Draft Renewal Assessment Report
under Regulation (EC) 1107/2009

CLOPYRALID

Volume 3 – B.7 (AS)

RMS: Finland
Co-RMS: Poland

May 2017

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Version History

When	What
2017/ May	DRAR- First version submitted to EFSA

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B.7 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED, AND PLANT METABOLISM

Introduction

Dow AgroSciences submitted the original clopyralid (CAS 1702-17-6) Annex I dossier on 30 April 2002.Draft Assessment Report (DAR) has been prepared by the rapporteur Member State (RMS) Finland under Directive 91/414/EEC. The Draft Assessment Report (DAR) was issued in December 2003 and the last Addendum was issued in September 2005. Peer review of the pesticide risk assessment of clopyralid was finalized on 14 Dec 2005 and sent to the EU Commission. The complete Review Report on clopyralid was issued on 04 Avril 2006 (SANCO/10012/2006/Rev. 3).

Clopyralid was included in Annex I of Directive 91/414/EC by Commission Directive 2006/64/EC, of 18 July 2006. Entry into force of Annex I listing was 1st May 2007. Annex I listing was extended to 30 April 2018 according to Commission regulation No 678/2014 of 19 June 2014.

Confirmatory Residue Addendum B.7 was issued in March 2014 by RMS Finland and published by EFSA on July 2014 (EFSA supporting publication 2014:EN-624. Title: 'Technical Report: Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment of confirmatory data for the active substance clopyralid').

According to Article 6 of the Regulation (EC) No 396/2005, Finland (Finnish Safety and Chemicals Agency) made an MRL application in order to modify existing MRLs for clopyralid in brassica vegetables, linseed, swedes and turnips and certain edible commodities derived from animals.

The MRL application made and evaluated by EMS Finland.

The MRL application has been sent under EU Regulation 396/2005 Art 10 on 25.1.2011 and processed by Evaluating Member State Finland (30 November 2011). The evaluation was published by EFSA (EFSA Journal 2011;9(10):2418). At the time of writing an Art 12 review was not available.

The toxicological profile of clopyralid was assessed in the framework of the peer revie wed under Directive 91/414/EEC and the data were sufficient to derive an ADI value of 0.15 mg/kg bw/day. Due to the low acute toxicity of the active substance the setting of an ARfD was considered not necessary.

It was a specific provision of the approval that Dow Agro Sciences was required to submit to the European Commission further studies to confirm the results on animal metabolism. Dow AgroSciences, submitted an updated dossier, which was evaluated by the designated RMS, Finland, in the form of an Addendum to the Draft Assessment Report. The submitted confirmatory data complete the missing information on the metabolism studies. This last review concluded that no changes were necessary to the consumer risk assessment presented in the EFSA Conclusion of 2005.

The Review report for clopyralid, finalized in the Standing Committee on the Food Chain and Animal Health, was issued on 04 April 2006 (SANCO/10012/2006/Rev. 3) which includes the relevant end points decided on for annex I inclusion.

The Review Report concluded on basis of data requirements applied at that time, that plant protection products containing clopyralid would fulfill the safety requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EC. The conclusion was reached on the basis of the evaluation of the representative uses as herbicide which comprising broadcast spraying to control a narrow spectrum of broad-leaved weeds in cereals, oilseed rape, sugar beet and pasture. The following particular conditions were stated as requiring consideration at Member State level in relation to the granting of authorizations of plant protection products containing clopyralid:

"On the basis of the proposed and supported uses (as listed in Appendix II), the following particular issues have been identified as requiring particular and short term attention from all Member States, in the framework of any authorisations to be granted, varied or withdrawn, as appropriate:

- Member States must pay particular attention to the protection of non-target plants and ground water under vulnerable conditions. Conditions of authorisation should include risk mitigation measures and monitoring programmes should be initiated to verify potential groundwater contamination in vulnerable zones, where appropriate."

