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This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850; submitted 02/01/2006). The DER has been reviewed by the HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

45710228 Gillis, N. (1997) Chlorothalonil Development and Validation of Methods of Analysis for the Determination of Chlorothalonil and its Metabolite 4-Hydroxy-2,5,6-Trichloro-1,3-Dicyanobenzene in Animal Tissues and Produce: Lab Project Number: VCM 49/962320. Unpublished study prepared by Huntingdon Life Sciences Ltd. 103 pages.

EXECUTIVE SUMMARY:

Vischim S.r.l has submitted a method for the determination of residues of chlorothalonil and its 4-hydroxy metabolite (4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile; referred to as 4-hydroxy-2,5,6-trichloro-dicyanobenzene by the petitioner) in livestock matrices. In the method, residues of chlorothalonil are determined by gas chromatography (GC) with mass-selective detection (MSD), and residues of the 4-hydroxy metabolite are determined by high-performance liquid chromatography/ultraviolet (HPLC/UV). Briefly, residues of chlorothalonil are extracted from egg, milk, liver, muscle, kidney, and fat using toluene (acidified with HCl for egg samples). The extract is evaporated to dryness, redissolved in acetonitrile, and partitioned with hexane. The acetonitrile phase is evaporated to dryness and redissolved in toluene for analysis by GC/MSD; egg extracts (acetonitrile phase) undergo a C-18 solid-phase extraction cleanup prior to analysis. The limit of quantitation (LOQ) is 0.01 ppm in each matrix.

Residues of the 4-hydroxy metabolite are extracted from livestock matrices using acidified ethyl acetate. The extracts are evaporated to dryness, redissolved in acetonitrile, mixed with NaCl solution, and partitioned with hexane. The aqueous phase is partitioned with ethyl acetate, and the ethyl acetate phase is evaporated to dryness and redissolved in toluene for HPLC/UV analysis. The LOQ is 0.01 ppm in each matrix.

The method was adequately validated using samples of livestock commodities. The overall average recoveries were 91 ± 8.7% for chlorothalonil and 85 ± 9.8% for the 4-hydroxy metabolite from samples of livestock liver, kidney, muscle, fat, egg, and milk fortified with each analyte at ~0.01 and ~1.0 ppm. Recoveries of chlorothalonil ranged 70-111% and recoveries of the 4-hydroxy metabolite ranged 70-109%.
The method did not include any confirmatory analysis procedures. No radiovalidation data were submitted.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, analytical method test data are classified as scientifically unacceptable. The petitioner should submit radiovalidation (extraction efficiency) data for the method; these data should demonstrate that aged residues of chlorothalonil and its metabolite 4-hydroxy chlorothalonil are extracted from livestock commodities using the extraction procedures of the method.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Chlorothalonil is a broad spectrum, non-systemic protectant pesticide mainly used as a fungicide to control fungal foliar diseases of vegetable, field, and ornamental crops. It is also used as a wood protectant, antimold and antimildew agent, bactericide, microbiocide, algaecide, insecticide, and acaricide. The exact mechanism of action is not known.

TABLE A.1. Test Compound Nomenclature.

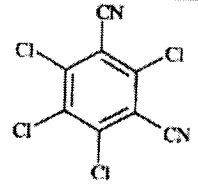
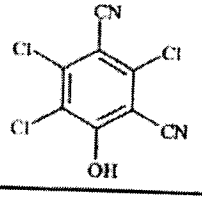
Compound	
Common name	Chlorothalonil
Company experimental name	N/A
IUPAC name	tetrachloroisophthalonitrile
CAS name	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
CAS registry number	1897-45-6
Compound	
Common name	4-hydroxy metabolite
Company experimental name	N/A
IUPAC name	2,4,5-trichloro-6-hydroxyisophthalonitrile
CAS name	4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile
CAS registry number	Not provided
End-use product (EP)	N/A

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Chlorothalonil.		
Parameter	Value	Reference
Melting point	250-251 °C	Chlorothalonil Reregistration Eligibility Decision, April 1999
Water solubility	0.6 ppm (25 °C)	Chlorothalonil Reregistration Eligibility Decision, April 1999
Solvent solubility	g/L at 25°C: acetone 20 dimethyl sulfoxide 20 cyclohexanone 30 dimethylformamide 30 kerosene <10 xylene 80	The Pesticide Manual, 8th ed.
Octanol/water partition coefficient, Log(K_{OW})	4.37×10^2	TOXNET database

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Vischim has submitted a method for the determination of residues of chlorothalonil and its metabolite 4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene (CAS name is 4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile) in livestock commodities. In the method, residues of chlorothalonil are determined by GC/MSD, and residues of the 4-hydroxy metabolite are determined by HPLC/UV. Briefly, residues of chlorothalonil are extracted from egg, milk, liver, muscle, kidney, and fat using toluene (acidified with HCl for egg samples). The extract is evaporated to dryness, redissolved in acetonitrile, and partitioned with hexane. The acetonitrile phase is evaporated to dryness and redissolved in toluene for analysis by GC/MSD; egg extracts (acetonitrile phase) undergo a C-18 solid-phase extraction cleanup prior to analysis. The LOQ is 0.01 ppm in each matrix.

