ANNEX B

Metalaxyl-M

B.4 Methods of analysis

B.4.1 Analytical methods for formulation analysis

B.4.1.1 Analytical methods for the determination of pure active substance in the active substance as manufactured (Annex IIA 4.1.1)

- AW-183/3 : Analytical method CGA 329351 tech. (Schneider, 1995a)
- Report on validation of Analytical Method AW-183/3 (Schneider, 1995b)

GLP :
The validation report was created in compliance with GLP.

Principle of the method :
1) Metalaxyl-M technical is dissolved in internal standard solution (adipic acid dibutyl ester in acetone), after which the *sum of the two enantiomers* (CGA 329351 (R) and CGA 351920 (S)) is determined by GC on a SE 54 wide-bore capillary using FID detection. Quantification by internal standard method.
2) For the determination of the *separate enantiomers*, metalaxyl-M technical is dissolved in a mixture of n-hexane and 2-propanol (90%:10%), followed by separation of CGA 329351 (R) and CGA 351920 (S) through HPLC on a chiral stationary phase (Chiralcel OJ) using UV detection at 230 nm. The content of CGA 329351 (R) and CGA 351920 (S) is calculated from the peak area ratio of the enantiomers, using the content determined according to 1) (= sum of both isomers).

Findings :

Specificity - interferences : 1) the GC-method is able to separate the a.s. (= sum of CGA 329351 (R) and CGA 351920 (S)) from its by-products, the internal standard and the solvent. No interferences were observed.
2) the HPLC-method is able to separate both enantiomers from each other, as well as from the by-products and the solvent. No interferences were observed.

Linearity : tested using 5 weights of pure metalaxyl-M reference substance ranging between 50% and 150% of the sample target weight
1) GC-method : r = 0.99999; y = 1.001 x - 3.260.
2) HPLC-method : for CGA 329351: r = 0.9999; y = 9118 x - 8462
for CGA 351920: r = 0.9994; y = 9300 x + 13941

Accuracy : established based on the findings for specificity and linearity.
For the GC-method, a mean recovery value of 99.7% can be calculated from the experimental data of the linearity test.

Repeatability : determined with 5 individual subsamples of the same batch of metalaxyl-M technical.
1) GC-method : RSD = 0.29%
2) HPLC-method : for CGA 329351 : RSD = 0.025%
for CGA 351920 : RSD = 1.469%

Conclusions :
The GC-method is suitable for determination of the sum of enantiomers CGA 329351 (R) and CGA 351920 (S) in metalaxyl-M technical, while the HPLC-method is suitable for determination of the separate enantiomers.
The applicant states that no CIPAC methods are available; no reference was made to CIPAC-method 365/TC/M/3 (determination of metalaxyl in metalaxyl technical).

B.4.1.2 Analytical methods for the determination of significant and/or relevant impurities and additives in the active substance as manufactured (Annex IIA 4.1.2)

- AK-183/2 : Analytical method CGA 329351 tech. - By-products and supplementary tests (Schneider, 1995c)
- Report on validation of Analytical Method AK-183/2 (Schneider, 1995d)

GLP :
The validation report was created in compliance with GLP.

Principle of the method :
1) Simultaneous determination of all *organic by-products* : metalaxyl-M technical is dissolved in tertiair butyl methyl ether containing benzoïc acid 2-naphthyl ester as internal standard, after which the by-products are separated by GC on a DB-1701 capillary column using FID detection. Quantification by internal standard method.
2) Determination of *water* using standard Karl Fischer method.

Findings :

Specificity - interferences : 1) the GC-method is able to separate the following organic by-products from one another and from the a.s., the internal standard and the solvent : CGA 226046, CGA 72649, CGA 145918, CGA 363736, CGA 92370, CGA 132538, CGA 132689, CGA 64188,

CGA 100645, CGA 59113, CGA 226048, CGA 226047 and CGA 226045. No interferences were observed.
2) the Karl Fischer method is widely used and known to give specific results

Linearity : 1) linearity of the GC-method was demonstrated for each impurity with spiked reference substance at 3 levels (4 injections each).
2) the Karl Fischer method is a widely used titration method known from literature.

Accuracy : 1) recovery of the GC-method was determined for each impurity with spiked reference substance at 3 levels (mean of 4 injections each)
2) recovery of the Karl Fischer method was determined with spiked metalaxyl-M technical at 3 levels

Repeatability : 1) repeatability of the GC-method was determined for each impurity with spiked reference substance at 3 levels (4 injections each)
2) repeatability of the Karl Fischer method was determined with spiked metalaxyl-M technical (4 weighings)

Data : see table B.4.1.2-1

Conclusions :

The GC-method is suitable for determination of the organic by-products in metalaxyl-M technical, while the Karl Fischer method is suitable for the determination of water.

The applicant states that no CIPAC methods are available; no reference was made to CIPAC-method 365/TC/M/4 (determination of 2,6-dimethylaniline in metalaxyl technical).

Table B.4.1.2-1 : Validation of method AK-183/2 (Schneider, 1995d)

By-product	Level (%)	Recovery (%)	Linearity	Repeatability (% RSD)	Limit of detection (%)
CGA 226046	0.1 0.2 0.5	80 88 94	r = 0.99966	0.69 0.59 1.36	< 0.1
CGA 72649	0.01 0.02 0.05	93 89 92	r = 0.99957	0.89 0.57 0.71	< 0.01
CGA 145918	0.1 0.2 0.5	89 91 95	r = 0.99980	0.09 0.50 0.41	< 0.1
CGA 363736	0.1 0.5 1.0	95 101 104	r = 0.9998	0.65 0.14 0.19	< 0.1
CGA 92370	0.1 0.2 0.5	93 96 99	r = 0.99996	0.22 0.20 0.37	< 0.1
CGA 132538	0.1 0.2 0.5	96 96 98	r = 0.99996	0.17 0.18 0.27	< 0.1
CGA 132689	0.09 0.14 0.19	96 95 96	r = 0.99990	0.35 0.13 0.32	< 0.1
CGA 64188	0.1 0.5 1.0	96 99 101	r = 0.99998	0.51 0.29 0.21	< 0.1
CGA 100645	0.1 1.0 2.1	101 102 105	r = 0.99985	0.49 0.37 0.16	< 0.1
CGA 59113	0.12 0.17 0.22	118 115 110	r = 0.99602	3.33 2.15 1.68	< 0.1
CGA 226048	0.1 2.0 4.0	113 111 116	r = 0.9993	1.10 0.13 0.07	< 0.1
CGA 226047	0.10 0.14 0.20	97 95 100	r = 0.99029	2.42 0.38 4.90	< 0.1
CGA 226045	0.13 0.18 0.23	129 119 115	r = 0.99899	0.72 1.14 1.24	< 0.1
water	0.1 0.25 0.5	116.7 98.6 99.9		 2.42	< 0.1

- AG-20/4 : Analytical method : Nitrosamines in agrochemicals - by chemical cleavage/chemiluminescence detection (Kreuzer & Wyden, 1995b)
- Report on nitrosamines (Kreuzer, 1995a)

GLP :
The validation report was created in compliance with GLP.
Principle of the method :
Metalaxyl-M technical is dissolved in 1,2-dichloroethane, after which HBr/Copper(II)acetate is added. The N-nitrosamines present react with HBr/catalyst to produce nitric oxide (NO) that is subsequently transferred with a purge gas to a chemiluminescence detector where it undergoes a chemiluminescent reaction with ozone. The emitted radiation is proportional to the nitrosamine content and quantification is accomplished by an external standard method using a N-nitroso-dimethylamine reference solution.
Findings :
Specificity - interferences : the method is not strictly specific since not only N-nitrosamines but also other N- nitroso and O-nitroso compounds undergo NO-elimination when treated with HBr/catalyst and chemiluminescent detection is not entirely specific for NO. In principle, interference with other N-containing compounds is thus possible. However, the low analytical results demonstrate that there is no significant interference.
Blank signals were determined to be low in comparison to the reference solution.

Linearity : the applied method is a widely used method, known from literature (Drescher & Frank, Anal. Chem. 50, 2118-2121 (1978)).

Accuracy - repeatability : duplicate analysis of 5 batches of metalaxyl-M technical, spiked with 0.248 µg/g N-nitroso-dimethylamine, gave the following recoveries :

sample 1 :	101% - 111%	(mean : 106%)
sample 2 :	98% - 105%	(mean : 101.5%)
sample 3 :	95% - 97%	(mean : 96%)
sample 4 :	95% - 92%	(mean : 93.5%)
sample 5 :	93% - 94%	(mean : 93.5%)

Conclusions :
The method is suitable to show that metalaxyl-M technical contains no nitrosamines above 0.5 µg/g

B.4.1.3 Analytical methods for the determination of pure active substance in plant protection products (Annex IIIA 5.1.1)

B.4.1.3.1 Determination of pure active substance in formulation A-9408 B

- AF-1176/1 : Analytical method CGA 329351 in formulation (Bourgeois, 1995a)
- Report on validation of Analytical Method AF-1176/1 (Bourgeois, 1995b)

GLP :
The validation report was created in compliance with GLP.
Principle of the method :
Formulation A-9408 B is dissolved in internal standard solution (adipic acid dibutyl ester in acetone), after which the *sum of the two enantiomers* (CGA 329351 (R) and CGA 351920 (S)) is determined by GC on a SE 54 wide-bore capillary using FID detection. Quantification by internal standard method.
Findings :
Specificity - interferences : the method is able to separate the a.s. (= sum of CGA 329351 (R) and CGA 351920 (S)) from its by-products and the co-formulants, the internal standard and the solvent. No interferences were observed.

Linearity : tested using 5 spiked formulation blanks with the amount of added a.s. ranging between 50 and 150% of the sample target weight : r = 0.99995; y = 1.009 x - 14.398.

Accuracy : established based on the findings for recovery, specificity and linearity.
Recovery was tested using 3 spiked formulation blanks (mean of 2 injections each) with the amount of added a.s. ranging between 75 and 125% of the sample target weight. A mean recovery value of 99.3% was found.

Repeatability : determined with 5 individual subsamples of the same batch of formulation A-9408 B
RSD = 0.133%

Conclusions :
The GC-method is suitable for determination of the sum of enantiomers CGA 329351 (R) and CGA 351920 (S) in formulation A-9408 B.
The applicant states that no CIPAC methods are available.

B.4.1.3.2 Determination of pure active substance in formulation A-9407 A

- AF-1167/1 : Analytical method CGA 329351and Mancozeb in formulation (Bourgeois, 1994)
- Report on validation of Analytical Method AF-1167/1 (Bourgeois, 1995c)

GLP :
The validation report was created in compliance with GLP.