The representative formulated product for the evaluation was "Lontrel 100 Herbicide", a soluble concentrate (SL) formulation containing 100 g/L clopyralid .

In the current application the representative formulation includes a different product (GF-1374) compared to the product evaluated for the first approval (Lontrel 100 Herbicide). GF-1374 is an emulsifiable concentrate containing the active substance clopyralid at 80 g a.i./L and 2 mixing partners namely Fluroxypyr meptyl 144 g/l (100 g as/L) and Florasulam 2.5 g/l.

New data and risk assessments are included in the supplementary dossier to reflect changes in legal requirements and changes in scientific and technical knowledge since the approval

Based on results from new studies which are currently underway (in response to new guidance documents) there is a potential for some endpoints to change.

Clopyralid has previously been evaluated in the following documents within the EU:

- EU Review report for the active substance clopyralid - SANCO/10012/2006 – rev. 3 4 April 2006
- EFSA Scientific Report (2005) 50, 1-65, Conclusion on the peer review of clopyralid, 14 December 2005)
- EFSA supporting publication 2014:EN-624, Technical report: Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment of confirmatory data for the active substance clopyralid, July 2014 – Confirmatory data - Residue

Two addendums to the DAR of the first approval containing residue assessments have been prepared (Addendum 1 Rapporteur Member State Finland 30.12.2004, and Addendum 2 Rapporteur Member State Finland 04.07.2005), and have been made available for the Member States and EFSA, but not been peer-reviewed.

The representative uses include two uses evaluated during the first approval (cereals and pasture) which also reflect changes in dosage of clopyralid containing products as doses have been reduced.

The representative formulation includes a different product (GF-1374) compared to the product evaluated for the first approval (Lontrel 100 Herbicide). GF-1374 is an emulsifiable concentrate containing the active substance clopyralid at 80 g a.i./L and 2 mixing partners namely Fluroxypyr mepthyl 144 g/l (100 g as/L) and Florasulam 2.5 g/l.

New data and risk assessments are included in the supplementary dossier to reflect the presence of two other actives.

Most relevant data and endpoints for the two other actives (mixing partners) are briefly summarized in the supplementary dossier. Main issue is whether the results obtained with new formulation are comparable with the results obtained with the previous formulation.

B.7.1 Storage Stability of Residues

The following table provides a justification for the use of additional studies to address storage stability of residues evaluated for the Active Approval.

Data point/Study	Rationale
B.7.1.1.1	An additional study was conducted in acidic and oily crop matrices in order to provide frozen storage stability data for all four major crop groupings (wet, dry, oily and acidic).
B.7.1.2.1	Additional frozen storage stability studies were conducted to demonstrate the stability of clopyralid in bovine fat, bovine muscle, bovine liver, bovine kidney, milk and egg.

B.7.1.1 Plants and Plant Products

B.7.1.1.1 Short Overview on Previously Submitted Data

Data to address stability in plant and plant edible commodities were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level for wet and dry crop groupings.

In the previous assessment made for the first approval frozen storage stability of clopyralid was studied in maize grain, forage and fodder according to the US requirements (EPA 171-4e).

Frozen storage stability studies in crop matrices submitted for the first clopyralid evaluation in 2002:

Report	IIA 6.0/01 Foster, DR, Blakeslee, BA, Rutherford, BS., Frozen Storage Stability of Clopyralid, 2,4 D in Corn Grain, Straw and Fodder 1996 DAS Report No.: GH-C 3779
Guidelines	US EPA 171-4 (e)
Analytical method	DowElanco Analytical Method ERC 94.8
GLP	Yes

Report	IIA 6.0/02 Clements, B, Bolton, A , Frozen Storage Stability of Clopyralid, 2,4 -D in Corn Grain, Straw and Fodder (1996) Dow AgroSciences unpublished report; RES93050.011996
Guidelines	US requirements (EPA 171-4e)
Analytical method	DowElanco Analytical Method GRM 94.03 The method GRM 94.03 is not found in the submission evaluated under the directive 91/414 EEC: ANALYTICAL METHODS; SECTION 2 TIER 2 – SUMMARY Analytical Method ERC 94.8
Matrixes	Corn Grain, Straw and Fodder
GLP	Y