Residues of the 4-hydroxy metabolite are extracted from livestock matrices using acidified ethyl acetate. The extracts are evaporated to dryness, redissolved in acetonitrile, mixed with NaCl solution, and partitioned with hexane. The aqueous phase is partitioned with ethyl acetate, and the ethyl acetate phase is evaporated to dryness and redissolved in toluene for HPLC/UV analysis. The LOQ is 0.01 ppm in each matrix.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Residues of Chlorothalonil and its 4-Hydroxy Metabolite in Livestock Matrices.

Method ID	Report No. VCM 49/62320	
Analyte(s)	Chlorothalonil	4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile
Extraction solvent/technique	**Liver, muscle, kidney, fat, milk:** Samples are extracted with toluene, and an aliquot of the extract is evaporated to dryness. The residue is redissolved in acetonitrile and partitioned with hexane. The acetonitrile phase is evaporated to dryness, and the residue is redissolved in toluene. **Egg:** Samples (without shells) are extracted with toluene containing concentrated HCl (120:1, v:v), and an aliquot of the extract is evaporated to dryness. The residue is redissolved in acetonitrile and partitioned with hexane. The acetonitrile phase is evaporated to dryness, and the residue is redissolved in methanol.	Samples are extracted twice with ethyl acetate and concentrated HCl (100:1, v:v); Celite is added to milk samples prior to extraction. The combined extracts are evaporated to dryness and redissolved in acetonitrile. Distilled water and saturated NaCl solution are added, and the mixture is partitioned twice with hexane. The aqueous phase is partitioned with ethyl acetate, and the ethyl acetate phase is evaporated to dryness and redissolved in methanol.
Cleanup strategies	**Liver, muscle, kidney, fat, milk:** None. **Egg:** The methanol extract is cleaned on a C-18 solid-phase extraction cartridge, using 10% ethyl acetate in hexane to elute residues. The eluate is evaporated to dryness and redissolved in toluene.	None.
Instrument/Detector	GC/MSD using a DB-5 column and monitoring ions 263.9 and 265.9.	HPLC/UV with two-column switching, using CN Nucleosil and NH_2 Nucleosil columns, and isocratic mobile phases of methanol, water, and orthophosphoric acid. Detector wavelength is 242 nm.
Standardization method	External standards, using linear regression of calibration curve.	External standards, using linear regression of calibration curve.
Stability of std solutions	Analytical standards are to be stored in the dark at 4 °C.	
Retention times	~8.6 minutes in milk; ~8.9-9.0 in tissues; ~10.3 minutes in egg	~17.7-21.2 minutes

The method was validated by Huntingdon Life Sciences Ltd. (Cambridgeshire, UK) using samples of milk, egg, liver, kidney, muscle, and fat provided by the laboratory or purchased commercially. The laboratory did not specify the animal source of the tissues used for validation. Samples were fortified with each analyte at ~0.01 and 1.0 ppm.

B.2. Enforcement Method

The petitioner has not proposed the submitted method for enforcement purposes.

An adequate GC/electron-capture detection (GC/ECD) method (CHW 6241-111) currently exists for the enforcement of tolerances for residues of the chlorothalonil metabolite SDS 3701 (4-hydroxy metabolite) in milk and beef tissues. This method is listed in the U.S. EPA Index of Residue Analytical Methods under chlorothalonil. In the method, residues are extracted from

samples using acidic acetone and then partitioned into diethyl ether. Residues of SDS 3701 are derivatized to the methyl ether, cleaned up by alumina column chromatography (optional), and then determined by GC/ECD. The LOQ is 0.01 ppm.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

The method validation recoveries of chlorothalonil and its 4-hydroxy metabolite using the above method were adequate from fortified samples of livestock matrices (Table C.1.1). The overall average recoveries were 91 ± 8.7% for chlorothalonil and 85 ± 9.8% for the 4-hydroxy metabolite from samples of livestock liver, kidney, muscle, fat, egg, and milk fortified with each analyte at ~0.01 and ~1.0 ppm. Recoveries of chlorothalonil ranged 70-111% and recoveries of the 4-hydroxy metabolite ranged 70-109%.

The extraction procedures of the method differ from those used in the goat metabolism study (MRID 45710226). In the goat metabolism study, the majority of the radioactivity (~52-82% TRR) in milk, liver, kidney, muscle, and fat was extracted using acetonitrile, acetonitrile/water, and/or acetone.

The method does not include any procedures for confirmatory analysis.

