TRR respectively) and as a major fraction in egg yolk (0.47 mg eq/kg, or 48% TRR in the 264 hour sample, but was not found in the 96 hour sample). In the extracts of liver, kidney, muscle and egg yolk (96 hour sample), regions of radioactive residue, accounting for >0.05 mg eq/kg could not be identified. As with goats, a significant portion of the radioactive residues were released by protease and pepsin hydrolysis, indicating incorporation into macromolecules, *via* biosynthetic pathways.

Summary of animal metabolism

Metabolism in ruminants was very limited. Parent chlormequat chloride was the only compound identified in kidney, liver and muscle (83, 42 and 76% TRR respectively). It was also the only identified compound in milk, although only accounting for <5% TRR in the 56 hour and 144 hour milk samples.

Parent chlormequat chloride was the only identified compound in the poultry metabolism study. It was identified in kidney, liver, and as a major fraction in the 264h sample of egg yolk, but not in the 96h yolk sample.

A similar pattern was observed in rats, with only parent chlormequat chloride, and two other components tentatively identified as other salts of chlormequat being found after oral administration.

Methods of analysis

The Meeting received information on analytical methods suitable for the determination of residues of chlormequat chloride in plant and animal matrices.

Plant matrices

A method (method 146) developed for the determination of chlormequat chloride in cereal matrices requires extraction with methanol and quantification using gas chromatography. Limits of quantification using this method were generally 0.05 mg/kg in cereal grains and 0.5 mg/kg for cereal straw.

Method 530/0 for the determination of chlormequat chloride in plant commodities, is based on extraction using water/ methanol/ hydrochloric acid, and quantification using LC-MS/MS. Limits of quantification (LOQ) using method 530/0 were generally 0.05 mg/kg in all plant matrices except for cereal straw (LOQ = 0.1 or 0.5 mg/kg). Method 530/0 was used for determination of residues in

the freezer storage stability study conducted on grapes, as well in the grape, wheat, barley, rye and oats residues trials.

Another LC-MS/MS method (CEN/TC 275/WG 4N), was used for determination of chlormequat chloride. This method involved extraction with methanol/water and quantification using a deuterated chlormequat internal standard (LOQ = 0.05 mg/kg for straw and 0.01 mg/kg for all other cereal matrices).

Animal matrices

Method 397 was developed for the determination of residues of chlormequat chloride in animal matrices. Samples are extracted using acetone/water, with determination using ion chromatography. Limits of quantification using method 397 were generally 0.05 mg/kg in all animal matrices except for milk (LOQ = 0.01 mg/kg). A modification of this method (397/0) employs LC-MS/MS. Limits of quantification using method 397/0 were 0.01 mg/kg in all animal matrices except for liver (LOQ = 0.05 mg/kg).

Stability of pesticide residues in stored analytical samples

The Meeting received information on the freezer storage stability of chlormequat chloride in plant and animal matrices.

A storage stability study showed that chlormequat chloride residues are stable for at least 24 months in grapes. A study for cereal matrices involved fortification of wheat grain and straw samples, and this demonstrated stability of chlormequat chloride residues in grain and straw samples for at least 24 months. The cereal study additionally re-analysed stored samples from processing studies, and demonstrated no significant changes in the residue levels over a further storage period of 13 months in wheat bran and wholegrain bread, 12 months in barley malt and 11 months in beer, when stored frozen at approximately -18 or -20°C. The storage periods in the storage stability studies covers the sample storage intervals in the residue trials.

A study in animal matrices showed that residues of chlormequat chloride are stable in cattle meat, milk and hen eggs for at least 12 months of frozen storage at -18 °C, covering the storage intervals in the animal feeding studies.

Definition of the residue

Plant commodities

In the metabolism study conducted on grapes using ^{14}C-chlormequat chloride, the parent compound was observed to be the major component of the radioactive residues, accounting for approximately 100 and 88% of the TRR in grapes and grape leaves respectively. In a wheat metabolism study, parent compound accounted for 67–86% TRR in forage, 78–81% TRR in straw and 28–30% TRR in grain. Parent compound was also the only component identified in the confined rotational crop study.

Validated analytical methods for parent compound in plant matrices are available.

The Meeting therefore considered that a residue definition of the chlormequat cation is appropriate for plant commodities for compliance with MRLs (enforcement). It is proposed to maintain the residue definition as applying to the cation, which is the current residue definition.

It is noted that parent chlormequat chloride was the predominant residue in plants in the metabolism studies and was the only measured component in the supervised field trials. Minor metabolites that were observed (choline, betaine, serine, and glycine) are not of toxicological concern, with most of these being biochemicals. A residues definition of parent only is therefore supported for dietary risk assessment in plant commodities.

