

# Course of Chlormequat Residue in 'Conference' Pears over a 9-Year Period Following Its Final Use in the Orchard

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**Keywords:** *Pyrus communis*, shoot growth, plant growth regulator, growth retardant, maximum residue limit

## Abstract

Chlormequat (CCC) was used for many years in the Netherlands as a chemical growth retardant to restrict vegetative growth and promote flower bud development in pears. After several years of annual applications, CCC residue levels in the fruits frequently exceeded the maximum residue limit (MRL) of 3.0 mg/kg. In 2001 the use of CCC in pears was banned and the MRL was reduced to 0.05 mg/kg. CCC is a very persistent chemical which seems to have accumulated in trees treated for many years with this growth retardant. Because of the expected carry over of these accumulated residues into fruits in the years following the last application of CCC, it was decided to decrease the MRL. In 2001 and 2002 a temporary MRL of 0.5 mg/kg was applied. From March 2003 until July 2006 the temporary MRL was reduced to 0.3 mg/kg. From August 2006 until July 2009 this was further reduced to 0.2 mg/kg. However, the final MRL will be reduced to 0.05 mg/kg within the shortest time possible. Pears sampled in years in which CCC was applied to the trees contained CCC residues that varied between 6.8 and 1.1 mg/kg, depending on the dosage and number of years of CCC application. During the first growing season without further CCC applications, the CCC residue of the fruits decreased on average by about 90%. During the following year without CCC the rates of decrease in CCC residues varied widely. In some trials a 60% decrease was noted in the second 'CCC free' year, while in other trials no further decrease was observed. In 2003 a further reduction was observed in most trees. Compared to 2002, the CCC levels had decreased by 6 to 80% and were all below the temporary MRL of 0.3 mg/kg. However, despite these decreases in CCC residue levels in trees grown for three to six years without any further CCC application, in 2003 the CCC residue in the fruits of most trees still exceeded the future MRL of 0.05 mg/kg. In 2006 only the fruits of one trial in Zeewolde still exceeded the MRL of 0.05 mg CCC/kg. In these trees which received their final CCC application in 1999, an average CCC level of 0.06 mg/kg was measured. Thus, even after 8 years of cultivation without CCC, pear trees which were amply treated with CCC in the past may produce fruits with a CCC residue level above the MRL of 0.05 mg/kg. Analysis of the wood of the trunks of these trees showed that this part of the trees still contained CCC levels up to 4.3 mg/kg. This CCC may have been partially transported into the fruits produced in 2007.

## INTRODUCTION

Chlormequat chloride (CCC) has been used for many years as a chemical growth retardant to restrict shoot growth and promote flower bud development in pears. The use of CCC allowed growers to grow pears in high density planting systems and to obtain quickly and thereafter maintain a good balance between shoot growth and fruit production after planting. CCC is also used in other crops. For example, in grain it is applied to reduce internode elongation. Shorter stems reduce the chance of a cereal crop being flattened by rain or wind. In floriculture it is used to grow compact and sturdy pot and bedding plants.

The mode of action of CCC in plants is the inhibition of the biosynthesis of gibberellins, a class of endogenous plant hormones involved in the control of cell elongation and flower development. CCC is a very persistent chemical in plants. Therefore, yearly applications of this growth retardant in perennial plants such as fruit trees may result in an accumulation of CCC. This stored CCC may act as a source of the chemical in future growing seasons and may be transported into the fruits. As a result CCC can be found in fruits several years after the trees have received their last CCC treatment.

Following a number of incidents within the European Community in which the maximum residue limit (MRL) of 3.0 mg/kg was exceeded, a preliminary decision was made in 2000 to reduce the MRL for pears from 3.0 to 0.05 mg/kg fresh weight. Due to the expected carry over of CCC accumulated in trees previously treated with CCC, growers were unlikely to obtain such a large reduction in MRL in only one or two years. In addition, more and more buyers were demanding CCC-free pears. Beginning in March 2001 the use of CCC in pears was no longer allowed.

