

PART 2

Section 2 Analytical methods

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. Although based on a real submission, the data included in the following summary and evaluation have been amended to protect the commercial interests of the owner of the data.

Applicants should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the country to which application is to be made.

IIA 4.1 Analytical standards and samples

.
.

.

.

IIA 4.3, 4.4, 4.5, 4.7, 4.8 Methods for the determination of residues

Table IIA 4-1 Summary table of analytical methods

Matrix	Analyte(s)	Method	LOQ	Reference
Wheat grain, straw and foliage	chemx and its chem2 labelled metabolites	HPLC with fluorescence detection after acid hydrolysis	0.01 mg/kg	IIA 4.3/01
Wheat grain and foliage	chemx and its chem2 labelled metabolites	HPLC with fluorescence detection after acid hydrolysis	0.01 mg/kg	IIA 4.3/02
Wheat grain and foliage	chemx and its chem2 labelled metabolites	HPLC / LSC (radio-validation) after acid hydrolysis	0.01 mg/kg	IIA 4.3/03
Soil	chemx and metabolite 2	HPLC with fluorescence detection after acid hydrolysis	0.0005 mg/kg	IIA 4.4.1/01
Soil	chemx and its chem2 labelled metabolites	HPLC with fluorescence detection after acid hydrolysis	0.0005 mg/kg	IIA 4.4.2/01
Soil	chemx	HPLC with fluorescence detection after acid hydrolysis	0.0005 mg/kg	IIA 4.4.3/01

Chemco September 1997 chemx (proposed ISO name) page of

Table IIA 4-1 Continued

Matrix	Analyte(s)	Method	LOQ	Reference
Water	chemx	HPLC with fluorescence detection after base hydrolysis	0.1 µg/l	IIA 4.5/01
Air	chemx	HPLC with UV/VIS detection	2.8 mg/l	IIA 4.7/01
Milk	chemx and its chem2 labelled metabolites	HPLC with fluorescence detection after acid hydrolysis	0.003 mg/kg	IIA 4.8/01
Meat	chemx and its chem2 labelled metabolites	HPLC with fluorescence detection after acid hydrolysis	0.005 mg/kg	IIA 4.8/01

IIA 4.3 Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs

Reports	IIA 4.3/01	Chemx residue method in wheat. Chemco Analytical Standard Operating Procedure. Document no XX-R/M-ASOP-155-0, February 1995.
	IIA 4.3/02	Smith BG "Independent method validation ruggedness trial for the determination of chemx in wheat matrices using Chemco method no. RES-082-94, version no. 2, entitled analytical method for the determination of chemx and its metabolites in wheat." Chemco report XX-14536, April 1996
	IIA 4.3/03	Jones P, Chapter IV Endogenous method validation (radio-validation) from the report entitled "Magnitude of the residues of chemx in Canadian wheat raw agricultural commodities." Chemco XX report 14397
	IIA 4.3/04	Jones P, Assessment of the suitability of multiresidue methods of analysis which are currently used for the determination of chemx residues in wheat. Chemco XX report 14398.

Multiresidue methods Multiresidue methods of analysis that are currently in common usage were not found to be suitable for the determination of chemx residues in wheat (reference IIA 4.3/04).

Single residue method

GLP : yes

Principle of the method

The analytical method involves the extraction of wheat samples with an acetonitrile/water mixture. Chemx [1] parent is quantitatively converted into Metabolite 7, upon hydrolysis with acid. After clean-up on aluminium oxide and florisil chromatographic columns, Metabolite 7 is quantified using HPLC with fluorescence detection. The residue is calculated as mg/kg of Metabolite 7 and is expressed in terms of chemx parent equivalent.

Residues not accounted for by the method are considered not to be of toxicological significant (reference IIA 6.2/01).

Recovery findings

Results obtained were within guideline requirements (70 - 110 %; RSD ≤ 20 %) except for two wheat foliage samples fortified at 0.02 mg/kg and 0.05 mg/kg, for which recoveries of 68 and 69 % respectively were obtained. Recovery data obtained on this matrix from field residue studies were satisfactory (reference IIA 6.3/03). The mean recovery for 23 foliage samples spiked with amounts in the range 0.01 to 1 mg/kg was 86 % with a standard deviation of 9 %. The results obtained are summarized in Table IIA 4.3-1.