A residue definition for plant commodities for both enforcement and dietary risk assessment of chlormequat cation is proposed.

Animal commodities

Parent was the only compound identified in goat kidney, liver and muscle (83, 42 and 76% TRR respectively). It was also the only identified compound in milk accounting for <5% TRR (0.002–0.011 mg eq/kg).

Parent chlormequat chloride was the only identified compound in the poultry metabolism study. It was found in kidney and liver and as a major component in egg yolk.

A residue definition of chlormequat cation is proposed for animal commodities for compliance with MRLs (enforcement) and for dietary risk assessment.

The octanol-water partition coefficient (log P_{ow}) at pH 7 (25 °C) is -3.47. There is no evidence from the feeding studies to suggest that there is significant potential for bioaccumulation in fat tissues.

Definition of the residue (for compliance with the MRL for plant and animal commodities and for estimation of dietary intake for plant and animal commodities): *Chlormequat cation*.

The residue is not fat soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for foliar application of chlormequat chloride to grapes, barley, oats, rye and wheat. European, Australian, South African, New Zealand, and South American GAP information for cereal crops, and Indian GAP information for grapes were provided.

All results listed below are for residues reported as chlormequat cation.

Grapes

The GAP in India for grapes is for 3 foliar applications per season, the first and second at 500 and 1000 g ai/ha after the 'April pruning' (which is conducted shortly after harvest of the crop), and the third at 250 g ai/ha, and made after the 'October pruning' (before flowering), with a PHI of 91 days. The 'October pruning' takes place in October and November and is restricted to that window by weather conditions. Harvest generally takes place in March-April.

A series of trials was conducted in accordance with the Indian GAP (two applications 4–5 days apart at 500 and 1000 g ai/ha in April, and a third application at 250 g ai/ha in October). Grapes were sampled at two intervals at each site, immature grapes at 79–117 days after the last application, and mature grapes at 120–150 days after the last application. The application and sampling timings are considered representative of viticultural practice in the hot tropical region of India, in which approximately 70% of the Indian grape crop is grown.

Residue of chlormequat cation in mature grapes at harvest after treatment in accordance with GAP were < 0.04 (6) mg/kg.

It is noted that at two additional trial sites, a fourth application of chlormequat chloride was made. However, as no residues were found above the LOQ (0.04 mg/kg expressed as chlormequat cation) in these trials, the results are still considered to be representative of the residues expected after treatment in accordance with GAP.

The Meeting estimated a maximum residue level of 0.04* mg/kg for chlormequat cation in grapes, together with an STMR and an HR of 0.04 mg/kg.

Oilseeds

Cottonseed

The Meeting decided to withdraw its previous recommendation of 0.5 mg/kg for cotton seed (SO 0691) as no GAP information or supporting residue data for cotton was provided.

Rape seed

The Meeting decided to withdraw its previous recommendation 5 mg/kg for rape seed (SO 0495) and the associated processed commodity rape seed oil, crude (OC 0495) as no GAP information or supporting residue data for rape seed was provided.

Cereals

A large residue data set for trials conducted in various countries in Europe across several growing seasons was available for barley, oats, rye and wheat.

Barley

No trials are available matching the GAP for barley in Ireland (2 × applications to winter barley, one in autumn at 562.5 g ai/ha and the second the following spring at 1500 g ai/ha, with application up to first node, BBCH 31).

The GAP in the UK for barley is for a single application at 1650 g ai/ha, with application recommended for mid tillering to just prior to first node detectable (BBCH 25–30). A harvest withholding period is not stated.

Trials in barley were conducted in Europe, with application at a target growth stage of BBCH 32 (second node visible, northern Europe trials) and 37 (flag leaf emergence, southern Europe trials). It is noted that these trials are conducted with slightly later applications than recommended on the label, however for barley, growth stage at application around the tillering and stem elongation stage does not appear to have a critical effect on residues in harvested grain, with residues after application at BBCH 37 not differing significantly from the residues after application at BBCH 32. Trials for both application timings are therefore considered representative of the residues expected in barley after treatment in accordance with UK GAP.

Residues of chlormequat (as the cation) in barley in trials conducted in accordance with GAP were < 0.04, 0.062, 0.12, 0.17, 0.31 (2), 0.32, 0.36, 0.38, 0.59, 0.60, 0.65, 0.71, 0.78, 0.93, and 1.1 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg for barley, confirming the previous recommendation, with an STMR of 0.37 mg/kg.

Oats

The critical GAP for chlormequat in oats is in Switzerland, with a single application at 1840 g ai/ha made at BBCH 30–33 (beginning of stem elongation to the third node). No harvest withholding period is stated.