In the study by Foster et al. frozen storage stability of clopyralid was tested in maize grain, forage and fodder according to the US requirements (EPA 171-4e). Samples were mixed and fortified at a concentration of 0.5 mg/kg of clopyralid and were stored in high-density polyethylene (HDPE) containers at – 20 ºC. Samples were analysed immediately (at day 0) and at 32, 82, 186, 385 days after fortification by using DowElanco Analytical Method GRM 94.03. At each time point, one untreated control sample, a reagent blank and three freshly spiked samples at 0.50 µg/l were analysed together with three replicates of stored samples.

The recovery rates of stored samples were corrected on the basis of freshly fortified samples. Uncorrected values were not available in the DAR.

In addition, the frozen storage stability of clopyralid was tested in incurred pasture samples. From a previously harvested treated pasture sample of known clopyralid residue, two sets of twenty 10 g replicate sub samples were weighed into plastic containers and placed at -18ºC. Samples were then analysed at 0, 34, 128, 332 and 520 days intervals by using DowElanco Analytical Method ERC 94.8. At each time point, one untreated control sample, a reagent blank and two freshly spiked samples at 10 or 20 mg/kg were analysed together with three replicates of stored samples.

A summary of results is shown in **Table B.7.1.1.1-2.** The results indicate that there is no significant degradation of clopyralid in pasture over a period of 520 days (18 months).

No significant degradation of clopyralid was found in maize grain, forage or fodder when stored frozen for a period of 385 days as seen in Table B.7.1.1.1-1:

To sum up these previously assessed studies two tables were extracted:

Table B.7.1.1.1-1 The frozen storage stability of clopyralid in maize fractions fortified with 0.50 mg as/kg

	Average recovery (%) of clopyralid *		
Days of storage	Grain	Forage	Fodder
0	108	103	101
32	99	99	89
82	97	101	94
186	99	101	102
385	92	98	95

Table B.7.1.1.1-2a The frozen storage stability of incurred clopyralid residues in pasture. Corrected values demonstrated in the DAR shown.

	Average recovery (%) of clopyralid	
Days of storage	Mean sample residue (mg/kg)*	% of mean Day 0 value
0	14.25	100
34	13.56	95.2
128	12.03	84.4
332	17.5	122.8
520	13.46	94.5

Table B.7.1.1.1-2 The frozen storage stability of incurred clopyralid residues in pasture. Uncorrected orginal values shown.

	Average recovery (%) of clopyralid	
Days of storage	Mean sample residue (mg/kg)	% of mean Day 0 value
0	11.61	100.0
34	10.67	91.9
128	9.81	84.5
332	12.31	106.0
520	9.60	82.6

Table B.7.1.1.1-3 Storage intervals between sampling and analysis of samples from supervised residue trials

Crop	Storage interval between sampling and analysis (days)
Oilseed rape	366 to 740
Sugar beet	170 to 513
Cereals	180 to 595
Pasture	178 to 540

In majority of the previously assessed residue trials the samples were analysed within 12 months from sampling. In a few residue trials the length of storage period exceeded that in the storage stability studies by about six months.

However, it was decided that as clopyralid has undergone very little degradation during storage it was concluded that it is unlikely that clopyralid has degraded to 70 % of the original in a further six months. In a one individual study, the length of storage was even 740 days but the residue level was still in same order of magnitude as in the other studies. The length of storage time was considered to have no effect on the validity of the results of residue trials

In order to support all four major crop groups (wet, dry, oily and acidic), an additional study has been conducted for the AIR3 procedure and is evaluated below for the frozen storage stability of clopyralid in acidic and oily crops.

The edible commodities covered by stability studies comprise of orange fruit (high acid content), olive fruit and olive oil (high oil content).