Table IIA 4.3-1 Recovery results from method validation of chemx in wheat

Wheat matrices	Fortification Level (mg/kg)	Number of tests	Average Recovery (%)	Standard Deviation	% Relative Standard Deviation
Grain	0.01	2	104	6	6
	0.02	2	98	4	4
	0.05	3	93	10	11
		Total = 7			Mean = 7
Straw	0.01	4	90	14	16
	0.05	3	87	12	14
	0.5	2	87	8	9
		Total = 9			Mean = 13
Foliage	0.01	2	82	3	4
	0.02	2	75	10	13
	0.05	2	73	5	7
	0.5	3	73	2	3
	5	4	88	4	5
		Total = 13			Mean = 6

(a) Fortifications were performed with chemx reference standard solutions

(b) The recoveries were calculated by comparison of the quantities of Metabolite 7 yielded upon hydrolysis of added chemx, with external reference standard solutions of Metabolite 7

Chemco	September 1997	chemx (proposed ISO name)	page of
--------	----------------	---------------------------	---------

Linearity

Good linearity was observed in the range of 0.0025 to 0.1 µg/ml for Metabolite 7 (external reference standard).

Specificity

The method determines parent chemx residues in wheat. The findings of the metabolism study (reference IIA 6.2/01) showed that the efficiency of extraction of chemx from straw and foliage matrices is approximately 100 % and it is expected that it is of a similar level for the less complex grain matrix.

Chemx is quantitatively converted into Metabolite 7, upon hydrolysis with acid. The results of hydrolysis reactions reported in the wheat metabolism study (reference IIA 6.2/01), indicate that treatment of chemx in grain, straw and foliage extracts with hydrochloric acid gives a quantitative yield of Metabolite 7.

A small amount of metabolites containing the Metabolite 6 moiety are extracted by the method which is therefore considered to overestimate the residue by approximately 10 %. By avoiding an additional cleanup step, this method provides a simple and fast enforcement method and can be considered acceptable for use for the determination of chemx (parent compound) residues in wheat.

There were no known interferences from wheat components or from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 0.01 mg/kg of Metabolite 7 expressed as chemx parent equivalent, for wheat grain, straw and foliage.

Repeatability

The relative standard deviations measured with respect to recoveries following fortification at the limit of quantification were 6 %, 16 % and 4 % for wheat grain, straw and foliage, respectively. The mean relative standard deviations at different fortification levels were 7 %, 13 % and 6 % for wheat grain, straw and foliage, respectively. The values obtained are indicative of the method having satisfactory repeatability.

Reproducibility

Method validation results from an independent laboratory were well within the guideline requirements, demonstrating the excellent reproducibility of the method. The results obtained are summarized in Table IIA 4.3-2.

Confirmation of the identity of residues

The results of endogenous method validation (radio-validation) (reference IIA 4.3/03) showed that the method accounts very well for chemx residues in straw and foliage wheat matrices. The results obtained are summarized in the Table IIA 4.3-3.

The residue levels present in grain were too low for accurate quantification. As the efficiency of the method was excellent for the more complex straw and foliage matrices, it is expected to be satisfactory for the grain matrix.

The identity of the Metabolite 7 analyte resulting from acid hydrolysis of chemx residues was confirmed with mass spectral data.

Conclusion

The residue method for the determination of chemx residues in wheat involves quantitative conversion of chemx into Metabolite 7, which are quantified using HPLC with fluorescence detection. A limit of quantification of 0.01 mg/kg of metabolite 7, expressed as chemx parent equivalent can be achieved, however given the complexity of the method it is proposed that the limit of quantification be specified, for control purposes, as being 0.02 mg/kg.