Trials in oats were conducted in Europe, with application at a target growth stage of BBCH 32 (second node visible, northern Europe trials) and 37 (flag leaf emergence, southern Europe trials). For oats, application timing does have an effect on residues at harvest, so only the trials within the application window stated on the label are considered to be in accordance with the GAP.

Residues of chlormequat (as the cation) in oats in trials with the application timing in accordance with the Swiss GAP were 0.54, 0.67, 0.90, 1.3, 1.6, 2.1, and 2.2 mg/kg.

As some of the trials were conducted with application rates outside ±25% of the GAP, the residues were adjusted proportionally for MRL estimation (adjustment factors ranged from 1.10–1.37×). After adjustment, residues of chlormequat cation were 0.68, 0.90, 1.0, 1.5, 2.0, 2.7, and 2.9 mg/kg.

The Meeting estimated a maximum residue level of 6 mg/kg for oats, together with an STMR of 1.5 mg/kg.

However, this GAP results in an exceedance of the ARfD, at 110% of the ARfD, for children in Canada consuming oat flakes.

The next most critical GAP is the UK GAP, with a single application at 1650 g ai/ha made at before the third node is detectable (BBCH 33). No harvest withholding period is stated.

Residues in trials matching the UK GAP are: 0.54, 0.67, 0.90, 1.3, 1.6, 2.1, and 2.2 mg/kg.

Therefore, the Meeting estimated a maximum residue level of 4 mg/kg, replacing the previous recommendation of 10 mg/kg, together with an STMR of 1.3 mg/kg.

Rye

The critical GAP for chlormequat chloride in rye is in Latvia, with a single application at 2250 g ai/ha made up to the second node stage (BBCH 21–32). A withholding period is not stated. No trials were conducted in accordance with this GAP, however trials with a lower application rate but the correct application timing are available and residues can be adjusted using the proportionality principle.

Trials in rye were conducted in Europe, with application at a target growth stage of BBCH 32 (second node visible, northern Europe trials) and 37 (flag leaf emergence, southern Europe trials). It is noted that the southern European trials are conducted with applications outside the growth stage window on the label, however for rye, growth stage at application around the tillering and stem elongation stage does not appear to have a critical effect on residues in harvested grain, with residues in mature grain after application at BBCH 37 not differing significantly from the residues after application at BBCH 32. Trials for both application timings in rye are therefore considered representative of the residues expected in rye grain after treatment in accordance with the Latvian GAP (after appropriate adjustment for proportionality).

Residues of chlormequat (as the cation) in rye at harvest maturity in trials conducted in Europe were 0.16, 0.25, 0.26, 0.29, 0.40, 0.52, 0.65, 0.69, 0.73, 0.78, 0.85, 0.93, 1.1, 1.5, 2.0, and 2.2 mg/kg.

After adjustment to the application rate specified in the Latvian GAP (proportionality factors of 1.36–1.84×) residues of chlormequat cation were 0.22, 0.37, 0.42, 0.43, 0.65, 0.71, 1.1 (4), 1.3, 1.4, 1.6, 2.8, 3.0, and 3.4 mg/kg.

The Meeting estimated a maximum residue level of 6 mg/kg for rye, replacing the previous recommendation of 3 mg/kg, together with an STMR of 1.1 mg/kg.

Wheat

No trials are available matching the GAP for Japan (a single application of 2300 g ai/ha made 10–20 days before heading [which corresponds to BBCH 51], at 40–60 cm plant height) as it is not clear the application timings in the trials corresponds to the GAP.

The GAP for Argentina is a single application of 2025 g ai/ha, made between tillering until the first node (BBCH 21–31), with no withholding period specified. Trials in accordance with this GAP are not available to the Meeting, however European trials with different application rates that can be considered using the proportionality principle are available.

Trials in wheat were conducted in Europe, with application at a target growth stage of BBCH 32 (second node visible, northern Europe trials) and 37 (flag leaf emergence, southern Europe trials). It is noted therefore that some trials are conducted with applications outside the growth stage window on the label, however for wheat, growth stage at application around the tillering and stem elongation stage does not appear to have a critical effect on residues in harvested grain, with residues in mature grain after application at BBCH 37 not differing significantly from the residues after application at BBCH 32. Trials for both application timings in wheat are therefore considered representative of the residues expected in wheat grain after treatment in accordance with the Argentine GAP (after appropriate adjustment for proportionality).

Residues of chlormequat (as the cation) as measured were < 0.05 (2), 0.05 (3), 0.078, 0.11, 0.16, 0.20, 0.23, 0.30, 0.34, 0.35 (2), 0.36, 0.47, 0.48 (3), 0.57 (3), 0.62 (2), 0.68, 0.74, and 1.0 mg/kg.