B.7.1.1.2 Studies on Frozen Storage Stability of Residues of Clopyralid in Crop Matrices submitted for the present evaluation

Reference	Allen, L.; 2013; Frozen Storage Stability of Residues of Clopyralid in Crop Matrices; CEM Analytical Services (CEMAS), North Ascot, Berkshire, UK; Lab Study No. CEMS-5745; DAS Study No. 120939; 25 November 2013; Unpublished
Guideline(s):	OECD 506, EC Guideline 1607/VI/97 rev.2, Appendix H 7032/VI/95 rev.5
US EPA Guideline(s):	OPPTS 860.1380
Deviations:	None
Test item and purity:	99.9% clopyralide
Dates of work:	28 November 2012 to 01 October 2013
Analytical method	Dow AgroSciences Study Number 120610 ABC Study Number 68930
Matrixes tested	Orange fruit, orange peel (high acid content) and olive fruit and olive oil (high oil content)
GLP status:	Yes

Materials and Methods

Analytical method

The analytical method used for the determination of clopyralid was described in Dow AgroSciences Study Number 120610 and ABC Study Number 68930, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS". This method is applicable for the quantitative determination of residues of clopyralid and picloram in acidic crop matrices (orange fruit and orange peel) and oily crop matrices (olive fruit and olive oil). The method has been validated over the concentration range of 0.01-1.0 mg/kg with a validated limit of quantitation of 0.01 mg/kg. The analytical method was validated with both analytes, picloram and clopryalid, but no with clopyralid conjugates.

Method Principle

Residues of clopyralid are extracted from crop samples with 100:1 methanol:10N sodium hydroxide by blending for approximately 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to stand at ambient temperature overnight. An aliquot of the

extract is submitted to a nitrogen stream to remove the methanol and reconstituted to volume with 1N sodium hydroxide. The cleanup for crops is affected by portioning the basic extract with dichloromethane (DCM). An aliquot of the extract is acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) cleanup and elution with DCM. After removal of the DCM using nitrogen stream, the samples is reconstituted in 10:90 methanol:0.1% formic acid. After filtering through a 0.2-µm PTFE filter and the final sample was analysed for clopyralid by liquid chromatography coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

Test Procedure

The residue level used in the storage stability study was 0.1 mg/kg. This is in accordance with guideline recommendations of at least 10 times limit of quantitation of the method.

The storage stability study was carried out on orange and olive specimens (whole orange fruit, orange peel, olive fruit and olive oil) purchased locally. The orange were peeled, and analysis was carried out on orange fruit and orange peel separately.

Pitted, jarred olives in brine were drained and thoroughly rinsed with cold water prior to preparation.

Aliquots of each specimen were placed in separate, labelled 250 mL centrifuge bottles. The samples that were fortified at the beginning of the study each received an aliquot of a fortification solution containing clopyralid to achieve the fortification level of 0.1 mg/kg. An additional twelve fortified specimens were prepared for each matrix at the start of the study, to allow for any required repeat analyses. The samples used as matrix controls, unfortified matrix to be spiked as procedural recovery samples and the stored fortified matrix samples, were all stored separately from each other in a freezer set to maintain a specimen temperature of <-18°C. The bulk unfortified control matrix specimens were also stored at <-18°C.

At the initial (day 0) time point, the analysis consisted of the following samples: 1 unfortified control sample of each matrix; 2 control samples of each matrix that were freshly fortified at 0.1 mg/kg immediately prior to extraction to be used for procedural recoveries; 3 stored samples of each matrix that were fortified at 0.1 mg/kg immediately prior to extraction to be used for day 0 stored recoveries; 1 reagent blank.