In the past ten years the Fruit Research Station in Randwijk and its former Experimental Garden Zeewolde in the Netherlands have carried out several experiments and practical trials to determine the efficacy of CCC in controlling shoot growth in pear trees. When it was announced that the use of CCC in pear orchards would be prohibited and the MRL for CCC-residue would be decreased, a monitoring program was started in which, after ending CCC applications, the CCC residue levels of the fruits of the same trees were assessed yearly at harvest in order to determine how many years it would take to produce fruits no longer exceeding the MRL for CCC-residue of 0.05 mg/kg.

## **MATERIALS AND METHODS**

### **Legal Directives for the Use of CCC in Pear Orchards**

Until March 2001 fruit growers were allowed to use CCC for controlling shoot growth and promoting flower bud development in pear. The latest date for legal application of the product was set at 3 months before harvest. Recommended dosages were 150 to 250 ml/100 L spray volume, using a product containing 750 g CCC/L. According to the instructions of the manufacturer, the first application should be given when the young shoots have developed 5 to 8 leaves, followed by a second application two to three weeks later, if necessary. The maximum residue limit (MRL) for CCC in fruits was, at this time, 3.0 mg/kg fresh weight. Based on EU regulations this MRL is currently reduced to 0.05 mg/kg. In 2001 and 2002 a 2-year transition period was in force, during which the maximum CCC level for pears was 0.5 mg/kg. From August 1, 2003 till July 31, 2006, a second transition period was in force with a maximum CCC residue level of 0.3 mg/kg fresh weight, followed by a third transition period from August 2006 till July 2009 with a MRL of 0.2 mg/kg fresh weight.

CCC-applications in the trials described in this paper were carried out by means of a knapsack sprayer. Trees were sprayed till runoff, equivalent to a total volume of approximately 1000 L/ha.

### **Experiments**

**1. Trial 1.** In spring 1989 two-year-old 'Conference' trees on quince MC rootstocks were planted in the Experimental Garden Zeewolde at a distance of 3.25 x 1.30 m (2367 trees/ha). From 1991 to 1997 the trees were sprayed annually with 2.3 kg CCC (two times 2.5 L/ha each year) using a product containing 460 g/L CCC. The treatment was carried out on plots of two trees and replicated four times. In 1998 the trees received 2.25 kg CCC (two times, 1.5 L/ha on May 12 and May 20) using a product containing 750 g/L CCC. In 1998 fruits were harvested on September 15. On February 19, 1999 a sample of these fruits was taken for CCC residue analysis. Samples of fruits were taken directly from the trees on September 22, 1999, August 29, 2000, September 5, 2001, September 2, 2002, September 8, 2003, September 11, 2004, September 12, 2005 and September 11,

2006.

**2. Trial 2.** In spring 1989 two-year-old ‘Conference’ trees on quince MC rootstocks were planted in the Experimental Garden Zeewolde at a distance of 3.25 x 1.30 m (2367 trees/ha). Each treatment was replicated eight times and contained one tree each. The treatments sampled for CCC residue analysis were: 1) CCC treatment from 1994–1997, no CCC from 1998 onwards; 2) CCC treatment from 1994–1998, no CCC application from 1999 onwards. From 1994 to 1997 both treatments were sprayed annually with 3.0 kg/ha CCC, using a product containing 460 g/L CCC. The dates on which CCC was sprayed in treatment 2 in 1998 were May 11 (2 L/ha), May 25 (1 L/ha) and June 8 (1 L/ha) with a product containing 750 g/L CCC, again up to a total amount of 3.0 kg/ha. In 1998 fruits were harvested on September 14 and stored. On January 29 and February 19, 1999 samples were taken for CCC residue analysis. In 1999 the trees of treatment 2 received a total amount of 3.75 kg/ha CCC, which was sprayed over 4 applications on May 3 (2 L/ha), May 17 (1 L/ha), May 31 (1 L/ha) and on June 11 (1 L/ha) using a product containing 750 g/L CCC. Fruits for CCC-residue analysis were sampled on September 22, 1999, August 29, 2000, September 5, 2001, September 2, 2002, September 8, 2003, September 11, 2004, September 12, 2005 and September 11, 2006.