After adjustment to the application rate specified in the Argentine GAP (proportionality factors of 1.21–2.89×, and excluding the two trials with <LOQ residues) residues of chlormequat cation were 0.06, 0.07, 0.09, 0.10, 0.14, 0.32, 0.33, 0.46, 0.47 (2), 0.58 (3), 0.61, 0.65 (2), 0.66, 0.74, 0.77 (2), 0.83 (2), 0.85, 0.95, and 1.3 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg for wheat, replacing the previous recommendation of 3 mg/kg, together with an STMR of 0.58 mg/kg.

Triticale

The critical GAP for triticale is in Ireland, with a single application of 1875 g ai/ha, with a recommended latest application timing of BBCH 31.

No data for triticale was available to the Meeting. However, the Meeting noted that rye, triticale and wheat are all in the Codex subgroup of wheat and similar grains. Residue data is available for rye and wheat, and this data has been proportionally adjusted for GAPs that involve higher application rates than the Irish GAP for triticale.

It was noted that, after proportional adjustment to the Irish GAP for triticale, residues in rye were higher than those in wheat.

After adjustment to the Irish GAP for triticale, the rye residue data set is: 0.18, 0.31, 0.35, 0.36, 0.54, 0.59, 0.92 (4), 1.1, 1.2, 1.3, 2.3, 2.5, and 2.8 mg/kg.

The Meeting estimated a maximum residue level of 5 mg/kg for triticale, replacing the previous recommendation of 3 mg/kg, together with an STMR of 0.92 mg/kg, based on the proportionally adjusted rye data.

Animal feeds – forages

Grazing of forage from cereal grain crops is not common practice in Europe and is precluded in conjunction with agricultural chemical use unless specifically allowed by label instructions. Noting the critical GAPs considered for barley (UK), oats (UK), rye (Latvia) and triticale (Ireland), median and highest residues for barley, oat, rye and triticale forage have therefore not been estimated.

Wheat forage

The GAP considered for wheat is in Argentina (1 × 2025 g ai/ha application at BBCH 21–31) and the label does not restrict grazing.

Residue data is available from trials conducted in Europe for wheat forage sampled at intervals of 0, 14, 28, and 42 days after application.

The Meeting considered that residues in forage sampled at 14 ± 2 days after application, the shortest interval for which data is available and at which grazing would be likely to occur in common agricultural practice, would give the most robust and realistic estimate of median and highest residues in forages.

Residues of chlormequat cation in wheat forage from trials conducted in Europe 14 ± 1 days after an application at 1000 or 1500 g ai/ha were 4.3, 4.4, 6.7, 7.8, and 13 mg/kg.

After proportionality adjustment to the Argentine GAP residues of chlormequat cation in wheat forage (fresh weight) were 5.2, 5.7, 8.7, 10, and 25 mg/kg.

The Meeting estimated a median and a highest residue of 8.7 and 25 mg/kg respectively for wheat forage (fresh weight basis).

Animal feeds – straws and fodders

Residue data is available from trials conducted across several seasons in Europe for barley, oat, rye and wheat straw collected at harvest after application at BBCH 32–39 at target rates of 700, 1000, or 1500 g ai/ha (the majority of trials were conducted at a target application rate of 1500 g ai/ha).

Barley straw

The critical GAP for barley is in the UK (1 × 1650 g ai/ha application at BBCH 25–30).

Residues of chlormequat (as the cation) in barley straw at harvest, from trials conducted in Europe, after an application in accordance with the UK GAP for barley were < 0.39, 0.62, 1.2 (2), 1.9, 2.6, 2.7, 3.2, 5.1, 5.2, 5.5, 5.9, 6.7, 7.1, 26, and 30 mg/kg (as received), or < 0.44, 0.70, 1.3 (2), 2.1, 2.9, 3.0, 3.6, 5.7, 5.8, 6.2, 6.6, 7.5, 8.0, 29, and 34 mg/kg (dry weight basis).

The Meeting estimated a maximum residue level of 50 mg/kg for barley straw and fodder, dry, together with median and highest residues of 4.15 and 30 mg/kg respectively.

Oat straw

The critical GAP for oats (Switzerland, 1 × 1840 g ai/ha application, BBCH 30–33 could not be used for estimation of maximum residue levels due to acute dietary intake exceedance) and the next highest GAP (UK, 1 × 1650 g ai/ha application at <BBCH 33) was used instead.