Principle of the method :
1) *Determination of metalaxyl-M* : same as for A-9408 B (see B.4.1.3.1)
2) *Determination of mancozeb* : formulation A-9407 A is suspended in a mixture of glacial acetic acid and 5 N sulphuric acid with heating, causing the extracted mancozeb to be immediately decomposed to carbon disulphide. The CS_2 formed is absorbed in methanolic potassium hydroxide solution with formation of potassium xanthate, after which the absorption solution is slightly acidified and subsequently titrated with 0.1 N iodine to determine the content of mancozeb.

Findings :
Specificity - interferences : 1) the GC-method is able to separate the a.s. (= sum of CGA 329351 (R) and CGA 351920 (S)) from mancozeb and the co-formulants, from its by-products, the internal standard and the solvent. No interferences were observed.
2) the recovery values obtained at the 3 concentration levels demonstrate that the iodometry method is not subject to interference from the formulation blank (including metalaxyl-M).

Linearity : tested using 5 spiked formulation blanks with the amount of added a.s. ranging between 50 and 150% of the sample target weight :
1) for metalaxyl-M : r = 0.99976; y = 1.014 x - 12.359
2) for mancozeb : r = 0.99999; y = 0.999 x + 0.138

Accuracy : established based on the findings for recovery, specificity and linearity.
Recovery was tested using 3 spiked formulation blanks with the amount of added a.s. ranging between 75 and 125% of the sample target weight (for GC-method : mean of 2 injections at each level; for iodometry method : mean of 2 weighings at each level). A mean recovery value of 100.1% was found for both a.s..

Repeatability : determined with 5 individual subsamples of the same batch of formulation A-9407 A :
1) for metalaxyl-M : RSD = 1.049%
2) for mancozeb : RSD = 0.168%

Conclusions :
The GC-method is suitable for determination of the sum of enantiomers CGA 329351 (R) and CGA 351920 (S) in formulation A-9407 A, while the iodometry method is suitable for the determination of mancozeb.
The applicant states that no CIPAC methods are available for the determination of metalaxyl-M; no reference was made to CIPAC-method 365/WP/M/3 (determination of metalaxyl in WP). With regard to mancozeb on the other hand, the applicant states that the CIPAC-method (34/3/M/6.3) is applicable to formulation A-9407 A.

B.4.1.3.3 Determination of enantiomeric purity of the a.s. in formulations

- Determination of the enantiomeric purity of CGA 329351 in formulations (Schneider, 1998a)

GLP :
No GLP-compliance stated

Principle of the method :
The optical purity of metalaxyl-M in formulations can be determined by 2 alternative procedures :

1) by HPLC : the formulation sample is dissolved in eluent by sonication, after which the mixture is clarified by centrifugation or filtration and the resulting solution is injected. CGA 329351 and its S-enantiomer CGA 351920 are separated on a chiral stationary phase (Chiralcel OJ) and detected by UV detection at 230 nm. Identification of the enantiomer peaks is done by comparison of the retention times with those of a reference solution (= metalaxyl-M or corresponding racemate of known content) and optical purity is calculated by comparison of peak areas (= same procedure as method AW-183/3).

2) by polarimetry : the formulation sample is dissolved in acetone by sonication, after which the mixture is clarified by centrifugation or filtration. The optical rotation of the resulting test solution and the reference solution (= metalaxyl-M of known content and optical purity) is measured at 25°C at 589 nm (10 cm path length, 10 s integration time) and optical purity is calculated by calculation of the enantiomeric excess of CGA 329351

In case of interferences from other active substances or formulation auxiliaries, a clean up of the formulation sample is recommended to separate CGA 329351 and CGA 351920 from the formulation matrix. However, the exact chromatographic clean up procedure depends on the composition of the formulation and must be elaborated for each formulation separately.

Findings/conclusions :

No validation data were presented, except for a test chromatogram obtained with the HPLC method. It is however not clear which formulation the chromatogram refers to and if the procedure used required any clean up steps.

B.4.1.4 Analytical methods for the determination of relevant impurities, additives and formulants in plant protection products (Annex IIIA 5.1.2)

No methods were submitted.

The applicant states :

- 'no toxicologically, ecotoxicologically or environmentally relevant impurities will be formed by the manufacturing processes of RIDOMIL GOLD 480 EC and RIDOMIL GOLD MZ 68 WP, or from degradation during storage'
- 'since all batches of CGA 329351 coming out of commercial production are checked for the presence of 2,6-dimethylaniline, Novartis Crop Protection respectfully submits that an analytical method for detection of this moiety in formulations is not necessary. Any production material that does not meet the stated specification limits, would not be released for formulation or sales.'
- 'RIDOMIL GOLD 480 EC and RIDOMIL GOLD MZ 68 WP contain no additives (e.g. stabilizers) and no formulants of toxicological or environmental significance'

Nevertheless, the notifier submitted a further statement concerning the determination of 2,6-dimethylaniline in formulations (Schneider, 1998b) : 'the determination of the content of 2,6-dimethylaniline in formulations may be performed according to analytical method AK-183/2. However, the weight of the test sample must be adapted taking into account the declared content of CGA 329351 in the formulation.

We recommend to check the specificity of the method by GC/MS. In case of interference from a different active ingredient or a formulation auxiliary, a modification of the temperature program is recommended.'

Conclusion :

Validation data demonstrating the applicability of method AK-183/2 for the determination of 2,6-dimethylaniline in formulations were not provided , although this impurity is considered to be of toxicological significance. The proposed method is however very similar to CIPAC method 365/WP/M/4 for determination of 2,6-dimethylaniline in metalaxyl WP formulations, the only difference being the solvent used (tertiair butyl methyl ether instead of carbon tetrachloride). The validation requirement is therefore considered to be sufficiently fulfilled.

B.4.2 Analytical methods for determination of residues

Metalaxyl-M is a mixture of the R-enantiomer CGA 329351 (min. 97%) and the S-enantiomer CGA 351920 (max. 3%) of the racemic compound metalaxyl (CGA 48988), a fungicide which has been used in a large number of applications world-wide for many years.
The analytical methodology described in this section was developed for residue determination of the racemate metalaxyl in various substrates and sample matrices, detecting metalaxyl as a single response signal (not enantiomer-selective). The applicant argues that the developed methods are also suitable for the determination of metalaxyl-M, since CGA 329351 and CGA 351920 are chemically not distinguishable from the racemate and thus exhibit the same analytical properties during residue analysis. Only four methods (REM 181.01, REM 181.02, REM 181.03 and total method AG-395) were validated through fortification of untreated samples with metalaxyl-M. However, these methods are no more enantiomer-selective than the other ones that were validated with metalaxyl.
Some reasons for not developing enantiomer-selective residue methods, as stated by the applicant, are :

- Chromatographic systems for the separation of enantiomers are known predominantly for the analysis of samples with high contents of the analyte(s). The use of such systems in cases where the analyte is present at sub-ppm level and in presence of large amounts of coextracted matrix compounds is not considered possible in routinely conducted analysis.
- Nothing is known sofar about the potential conversion of an applied enantiomer (i.e. CGA 329351) into the racemate (i.e. CGA 48988) (or vice versa) during the time until harvest. Using a non-selective method will provide a "sum of all" result and thus account for all parent active substance present.
- From the viewpoint of enforcement analysis, the availability of a non-selective and rugged racemate residue method is a clear advantage.

B.4.2.1 Analytical methods (residue) for food and feed (Annex IIA 4.2.1; Annex IIIA 5.2.1)

B.4.2.1.1 Analytical methods (residue) for target crops

B.4.2.1.1.1 Residue methods for analyzing parent compound in target crops

Several GC-methods for the determination of parent metalaxyl in target crops were submitted. REM 16/76 was the first method developed and subsequent parent methods are in principle adaptations of REM 16/76. As already mentioned above, REM 181.01, REM 181.02 and REM 181.03 are the only methods that were validated through fortification of untreated samples with metalaxyl-M.
Basically, the methodology consists of extraction of crop material, clean-up steps of the extract involving partitioning and adsorption chromatography (column or cartridge), and/or preparative Liquid Chromatography, followed by final determination by GC (on packed or wide bore columns) using a N-specific detector.

- REM 16/76 : CGA 48988 - Gas Chromatographic determination of residues in soil, vegetable and grapes (Ramsteiner, 1976a)

GLP :
No GLP-compliance stated.
Principle of the method :
The plant material is extracted with methanol in a high speed homogenizer, after which the methanol extracts are diluted with water and cleaned up by water-methanol/dichloromethane partitioning. The dichloromethane phases are evaporated and the remaining residues are cleaned up by alumina column chromatography before the final determination of metalaxyl (CGA 48988) by GC with alkali flame ionisation detector (AFID) or Coulson electrolytic conductivity detector (CECD, N-mode).
Some submitted residue trial reports were analyzed with a modified version of REM 16/76. Modifications are basically : 1) replacement of alumina clean-up by preparative HPLC on LiChrosorb Si 100 (10 µm) column.
2) determination by GC using a wide bore fused silica column (530 µm, coated with 50% phenyl-methyl silicone) and a nitrogen specific detector (NPD)

Findings :
Specificity - interferences : untreated samples of potatoes, beans and grapes showed no significant peaks interfering with that of CGA 48988.
Recovery - precision : for vegetables (potatoes, beans), grapes and soil, the overall mean recovery for fortification levels ranging from 0.05 to 0.5 mg/kg equals 98% (N = 17; RSD = 12%).
Using the modified version of REM 16/76 (supervised residue trials with various crops), the

overall mean recovery for fortification levels ranging from 0.04 to 0.8 mg/kg equals 108% (N = 128; RSD = 12%).

Limit of determination (LOQ) : a value of 0.05 mg/kg is stated

Conclusions :

The summary validation data indicate that in terms of interferences, accuracy (overall mean recovery between 70 and 110%) and precision (overall RSD lower than 20%), the method is suitable for metalaxyl residue analysis in vegetables, grapes and soil. The available data do however not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level of 0.05 mg/kg were not reported. Validation by an independent laboratory was not discussed.

- Anal. Proc. 156 : Gas Chromatographic determination of residues of CGA 48988 in vegetables, grapes and tobacco (fresh leaves) (Anonymous, 1977)

GLP :

No GLP-compliance stated.

Principle of the method :

The method is basically an adaptation of REM 16/76. The plant material is extracted with methanol in a high speed homogenizer and after filtration, the methanol extract is diluted with water and cleaned up by water-methanol/dichloromethane partitioning. The dichloromethane phase is evaporated to dryness and the residue is cleaned up on an alumina column, prior to the final determination of metalaxyl (CGA 48988) by GC with flame thermionic detector. For crops containing waxes and oil (e.g. onions, tobacco), the residue is further cleaned up by hexane/acetonitrile partitioning prior to alumina column chromatography.

Findings :

Specificity - interferences : not discussed by the applicant; no chromatograms shown

Recovery - precision : see table B.4.2.1.1.1-1

For vegetables (lettuce, onions), grapes and tobacco (fresh leaves), the overall mean recovery for fortification levels ranging from 0.1 to 1.0 mg/kg equals 96.4% (N = 8; range = 86-100%; RSD = 5.8%).