**3. Trial 3.** In spring 1992 eight-year-old ‘Conference’ trees on quince MC rootstocks were planted in the Experimental Garden Zeewolde at a distance of 3.25 x 1.20 m (2564 trees/ha). Each treatment consisted of plots of four trees planted in four replicates. The treatments sampled for CCC residue analysis were: 1) Untreated control trees, 2) Trees treated twice with 0.5 L/ha CCC, 3) Trees treated twice with 1 L/ha CCC, and 4) Trees treated twice with 1.5 L/ha CCC. These CCC-treatments were carried out in 1999 only and took place on May 4 and 17, using a product containing 750 g/L CCC and giving total dosages of 0.75, 1.5 and 2.25 kg/ha, respectively. Fruits were sampled on September 22, 1999, August 29, 2000, September 5, 2001, September 2, 2002, September 8, 2003, September 11, 2004, September 12, 2005 and September 11, 2006.

### **CCC Residue Analysis**

For each sample 10 to 20 randomly harvested fruits from a single treatment were homogenised. Samples of the wood of the trunks of trees of trial 2 were taken on September 11, 2006 using a drill with a 16-mm diameter bore and collecting ca. 50 g of drillings per sample from holes drilled into the trunk at approx. 20 to 50 cm above the graft union. In 1998, 1999 and from 2001 to 2006 the CCC residue analyses were carried out by the Soil, Crop and Environmental Laboratory “Zeeuws Vlaanderen BV” in Graauw (Netherlands). The samples taken in 2000 were analysed by TNO Nutrition in Zeist (Netherlands). Both laboratories use similar procedures for extraction, purification, detection and quantification of the CCC-content of the fruits (LC-MSMS, combined liquid chromatography and mass spectrometry). The analytical method (European standard NEN 15055) warrants a very sensitive and reproducible determination of CCC. Since 2002 the sensitivity of the CCC analysis method has been improved. The detection limit of the method has been reduced from 0.05 to 0.01 mg CCC/kg fresh product.

### **Growth and Production**

The growth and production of the trees was determined in 1998 and 1999. Shoot growth was determined as total shoot length (m/tree). Production was measured by weighing of all fruits of the observation trees at harvest.

## **RESULTS**

### **CCC Residues**

Tables 1 to 4 summarize the results of the CCC residue analyses carried out in the years CCC was applied to the trees, as well as up to six years after the final CCC application to the trees.

**1. Trial 1.** In 1998, the year in which the trees of trial 1 received their last CCC treatment,

the average CCC residue content of the fruits was 6.8 mg/kg fruit (Table 1). This residue level was more than two times the maximum residue limit for CCC of 3 mg/kg fruit (MRL valid up to March 2001). Fruits sampled from the same trees in 1999 and 2000 contained significantly less CCC. In 1999, one year after the last CCC application, the average CCC residue level had decreased to 0.5 mg/kg. In 2000 the average residue level had further decreased to 0.17 mg/kg. Thus, during the first growing season after termination of the CCC applications, the CCC residue level decreased by more than 92%, followed by another 66% decrease in the following growing season. Surprisingly, in 2001 no further decrease in the CCC residue was observed. On the contrary, the average residue level of 0.27 mg/kg in fruits grown in 2001 was even higher than the level of 0.17 mg/kg in 2000. From 2002 to 2004 the average CCC level in the fruits of the treated trees steadily decreased from 0.19 to 0.04 mg/kg. However, in 2005 no further decrease in CCC level was observed. In one out of three replicates the CCC level was equal to that of 2004 in the other two replicates increases of 0.01 to 0.05 mg/kg were determined. In 2006 the average CCC residue level of 0.04 mg/kg was similar to that in 2004 and all samples from this trial contained less than 0.05 mg CCC/kg fresh weight.