Residues of chlormequat cation in oat straw at harvest from trials conducted in Europe, and matching the timing and application rate for the UK GAP () were < 0.16, 0.39, 0.43, 0.93, 1.8, 2.4, and 3.5 mg/kg (as received), or < 0.18, 0.43, 0.48, 1.0, 2.0, 2.7, and 3.9 mg/kg (dry weight basis).

The Meeting estimated a maximum residue level of 7 mg/kg for oat straw and fodder, dry, together with median and highest residues of 0.93 and 3.5 mg/kg respectively.

Rye straw

The critical GAP for rye is in Latvia (1 × 2250 g ai/ha application, at BBCH 21–32).

Residues of chlormequat cation in rye straw at harvest from trials conducted in Europe 14 ± 1 days after an application at a target rate of 1500 g ai/ha, residues were 0.56, 1.1 (2), 1.2, 1.3, 2.4, 2.6 (2), 2.7, 3.3, 3.6, 3.7, 4.7, 4.9, 5.5, and 6.0 mg/kg (as received).

After proportional adjustment of the residues to the Latvian GAP for rye, residues of chlormequat cation in rye straw were 0.84, 1.6 (2), 1.9, 2.1, 3.7, 4.0, 4.2 (2), 5.0, 5.3, 5.6, 6.6, 7.5, 7.9, and 8.9 mg/kg (as received), or 0.95, 1.8 (2), 2.2, 2.4, 4.2, 4.5, 4.8 (2), 5.7, 6.0, 6.4, 7.5, 8.5, 9.0, and 10 mg/kg (dry weight basis).

The Meeting estimated a maximum residue level of 20 mg/kg for rye straw and fodder, dry, together with median and highest residues of 4.2 and 8.9 mg/kg respectively.

Triticale straw

The critical GAP for triticale is in Ireland (1 × 1875 g ai/ha application, up to BBCH 31).

Residue data for triticale straw is not available. However, data is available for rye and wheat straw.

Residues of chlormequat cation at harvest in wheat straw (adjusted to the Irish GAP for triticale and eliminating the < LOQ residues) are 0.58, 2.7, 3.8, 4.0 (2), 5.7, 5.9, 7.0, 8.5, 8.9, 10, 12, 14, 15, 19 (2), 22, 26 (2), 30 (2), 34, and 51 mg/kg (as received), or 0.65, 3.0, 4.2, 4.4 (2), 6.4, 6.6, 7.8, 9.5, 9.9, 11, 13, 15, 16, 21 (2), 25, 29 (2), 33 (2), 38, and 57 mg/kg (dry weight).

The Meeting estimated a maximum residue level of 80 mg/kg for triticale straw and fodder, dry, together with median and highest residues of 12 and 51 mg/kg respectively.

Wheat straw

The critical GAP for wheat is in Argentina (1 × 2025 g ai/ha application, BBCH 21–31).

Residues of chlormequat cation in wheat straw at harvest from trials conducted in Europe after an application at 700, 1000, 1500 g ai/ha were < 0.39 (5), 0.47, 1.5, 3.2 (3), 3.3, 4.8, 6.3, 7.0, 7.3, 7.4, 7.8, 10, 12, 13, 15 (2), 19, 21 (2), 24 (2), and 41 mg/kg (as received).

After proportionality adjustment to the Argentine GAP (eliminating the <LOQ residues) residues of chlormequat cation in wheat straw were 0.63, 2.9, 4.1, 4.3 (2), 6.2, 6.4, 7.6, 9.2, 9.6, 11, 13, 15, 16, 20 (2), 24, 28 (2), 32 (2), 37, and 55 mg/kg (as received), or 0.72, 3.3, 4.7, 4.9 (2), 7.0, 7.3, 8.6, 10, 11, 13, 15, 17, 18, 23 (2), 27, 32 (2), 36 (2), 42, and 63 mg/kg (dry weight basis).

The Meeting estimated a maximum residue level of 80 mg/kg for wheat straw and fodder, dry, together with median and highest residues of 13 and 55 mg/kg respectively.

The Meeting withdrew the previous recommendation of 30 mg/kg for cereal straw and fodder, dry.

Maize fodder

The current MRL of 5 mg/kg for maize fodder (dry) (AS 0645) should be withdrawn as no GAP information for maize or supporting residue data was provided.

Fate of residues during processing

Barley

A processing study for chlormequat chloride in barley was provided to the Meeting. The processing factors determined from that study are tabulated below.

Processing factors for chlormequat chloride in barley

Processed fraction	Processing factor (parent)	Best estimate PF	RAC STMR	STMR-P
Pearl (pot) barley	*0.06*, 0.8, 0.9, 1.0, 1.0	0.9	0.37	0.33
Malt	*0.69*, 0.9, 0.9, 0.9, 1.0	0.9		0.33
Spent grain	0.01, 0.02, 0.02, 0.03	0.02		0.007
Beer	*0.015*, 0.1, 0.2, 0.2, 0.2	0.2		0.074

Processing factors in italics were obtained from studies considered by the 1994 and/or 2000 JMPRs.