Limit of determination (LOQ) : the applicant states the limit of determination to be 0.04 mg/kg, but this value was not substantiated with recovery trials at the corresponding concentration level. Based on the recovery results that were submitted, a limit of determination of 0.1 mg/kg seems appropriate.

Table B.4.2.1.1.1-1 : Validation of method Anal. Proc. 156 (Anonymous, 1977)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
grapes	metalaxyl	0.1 - 1.0	2	95 - 100	97.5	-
lettuce	metalaxyl	0.1 - 1.0	2	100 - 100	100.0	-
onions	metalaxyl	0.1 - 1.0	2	86 - 100	93.0	-
tobacco (fresh leaves)	metalaxyl	0.1 - 1.0	2	90 - 100	95.0	-

Conclusions :

In terms of accuracy (mean recoveries between 70 and 110%) and precision (overall RSD lower than 20%), the method appears suitable for metalaxyl residue analysis in vegetables, grapes and tobacco (fresh leaves) with a LOQ of 0.1 mg/kg. Specificity/interferences and validation by an independent laboratory were not discussed.

Method n° CG-123 : CGA 48988-metalaxyl Gas Chromatographic determination of residues in soil, strawberries, tomatoes, radishes, peppers, onions, sugarbeet, water cress, potatoes, peas and broad beans (Upson, 1980)

GLP :

No GLP-compliance stated.
Principle of the method :
The method is basically an adaptation of REM 16/76. Liquidised plant samples are extracted by shaking with methanol and after filtration, the methanol extracts are diluted with water and cleaned up by water-methanol/chloroform partitioning. The chloroform phases are evaporated to dryness and the remaining residue is cleaned up by alumina column chromatography if interfering peaks are observed in the sample chromatogram. If the latter is not the case, the residue is immediately dissolved in acetone and metalaxyl (CGA 48988) is determined by GC using a nitrogen/phosphorus detector (NPD).
Findings :
Specificity - interferences : not discussed by the applicant; no chromatograms shown
Recovery - precision : for crops (strawberries, tomatoes, radishes, peppers, onions, sugarbeet, water cress, potatoes, peas and broad beans) and soil, recoveries were found to fall in the range of 75 to 100% (fortification levels ranging from 0.05 to 0.5 mg/kg).
The overall mean recovery and RSD are not mentioned in the report, nor can they be calculated (individual recoveries are not listed).
Validation by an independent laboratory is not discussed.
Limit of determination (LOQ) : the applicant states the limit of determination to be 0.01 mg/kg for soil to 0.1 mg/kg for water cress, but the first value was apparently not substantiated with recovery trials at the corresponding concentration level. Based on the summary recovery results that were submitted, a LOQ-range of 0.05 mg/kg to 0.1 mg/kg seems appropriate, although the mean recovery and RSD obtained at these concentration levels were not reported.
Conclusions :
The method is poorly validated.

RES 03/93 (ed.2) : CGA 48988 - Détermination des résidus de CGA 48988 dans les céréales, végétaux et vin par chromatographie phase gazeuse (Bussy, 1995)
GLP :
No GLP-compliance stated.
Principle of the method :
The method is basically an adaptation of REM 16/76. Metalaxyl is extracted from the sample by shaking in the presence of methanol and water. After evaporating the methanol phase, metalaxyl is partitioned into dichloromethane and purified by alumina column chromatography. Final determination is accomplished by capillary GC using a nitrogen/phosphorus detector (NPD).
Findings :
Specificity - interferences : not discussed by the applicant; only chromatograms of wine samples are shown (control appears to exhibit peak at retention time of CGA 48988)
Recovery - precision : for cereals, vegetables and wine, recoveries were found to fall in the range of 70 to 110% (fortification levels ranging from 0.04 to 0.4 mg/kg for plant material and from 0.02 to 0.2 mg/l for wine). The overall mean recovery and RSD are not mentioned in the report, nor can they be calculated (individual recoveries are not listed).
For cucumbers, melons, onions and strawberries (recovery data from reported residue trials), the overall mean recovery for fortification levels ranging from 0.04 to 0.2 mg/kg equals 96% (N = 14; range = 68-122%, RSD = 18%) (see Table B.4.2.1.1.1-2).
Limit of determination (LOQ) : the applicant states the limit of determination to be 0.02 mg/kg for plant material and 0.01 mg/l for wine, but these values were not substantiated with recovery trials at the corresponding concentration levels. Based on the recovery results that were submitted, a limit of determination of 0.04 mg/kg for crops and 0.02 mg/l for wine seems appropriate, although the mean recovery and RSD obtained at the latter concentration level were not reported.

Table B.4.2.1.1.1-2 : Validation of method RES 03/93 (ed. 2) (supervised residue trials)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)

cucumbers, melons, onions, strawberries	metalaxyl	0.04	7	68-110	91	19
		0.2	7	80-122	100	17

Conclusions :
In terms of accuracy (mean recoveries between 70 and 110%) and precision (RSD's lower than 20%), the method appears suitable for residue analysis in cucumbers, melons, onions and strawberries with a LOQ of 0.04 mg/kg. Specificity/interferences and validation by an independent laboratory were not discussed.
The available data do not permit to evaluate the applicability of the method to wine matrices.

REM 181.01 : CGA 329351 - Determination of parent compound by Gas Chromatography (GC) (Plant material) (Kühne, 1995b)
GLP :
The validation report was created in compliance with GLP.
Principle of the method :
Method REM 181.01 is a modified version of method REM 16/76. The homogenized samples are extracted by shaking with methanol, after which the extract is diluted with water and the analyte is partitioned into dichloromethane. After evaporation of the solvent and redissolving the residue in n-hexane, an aliquot of the solution is cleaned up by normal phase preparative HPLC on a silica column. In case of strong interferences, an optional alumina column chromatographic clean-up step may be performed prior to the preparative HPLC clean-up step. After evaporation of the solvent and redissolving the residue in iso-octane/diethylene glycol diethyl ether (98 vol. + 2 vol.), metalaxyl-M (CGA 329351 + CGA 351920) is quantitated by GC on a wide bore column, using a nitrogen-phosphorus detector (NPD). The results may be confirmed using GC-MS quantitation.
Findings :
Specificity - interferences : untreated samples of tomatoes, grapes and potatoes showed no significant peaks interfering with that of metalaxyl-M.
Recovery - precision : see Table B.4.2.1.1.1-3
For tomatoes, grapes and potatoes, the overall mean recovery for fortification levels ranging from 0.02 to 0.2 mg/kg equals 100% (N = 31; range = 84-114%; RSD = 7%)
Limit of determination (LOQ) : for all three matrices, LOQ = 0.02 mg/kg
Repeatability : for tomatoes at LOQ : _ min/max = 3% (N = 8) - single operator
Reproducibility : for tomatoes at LOQ : _ min/max = 16% (N = 11) - two operators from different laboratory groups using different equipment.
Conclusions :
The method is suitable for metalaxyl-M residue analysis in tomatoes, grapes and potatoes with a LOQ of 0.02 mg/kg.

Table B.4.2.1.1.1-3 : Validation of method REM 181.01 (Kühne, 1995b)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation by laboratory group 1						
tomatoes	metalaxyl-M	0.02 0.2	8 8	97 - 100 94 - 114	99 100	1 8
grapes	metalaxyl-M	0.02 0.2	3 3	96 - 107 104 - 106	102 105	5 1
potatoes	metalaxyl-M	0.02 0.2	3 3	84 - 98 107 - 112	92 110	8 3
Validation by laboratory group 2						
tomatoes	metalaxyl-M	0.02	3	90 - 106	96	9

REM 181.02 : CGA 329351 - Determination of parent compound by Gas Chromatography (GC) (must and wine) (Kühne, 1995c)

GLP :

The validation report was created in compliance with GLP.

Principle of the method :

Essentially the same as for REM 181.01, but without the optional alumina column chromatographic clean-up step.

Findings :

Specificity - interferences : untreated samples of must and wine showed no significant peaks interfering with that of metalaxyl-M.

Recovery - precision : see Table B.4.2.1.1.1-4
For grape fractions (must and wine), the overall mean recovery for fortification levels ranging from 0.02 to 0.2 mg/kg equals 97% (N = 27; range = 83-116%; RSD = 10%)

Limit of determination (LOQ) : for the two matrices, LOQ = 0.02 mg/kg

Repeatability : for wine at LOQ : _ min/max = 12% (N = 8) - single operator

Reproducibility : for wine at LOQ : _ min/max = 16% (N = 11) - two operators from different laboratory groups using different equipment.

Conclusions :

The method is suitable for metalaxyl-M residue analysis in must and wine with a LOQ of 0.02 mg/kg.

Table B.4.2.1.1.1-4 : Validation of method REM 181.02 (Kühne, 1995c)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation by laboratory group 1						
grapes (wine)	metalaxyl-M	0.02 0.2	8 8	83 - 95 93 - 116	88 107	5 7
grapes (must)	metalaxyl-M	0.02 0.2	3 5	87 - 97 90 - 103	94 100	6 6
Validation by laboratory group 2						
grapes (wine)	metalaxyl-M	0.02	3	95 - 99	97	2

REM 181.03 : CGA 329351 - Determination of parent compound by Gas Chromatography (GC) (tobacco) (Kühne, 1995d)

GLP :

The validation report was created in compliance with GLP.

Principle of the method :

The homogenized samples are extracted by shaking with methanol/0.1% phosphoric acid (9 vol. + 1 vol.), after which a solid-liquid partition clean-up is performed by shaking with cation exchange resin. The filtrate is diluted with water and the analyte is partitioned into dichloromethane, after which the method follows the same steps as REM 181.01 (alumina clean-up step is mandatory for dried leaves).

Findings :

Specificity - interferences : some control specimens showed a little peak at the characteristic retention time of metalaxyl-M. In these cases, the recoveries were corrected for the control value.

Recovery - precision : see Table B.4.2.1.1.1-5
For tobacco (green and dried leaves), the overall mean recovery for fortification levels ranging from 0.1 to 2.0 mg/kg equals 86% (N = 40; range = 65-108%; RSD = 13%)

Limit of determination (LOQ) : for tobacco (green leaves) : LOQ = 0.1 mg/kg
for tobacco (dried leaves) : LOQ = 0.2 mg/kg

Repeatability : for green leaves at LOQ : _ min/max = 14% (N = 8) - single operator

Reproducibility : for green leaves at LOQ : _ min/max = 22% (N = 11) - two operators from different laboratory groups using different equipment.

Conclusions :

The method is suitable for metalaxyl-M residue analysis in tobacco with a LOQ of 0.1 mg/kg for green leaves and 0.2 mg/kg for dried leaves.