Table IIA 4.3-2 Recovery results obtained by an independent laboratory for the determination of chemx residues in wheat matrices (reference IIA 4.3 /02)

Wheat matrices	Fortification Level (mg/kg)	Number of tests	Average Recovery (%)	Standard Deviation	% Relative Standard Deviation
Grain	0.01	2	96	5	5
	0.05	2	82	3	3
		Total = 4			Mean = 4
Foliage	4	2	88	1	2
	20	2	89	3	3
		Total = 4			Mean = 2

(a) Fortifications were performed with chemx reference standard solutions

(b) The recoveries were calculated by comparison of the quantities of Metabolite 7 yielded upon hydrolysis of added chemx, with external reference standard solutions of Metabolite 7

Table IIA 4.3-3 Comparison of the results obtained with respect to recoveries obtained in endogenous method validation and in the metabolism study

Matrix	Results from metabolism study (% TRR)	Recovery corrected method accountability
Forage (post-emergence treated)	80.4 %	78 %
Straw (pre-emergence treated)	60.5 %	64 %
Straw (post-emergence treated)	57.4 %	56 %

Chemco	September 1997	chemx (proposed ISO name)	page of
--------	----------------	---------------------------	---------

Linearity

Good linearity was observed in the range of 0.001 to 0.05 µg/ml for Metabolite 7 reference standard.

Specificity

The method determines parent chemx residues and its Metabolite 2 residues in soil as Metabolite 7 analyte, obtained by acid hydrolysis. The residue is calculated as mg/kg of Metabolite 7, expressed as chemx parent equivalent.

There were no known interferences from soil components or from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 0.0005 mg/kg of Metabolite 7 expressed as chemx parent equivalent.

Repeatability

The relative standard deviation of recoveries at the limit of quantification was 20 %. The mean relative standard deviation obtained at different fortification levels was 10 %. Those values are indicative of the method having satisfactory repeatability.

Reproducibility

The reproducibility of the method was not estimated as identical samples were not evaluated by Chemco and an independent laboratory. However, based on the performance of the method, its reproducibility is expected to be good.

Conclusion

The residue method for the determination of chemx and Metabolite 2 residues in soil involves separation of chemx on chromatographic columns and quantitative conversion of chemx and Metabolite 2 into Metabolite 7, by means of acid hydrolysis. Quantification of Metabolite 7 residues is achieved using HPLC with fluorescence detection. The residue accounting for chemx and Metabolite 2 is calculated as mg/kg of Metabolite 7 and is expressed as chemx parent equivalent.

IIA 4.4.2 Analytical method for determination of residues of chemx and its metabolites in soil

Reports	IIA 4.4.2/01	Residue method for determination of chemx parent and its metabolites in soil. Chemco Analytical Standard Operating Procedure. Document no XX-ES-ASOP-170-0. April 1996
----------------	--------------	--

GLP : yes

Principle of the method

The analytical method involves the extraction of soil samples with a methanol / hydrochloric acid mixture. Chemx, Metabolite 2 and Metabolite 1 are quantitatively converted upon acid hydrolysis into a single analyte, Metabolite 7. After clean-up on aluminium oxide and florisil chromatographic columns, Metabolite 7 is quantified by HPLC with fluorescence detection. The residue accounting for chemx, Metabolite 2 and Metabolite 1 is calculated as mg/kg of Metabolite 7 expressed as chemx parent equivalent and is defined as the total residue.

Recovery findings

Results obtained were within guideline requirements (70 - 110 %; RSD \leq 20 %) except for one sample for which a recovery of 68 % at the 0.001 mg/kg fortification level was measured.

Soil samples were spiked with known amounts of chemx and the recoveries were calculated by comparison of Metabolite 7 yielded upon hydrolysis of added chemx, with external reference standard solutions of Metabolite 7. The recoveries obtained can be considered as indicative of the efficiency of the quantitative conversion of chemx into Metabolite 7. Metabolite 2 and Metabolite 1 have chemical structures derived from chemx by elimination of a functional group. The efficiency of conversion of those metabolites into Metabolite 7, upon acid hydrolysis, is expected to be similar to that for chemx. The residue of the metabolites is calculated by the subtraction of the levels obtained from the chemx parent method from those obtained from the total residue method.

The results obtained are summarized in Table IIA 4.4.2-1.