**2. Trial 2.** Despite the higher amounts of CCC applied in this trial, the CCC residue levels in the fruits were lower than those in trial 1. In this second trial, the residue-levels in 1998 in treatment 2 remained below 3.0 mg/kg (Table 2). Continuation of the CCC treatments in 1999 increased the residue levels in this year to an average value of 3.2 mg/kg. In 2000 CCC was no longer applied, which resulted in residue levels that were only about 10% of those in the previous year. In 2001 no further decrease in CCC residue levels was observed but in 2002 they showed a further decrease to 0.24 mg/kg for the trees of treatment 2. However, in 2003 the residue level remained almost similar to that in 2002 and amounted to 0.23 mg/kg. In 2004 a further decrease in the level of CCC to 0.12 mg/kg was noted. However, in 2005 the CCC residue level showed no further decrease in two out of three replicates, yielding an average residue level of 0.11 mg/kg, almost equal the 0.12 mg/kg measured in 2004. Residue levels in 2006 were lower than in 2005 and had decreased to 0.06 mg CCC/kg. The trees of treatment 1 received their last CCC application in 1997. In 1998 the CCC residue level in their fruits was 0.4 mg/kg. In 2000, after 3 years without CCC applications this level had decreased to 0.10 mg/kg. As observed in treatment 2 a slightly higher level of 0.14 mg/kg was observed in 2001. In 2002, 5 years after the last application of CCC in this treatment, the average level of CCC in the fruits was still 0.10 mg/kg. One year later, in 2003, again a residue level of 0.10 mg/kg was measured. However, in 2004 this level had decreased to 0.03 mg/kg. In 2005 no further decrease was observed. In fact in two out of three replicates the CCC levels were 0.06 mg/kg above those in 2004 while in the other replicate a 0.01 mg/kg reduction was observed. The average CCC residue level of treatment 1 in 2005, 8 years after the last treatment with CCC, amounted to 0.07 mg/kg, i.e., 0.02 mg/kg above the final MRL of 0.05 mg/kg. In 2006 the average residue level in the fruits had decreased to 0.03 mg CCC/kg. In only one of the three replicates a residue level was determined which was 0.01 mg/kg above the final MRL of 0.05 mg CCC/kg.

**3. Trial 3.** This trial was conducted to examine whether a satisfactory degree of growth control in pear trees could be achieved by applying lower dosages of CCC and how this would affect the residue levels in the fruits. The CCC residue data listed in Table 3 clearly demonstrate that lowering the dosage of CCC reduces the CCC residue in the fruits. In the year of application, CCC residue levels in fruits of trees treated with 0.75, 1.5 or 2.25 kg/ha CCC was 1.1, 1.7 and 2.0 mg/kg, respectively. In 2001 and 2002, two and three years after the final CCC treatment, these residue levels had decreased to 0.15, 0.20 and 0.22 mg/kg and to 0.08, 0.12 and 0.17 mg/kg, respectively. Despite these large decreases in residue levels of 88% or more, the future maximum residue limit of 0.05 mg/kg was still exceeded in all treatments. In 2003 and 2004 a further decrease in CCC levels was noted to 0.06, 0.10 and 0.05 mg/kg and to 0.03, 0.02 and 0.02 mg/kg, respectively. Surprisingly, this decrease did not continue in 2005. On the contrary, the average CCC residue level in the trees treated with 2 times 1.5 L/ha CCC in 1999 even increased from 0.02 mg/kg in

2004 to 0.07 mg/kg in 2005. Unfortunately, in 2000 only trees treated with 2.25 kg/ha CCC in 1999 was sampled for CCC residue analysis. Comparing the residue levels of 2000 to 2002 of fruits from this treatment shows that the greatest reduction in residue level took place during the first growing season without CCC application and that the decrease continued at a much slower pace during the following growing seasons.

In 2006, seven to nine years after the last application of CCC to the trees of trial 3, the wood of the trunks still contained considerable amounts of CCC (Table 4). On average the wood of trees that were last sprayed with CCC in 1997 or 1999 contained 2.6 and 3.7 mg chlormequat/kg, respectively.

### **Growth and Production**

The growth of the trees was significantly inhibited by the amounts of CCC applied to the trees (Table 5). In trial 1 tree growth in 1998, the last year of CCC-treatment, was reduced by more than 75% as compared with untreated trees. In 1999, the first year without further CCC-application, growth of the CCC treated trees was still strongly reduced although the extent of growth reduction had decreased to 40%. Fruit production was slightly higher in the CCC-treated trees. In trial 2 growth of the trees of treatment 1 (last CCC-application in 1997) was stronger than that of the trees of treatment 2 (last CCC-application in 1999) in both 1998 and 1999. However, in the same years only very small differences in fruit production were observed between both treatments.