Table B.4.2.1.1.1-5 : Validation of method REM 181.03 (Kühne, 1995d)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation by laboratory group 1						
tobacco (green leaves)	metalaxyl-M	0.1 1.0	8 8	90 - 104 79 - 94	97 89	4 6
tobacco (dried leaves)	metalaxyl-M	0.2 2.0	11 10	65 - 88 66 - 99	78 80	11 14
Validation by laboratory group 2						
tobacco (green leaves)	metalaxyl-M	0.1	3	86 - 108	99	12

REM 21/76 : CGA 48988 - Gas Chromatographic determination of residues in hops and tobacco (Ramsteiner, 1976b)

GLP :

No GLP-compliance stated.

Principle of the method :

The plant material is extracted by shaking with acetone, after which the acetone is evaporated to dryness. The oily residue is dissolved in methanol and the interfering coextractives are precipitated with Celite and $FeCl_3$/$CuSO_4$-solutions. After filtration, the a.s. is partitioned into toluene and the toluene phase is evaporated. The residue is cleaned up by alumina column chromatography before the final determination of metalaxyl (CGA 48988) by GC with alkali flame ionisation detector (AFID) or Hall electrolytic conductivity detector (HECD).

Findings :

Specificity - interferences : when using AFID, dried tobacco samples showed a significant peak interfering with that of metalaxyl, but below the detection limit. When using HECD however, none of the untreated hops and tobacco samples showed any peak interfering with that of metalaxyl.

Recovery - precision : for tobacco and hops, the overall mean recovery for fortification levels ranging from 0.2 to 1.0 mg/kg equals 83% (N = 8; RSD = 18%).

Limit of determination (LOQ) : the applicant states the limit of determination to be 0.1 mg/kg (except for hops detected with AFID where the LOQ is 0.2 mg/kg), but this value was not substantiated with recovery trials at the corresponding concentration level. An overall LOQ of 0.2 mg/kg seems appropriate.

Conclusions :
The summary validation data indicate that in terms of interferences, accuracy (overall mean recovery between 70 and 110%) and precision (overall RSD lower than 20%), the method is suitable for metalaxyl residue analysis in tobacco and hops. The available data do however not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level of 0.2 mg/kg were not reported.Validation by an independent laboratory was not discussed.

REM 1/80 : CGA 48988 - Gas Chromatographic determination of residues in wine and beer (Büttler, 1980)
GLP :
No GLP-compliance stated.
Principle of the method :
The sample is extracted by partition chromatography with hexane on a EXTRELUT-column, after which the eluate is evaporated to dryness. The residue is redissolved in toluene and cleaned up by alumina column chromatography before the final determination of metalaxyl (CGA 48988) by GC using a nitrogen-phosphorus flame ionisation detector (NPID).
Findings :
Specificity - interferences : untreated samples showed no measurable peak interfering with that of metalaxyl.

Recovery - precision : for wine and beer, the overall mean recovery for fortification levels ranging from 0.01 to 0.5 mg/kg equals 102% (N = 12; RSD = 7.8%).

Limit of determination (LOQ) : the applicant states the limit of determination to be 0.005 mg/kg, but this value was not substantiated with recovery trials at the corresponding concentration level. A LOQ of 0.01mg/kg seems appropriate.

Conclusions :
The summary validation data indicate that in terms of interferences, accuracy (overall mean recovery between 70 and 110%) and precision (overall RSD lower than 20%), the method is suitable for metalaxyl residue analysis in wine and beer. The available data do however not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level of 0.01 mg/kg were not reported. Validation by an independent laboratory was not discussed.

B.4.2.1.1.2 Residue methods for analyzing total residues (parent compound + metabolites containing the 2,6-dimethylaniline moiety) in target crops

Several methods (GC and HPLC) for total metalaxyl residue analysis in target crops by determination of the common moiety 2,6-dimethylaniline (DMA) were submitted. AG-348 was the first method developed, involving derivatization of the formed aniline, while the other methods are improvements and simplifications of AG-348. Method AG-395 is the only method that was also validated through fortification of untreated samples with metalaxyl-M.

As these methods are common-moiety methods, they are not specific for metalaxyl and its metabolites forming DMA upon hydrolysis; other compounds containing the DMA-moiety will be detected too.

- AG-348 : Analytical method for the determination of total residues of metalaxyl in crops as 2,6-dimethylaniline (Balasubramanian, 1980a)
GLP :
No GLP-compliance stated.
Principle of the method :
Crop samples with high moisture content (e.g. potatoes, lettuce, cabbage, fruits, broccoli, etc.) are extracted by blending with 20% water/methanol, while dry crop samples (e.g. cottonseed, soybeans, etc.) are extracted by refluxing with 20%

water/methanol. Cottonseed extracts are partitioned between acetonitrile and hexane in order to remove the oils and fats which cause interferences in the method.
The sample extract is then evaporated and refluxed overnight with phosphoric acid in the presence of cobalt chloride, after which the solution is basified and the 2,6-dimethylaniline (DMA) formed is steam distilled. The steam distilled product is immediately derivatized with trichloroacetylchloride, after which the derivative is cleaned up by alumina column chromatography and analyzed by GC using an alkali flame ionisation detector (AFID) operating in the N-specific mode. Cottonseed samples are subjected to an additional silica gel column clean-up before the GC analysis. Samples that are subject to interferences in the AFID detector (e.g. cabbage, cauliflowers, onions) are analyzed by GC-MS, which can also be used to confirm the residues analyzed by AFID.
Findings :
Specificity - interferences : when using AFID, selected samples of cole crops showed an interference peak. However, when these samples were analyzed by GC-MS, no interference was seen.
Recovery - precision : see Table B.4.2.1.1.2-1
For a range of crops, the overall mean recovery for fortification levels ranging from 0.05 to 1.0 mg/kg equals 68% (N = 76; RSD = 26%).
Limit of determination (LOQ) : a value of 0.05 mg/kg is stated.

Table B.4.2.1.1.2-1 : Validation of method AG-348 (Balasubramanian, 1980a)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
potatoes	total metalaxyl	0.05 - 1.0	10	60 - 83	72	13.9
winter wheat, soybeans, corn, lettuce, sweet potatoes, sugarbeet, rye	total metalaxyl	0.05 - 1.0	24	-	72	15.3
cucurbits	total metalaxyl	0.05 - 0.4	18	53 - 95	72	15.3
cole crops	total metalaxyl	0.05 - 0.5	24	42 - 79	59	18.6

Conclusions :
The summary validation data indicate that in terms of interfering blanks, accuracy (mean recoveries between 70 and 110%) and precision (RSD's lower than 20%), the method is suitable for total metalaxyl residue analysis in potatoes, winter wheat, soybeans, corn, lettuce, sweet potatoes, sugarbeet, rye and cucurbits. In the case of cole crops on the other hand, the method doesn't meet all requirements (mean recovery < 70%), which is also reflected in the overall mean recovery (68%) and RSD (26%).
The available data do not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level of 0.05 mg/kg were not reported. Validation by an independent laboratory was not discussed.

- AG-395 : Improved method for the determination of total residues of metalaxyl in crops as 2,6-dimethylaniline (Balasubramanian & Perez, 1982)
- Validation of analytical method AG-395 using ^{14}C-metalaxyl treated lettuce (Perez, 1983)
- Validation of analytical methodology for CGA-329351 with lettuce samples treated with ^{14}C-CGA-329351 (Eudy, 1996)

GLP :
GLP-compliance stated for study by Eudy (1996).
Principle of the method :
Method AG-395 is a modification of method AG-348. Crops with high moisture content (e.g. lettuce, cabbage, fruit,

broccoli, cucumber, onion etc.) are extracted by blending with 80% (v/v) methanol/water, while dry crops (e.g. cottonseed, grain, dry straw etc.) are extracted by refluxing with 80% (v/v) methanol/water. An aliquot of the sample extract is evaporated to dryness, after which the residue is redissolved in water and the sample is refluxed for 15 min with methanesulfonic acid (refluxing for more than 20 min may cause losses through degradation of 2,6-dimethylaniline (DMA)). After addition of water, the solution is basified and the 2,6-dimethylaniline formed in the reaction is steam distilled. In the case of nut meat samples, the solution is partitioned with hexane prior to basification.
The steam distilled product is next cleaned up using a Silica Sep-Pak cartridge, after which trifluoroacetic acid (TFA) is added to the eluate to form the DMA-TFA salt and the sample is evaporated to dryness. Analysis of the sample is performed by capillary GC using a nitrogen/phosphorous detector (NPD) operating in the N-specific mode. Excess TFA is removed by partitioning with dilute aqueous base prior to GC, to avoid adsorption effects in the injection port. GC-MS can be used for confirmation of the results.
Findings :
Specificity - interferences : typical chromatograms are shown.
Recovery - precision : see Table B.4.2.1.1.2-2
Limit of determination (LOQ) : for the crops tested, LOQ = 0.05 mg/kg.

Table B.4.2.1.1.2-2 : Validation of method AG-395

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation 1 (Balasubramanian & Perez, 1982; Perez, 1983)						
avocado, asparagus, beans, broccoli, cabbage, cantaloupes, cauliflower, cucumbers, peanuts, peas, squash	total metalaxyl	0.05 0.10 0.20 0.50 1.0 2.0 5.0	12 21 13 20 14 7 2	- - - - - - -	90.1 93.1 89.0 92.2 76.9 84.1 113.0	13.3 18.3 20.2 22.8 32.5 14.3 -
		0.05 - 5.0	89	-	89	19
Validation 2 (Eudy, 1996)*						
lettuce	total metalaxyl-M	0.05 1.0 5.0	3 2 1	80 - 116 73 - 87 74	97 80 -	19 - -
		0.05 - 5.0	6	73 - 116	88	19

* no addition of TFA

Conclusions :
The summary validation data indicate that in terms of accuracy (overall mean recovery between 70 and 110%) and precision (overall RSD lower than 20%), the method is suitable for total metalaxyl residue analysis in avocado, asparagus, beans, broccoli, cabbage, cantaloupes, cauliflower, cucumbers, peanuts, peas and squash with a LOQ of 0.05 mg/kg. Only at fortification levels 0.5 mg/kg and 1.0 mg/kg (RSD > 20%) and 5 mg/kg (mean recovery > 110%), the method doesn't meet all requirements.
Applicability of the method for total metalaxyl-M residue analysis was demonstrated in lettuce.
Validation by an independent laboratory was not discussed.

- REM 143.01 : Metalaxyl (CGA 48988) - Determination of parent compound and metabolites by High

Performance Liquid Chromatography (HPLC), after conversion into 2,6-dimethylaniline (common moiety) (plant material) (Kühne, 1995a)

GLP :

The analytical part of the recovery studies was performed in a GLP-certified facility, although GLP-compliance was only stated for the studies in group A (fortification levels from 0.04 - 0.4 mg/kg).