Linearity

Good linearity was observed in the range of 0.001 to 0.05 $\mu\text{g/ml}$ for Metabolite 7 reference standard.

Table IIA 4.4.2-1 Recovery results from method validation of chemx parent and its metabolites in soil (reference IIA 4.4.2 /01)

Fortification Level (mg/kg) (a)	Number of tests	Average Recovery (%)	Standard Deviation	% Relative Standard Deviation
0.0005 (b)	6	108	26	24
0.001	4	84	11	13
0.01	2	89	4	4
0.05	2	77	3	4
	Total = 14			Mean = 11

(a) Fortifications were performed with chemx reference standard solutions.

(b) For the 0.0005 mg/kg fortification level, the recovery data were obtained from the field soil dissipation studies (Reference 7.1.1.2.2 /02)

Chemco	September 1997	chemx (proposed ISO name)	page of
--------	----------------	---------------------------	---------

Specificity

The method determines residues of parent chemx, Metabolite 2 and Metabolite 1 as a single analyte, Metabolite 7, obtained by acid hydrolysis. The residue defined as the total residue is calculated as mg/kg of Metabolite 7 expressed as chemx parent equivalent.

There were no known interferences from soil components or from reagents, solvents and glassware used.

Limit of Quantification

Recovery data obtained from field soil dissipation studies (Reference 7.3.1/02) were acceptable at the fortification level of 0.0005 mg/kg - mean recovery on 6 samples fortified at the 0.0005 mg/kg level was 108 % with a relative standard deviation of 24 %.

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 0.0005 mg/kg of Metabolite 7 expressed as chemx parent equivalent.

Repeatability

The relative standard deviation from recoveries at the limit of quantification was 24 % and the mean relative standard deviation obtained at different fortification levels was 11 %. Those values demonstrate that the method has satisfactory repeatability.

Reproducibility

The reproducibility of the method was not determined, as identical samples were not evaluated by Chemco and an independent laboratory. However, based on the performance of the method, reproducibility is expected to be good.

Conclusion

The residue method for the determination of residues of chemx, Metabolite 2 and Metabolite 1 involves the quantitative conversion of chemx and the metabolites into a single analyte, Metabolite 7, by means of acid hydrolysis. Following clean-up using aluminium oxide and florisil chromatographic columns, the amount of Metabolite 7 present is quantified using HPLC with fluorescence detection. The residue accounting for chemx, Metabolite 2 and Metabolite 1 is calculated as mg/kg of Metabolite 7, expressed as chemx parent equivalent and is defined as the total residue.

The residue of the metabolites is calculated by the subtraction of the levels obtained from the chemx parent method from those obtained from the total residue method.

Chemco	September 1997	chemx (proposed ISO name)	page	of
--------	----------------	---------------------------	------	----

IIA 4.4.3 Analytical method for determination of parent chemx residues in soil

Reports	IIA 4.4.3/01	Residue method for determination of Chemx Parent and Metabolite 2 in soil. Chemco Analytical Standard Operating Procedure. Document no XX-ES-ASOP-185-0. March 1997.
----------------	--------------	--

GLP : yes

Principle of the method

The analytical method involves the extraction of soil samples with a methanol / hydrochloric acid mixture. After clean-up on an aluminium oxide column, followed by a florisil chromatographic column, chemx is quantitatively converted into Metabolite 7, by means of hydrolysis with acid. After being made basic (pH 12-14), the aqueous solution is extracted into dichloromethane. The dichloromethane extract is eluted through a florisil chromatographic column prior to HPLC injection. Metabolite 7 is quantified using HPLC with fluorescence detection. The residue is calculated as mg/kg of Metabolite 7 and is expressed as chemx parent equivalent.

Recovery findings

Results obtained were within guideline requirements (70 - 110 %; RSD ≤ 20 %). The recovery data used were obtained from the field dissipation studies (reference IIA 7.3.1/01).

A long validation procedure was not conducted for the determination of chemx parent. The difference between this method and the method described in point IIA 4.4.1/01 consists of the inclusion of a clean-up using a florisil chromatographic column, before the hydrolysis step.