TABLE C.1.1. Recovery Results from Method Validation of Livestock Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
			Mean	SD	CV
Chlorothalonil - GC/MSD					
Liver	0.0101	92, 85, 89, 80, 76, 93	91	8.4	9.3
	1.0064	89, 101, 87, 92, 106, 97			
Kidney	0.0101	105, 97, 95, 85, 79, 77	95	10.5	11.0
	1.0064	94, 96, 94, 111, 104, 104			
Muscle	0.0101	95, 70, 79, 83, 92, 101	87	9.2	10.6
	1.0064	76, 88, 84, 96, 95, 86			
Fat	0.0101	110, 99, 104, 79, 103, 101	99	7.6	7.7
	1.0064	98, 103, 98, 102, 93, 99			
Egg	0.0101	91, 94, 85, 83, 85, 85	87	4.1	4.8
	1.0139	80, 85, 83, 89, 92, 86			
Milk	0.0101	86, 94, 83, 89, 82, 81	88	4.6	5.2
	1.0109	90, 90, 89, 96, 87, 91			

TABLE C.1.1. Recovery Results from Method Validation of Livestock Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Recovery (%)		
			Mean	SD	CV
4-Hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile - HPLC/UV					
Liver	0.0104	82, 82, 84, 85, 87, 83	90	6.3	7.0
	1.0438	96, 97, 96, 95, 94, 95			
Kidney	0.0104	73, 70, 74, 78, 78, 77	91	16.6	18.3
	1.0438	103, 103, 107, 107, 109, 109			
Muscle	0.0104	93, 90, 87, 92, 91, 88	89	3.5	3.9
	1.0438	93, 90, 85, 87, 85, 82			
Fat	0.0104	73, 72, 74, 73, 74, 81	78	4.5	5.7
	1.0438	80, 83, 82, 81, 82, 83			
Egg	0.0104	71, 73, 73, 89, 74, 77	76	4.6	6.0
	1.0438	74, 75, 76, 75, 78, 77			
Milk	0.0104	78, 79, 83, 86, 89, 86	87	5.5	6.3
	1.0438	93, 93, 93, 94, 85, 88			

[1] Chlorothalonil standards were prepared in toluene, and 4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile standards were prepared in methanol.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Residues of Chlorothalonil and its 4-Hydroxy Metabolite in Livestock Matrices.

Analyte(s)	Chlorothalonil	4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile
Equipment ID	HP5890 Series II GC with HP5971 MSD; DB-5 column (15 mm x 0.25 mm)	HPLC two-column switching system, with Spectra 100 UV detector, CN Nucleosil column (5 µm, 25 cm x 4.6 mm), NH_2 Nucleosil column (5 µm, 15 cm x 4.6 mm)
LOQ	0.01 ppm; lower limit of method validation	0.01 ppm; lower limit of method validation
Limit of detection (LOD)	0.0028 ppm for liver, 0.0058 ppm for kidney, 0.0049 ppm for muscle, 0.0039 ppm for fat, 0.0024 ppm for egg, and 0.0021 ppm for milk; calculated using recoveries at 0.01 ppm.	7.9×10^{-4} ppm for liver, 0.0016 ppm for kidney, 9.9×10^{-4} ppm for muscle, 0.0016 ppm for fat, 0.0033 ppm for egg, and 0.0017 ppm for milk; calculated using recoveries at 0.01 ppm.
Accuracy/Precision	Recoveries of chlorothalonil ranged 70-111% (coefficient of variation = 9.6%) indicating acceptable accuracy/precision from livestock commodities at spiking levels of 0.01 and 1.0 ppm.	Recoveries of 4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile ranged 70-109% (coefficient of variation = 11.5%) indicating acceptable accuracy/precision from livestock commodities at spiking levels of 0.01 and 1.0 ppm.
Reliability of the Method [ILV]	No ILV data have been submitted.	
Linearity	The method/detector response was linear (coefficient of determination, $r^2>0.99$) within the range of 0.025-0.25 µg/mL.	The method/detector response was linear (coefficient of determination, $r^2>0.99$) within the range of 0.025-0.50 µg/mL.
Specificity	The control chromatograms generally have no peaks above the chromatographic background near the retention time of the analyte, and the spiked sample chromatograms contain only the analyte peak of interest at the retention time of the analyte. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.	

C.2. Enforcement Method

The petitioner has not proposed the submitted method for enforcement purposes.

C.3. Independent Laboratory Validation

No independent laboratory validation data were submitted.

D. CONCLUSION

HED is unable to determine whether the submitted method (Report No. VCM 49/62320) is adequate for determining residues of chlorothalonil and its 4-hydroxy metabolite (4-hydroxy-2,5,6-trichloro-1,3-benzenedicarbonitrile) in livestock commodities. Adequate validation data have been submitted at fortification levels of 0.01 and 1.0 ppm for each analyte in liver, kidney, muscle, fat, egg, and milk. However, no radiovalidation data were submitted; these data are required.

E. REFERENCES

45710226 Shaw, D. (1997) Chlorothalonil Metabolism in the Lactating Goat: Lab Project Number: VCM 73/961389. Unpublished study prepared by Huntingdon Life Sciences Ltd. 143 p.

F. DOCUMENT TRACKING

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