Principle of the method :

Method REM 143.01 is a modification of method AG-395 : it comprises essentially the same procedures for extraction, hydrolysis and steam distillation, but involves a different approach for the chromatographic determination. The homogenized samples are extracted by refluxing with methanol/water (80% v/v). An aliquot of the extract is, after evaporation of the solvent, subjected to hydrolysis through refluxing with methanesulfonic acid for 15 min, after which the solution is alkalinized. The 2,6-dimethylaniline formed is isolated by steam distillation and partitioned into n-hexane. After partition into methanol-0.2 M HCl (2 + 8), the analyte is further cleaned up and quantitated using a two-column switching HPLC-system with electrochemical detection.

Findings :

Specificity - interferences : most control specimens used for the recovery experiments show a little peak at the characteristic retention time of dimethylaniline (DMA) (height between 8 and 31% of the corresponding value obtained at the lowest fortification level). It has not been investigated whether this peak is due to an interference, to contamination with metalaxyl or to the presence of other compounds which can be converted to DMA under the present conditions. All recoveries were thus corrected for the control value.

Recovery - precision : see Table B.4.2.1.1.2-3

For a range of crops, the overall mean recovery for fortification levels ranging from 0.04 to 2.0 mg/kg equals 80% (N = 65; range = 56-99%; RSD = 11%).

Apparently, the data were generated by several operators from different laboratory groups, but it is not indicated which data were generated by which group.

Limit of determination (LOQ) : according to the applicant, the limit of determination was set at 0.02 mg/kg for some types of plant material (first set of matrices in Table B.4.2.1.1.2-3) and 0.04 mg/kg for other types (second set of matrices). However, these values were not substantiated with recovery trials at the corresponding concentration levels. A LOQ of 0.04 mg/kg for the first set of plant types, 0.08 mg/kg for the second set and 0.2 mg/kg for grapes seems appropriate.

Table B.4.2.1.1.2-3 : Validation of method REM 143.01 (Kühne, 1995a)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
potatoes, apples, alfalfa, cauliflower, sugarbeets, broccoli, soybeans, rapeseed, peas (dried seeds),	total metalaxyl	0.04	18	61 - 99	78	11
		0.4	21	61 - 90	78	9

broad beans (seeds), strawberries						
peas (haulms & empty pods), broad beans (whole plant)	total metalaxyl	0.08 0.8	5 6	78 - 94 80 - 88	87 84	8 3
grapes	total metalaxyl	0.2 2.0	7 8	75 - 98 56 - 83	86 74	10 13

Conclusions :
In terms of interfering blanks, accuracy (mean recoveries between 70 and 110%) and precision (RSD's lower than 20%), the method is suitable for total metalaxyl residue analysis in potatoes, apples, alfalfa, cauliflower, sugarbeets, broccoli, soybeans, rapeseed, peas (dried seeds), broad beans (seeds) and strawberries with a LOQ of 0.04 mg/kg; in peas (haulms & empty pods) and broad beans (whole plant) with a LOQ of 0.08 mg/kg and in grapes with a LOQ of 0.2 mg/kg. Validation by an independent laboratory was not discussed as such in the report.

- REM 16/80 : CGA 48988 (metalaxyl) - Determination of the active ingredient of RIDOMIL and its metabolites convertible to 2,6-dimethylaniline (CGA 72649) in green plant material (Ramsteiner, 1980)

GLP :
No GLP-compliance stated.
Principle of the method :
Samples are extracted by homogenizing with 80% (v/v) methanol-water, after which the extract is evaporated to dryness. The residue is subjected to acidic hydrolysis with 6 N HCl for 12 h at 460 K, after which the acidic aqueous solution is alkalinized and the generated 2,6-dimethylaniline is isolated by steam distillation and partitioning into iso-octane. The 2,6-dimethylaniline is derivatized with 3,4-dichlorobenzoylchloride in pyridine and the derivative is cleaned up by alumina column chromatography prior to the GC determination using an AFID detector in the N-specific mode.
Findings :
Specificity - interferences : control samples contained an interfering peak at levels of 0.1 mg/kg
Recovery - precision : for grape leaves and lettuce, recoveries were found to fall in the range of 50 to 75% (fortification levels ranging from 0.5 to 1.0 mg/kg). The overall mean recovery and RSD are not mentioned in the report, nor can they be calculated (individual recoveries are not listed). Validation by an independent laboratory is not discussed.
Limit of determination (LOQ) : the applicant states the limit of determination to be 0.2 mg/kg, but this value was not substantiated with recovery trials at the corresponding concentration level
Conclusions :
The method is poorly validated.

B.4.2.1.2 Analytical methods (residue) for food of animal origin

Two GC-methods for total metalaxyl residue analysis in animal products by determination of the common moiety 2,6-dimethylaniline (DMA) were submitted. AG-349 was the first method developed, involving derivatization of the formed aniline, while AG-576 is an improvement of AG-349.

As these methods are common-moiety methods, they are not specific for metalaxyl and its metabolites forming DMA upon hydrolysis; other compounds containing the DMA-moiety will be detected too.

- AG-349 : Analytical method for the determination of total residues of metalaxyl in animal tissues, milk and eggs as 2,6-dimethylaniline (Balasubramanian, 1980b)

GLP :
No GLP-compliance stated.
Principle of the method :
Milk is extracted by shaking with acetonitrile, tissue samples by blending with 20% water/acetonitrile and egg samples by blending with acetonitrile, while fat samples are extracted by blending with hexane. An aliquot of the extract is

partitioned between acetonitrile and hexane to remove the oils and fats which cause interferences in the method, after which the sample extract is evaporated and refluxed overnight with phosphoric acid in the presence of cobalt chloride. The solution is basified and the 2,6-dimethylaniline formed is steam distilled.
The steam distilled product is derivatized with trichloroacetyl chloride, after which the derivative is cleaned up by alumina column chromatography and analyzed by GC using an alkali flame ionization detector (AFID) operating in the N-specific mode. Liver and kidney samples are subjected to an additional silica gel column clean-up before the GC-analysis. Milk and other samples showing interferences in the AFID detector are analyzed by GC-MS.
Findings :
Specificity - interferences : an interference peak was seen in some liver and kidney samples using the AFID detector, while these samples analyzed by GC-MS showed < 0.1 mg/kg interference.
Recovery - precision : see Table B.4.2.1.2-1
Limit of determination (LOQ) : the applicant states the LOQ to be 0.05 mg/kg for muscle and fat tissues and eggs, but no validation data were provided with respect to these substrates. For milk (0.01 mg/kg) and liver (0.1mg/kg) the lower fortification level of the submitted recovery trials was proposed.

Table B.4.2.1.2-1 : Validation of method AG-349 (Balasubramanian, 1980b)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
milk	total metalaxyl	0.01 - 0.10	10	52 - 76	66	12
liver	total metalaxyl	0.1 - 0.4	5	54 - 116	83	28

Conclusions :
The summary validation data indicate that in terms of accuracy and precision, the method doesn't meet all requirements for total metalaxyl residue analysis in milk (mean recovery < 70%) and liver (RSD > 20%). Applicability of the method to muscle and fat tissues and eggs could not be evaluated.
The available data do not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level (0.01 mg/kg for milk and 0.1 mg/kg for liver) were not reported. Validation by an independent laboratory was not discussed.

- AG-576 : Improved analytical method for the determination of total residues of metalaxyl in poultry tissues and eggs as 2,6-dimethylaniline (Cudd & Eudy, 1991)
- Validation of analytical method AG-576 for the determination of total residues of metalaxyl in goat tissues, milk, poultry tissues and eggs (Yokley & Mc. Killican, 1991)

GLP :
The validation report was created in compliance with GLP.
Principle of the method :
Method AG-576 is an improved version of method AG-349. Tissue samples are extracted by homogenizing with 20% water/acetonitrile and egg/milk samples by homogenizing with acetonitrile, while fat and skin samples are extracted by homogenizing with hexane (for goat omental fat : extraction with 20% water/acetonitrile). An aliquot of the extract is partitioned between acetonitrile and hexane to remove the oils and fats which cause interferences in the method, after which the sample extract is evaporated to dryness and refluxed for 12-15 min after addition of methanesulfonic acid (refluxing for more than 15-20 min may cause losses through degradation of DMA). The solution is basified and the 2,6-dimethylaniline formed is steam distilled.
The steam distilled product is cleaned up with a silica Sep-Pak cartridge prior to analysis by capillary GC using a nitrogen/phosphorus detector (NPD). GC-MS can be used for confirmation of the results.
Findings :
Specificity - interferences : interferences were observed in several untreated liver control samples. Apparently natural products in liver samples produced an analyte that co-chromatographed with

dimethylaniline. In these cases, recoveries were corrected for the control value.

Recovery - precision : see Table B.4.2.1.2-2
For poultry matrices (eggs, fat/skin, liver, breast) and goat matrices (milk, omental fat, liver, leg muscle), the overall mean recovery for fortification levels ranging from 0.01 to 2.0 mg/kg equals 98% (N = 76; range = 52-194%; RSD = 20%).
Validation by an independent laboratory was not discussed as such, but 2 sets of poultry validation data were submitted. According to the applicant, data were generated by different laboratory groups at different times.

Limit of determination (LOQ) : the applicant states the LOQ to be 0.01 mg/l for milk and 0.05 mg/kg for the other substrates (= lower fortification levels of submitted recovery trials)

Table B.4.2.1.2-2 : Validation of method AG-576 (Cudd & Eudy, 1991; Yokley & Mc. Killican, 1991)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation 1 (Cudd & Eudy, 1991)						
poultry eggs	total metalaxyl	0.05 - 1.0	14	82 - 118	98	13
poultry fat/skin	total metalaxyl	0.05 - 1.0	16	92 - 125	106	9
poultry liver	total metalaxyl	0.1 - 2.0	14	64 - 125	93	21
poultry breast	total metalaxyl	0.05 - 1.0	8	85 - 104	94	7
Validation 2 (Yokley & Mc. Killican, 1991)						
poultry eggs	total metalaxyl	0.05 - 0.3	3	87 - 93	90	3
poultry fat/skin	total metalaxyl	0.05 - 0.5	3	82 - 96	88	8
poultry liver	total metalaxyl	0.05 - 1.50	3	103 - 194	135*	38*
poultry breast	total metalaxyl	0.05 - 0.5	3	75 - 115	93	22
goat milk	total metalaxyl	0.01 - 0.05	3	88 - 159	112**	36**
goat omental fat	total metalaxyl	0.05 - 0.067	3	83 - 127	101	23
goat liver	total metalaxyl	0.05 - 2.0	3	52 - 101	73***	35***
goat leg muscle	total metalaxyl	0.05 - 0.2	3	88 - 104	94	9

* when omitting the most extreme recovery (194) as outlier : mean = 105%
** when omitting the most extreme recovery (159) as outlier : mean =89%
*** when omitting the most extreme recovery (52) as outlier : mean =83.5%

Conclusions :
The method is suitable for total metalaxyl residue analysis in eggs, muscle tissue and fat/skin with a LOQ of 0.05 mg/kg, but in the case of liver and milk samples, mean recovery and RSD do not always meet the requirements. Only if the most extreme recoveries are omitted as outliers, as suggested by the applicant, mean recoveries fall within the required range (70 - 110%) with the corresponding RSD's being almost entirely less than 20%.
The available data do not permit to establish the LOQ for milk unequivocally, since only 2 recovery tests were performed at the lower fortification level of 0.01 mg/l, one of which was considered to be an outlier. For liver a LOQ of 0.1 mg/kg seems defensible.