Soil samples were spiked with known amounts of chemx. Recoveries were calculated by comparison of amounts of Metabolite 7 yielded upon hydrolysis of added chemx, with external reference standard solutions of Metabolite 7. The recoveries obtained can be considered indicative of the efficiency of the quantitative conversion of chemx into Metabolite 7. The results obtained are summarized in Table IIA 4.4.3-1.

Table IIA 4.4.3-1 Recovery results from method validation of chemx (reference IIA 4.4.3 /01)

Fortification Level (mg/kg) (a)	Number of tests	Average Recovery (%)	Standard Deviation	% Relative Standard Deviation
0.0005	4	93	12	13
0.0010	1	88	na	na
0.0020	1	77	na	na
0.0050	3	75	2	3
0.0150	1	89	na	na
	Total = 10			Mean = 8

(a) Fortifications were performed with chemx reference standard solutions

Chemco	September 1997	chemx (proposed ISO name)	page of
--------	----------------	---------------------------	---------

Linearity

Good linearity was observed in the range of 0.001 to 0.05 µg/ml for Metabolite 7 reference standard.

Specificity

The method determines parent chemx residues in soil as the Metabolite 7 analyte, obtained by acid hydrolysis. The residue is calculated as mg/kg of Metabolite 7, expressed as chemx parent equivalent.

There were no known interferences from soil components or from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 0.0005 mg/kg of Metabolite 7, expressed as chemx parent equivalent.

Repeatability

The relative standard deviation from recoveries at the limit of quantification was 13 %. The mean relative standard deviation obtained at different fortification levels was 8 %. Those values are indicative of the method having satisfactory repeatability.

Reproducibility

The reproducibility of the method was not estimated as identical samples were not evaluated by Chemco and an independent laboratory. However, based on the performance of the method, reproducibility is expected to be good.

Conclusion

The residue method for the determination of chemx residues in soil involves separation of chemx on chromatographic columns and the quantitative conversion of chemx into Metabolite 7, by means of acid hydrolysis. Quantification of Metabolite 7 is achieved using HPLC with a fluorescence detector. The method has a limit of quantification of 0.0005 mg/kg for Metabolite 7, expressed as chemx parent equivalent.

IIA 4.5 Residues in water (including drinking water, ground water and surface water)

Report	IIA 4.5/01	Reilly TT and Smith BG "Independent method validation Ruggedness trial for the determination of chemx in water using Chemco method no. RES-093-95, version no. 0, entitled analytical method for the determination of chemx and its metabolites in water." Chemco report XX 14535.
---------------	------------	--

GLP: yes

Chemco	September 1997	chemx (proposed ISO name)	page	of
--------	----------------	---------------------------	------	----

Principle of the method

The analytical method for the determination of residues of chemx in water involves quantitative conversion of chemx into rearranged chemx using base hydrolysis. Quantification of residues of rearranged chemx residues is achieved using HPLC with fluorescence detection. The method has a limit of determination of 0.1 µg/l of rearranged chemx, expressed as chemx parent equivalent.

Recovery findings

Results obtained were within guideline requirements (70 - 110 %; RSD ≤ 20 %), except in one instance when a recovery of 61 % was obtained at the limit of determination (0.1 µg/l).

The results obtained are summarized in Table IIA 4.5-1.

Table IIA 4.5-1 Recovery results from method validation of chemx in water (reference IIA 4.5/01)

Fortification Level (µg/l)	Number of tests	Average Recovery (%)	Standard Deviation	% Relative Standard Deviation
0.04	5	80	14	18
0.1	5	80	11	14
1	5	94	4	4
	Total : 15			Mean : 12

- (a) Fortifications were performed with chemx reference standard solutions.
 (b) The recoveries were calculated by comparison of rearranged chemx yielded upon base hydrolysis of added chemx, with external reference standard solutions of rearranged chemx

Linearity

Good linearity was observed over the range of 0.001 µg/ml to 0.2 µg/ml for rearranged chemx.

