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Study on the Potential Distribution, Accumulation and Metabolite Products of Chlorocholine Chloride (CCC) in Tissues of Laying Hens receiving Diets with varying Levels of 15N-CCC

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STUDY ON THE POTENTIAL DISTRIBUTION, ACCUMULATION AND METABOLITE PRODUCTS OF CHLOROCHOLINE CHLORIDE (CCC) IN TISSUES OF LAYING HENS RECEIVING DIETS WITH VARYING LEVELS OF ^{15}N-CCC

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1. INTRODUCTION

Chlorocholine chloride (CCC) is the common name of 2-chloro-N,N,N-trimethylammonium salts. Usually the salt of chlorocholine chloride is used as a plant growth regulator. CCC is mostly used commercially in agriculture as well as in horticulture in Europe and in the United States. It is applied on several food crops (cereals, fruits and vegetables), grass and ornamental plants. As a controller of plant growth, CCC is applied to improve plant shape, size and grain yield. It is also used to promote flower formation, improve fruit setting and prevent premature drop of fruit. Due to its ability to inhibit gibberellic acid (GA) biosyntheses, CCC also can reduce straw growth and, consequently, increase lodging resistance which often has a beneficial effect on quantity and quality of the harvested crop. TOMLIN (1997) stated that these beneficial effects are due to the chlorine ion (Cl^-) in the structural formula of CCC. However, this ion also prevents CCC structural alteration during metabolism in plants. As a result, presence of CCC residues in crops is unavoidable. The CCC residues are found in different amounts in different plants or plant products. It depends upon several factors such as concentration used, season, weather conditions, cultivars, type of soil, level of nitrogen fertilization, application time, methods or modes of application, amount of CCC application and harvesting time (ACKERMANN et al., 1975; SACHSE, 1977; TORNER et al., 1999). The residual CCC can be transferred to the animal products when the CCC-treated plants are mixed into the rations and offered to animals. It is possible also to transfer it along the food chain to humans either directly through their consumption of products of treated plants or indirectly through their consumption of products from animals which have been fed diets made up of ingredients from CCC-treated plants.

Several research studies have been conducted to determine the residue of CCC in plants or plant products using different methods since the 60's. However, the given problem is rather complex, therefore a number of different approaches have been applied to tackle this problem. The number of publications devoted to elucidating the issue continue to grow from year to year. The first method was developed by MOONEY and PASARELA (1967). These authors used non specific spectrometry to determine CCC residues in wheat grain, straw and green wheat foliage with recoveries of 76.0, 90.0 and 86.4 %, respectively. A recent method was developed and reported by VAHL et al. (1998). These authors described a Liquid Chromatography/Mass Spectrometry (LC-MS) procedure to determine the CCC in cereals. This procedure was then modified by other workers who determined the residue of CCC in other material samples or with different instruments. However, it is still unclear which method can be applied to determine the residual CCC in animal products. Due to the presence of similar natural substances such as choline, determination of CCC residues in animal materials by non-specific detection is problematic. Besides, publications of monitoring and surveillance results for CCC residues in animal products are lacking. The reasons for this may be that most methods for analysis of residues in animal products are very time-consuming and laborious. Higher content of fat, peptides or proteins in animal products should be considered in extraction and isolation procedures which make the analytical methods less suitable for routine analysis.

Moreover, although CCC has been used for a long time, its metabolism in both plants and animals is still unclear. Several workers have reported that no CCC breakdown takes place in plants. They found a very high stability of CCC in plants (BOHRING, 1972; 1982) as well as in animals (BLINN, 1967). Also a low mobility within plant compartments was found as well as insignificant CCC metabolism in mature kernels during storage (BOHRING, 1982). In contrast, other workers have found that CCC breakdown occurs in both plants (JUNG and EL-FOULY, 1966; SCHNEIDER, 1967; STEPHAN and SCHÜTTE, 1970; DEKHUIJZEN and VONK, 1974; SEIBEL et al., 1975) and animals (ROMANOWSKI, 1972). CCC can be broken down to choline or its derivative fractions in protein fractions, or in other chemical fractions. EVANS et al. (2000[b]; 2001[b]) reported that determination of CCC using mass spectrometer showed that CCC could fragment to form other cluster formations with different and smaller mass of charge ratio (m/z).

After use of radioactive-labelled CCC in feed, radioactivity was detected in several organs in rats (BLINN, 1967; ROMANOWSKI, 1972), cows (LAMPETER and BIER, 1970), in ovaries of pigs (AZEM, 1996) and in oviducts of laying hens (LANDAZURI et al., 1993). SONGSANG (2001); SONGSANG et al. (2002) used ^{15}N-labelled CCC (^{15}N-CCC) in feed of laying hens, at levels of 5, 50 and 250 ppm. These authors found significant increases in $\delta^{15}N$ from hens on 50 and 250 ppm ^{15}N-CCC in both egg yolk and albumen fractions. Whether the differences in estimated ^{15}N-CCC and measured CCC represent breakdown products of CCC is not known. The authors also found differences in accumulation of ^{15}N-

CCC between the yolk and albumen, which could have arisen from differences perhaps in the mode of yolk and albumen formation. However, they did not mention in which compound ^{15}N was bound.

This study was carried out using laying hens with the general objective of identifying the form in which the metabolic product of CCC appears in eggs and meat when the hens were offered diets with ^{15}N-CCC inclusion.