The Meeting estimated STMR-P values of 0.33, 0.33, 0.007, and 0.074 mg/kg for pearl (pot) barley, malt, spent grain, and beer respectively.

Oats

A processing study for chlormequat chloride in oats was provided to the Meeting. The processing factors determined from that study are tabulated below.

Processing factors for chlormequat chloride in oats

Processed fraction	Processing factor (parent)	Best estimate PF	RAC STMR	STMR-P
Oat kernels	1.0	1.0	1.3	1.3
Oat flakes	*0.1*, *0.25*, *0.27*, 0.8, 0.9, 1.0, 1.2	0.80		1.04

Processing factors in italics were obtained from studies considered by the 1994 and/or 2000 JMPRs.

The Meeting estimated an STMR-P of 1.04 mg/kg for oat flakes, based on the UK GAP.

Rye

A processing study for rye was not provided to the Meeting. Key processing factors for rye from studies supplied to the 1994 JMPR are tabulated below.

Processing factors for chlormequat chloride in rye

Processed fraction	Processing factor (parent)	RAC MRL	Processed commodity MRL	RAC STMR	STMR-P
Rye bran	3.2	6	20	1.1	6.6
Rye flour	0.99		-		1.1
Rye wholemeal	1.3		8		1.4
Rye wholemeal bread	0.95		-		1.0

The Meeting estimated a maximum residue level of 20 mg/kg for rye bran, unprocessed, replacing the previous MRL of 10 mg/kg, together with an STMR-P of 6.6 mg/kg.

The Meeting estimated a maximum residue level of 8 mg/kg for rye wholemeal, replacing the previous recommendation of 4 mg/kg, together with an STMR of 1.4 mg/kg.

The Meeting withdrew the previous recommendation of 3 mg/kg for rye flour, as residues do not concentrate in rye flour and will be covered by the MRL for the raw commodity.

The Meeting estimated new STMR-P values of 1.1 and 1.0 mg/kg for rye flour and rye wholemeal bread respectively.

Wheat

A processing study for chlormequat chloride in wheat was provided to the Meeting. The processing factors determined from that study are tabulated below.

Processing factors for chlormequat chloride in wheat

Processed fraction	Processing factor (parent)	Best estimate PF	RAC MRL	Processed commodity MRL	RAC STMR	STMR-P
Flour (type 550)	0.19, 0.28, 0.29, 0.30, *0.41*	0.29	2	-	0.58	0.17
Bran	*2.5*, 2.8, 2.9, 3.1, 3.4, *4.6*	3.0		7		1.7
Wholemeal flour	0.86, 0.91, 1.0, 1.1	0.955		-		0.55
Wholemeal	*1.0, 1.4*	1.2		-		0.70
Wholemeal bread	0.49, 0.51, 0.53, 0.55, *0.63, 0.79*	0.54		-		0.31

Processing factors in italics were obtained from studies considered by the 1994 and/or 2000 JMPRs.

The Meeting estimated a maximum residue level of 7 mg/kg for wheat bran, unprocessed, replacing the previous recommendation of 10 mg/kg, together with an STMR-P of 1.7 mg/kg.

The Meeting withdrew the previous recommendations of 2 and 5 mg/kg for wheat flour and wheat wholemeal respectively, as residues do not concentrate in these commodities.

The Meeting estimated STMR-P values of 0.17, 0.55, 0.70, and 0.31 mg/kg for type 550 (white) flour, wholemeal flour, wheat wholemeal, and wholemeal bread respectively.

Farm animal dietary burden

Farm animal feeding studies in lactating cattle and laying hens were provided to the Meeting.

Lactating cattle

Groups of three lactating cows were given chlormequat chloride in the diet twice daily at a dose of 240, 720, or 2400 mg/animal per day, equivalent to 0.4, 1.3, and 4 mg/kg bw per day or 12, 36, and 120 ppm on a dry weight basis, for 28 consecutive days. Two additional animals were treated at the high dose for 28 days and slaughtered 2 and 7 days after the last dose. The doses were equivalent to 0.31, 1, and 3.1 mg/kg bw per day (or 9.3, 28, and 93 ppm), calculated as chlormequat cation. At the lowest dose, the average concentrations of chlormequat chloride residues were 0.029 mg/kg in milk,

0.1 mg/kg in liver, and 0.2 mg/kg in kidney. No residues were found in meat or fat. At the medium and high doses, the plateau concentrations of chlormequat chloride residue in milk were 0.1 and 0.2 mg/kg. Concentrations up to 0.11 mg/kg were determined in some meat and fat samples. The concentrations were 0.1 and 0.4 mg/kg in liver and 0.4 and 0.8 mg/kg in kidney at the two doses, respectively, indicating that the values in kidney were at least twice as high in liver.