Specificity

The method determines parent chemx residues in water as rearranged chemx, expressed as chemx parent equivalent. There were no known interferences from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 0.1 µg/l of rearranged-chemx expressed as chemx parent equivalent.

Chemco	September 1997	chemx (proposed ISO name)	page of
--------	----------------	---------------------------	---------

Repeatability

The relative standard deviation of the recoveries obtained with five fortified control samples, at the limit of quantification, was 14 %. The mean relative standard deviation obtained at different fortification levels was 12 %. Those values demonstrate that the method has satisfactory repeatability.

Reproducibility

The reproducibility of the method was not estimated as identical samples were not evaluated by Chemco and an independent laboratory. However, based on performance of the method, reproducibility is expected to be good.

Conclusion

The analytical method for the determination of chemx residues in water, involves quantitative conversion of chemx into rearranged chemx. Quantification of rearranged chemx residues is done using HPLC with a fluorescence detector. The method has a limit of quantification of 0.1 µg/l of rearranged-chemx, expressed as chemx parent equivalent.

IIA 4.7 Residues in sediment

.
. .

IIA 4.7 Residues in air

On the basis of chemx's vapour pressure and Henry's Law constant, it is clear that it is quite unlikely that chemx would be found in air. Consequently, exposure of operators workers and bystanders by the inhalation route will be minimal. It is therefore contended that an analytical method for air is not required. However as analysis of air was conducted in the context of rat inhalation study, details of the methodology used is provided below, for information purposes. As it is claimed that such an analytical method is not required, not all of the usual parameters are reported.

Report	IIA 4.7/01	Smith CL, Analytical procedure for determination of chemx in air, Report XX 94004, July 1994
---------------	------------	--

GLP : yes

Principle of the method

Chemx in a measured quantity of air is trapped on a filter paper. Following extraction with acetone, chemx residues are determined using HPLC with UV/VIS detection at 254 nm.

Chemco	September 1997	chemx (proposed ISO name)	page of
--------	----------------	---------------------------	---------

Recovery findings

The analysis of the quality control sample showed a divergence of 3.8 % from the expected value, indicating that the analytical method used was capable of giving precise and accurate data.

Linearity

The method involved use of standard solutions in the range 20-100 mg/l

Specificity

Not reported

Limit of Quantification

The method was used to measure chemx concentrations in air at levels that ranged from 2.8 to 3.0 mg/l

Repeatability

Not reported

Reproducibility

Independent laboratory validation has not been conducted with the method

Conclusion

The residue method for the determination of chemx residues in a measured quantity of air involves trapping on filter paper, extraction of the filter paper with acetone and analysis by HPLC using UV/VIS detector at 254 nm.

IIA 4.8 Residues in body fluids and tissues

As chemx is neither classifiable as being *Toxic* or *Very Toxic* an analytical method for residues in body fluids and tissues is not required. However as analysis of body tissues was included in the dairy cow feeding study (Point IIA 6.4.1), details of the methodology used is provided, for information purposes. As it is claimed that the method is not a mandatory requirement, not all of the usual parameters are reported.

Report	IIA 4.8/01	Jones RS, Analytical Method for the Determination of Residues of chemx and the chem2 Class of Metabolites in Dairy Cow Milk and Meat tissues. Chemco Report No RES-095-96, Version Number 0, March 1996
---------------	------------	---

GLP : Yes

Chemco September 1997 chemx (proposed ISO name) page of

Principle of the method

Chemx is extracted from milk and edible tissues of lactating dairy cattle, using acetonitrile/water mixtures. It is converted into Metabolite 7, using acid hydrolysis. Following extraction into dichloromethane, the extract is cleaned-up using Florisil. The residue is quantified using HPLC with fluorescence detection.

Recovery findings

Results obtained were within guideline requirements (70 - 110 %; RSD ≤ 20 %). The results obtained are summarized in Table IIA 4.8-1.

Linearity

Not reported

Table IIA 4.8-1 Recovery results from method validation of chemx in dairy cow milk and meat tissues (reference IIA 4.8/01).