B.4.2.2 Analytical methods (residue) in soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2.2 to 5.2.4)

B.4.2.2.1 Analytical methods for soil (Annex IIA 4.2.2; Annex IIIA 5.2.2)

Three GC-methods for metalaxyl residue analysis in soil were submitted : two nearly identical methods (REM 16/76 and Method n° CG-123) for the determination of parent metalaxyl and a GC-method (REM 7/77) that allows to determine parent CGA 48988 as well as its major metabolite CGA 62826 (metalaxyl acid).

- REM 16/76 : CGA 48988 - Gas Chromatographic determination of residues in soil, vegetable and grapes (Ramsteiner, 1976a)

GLP :
No GLP-compliance stated.
Principle of the method :
Original method: homogenized soil samples are extracted with methanol in a hot extractor, after which the methanol extracts are diluted with water and cleaned up by water-methanol/dichloromethane partitioning. The dichloromethane phases are evaporated and the remaining residues are cleaned up by alumina column chromatography before the final determination of metalaxyl (CGA 48988) by GC with alkali flame ionisation detector (AFID) or Coulson electrolytic conductivity detector (CECD, N-mode).
Some submitted residue trial reports were analyzed with a *modified version* of REM 16/76. Modifications are basically :
1) replacement of alumina clean-up by preparative HPLC on LiChrosorb Si 100 (10 µm) column.
2) determination by GC using a wide bore fused silica column (530 µm, coated with 50% phenyl-methyl silicone) and a nitrogen specific detector (NPD)

Findings :
Specificity - interferences : untreated samples of soil showed no significant peaks interfering with that of CGA 48988 when using the original method.
Recovery - precision : • original method : for soil, vegetables (potatoes, beans) and grapes, the overall mean recovery for fortification levels ranging from 0.05 to 0.5 mg/kg equals 98% (N = 17; RSD = 12%).
• modified version (supervised residue trials with soil) : see Table B.4.2.2.1-1
Limit of determination (LOQ): a value of 0.05 mg/kg is stated for the original method, while for the modified version a LOQ of 0.02 mg/kg is proposed by the notifier. The latter value is however not substantiated with recovery tests at the corresponding concentration level.

Table B.4.2.2.1-1 : Validation of method REM 16/76 (supervised residue trials)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	metalaxyl	0.04 0.4	11 7	90 - 130 87 - 125	112 115	11 11
		0.04 - 0.4	18	87 - 130	114	11

Conclusions :
The summary validation data indicate that in terms of interfering blanks, accuracy (overall mean recovery between 70 and 110%) and precision (overall RSD lower than 20%), the *original method* is suitable for metalaxyl residue analysis in vegetables, grapes and soil. The available data do however not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level of 0.05 mg/kg were not reported.
The *modified version* doesn't meet the requirements in terms of accuracy (mean recoveries slightly above 110%). According to the notifier this may be explained by an increase in the peak height due to soil coextractives, although the latter should normally be covered by subtraction of the corresponding control values. Apart from that, the proposed LOQ of 0.04 mg/kg appears defensible. Chromatograms were not shown.

- REM 7/77 : Gas Chromatographic residue determination of CGA 48988 and its main metabolite CGA 62826 in soil (provisional) (Ramsteiner, 1977)

GLP :

No GLP-compliance stated.

Principle of the method :

Original method: homogenized soil samples are extracted with methanol and aqueous buffer solution (pH 10), after which the extract is diluted with water and CGA 48988 is partitioned from this alkaline solution into dichloromethane. The organic phase is evaporated to dryness and the residues are cleaned up by alumina column chromatography before the final determination by GC with AFID or CECD or HECD.

The aqueous phase is acidified to pH 3 with 0.1 N HCl, after which the metabolite CGA 62826 is partitioned into dichloromethane. This dichloromethane phase is evaporated and the residues methylated with diazomethane. The resulting derivative (= CGA 48988) is cleaned up by alumina column chromatography before the final determination by GC.

Some supervised trials were analyzed with a *modified version* of REM 7/77. The main modifications are :

- reduction of the subsample size for analysis (25 g instead of 50 g), with corresponding reduction of amount of solvent
- replacement of alumina clean-up by preparative HPLC on LiChrosorb Si 100 (10 µm) column.
- determination by GC using a wide bore fused silica column (530 µm, coated with 50% phenyl-methyl silicone) and a nitrogen specific detector (NPD)

Findings :

Specificity - interferences : untreated samples of soil showed no significant peaks interfering with that of CGA 48988 when using the original method.

Recovery - precision :
- original method : for soil, the overall mean recovery for fortification levels ranging from 0.05 to 0.5 mg/kg equals 98% for metalaxyl and 80% for CGA 62826 (N = 17; RSD for metalaxyl = 12%, RSD for CGA 62826 not reported)
- modified version (analyses of supervised trials) : see Table B.4.2.2.1-2

Limit of determination (LOQ) : for both substances a value of 0.05 mg/kg is stated for the original method, while for the modified version the notifier proposes a LOQ of 0.02 mg/kg. The latter value is however not substantiated with recovery tests at the corresponding concentration level.

Table B.4.2.2.1-2 : Validation of method REM 7/77 (supervised residue trials)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
soil	metalaxyl	0.04 0.4	37 37	78 - 138 84 - 132	108 107	13 12
		0.04 - 0.4	74	78 - 138	108	13
	CGA 62826	0.04 0.4	36 36	65 - 129 56 - 134	103 101	17 14
		0.04 - 0.4	72	56 - 134	102	16

Conclusions :

The summary validation data indicate that in terms of interfering blanks, accuracy (overall mean recoveries between 70 and 110%) and precision (overall RSD for metalaxyl lower than 20%), the *original method* is suitable for residue analysis in soil. For acid metabolite CGA 62826, RSD was however not mentioned. The available data do not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the lower fortification level of 0.05 mg/kg were not reported.

The *modified version* is suitable for residue analysis of metalaxyl and CGA 62826 in soil with a LOQ of 0.04 mg/kg.

- Method n° CG-123 : CGA 48988-metalaxyl - Gas Chromatographic determination of residues in soil, strawberries, tomatoes, radishes, peppers, onions, sugarbeet, water cress, potatoes, peas and broad beans (Upson, 1980)

GLP :
No GLP-compliance stated.
Principle of the method :
The method is basically an adaptation of REM 16/76. Sieved, homogenized soil samples are extracted with methanol and after filtration, the methanol extracts are diluted with water and cleaned up by water-methanol/chloroform partitioning. The chloroform phases are evaporated to dryness and the remaining residue is cleaned up by alumina column chromatography if interfering peaks are observed in the sample chromatogram. If the latter is not the case, the residue is immediately dissolved in acetone and examined by GC using a nitrogen/phosphorus detector (NPD).
Findings :
Specificity - interferences : not discussed by the applicant; no chromatograms shown
Recovery - precision : for the different types of substrate (both cleaned-up and non-cleaned-up samples), recoveries were found to fall in the range of 75 to 100% for fortification levels ranging from 0.05 to 0.5 mg/kg.
The mean recoveries and RSD's (overall, as well as for soil samples as such) are not mentioned in the report, nor can they be calculated (individual recoveries are not listed).
Limit of determination (LOQ) : the applicant states the limit of determination to be 0.01 mg/kg for soil, but this value was not substantiated with recovery trials at the corresponding concentration level. Based on the summary recovery results that were submitted, a limit of determination of 0.05 mg/kg seems appropriate, although the mean recovery and RSD obtained for soil at this concentration level were not reported.
Conclusions :
The method is poorly validated.

B.4.2.2.2 Analytical methods for water (Annex IIA 4.2.3; Annex IIIA 5.2.3)

Two HPLC-methods for the determination of parent metalaxyl and/or major soil metabolite CGA 62826 in water were submitted : one method (REM 2/86) determining CGA 48988 and CGA 62826 as single compounds and another method (REM 12/87) determining the sum of both compounds.

- REM 2/86 : Metalaxyl (CGA 48988) - Water : Determination of residues of parent compound and metalaxyl acid (CGA 62826) by High Performance Liquid Chromatography (HPLC) (Formica & Giannone, 1986)

GLP :
No GLP-compliance stated.
Principle of the method :
The entire water sample is acidified with 85% o-phosphoric acid and passed through a Bond Elute C_{18} cartridge by suction. Metalaxyl and metalaxyl acid are eluted with acetonitrile and determined during the same analytical run by HPLC using UV detection at 210 nm.
Findings :
Specificity - interferences : the applicant states that interfering signals from similar compounds or coextracted matrix components were not observed sofar; confirming chromatograms are shown.
Recovery - precision : see Table B.4.2.2.2-1
For water (HPLC-grade), the overall mean recovery for fortification levels ranging from 0.1 to 2 µg/l equals 104 % for metalaxyl (N = 12; range = 96-110%; RSD = 5%) and 98% for metalaxyl acid (N = 12; range = 87-104%; RSD = 4%).
Limit of determination (LOQ) : the applicant states the limit of determination to be 0.05 µg/l for each compound, but this value was not substantiated with recovery trials at the

corresponding concentration level. A LOQ of 0.1 µg/l for each compound seems appropriate.

Table B.4.2.2.2-1 : Validation of method REM 2/86 (Formica & Giannone, 1986)

Matrix	Analyte	Fortification level (µg/l commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
water (HPLC-grade)	parent metalaxyl	0.1 0.5 2.0	6 2 4	105 - 110 98 - 100 96 - 100	109 99 98	2 - 2
	metalaxyl acid	0.1 0.5 2.0	6 2 4	87 - 104 98 - 100 97 - 98	98.5 99 97.5	6 - 0.6

Conclusions :
The method is suitable for metalaxyl and metalaxyl acid residue analysis in drinking water with a LOQ of 0.1 µg/l.