During the first year of treatment 3, two applications of 1.5 L/ha (2.25 kg/ha) of CCC reduced the shoot growth of the trees in trial 3 by almost 40% without any effect on fruit production.

### **DISCUSSION**

A number of the observations which were made in this study are difficult to explain. In 1998 almost three times less CCC was found in the fruits of trial 2, which had received 3 kg CCC/ha, than in the fruits of trial 3, which had received a lower dosage of CCC of 2.3 kg/ha. The most logical factors that may explain this discrepancy in CCC residue levels are differences in tree vigour and number of years of CCC treatment. The trees of trial 3 which showed the highest levels of CCC residue in 1998 had been sprayed with CCC for 8 years, while those of trial 2 had only received CCC for 5 years in 1998. A comparison of the residue levels in trial 2 for 1998 and 1999 strongly suggests an accumulation of CCC residue during the years of CCC treatment. In 1999, after 6 years of CCC application, the residue level had increased from 2.3 mg/kg in 1998 to 3.2 mg/kg in 1999. Similar observations, based on analyses of retail samples of pears in the UK between 1997 and 2002, have been reported recently (Reynolds et al., 2004). Since CCC decomposition soils takes place within approx. 3 weeks (Cathey and Stuart, 1961) CCC residues in the soils are unlikely to have influenced the CCC levels in the fruits in the years after the final application of CCC to the trees.

The degree of shoot growth decreases with the number of years of CCC treatment. The data on shoot growth presented in Table 3 clearly show that the growth of the trees in trial 3 was significantly less than that of the trees in trial 2. Consequently, more of the CCC may be transported into the fruits of the trees with the weaker growth instead of being diluted into new shoot growth. Finally, it cannot be excluded that differences in climatic conditions between the dates of CCC application in both trials may have resulted in differences in the rates of CCC uptake and subsequently have led to differences in CCC residue in the fruits. The slightly higher CCC residues in 2001 as compared to 2000 may be related to the much lower fruit load in 2001 as opposed to 2000, resulting in a relatively higher availability of the remaining CCC in the tree per fruit.

### **CONCLUSIONS**

The results of the CCC residue analyses of pears harvested in the year of CCC application and in the years thereafter clearly demonstrate that it may take at least six years of cultivation without CCC applications to produce fruits with a CCC residue level

below the maximum residue limit of 0.05 mg/kg. In all trials used for this study, a very large initial decrease in CCC residue level was observed in fruits grown in the first years after ending the CCC applications. Compared to the residue levels in the last year of CCC treatment, the levels after one year without any further application of CCC had decreased by about 90%. In the second year without CCC application, CCC residues decreased by 66% and 42% in the trials 1 and 4, respectively. However, no further or only a very small decrease was observed in the second year without CCC in the other trials. Despite these large initial decreases, the CCC residue levels in most of the trials sampled after two to five years without further CCC treatments still exceeded the value of 0.05 mg/kg but were below the temporary MRL of 0.3 mg/kg. Even after six years without CCC, some trees produced fruits containing 0.12 mg CCC/kg.

Therefore, it can be concluded that for orchards which have been treated for many years with CCC more than six growing seasons without CCC applications are needed to obtain fruit with a CCC residue level below 0.05 mg/kg. The exact number of years required to reach this maximum residue limit of 0.05 mg/kg most likely depends on the amount of CCC that has been accumulated in the trees during the years that their growth was controlled by CCC and on the rate at which the accumulated CCC is transported into fruits and shoots that will be removed during harvesting and pruning of the trees.

#### ACKNOWLEDGEMENT

This research was financed by the Dutch Product Board for Horticulture.

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#### Tables

Table 1. CCC residues detected in ‘Conference’ fruits (mg chlormequat/kg fresh weight) in trial 1.

Treatment	Replicates			Average
	A	B	C	
CCC 1990-1998				
1998	6.4	6.7	7.4	6.8
1999	0.5	0.4	0.5	0.5
2000	0.19	0.15	0.17	0.17
2001	0.19	0.43	0.19	0.27
2002	0.12	0.25	0.20	0.19
2003	0.07	0.12	0.14	0.11
2004	0.02	0.05	0.04	0.04
2005	0.03	0.09	0.04	0.05
2006	0.04	0.04	0.04	0.04

Table 2. CCC residues detected in ‘Conference’ fruits (mg chlormequat/kg fresh weight) in trial 2.