Matrix	Fortification Level (mg/kg)	Number of fortified samples	Average % Recovery	% Standard Deviation	% Relative Standard Deviation
Milk	0.003	14	92	8	9
	0.01	16	90	7	8
	0.05	9	88	5	5
	0.1	2	94	nr	nr
	1.0	2	95	nr	nr
	2.0	2	98	nr	nr
Fat	0.005	4	94	14	14
	0.01	4	86	9	10
	0.05	2	85	nr	nr
	0.1	1	91	nr	nr
	2.0	1	91	nr	nr
Muscle	0.005	4	100	7	7
	0.01	4	100	9	9
	0.05	2	82	nr	nr
	0.1	1	87	nr	nr
	2.0	1	99	nr	nr
Liver	0.005	4	105	13	12
	0.01	4	90	4	5
	0.05	2	92	nr	nr
	0.1	1	97	nr	nr
	2.0	1	100	nr	nr
Kidney	0.005	2	90	nr	nr
	0.01	2	94	nr	nr
	0.05	1	96	nr	nr
	0.1	1	91	nr	nr
	2.0	1	97	nr	nr

nr : Method performance was evaluated by determining residues in check and fortified check milk and meat tissue samples, analysed in ten sets, processed on separate days. Statistical parameters were evaluated only when the number of samples (n) is ≥ 4.

Specificity

The method is specific for residues containing the chem2 portion of chemx.

Limit of Quantification

The limit of quantification, defined as the lowest concentration at which an acceptable recovery is obtained, is 0.003 mg/kg for milk samples and 0.005 mg/kg for non-milk samples, for Metabolite 7, expressed as chemx parent equivalent.

Repeatability

The relative standard deviation of the recoveries obtained with fortified control samples at the limit of quantification was 9 % for milk, 14 % for fat, 7 % for muscle and 12 % for liver. The mean of the relative standard deviation of recoveries obtained from two (minimum) different fortification levels for all matrices was 9 %, except in the case of kidney where the RSD value was not reported. Those values demonstrate that the method has satisfactory repeatability.

Reproducibility

Independent laboratory validation of the method was not carried out.

Conclusion

The residue method for the determination of chemx residues in milk and edible tissues of lactating dairy cattle involves extraction with acetonitrile/water solutions, quantitative conversion of chemx into Metabolite 7, using acid hydrolysis. Quantification of Metabolite 7 residues is done using HPLC with fluorescence detection.

The limit of quantification for Metabolite 7, expressed as chemx parent equivalent, is 0.003 mg/kg for milk samples and 0.005 mg/kg for edible tissues.

Attachment**CHEMX METABOLITE KEY : NAMES, STRUCTURES & STUDIES WHERE FOUND**[Number] in *Tier II* text refers to ID Number below

ID No	Trivial Name	Chemical Abstracts Name	Structure	Where Found
1	Chemx			Wheat forage, wheat straw
2	Metabolite 2			Hydrolysis, aerobic soil, aquatic sediment, wheat forage, wheat straw, rotation crops, rat (urine and faeces)
3	Metabolite 3			Hydrolysis, aerobic soil, aquatic sediment, aqueous photolysis
4	Metabolite 1			Aerobic soil, aquatic sediment, wheat forage, wheat straw, rat (urine and faeces)
5	Metabolite 10			Aerobic soil, aqueous photolysis
6	Metabolite 6			Lysimeter leachate, wheat forage, wheat straw
7	Metabolite 5			Aqueous photolysis, soil photolysis, wheat forage, wheat straw
8	Metabolite 7			Aqueous photolysis, Common chemophore or analyte for residue method

Attachment (Continued)

ID No	Trivial Name	Chemical Abstracts Name	Structure	Where Found
9	Metabolite 10			Aqueous photolysis, wheat forage, wheat straw
10	Metabolite 8			Aqueous photolysis, wheat forage, wheat straw
11	Metabolite 9			Aqueous photolysis
12	N-hydroxy Metabolite 5			Aqueous photolysis
13	5-Hydroxy-OEC 1000			Rat (urine and faeces)
14	Rearrangement product			Chemophore (analyte) for analysis of OEC 1000 in water and in soil storage stability
15	Metabolite 11			Rat (urine)