- REM 12/87 : Metalaxyl (CGA 48988) - Potable Water : Determination of residues of parent compound and/or metalaxyl acid (CGA 62826) by High Performance Liquid Chromatography (HPLC) (Formica & Giannone, 1987)

GLP :
No GLP-compliance stated.
Principle of the method :
The entire water sample is acidified with 85% o-phosphoric acid and passed through a Bond Elute C_{18} cartridge by suction. Metalaxyl and metalaxyl acid are eluted with acetonitrile, after which the metalaxyl acid in the eluate is methylated with diazomethane to yield metalaxyl. The sum of both compounds is determined as metalaxyl by HPLC (2 column switch) using UV detection at 220 nm.
Findings :
Specificity - interferences : the applicant states that interfering signals from similar compounds or coextracted matrix components were not observed sofar; confirming chromatograms are shown.
Recovery - precision : see Table B.4.2.2.2-2
For water (HPLC-grade), the overall mean recovery of metalaxyl and metalaxyl acid (determined together as metalaxyl) for fortification levels ranging from 0.05 to 0.25 µg/l of each compound, equals 97% (N = 14; range = 91-105%; RSD = 3.3%).
Limit of determination (LOQ) : the applicant states the limit of determination to be 0.05 µg/l for the sum of both compounds. However, in the submitted recovery tests the lower fortification level was set at a concentration of 0.05 µg/l of each compound, thus a total concentration of "metalaxyl measured" of 0.1 µg/l. We therefore propose a LOQ of 0.1 µg/l.

Table B.4.2.2.2-2 : Validation of method REM 12/87 (Formica & Giannone, 1987)

Matrix	Analyte	Fortification level (µg/l commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
water (HPLC-grade)	parent metalaxyl and metalaxyl acid (determined as metalaxyl)	0.05 each	7	91 - 105	97	5
		0.25 each	7	96 - 98	97	1

Conclusions :
The method is suitable for "sum of metalaxyl and metalaxyl acid" residue analysis in drinking water with a LOQ of 0.1 µg/l.

In addition, a GC-method (REM 181.04) for the determination of metalaxyl-M in potable and surface water was provided. Like the other methods, REM 181.04 is however not enantioselective.

- REM 181.04 : Metalaxyl-M (CGA 329351) - Water : Determination of parent compound by Gas Chromatography (Tribolet, 1998a)
- Report on special study 223/98 : Validation of method REM 181.04 by analysis of fortified water specimens for metalaxyl-M (CGA 329351) and evaluation of recoveries (Tribolet, 1998b)
GLP :
GLP-compliance stated for the validation study (Tribolet, 1998b)
Principle of the method :
After addition of some methanol, the water sample is sucked through a C-18 bonded solid phase extraction column (SPE) to concentrate the analyte. The eluate containing metalaxyl-M is evaporated and the residue is redissolved for quantification.
Final clean up and quantification of metalaxyl-M (CGA 329351 + CGA 351920) is performed by GC (DB-1; 1.5 µm) with nitrogen phosphorous detection (NPD); quantification by external standardization. GC-MS (HP-5MS; 0.25 µm) is proposed as confirmatory technique, MS operated in SIM mode (monitored mass : 249 amu).
Findings :
Specificity - interferences : the report states that no interferences were observed sofar; confirming chromatograms are shown. Control values are reported to be < 0.05 µg/l for potable water and < 0.1 µg/l for surface water
Recovery - precision : see Table B.4.2.2.2-3
Limit of determination (LOQ) : 0.05 µg/l for potable water
0.10 µg/l for surface water

Table B.4.2.2.2-3 : Validation of method REM 181.04 (Tribolet, 1998b)

Matrix	Analyte	Fortification level (µg/l commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
potable water	metalaxyl-M	0.05 0.5	5 5	93 - 109 95 - 96	102 95	7.1 0.5
		0.05 - 0.5	10	93 - 109	98	6.0
surface water		0.1 1.0	5 9	63 - 92 79 - 102	84 94	14 7.2
		0.1 - 1.0	14	63 - 102	91	11

Conclusions :
The method is suitable for metalaxyl-M residue analysis in water with a LOQ of 0.05 µg/l for potable water and 0.1 µg/l for surface water.

B.4.2.2.3 Analytical methods for air (Annex IIA 4.2.4; Annex IIIA 5.2.4)

A GC-method (REM 143.02) for the determination of parent metalaxyl in air (vapors, aerosols and coarse dusts) was submitted.

- REM 143.02 : Metalaxyl (CGA 48988) - Sampling of air and determination of residues of parent compound by Gas Chromatography (Tribolet, 1993)
- Report on special study 135/96 - Validation of method REM 143.02 in air : Validation by analysis of fortified specimens and determination of recoveries (Tribolet, 1996)

GLP :
GLP-compliance was stated for the method validation.
Principle of the method :
A defined volume of air is sucked through a sorbent tube using an air sampler pump. The different layers of the tube are separated and CGA 48988 is extracted from the adsorptive matrix with methanol using an ultrasonic bath. The methanol is evaporated and the residue is redissolved in hexane, after which CGA 48988 is determined by GC (SPB-5) using a P/N detector.
Findings :
Specificity - interferences : the applicant states that interfering signals from similar compounds or coextracted matrix components were not observed sofar; confirming chromatograms are shown.
Recovery - precision : see Table B.4.2.2.3-1
No break through onto the second set of layers was observed (< 2%)
Limit of determination (LOQ) : 2 µg/m³

Table B.4.2.2.3-1 : Validation of method REM 143.02

Matrix	Analyte	Fortification level (µg/m³ commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Validation 1 (Tribolet, 1993)						
indoor air (22°C, 29% RH)	metalaxyl	10 - 100	3	80 - 118	102	19
outdoor air (4 °C, 90% RH)		10 - 100	3	76 - 113	97	20
Validation 2 (Tribolet, 1996)						
air (36°C, 83%RH)	metalaxyl	2 20 50	8 8 3	107 - 113 88 - 92 76 - 93	110 90 82	2 2 12
		2 - 50	19	76 - 113	97	12

Conclusions :
The method is suitable for metalaxyl residue analysis in air with a LOQ of 2 µg/m³.

B.4.2.3 Analytical methods (residue) wildlife and for use in support of diagnostic and therapeutic regimes (Annex IIA 4.2.5; Annex IIIA 5.2.5)

A GC-method (AG-438) for total metalaxyl residue analysis in urine by determination of the common moiety 2,6-dimethylaniline (DMA) was submitted.
As this method is a common-moiety method, it is not specific for metalaxyl and its metabolites forming DMA upon hydrolysis; other compounds containing the DMA-moiety will be detected too.

For methods for the determination of total metalaxyl residues in animal tissues, see point B.4.2.1.2.

- AG-438 : Determination of total residues of metalaxyl in human urine as 2,6-dimethylaniline (Perez & Vincent,

1984)

GLP :

No GLP-compliance stated.

Principle of the method :

Method AG-438 is an adaptation of method AG-395, developed for the analysis of crop substrates. Urine samples are refluxed for 15 min with methanesulfonic acid. The resulting extracts are basified after addition of water, after which the 2,6-dimethylaniline formed is steam distilled.

The steam distilled product is next cleaned up using a silica Sep-Pak cartridge, after which trifluoroacetic acid (TFA) is added to the eluate to form the DMA-TFA salt and the sample is evaporated to dryness. Analysis of the sample is performed by capillary GC using a nitrogen/phosphorus detector (NPD) operating in the N-specific mode. Excess TFA is removed by partitioning with dilute aqueous base prior to GC, to avoid adsorption effects in the injection port.

Findings :

Specificity - interferences : an average background of 0.14 ± 0.07 mg/l (N = 10) has been found in unfortified control samples from California, North Carolina and Ohio, in which the presence of dimethylaniline was confirmed through analysis by GC-MS. Recoveries were corrected for the control value.

Recovery - precision : for human urine, the overall mean recovery for fortification levels ranging from 0.2 to 2 mg/l equals 89% (N = 8; RSD = 26%).

Limit of determination (LOQ) : the applicant reports LOQ to be 0.3 mg/l, taking into account the average background level (0.14 mg/l) found in control samples.

Conclusions :

The summary validation data indicate that in terms of accuracy (overall mean recovery between 70 and 110%), the method is suitable for total metalaxyl residue analysis in human urine. In terms of precision on the other hand, the method doesn't meet the requirements (overall RSD exceeds 20%). The applicant argues this deviation to be tolerable. The available data do not permit to establish the LOQ of the method unequivocally, since the mean recovery and RSD obtained at the proposed LOQ of 0.3 mg/l were not reported.

B.4.3 Evaluation and assessment

B.4.3.1 Evaluation and assessment of analytical methods for technical active substance and formulation analysis

Table B.4.3.1-1 : Summary of analytical methods for technical active substance and formulation analysis

Matrix	Analyte	Type of method	Validation	References
technical active substance	CGA 329351 (R) + CGA 351920 (S) (sum of 2 enantiomers)	GC with FID	full	AW-183/3 (Schneider, 1995a) (Schneider, 1995b)
	CGA 329351 CGA 351920 (separate enantiomers)	HPLC (chiral) with UV detection	full	
technical active substance	organic by-products	GC with FID	full	AK-183/2 (Schneider, 1995c) (Schneider, 1995d)
	water	Karl Fischer	full	
	nitrosamines	chemical cleavage with chemiluminescence detection	full	AG-20/4 (Kreuzer & Wyden, 1995b) (Kreuzer, 1995a)
formulation A-9408 B (EC)	CGA 329351 (R) + CGA 351920 (S) (sum of 2 enantiomers)	GC with FID	full	AF-1176/1 (Bourgeois, 1995a) (Bourgeois, 1995b)
formulation A-9407 A (WP)	CGA 329351 (R) + CGA 351920 (S) (sum of 2 enantiomers)	GC with FID	full	AF-1167/1 (Bourgeois, 1994) (Bourgeois, 1995c)
	mancozeb	iodometry	full	

Evaluation :
The methods submitted allow to determine the purity (sum of 2 enantiomers, as well as separate enantiomers) and the impurities of the technical a.s., as well as the a.s. content (sum of 2 enantiomers) of formulations (EC and WP). Methods that allow to determine the enantiomer ratio in formulations were not submitted, but they are required to enable the a.s. in the formulation to be distinguished from metalaxyl.
Validated analytical methods for the determination of 2,6-dimethylaniline in formulations also remain to be provided, as this impurity is considered to be of toxicological significance.

The applicant stated that no CIPAC-methods are available for the determination of metalaxyl-M in the technical compound or in formulations; no reference was made to CIPAC-methods 365/TC/M/3 and 365/WP/M/3 (resp. for determination of metalaxyl in metalaxyl technical and WP-formulations).

B.4.3.2 Evaluation and assessment of analytical methods for determination of residues

The submitted analytical methods were developed for determination of residues of the racemate metalaxyl, detecting metalaxyl as a single response signal (not enantiomer-selective). Since metalaxyl-M exhibits the same analytical properties as metalaxyl under these conditions, the developed methods are also suitable for the determination of metalaxyl-M residues.
Consequently the submitted methods do not permit to distinguish between both compounds, which implies that residues resulting from the use of metalaxyl formulations cannot be distinguished from those resulting from the use of metalaxyl-M preparations.