The concentrations of chlormequat chloride in skim milk were similar to those in whole milk, but they were two times lower than those in cream because of the solubility of the compound in water.

The concentration of chlormequat residues in milk reached a plateau 10–11 days after the first treatment with the medium dose, but after 3–4 days with the low and high doses. The residues were cleared rapidly from meat, fat, and liver, and none could be determined in these tissues 2 days after the end of dosing. The concentrations in milk and kidney fell to about 20% of their plateau values. After 7 days, the values for milk were below the LOQ of 0.01 mg/kg, but 0.09 mg/kg remained in kidney. Although milk and tissue samples were frozen on the day of sampling, they were analysed in part 1 year later. No adequate information on stability was provided to the 2000 JMPR.

However, a storage stability study in animal commodities was provided to the current Meeting, and this confirmed that residues of chlormequat chloride were stable in cattle meat, milk and eggs for up to 12 months of frozen storage. Therefore, the results of the cattle feeding study are unlikely to have been adversely affected by sample degradation during storage.

Laying hens

Three groups of four hens were given capsules containing chlormequat chloride at a dose of 0.72, 2.1, or 7.2 mg/bird per day, equal to 6, 18, and 60 ppm on a dry weight basis, for 28 consecutive days. Two additional groups of 12 hens were treated with the high dose for 28 days and slaughtered 2 or 7 days after the last dose. The doses were equivalent to 4.6, 14, and 46 ppm when calculated as chlormequat cation.

The lowest dose resulted in concentrations of chlormequat chloride residues in eggs at or above the LOQ of 0.05 mg/kg, while 0.05 mg/kg was found in liver and none in meat or fat. Plateau concentrations of 0.06 and 0.1 mg/kg were found in eggs of hens treated with the two higher doses after 1 week of dosing. The concentrations in meat and fat samples were below the LOQ of 0.05 mg/kg, while those in liver were 0.07 mg/kg at the medium dose and 0.18 mg/kg at the high dose.

The residues were cleared rapidly from meat, fat, and liver. No chlormequat chloride was determined in meat or fat. The concentrations in liver had fallen to 0.05 mg/kg 2 days after the end of dosing and to below the LOQ after 7 days. After 2 and 7 days, the residues in eggs had fallen to values below the LOQ of 0.05 mg/kg.

Egg and tissue samples were frozen on the day of sampling but were analysed in part 3 months (tissues) or 10 months (eggs) later. No adequate information on stability was provided to the 2000 JMPR.

However, a storage stability study in animal commodities was provided to the current Meeting, and this confirmed that residues of chlormequat chloride were stable in cattle meat, milk and eggs for up to 12 months of frozen storage. Therefore, the results of the laying hen feeding study are unlikely to have been adversely affected by sample degradation during storage.

Livestock dietary burden

Dietary burden calculations for cattle and poultry are provided below. The dietary burdens were estimated using the OECD diets listed in Appendix IX of the 2016 edition of the FAO Manual.

Summary of livestock dietary burden (ppm chlormequat cation)

	USA-Canada		EU		Australia		Japan	
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Beef cattle	10.5	3.34	24.5	8.59	100[a]	34.8[b]	1.72	1.72

	USA-Canada		EU		Australia		Japan	
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Dairy cattle	21.3	8.02	24.5	8.59	66.8[c]	22.8[d]	1.09	1.09
Broiler hens	1.70	1.70	1.41	1.41	0.60	0.60	0.097	0.097
Laying hens	1.70	1.70	11.4[e]	4.89[f]	0.60	0.60	0.58	0.58

[a] Highest maximum dietary burden for beef cattle suitable for estimation of MRLs for mammalian meat and offal.

[b] Highest mean dietary burden for beef cattle suitable for estimation of STMRs for mammalian meat and offal.

[c] Highest maximum dietary burden for dairy cattle suitable for estimation of MRLs for milk.

[d] Highest mean dietary burden for dairy cattle suitable for estimation of STMRs for milk.

[e] Highest maximum dietary burden for broiler and layer poultry suitable for estimation of MRLs for poultry meat, offal and eggs.

[f] Highest mean dietary burden for broiler and layer poultry suitable for estimation of STMRs for poultry meat, offal and eggs.