2. LITERATURE REVIEW

2.1. Chlorocholine chloride (CCC) properties

Chlorocholine chloride (CCC) is well known with common names as chlormequat chloride, Chlorcholine chloride, Cycocel, Cycogan, Increcel, Retacel and Stabilan. It has some chemical names, namely, 2-Chloroethyl-N,N,N-trimethyl-ammonium chloride, N-(2-Chloroethyl) trimethylammonium chloride and 2-Chloroethyl trimethyl-ammonium chloride (DATABASE of NIST62 LIBRARY). The Chemical Abstract Service (CAS) number is 999-81-5. CCC is a chemical family of quaternary ammonium compounds. The structural formula is shown in Figure 2.1.

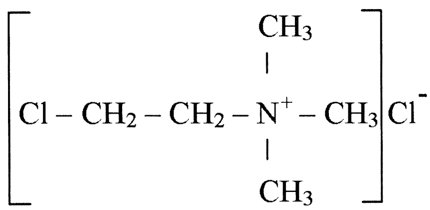

Figure 2.1. Chemical structure of Chlorocholine chloride (RÖDEL and SIEBERS, 1998).

Chlorocholine chloride is one of choline analogues besides (2-bromoethyl) trimethyl ammonium chloride (BCC, bromocholine chloride) and (2-bromoethyl) trimethyl ammonium bromide (BCB, bromocholine bromide). The difference is a substitution of the hydroxyl atom on the second carbon of choline into chlorine on CCC and BCC, and into bromide on BCB as shown in Figure 2.2 (SCHNEIDER, 1967). By-products of CCC and choline were trimethylamine hydrochloride and trimethylvinylammonium hydroxide (WYCHERLEY et al., 1996). CCC is primarily used as anti lodging agent in cereal production to reduce excessive vegetative growth without any adverse effects to the plants (RADEMACHER, 2000). CCC is a very polar compound with high melting point (245°C). It is crystalline and is of relatively low molecular weight (BAKER et al., 1992). The general physical and chemical properties of CCC are described in Table 2.1.

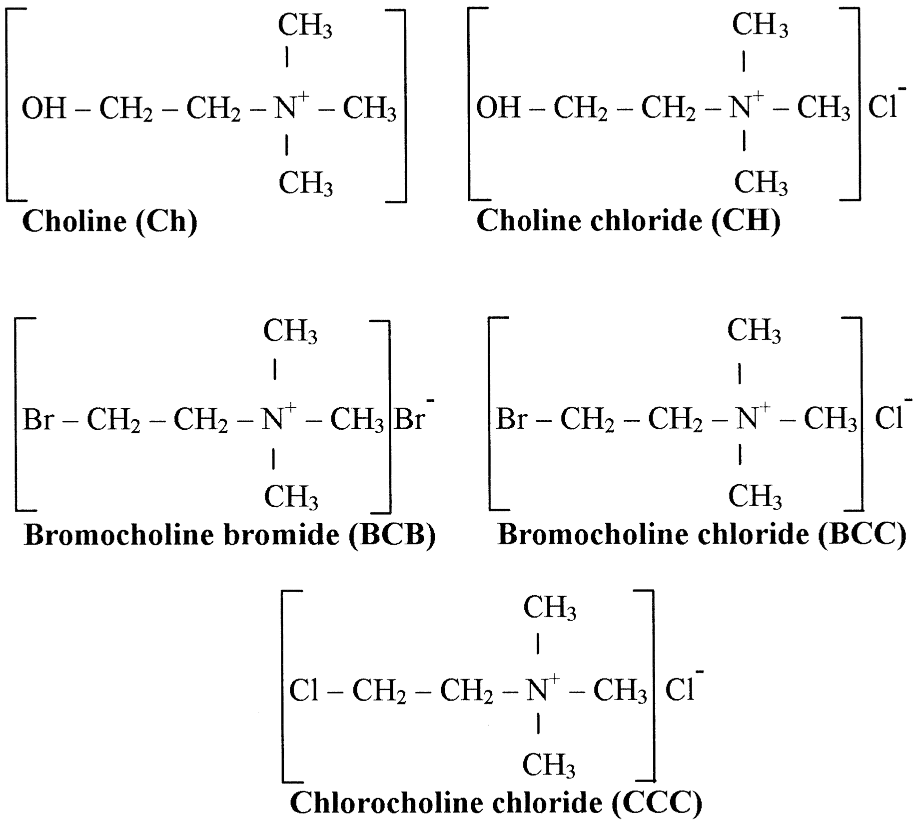

Figure 2.2. Chemical structure of choline, choline chloride, bromocholine bromide, bromocholine chloride and chlorocholine chloride (SCHNEIDER, 1967).

Table 2.1. Physical and chemical properties of chlorocholine chloride compound.

Item	**Description**	**References**
Appearance	Colourless to pale yellow liquid White crystalline solid	HARTLEY and KIDD (1987) SPENCER (1982)
Odour	Slight fishy odour Typical amine odour	PASARELA and ORLOSKI (1973) SPENCER (1982)
Melting point	245°C, 473°F	PASARELA and ORLOSKI (1973)
Vapour pressure	$<$ 10 μPa at 20 – 25°C	PICÓ et al. (2000[a])
Solubility	In lower alcohol such as methanol In acetone, 0.3 g kg^{-1} acetone	SPENCER (1982) HARTLEY and KIDD (1987)