Treatment 1 CCC 1994-1997	Replicates			Average	Treatment 2 CCC 1994-1999	Replicates			Average
	A+B	C+D	E+F			A+B	C+D	E+F	
1998	0.4	0.5	0.3	0.4	1998	2.5	2.2	2.2	2.3
1999	0.3	0.3	0.2	0.3	1999	3.6	3.6	2.5	3.2
2000	0.09	0.12	0.09	0.10	2000	0.38	0.27	0.41	0.35
2001	0.11	0.09	0.21	0.14	2001	0.44	0.25	0.53	0.41
2002	0.16	0.11	0.04	0.10	2002	0.28	0.21	0.22	0.24
2003	0.12	0.10	0.07	0.10	2003	0.19	0.19	0.31	0.23
2004	0.04	0.03	0.03	0.03	2004	0.14	0.09	0.14	0.12
2005	0.10	0.09	0.02	0.07	2005	0.09	0.10	0.15	0.11
2006	0.06	0.01	0.02	0.03	2006	0.06	0.05	0.08	0.06

Table 3. CCC residues detected in ‘Conference’ fruits (mg chlormequat/kg fresh weight) in trial 3.

Treatment 1999	Residue in year	Replicates			Average
		A	B	C	
2 x 0.5 L/ha	1999	-	-	-	1.1
2 x 1.0 L/ha		-	-	-	1.7
2 x 1.5 L/ha		-	-	-	2.0
2 x 0.5 L/ha	2000	-	-	-	-
2 x 1.0 L/ha		-	-	-	-
2 x 1.5 L/ha		0.18	0.21	0.21	0.20
2 x 0.5 L/ha	2001	0.18	0.09	0.17	0.15
2 x 1.0 L/ha		0.26	0.17	0.18	0.20
2 x 1.5 L/ha		0.32	0.23	0.10	0.22
2 x 0.5 L/ha	2002	0.07	0.06	0.11	0.08
2 x 1.0 L/ha		0.08	0.14	0.13	0.12
2 x 1.5 L/ha		0.22	0.10	0.18	0.17
2 x 0.5 L/ha	2003	0.01	0.09	0.07	0.06
2 x 1.0 L/ha		0.09	0.11	0.09	0.10
2 x 1.5 L/ha		0.05	0.04	0.07	0.05
2 x 0.5 L/ha	2004	0.02	0.0 (<0.01)	0.04	0.03
2 x 1.0 L/ha		0.0 (<0.01)	0.02	0.0 (<0.01)	0.02
2 x 1.5 L/ha		0.03	0.02	0.02	0.02
2 x 0.5 L/ha	2005	0.0 (<0.01)	0.02	0.02	0.01
2 x 1.0 L/ha		0.04	0.01	0.01	0.02
2 x 1.5 L/ha		0.11	0.03	0.06	0.07
2 x 0.5 L/ha	2006	0.03	0.02	0.03	0.03
2 x 1.0 L/ha		0.04	0.02	0.01	0.02
2 x 1.5 L/ha		0.03	0.03	0.04	0.03

Table 4. CCC-residues in 2006 in trunks of ‘Conference’ trees (mg chlormequat/kg fresh weight).

Treatment	Replicates			Average
	A+B	C+D	E+F	
CCC 1994-1997	2.9	2.9	2.1	2.6
CCC 1994-1999	4.3	2.9	3.9	3.7

Table 5. Growth and production of untreated and CCC-treated ‘Conference’ trees.

Treatment	Trial	1998		1999	
		Production (kg/tree)	Growth (m/tree)	Production (kg/tree)	Growth (m/tree)
Untreated	1	18.4	29	25.7	43
CCC 1990-1998		20.0	7	27.3	26
CCC 1994-1997	2	30.3	36	20.6	46
CCC 1994-1999		29.4	21	21.4	17
Untreated	3	-	-	22.3	31
CCC 1999		-	-	22.0	19