Animal commodity maximum residue levels

Mammals

The highest maximum dietary burden for dairy cattle was 66.8 ppm while the highest mean dietary burden was 22.8 ppm (both numbers as chlormequat cation).

	Feed level (ppm, as the cation)	Residues in milk (mg/kg, as the cation)
MRL dairy cattle		
Feeding study	28	0.15
	93	0.26
Dietary burden and highest residue	66.8	0.22
STMR dairy cattle		
Feeding study	9.3	0.039
	28	0.15
Dietary burden and mean residue	22.8	0.12

The Meeting estimated a maximum residue level of 0.3 mg/kg for milk, together with an STMR of 0.12 mg/kg. The Meeting withdrew the previous recommendation of 0.5 mg/kg for milk of cattle, goats and sheep.

The highest maximum dietary burden for beef cattle was 100 ppm while the highest mean dietary burden was 34.8 ppm.

	Feed level (ppm, as the cation)	Residues (mg/kg as chlormequat cation)			
		Meat	Fat	Liver	Kidney
MRL beef cattle					
Feeding study	93	0.085[a]	0.078	0.39	0.82
Dietary burden and highest residue	100	0.091	0.083	0.42	0.88
STMR beef cattle					
Feeding study	28	< 0.04	< 0.04	0.062	0.31
	93	< 0.04	0.08	0.29	0.59
Dietary burden and mean residue	34.8	< 0.04	0.04	0.086	0.34

[a] This value is from the mid dose (28 ppm as the cation) feeding level, as a higher highest residue was observed for the mid dose feeding level than for the high dose feeding level.

The Meeting estimated a maximum residue level of 0.2 mg/kg for meat from mammals other than marine mammals, together with an STMR and an HR of 0.04 and 0.091 mg/kg respectively.

The Meeting withdrew the previous recommendations of 0.2 mg/kg for meat of cattle, pigs and sheep, and for goat meat.

The Meeting estimated a maximum residue level of 0.1 mg/kg, for mammalian fat together with an STMR and an HR of 0.04 and 0.083 mg/kg respectively.

Based on the data for kidney, the Meeting estimated a maximum residue level of 1 mg/kg for edible offal, mammalian, together with an STMR and an HR of 0.086 and 0.42 mg/kg for liver and an STMR and an HR of 0.34 and 0.88 mg/kg for kidney.

The Meeting withdrew the previous recommendations of 0.1 and 0.5 mg/kg for liver and kidney.

Poultry

The highest maximum dietary burden for poultry was 11.4 ppm, while the highest mean dietary burden was 4.89 ppm, for estimation of MRLs and dietary parameters for both meat and eggs (both values expressed as the cation).

No residues of chlormequat chloride above the LOQ (0.05 mg/kg in the study) were found at feeding levels of 6, 18 or 60 ppm (as the chloride, or 4.65, 14, or 46.5 ppm as the cation) in the meat or fat of laying hens.

The Meeting therefore estimated maximum residue levels of 0.04* mg/kg for poultry meat (confirming the previous recommendation), and 0.04* mg/kg for poultry fats, together with STMR and HR values of 0.04 mg/kg for both meat and fat (these values are for the cation).

	Feed level (ppm, as the cation)	Residues in liver (mg/kg, as the cation)	Residues in eggs (mg/kg, as the cation)
MRL poultry			
Feeding study	4.65	0.07	0.046
	14	0.078	0.093
Dietary burden and highest residue	11.4	0.072	0.079
STMR poultry			
Feeding study	4.65	0.039	< 0.039
	14	0.054	0.078
Dietary burden and mean residue	4.89	0.04	0.04

The Meeting estimated a maximum residue level of 0.1 mg/kg, confirming the previous recommendation, together with an STMR and HR of 0.04 and 0.072 mg/kg respectively, for poultry edible offal.

The Meeting estimated a maximum residue level of 0.1 mg/kg, confirming the previous recommendation, together with an STMR and an HR of 0.04 and 0.079 mg/kg respectively, for eggs.

RECOMMENDATIONS

On the basis of the data from supervised trials, the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IESTI and IEDI assessments.

The residue definition (for compliance with the MRL and dietary risk assessment) in plant and animal commodities remains as previously recommended: *chlormequat cation*.

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The International Estimated Daily Intakes (IEDIs) of chlormequat chloride were calculated for the 17 GEMS/Food cluster diets using STMRs/STMR-Ps estimated by the current Meeting. The results are shown in Annex 3 of the 2017 Report.

The ADI for chlormequat chloride is 0–0.05 mg/kg bw/day (or 0–0.0388 mg/kg bw/day expressed as chlormequat cation). The calculated IEDIs for chlormequat chloride were 1–7% of the