B.4.3.2.1 Analytical methods (residue) for food and feed

Table B.4.3.2.1-1 : Summary of analytical methods (residue) for target crops - parent compound

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation	References
vegetables, grapes, (soil)	metalaxyl	GC with AFID or CECD	0.05**-0.5	summary data provided (no ILV; no validated LOQ)	REM 16/76 (Ramsteiner, 1976a)
vegetables, grapes, tobacco (fresh leaves)	metalaxyl	GC with NPD	0.1*-1.0	data provided (no ILV; no chromatograms)	Anal.Proc.156 (Anonymous, 1977)
strawberries, tomatoes, radishes, peppers, onions, sugarbeet, water cress, potatoes, peas, broad beans	metalaxyl	GC with NPD	0.05-0.5 (0.05-0.1)**	insufficient	Method CG-123 (Upson, 1980)
(cereals), vegetables (cucumbers, onions), strawberries, melons	metalaxyl	GC with NPD	0.04*-0.4	data provided (no ILV; no chromatograms)	RES 03/93 (ed2) (Bussy, 1995)
wine			0.02**-0.2	insufficient	
tomatoes, grapes, potatoes	metalaxyl-M	GC with NPD	0.02*-0.2	full	REM 181.01 (Kühne, 1995b)
wine, must	metalaxyl-M	GC with NPD	0.02*-0.2	full	REM 181.02 (Kühne, 1995c)
tobacco (green leaves)	metalaxyl-M	GC with NPD	0.1*-1.0	full	REM 181.03 (Kühne, 1995d)
tobacco (dried leaves)			0.2*-2.0		
tobacco, hops	metalaxyl	GC with AFID or HECD	0.2**-1.0	summary data provided (no ILV; no validated LOQ)	REM 21/76 (Ramsteiner, 1976b)
wine, beer	metalaxyl	GC with NPD	0.01**-0.5	summary data provided (no ILV; no validated LOQ)	REM 1/80 (Büttler, 1980)

* LOQ = limit of determination, unequivocally established

** provisional LOQ = lower fortification level from recovery tests, for which the mean recovery and RSD were not reported

ILV = independent laboratory validation

Evaluation :

The GC-methods submitted allow to determine parent metalaxyl/metalaxyl-M in food matrices of plant origin. Methods REM 181.01, REM 181.02 and REM 181.03 were fully validated for use in tomatoes, grapes, potatoes, wine, must and tobacco, and appear suitable for enforcement.

Table B.4.3.2.1-2 : Summary of analytical methods (residue) for target crops - total residues

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation	References
potatoes, winter wheat, soybeans, corn, lettuce, sweet potatoes, sugarbeet, rye	total metalaxyl (as DMA derivative)	GC with AFID (or GC-MS)	0.05**-1.0	summary data provided (no ILV; no validated LOQ)	AG-348 (Balasubramanian, 1980a)
cucurbits			0.05**-0.4		
cole crops			0.05**-0.5	*mean < 70% (cole crops)*	
avocado, asparagus, beans, broccoli, cabbage, cantaloupes, cauliflower, cucumbers, peanuts, peas, squash	total metalaxyl (as DMA)	GC with NPD	0.05*-5.0	summary data provided (no ILV)	AG-395 (Balasubramanian & Perez, 1982) (Perez, 1983)
lettuce	total metalaxyl-M (as DMA)	GC with NPD	0.05*-5.0	data provided (no ILV)	AG-395 (Eudy, 1996)
potatoes, apples, alfalfa, cauliflower, sugarbeets, broccoli, soybeans, rapeseed, peas (dried seeds), broad beans (seeds), strawberries	total metalaxyl (as DMA)	HPLC (2-column switch) with electrochemical detection	0.04*-0.4	full, except for ILV	REM 143.01 (Kühne, 1995a)
peas (haulms & empty pods), broad beans (whole plant)			0.08*-0.8		
grapes			0.2*-2.0		
grape leaves, lettuce	total metalaxyl (as DMA derivative)	GC with AFID	0.5**-1.0	insufficient	REM 16/80 (Ramsteiner, 1980)

* LOQ = limit of determination, unequivocally established

** provisional LOQ = lower fortification level from recovery tests, for which the mean recovery and RSD were not reported

ILV = independent laboratory validation

Evaluation :

The GC- and HPLC (2-column switch)-methods submitted, being based on hydrolysis of the residues and subsequent determination of the 2,6-dimethylaniline (DMA) formed, are not specific for metalaxyl/metalaxyl-M and their

metabolites forming DMA upon hydrolysis, but will also detect other compounds containing the DMA-moiety. Methods AG-395 and REM 143.01, although sufficiently validated (except for ILV), can thus not be recommended for enforcement.
Additional specific methodology for the determination of metalaxyl-M and metabolites in crops is however not required, since the proposed MRL residue definition for metalaxyl-M mentions only parent compound as the relevant residue in target crops, and for this suitable methods are available.

Table B.4.3.2.1-3 : Summary of analytical methods (residue) for animal products - total residues

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation	References
milk liver	total metalaxyl (as DMA derivative)	GC with AFID (or GC-MS)	0.01**-0.1 0.1**-0.4	summary data provided (no ILV; no validated LOQ) *RSD > 20% (liver) mean < 70% (milk)*	AG-349 (Balasubramanian, 1980b)
eggs fat/skin muscle tissue liver milk	total metalaxyl (as DMA)	GC with NPD	0.05*-1.0 0.05-2.0 (0.1*) 0.01**-0.05	full, except for validated LOQ for milk	AG-576 (Cudd & Eudy, 1991) (Yokley & Mc. Killican, 1991)

* LOQ = limit of determination, unequivocally established
** provisional LOQ = lower fortification level from recovery tests, for which the mean recovery and RSD were not reported or at which an insufficient number of samples was tested
ILV = independent laboratory validation

Evaluation :
The GC- methods submitted are based on hydrolysis of the residues and subsequent determination of the 2,6-dimethylaniline (DMA) formed, meaning that they are not specific for metalaxyl/metalaxyl-M and their metabolites forming DMA upon hydrolysis, but will also detect other compounds containing the DMA-moiety. Method AG-576, although sufficiently validated (except for milk), can thus not be recommended for enforcement.

B.4.3.2.2 Analytical methods (residue) in soil, water and air

Table B.4.3.2.2-1 : Summary of analytical methods (residue) for soil

Matrix	Analyte	Type of method	Method range (mg/kg commodity)	Validation	References
soil, (vegetables, grapes)	metalaxyl	GC with AFID or CECD	0.05**-0.5	summary data provided (no validated LOQ)	REM 16/76 (Ramsteiner, 1976a)
soil, (plant material)	metalaxyl	GC with NPD	0.05**-0.5	insufficient	Method CG-123 (Upson, 1980)
soil	metalaxyl acid metabolite (CGA 62826)	GC with AFID or CECD or HECD	0.05**-0.5	summary data provided (no validated LOQ, no RSD for CGA 62826)	REM 7/77 (Ramsteiner, 1977)

** provisional LOQ = lower fortification level from recovery tests, for which the mean recovery and RSD were not reported

Evaluation :
GC-methods REM 16/76 and REM 7/77 both allow the determination of parent metalaxyl/metalaxyl-M in soil, while REM 7/77 also determines acid metabolite CGA 62826.

Both methods can be recommended for enforcement, provided that the proposed LOQ is confirmed by additional validation data and precision data are submitted for the acid metabolite.

Table B.4.3.2.2-2 : Summary of analytical methods (residue) for water

Matrix	Analyte	Type of method	Method range (µg/l commodity)	Validation	References
water (HPLC-grade)	parent metalaxyl metalaxyl acid (CGA 62826)	HPLC with UV detection	0.1*-2.0 0.1*-2.0	full	REM 2/86 (Formica & Giannone, 1986)
water (HPLC-grade)	parent metalaxyl + metalaxyl acid (CGA 62826) (sum as metalaxyl)	HPLC (2 column switch) with UV detection	0.1*-0.5	full	REM 12/87 (Formica & Giannone, 1987)

* LOQ = limit of determination, unequivocally established

Evaluation :
HPLC- methods REM 2/86 and REM 12/87 allow to determine parent metalaxyl/metalaxyl-M and major soil metabolite metalaxyl acid in drinking water, resp. as single compounds or as the sum of both.

Both methods were fully validated and appear suitable for enforcement where drinking water is concerned. Data demonstrating the applicability of the methods to surface water remain to be provided.

Table B.4.3.2.2-3 : Summary of analytical methods (residue) for air

Matrix	Analyte	Type of method	Method range (µg/m³ commodity)	Validation	References
air	parent metalaxyl	GC with NPD	10**-100	full, except for validated LOQ	REM 143.02 (Tribolet, 1993)

** provisional LOQ = lower fortification level from recovery tests, at which insufficient number of samples was tested

Evaluation :
GC-method REM 143.02 allows the determination of parent metalaxyl/metalaxyl-M in air and can be recommended for monitoring, provided that the proposed LOQ is confirmed by additional validation data.

B.4.3.2.3 Analytical methods (residue) wildlife and for use in support of diagnostic and therapeutic regimes

Table B.4.3.2.3-1 : Summary of analytical methods (residue) for body fluids

Matrix	Analyte	Type of method	Method range (mg/l commodity)	Validation	References
urine	total metalaxyl (as DMA)	GC with NPD	0.2-2.0 (0.3**)	summary data provided (no validated LOQ) *RSD > 20%*	AG-438 (Perez & Vincent, 1984)

** proposed LOQ for which the mean recovery and RSD were not reported

Evaluation :
The GC- method submitted, being based on hydrolysis of the residues and subsequent determination of the 2,6-dimethylaniline (DMA) formed, is not specific for metalaxyl/metalaxyl-M and their metabolites forming DMA upon hydrolysis, but it also detects other compounds containing the DMA-moiety.
However, as metalaxyl-M is not classified as toxic or highly toxic, analytical methods for residue analysis in body fluids and tissues are not required.

B.4.4 References relied on

Methods of analysis for the active substance (Annex IIA 4)

Annex point(s) 91/414/EEC	Author, title, report number, test institute, date of report Owner of the report (company or organisation) Submitted by (company or organisation) For publications: reference	Ciba file N°	GLP GEP	Published Protected
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Methods of analysis for the formulation RIDOMIL GOLD 480 EC (Annex IIIA *5*)

Annex point(s) 91/414/EEC	Author, title, report number, test institute, date of report Owner of the report (company or organisation) Submitted by (company or organisation) For publications: reference	Ciba file N°	GLP GEP	Published Protected
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Methods of analysis for the formulation RIDOMIL GOLD MZ 68 WP (Annex IIIA *5*)

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