At the 1 month (± 1 week), 2 months (± 2 weeks), 3 months (± 2 weeks) and 6 months (± 1 month) and 10 months (+ 1 month) time points, the analysis consisted of the following samples: 1 unfortified control sample of each matrix; 2 control samples of each matrix that were freshly fortified at 0.1 mg/kg immediately prior to extraction to be used for procedural recoveries; 3 stored samples of each matrix that were fortified at 0.1 mg/kg prior to storage; 1 reagent blank.

Results and Discussion

Method Performance

The validated method performance was further demonstrated by virtue of concurrent recovery samples analysed in this study. Mean concurrent recovery values were 70-120%; RSD ≤ 20%. The storage stability sample concentrations were corrected for the mean recovery values of the procedural samples. The results obtained are summarised in the following table.

Table B.7.1.1.2-1: Summary of analytical recovery of clopyralid (m/z 189.8/146.1)

Matrix group	Matrix	Fortification level	Recovery (%)		RSD	n
		(mg/kg)	mean	range	(%)	
Acidic Crops	**Orange Fruit**	**0.1**	**81**	**71-89**	**8.1**	**12**
Acidic Crops	**Orange Peel**	**0.1**	**79**	**71-89**	**7.1**	**12**
Oily Crops	**Pitted jarred olive fruit in brine**	**0.1**	**91**	**76-98**	**6.8**	**12**
Oily Crops	**Olive Oil**	**0.1**	**86**	**71-96**	**8.7**	**12**

Stability

The data indicates that residues of the parent compound, clopyralid, are stable for at least 305 days (10 months) in orange peel, orange fruit, olive oil and olive fruit stored under frozen conditions.

Storage stability of clopyralid was demonstrated for up to 305 days (10 months) in orange peel, orange fruit, olive oil and olive fruit, see data presented in the Tables from **Table B.7.1.1.2-2** to **Table B.7.1.1.2-5.**

In order to demonstrate storage stability of commodities with high oil content, pitted jarred olive fruit in brine were used. This commodity can be considered as processed, it may have a different pH than fresh olive fruits. Olives are consumed after different kinds of processing, but question remains wether the jarred olives truly represents edible commodities with high oil content in general. pH values (during and) after processing is an important piece of information. Any data indicating the pH of the brine or decribing the whole procedure applied, prior to purchasing the jarred olives with brine from a supermarket, were not available. Stability in basic environment is not covered in the hydrolysis studies.

Table B.7.1.1.2-2 Results of frozen storage stability samples for clopyralid residues – orange fruit

Days of storage	Spike level mg/kg	Uncorrected mg/kg found	Recovery (%)	Mean (%)	RSD (%)	Corrected mg/kg found	Recovery (%)	Mean (%)	RSD (%)
0	0.1	0.0868	86	86	10.5	0.1059	105	105	10.5
0	0.1	0.0951	95			0.1160	116		
0	0.1	0.0780	77			0.0951	94		
35	0.1	0.0769	76	75	1.3	0.1068	106	104	1.5
35	0.1	0.0761	75			0.1057	104		
35	0.1	0.0747	74			0.1038	103		
61	0.1	0.0826	82	78	5.2	0.1087	108	102	5.5
61	0.1	0.0750	74			0.0987	97		
61	0.1	0.0774	77			0.1018	101		
82	0.1	0.0788	78	78	3.2	0.0944	93	93	3.2
82	0.1	0.0755	75			0.0904	90		
82	0.1	0.0801	80			0.0959	96		
181	0.1	0.0903	90	87	3.5	0.1115	111	107	3.4
181	0.1	0.0845	84			0.1043	104		
181	0.1	0.0869	86			0.1073	106		
305	0.1	0.0821	80	80	0.0	0.0933	91	91	0.0
305	0.1	0.0813	80			0.0924	91		
305	0.1	0.0814	80			0.0925	91		

Table B.7.1.1.2-3 Results of frozen storage stability samples for clopyralid residues – orange peel

Days of storage	Spike level mg/kg	Uncorrected mg/kg found	Uncorrected Recovery (%)	Mean (%)	RSD (%)	Corrected mg/kg found	Recovery (%)	Mean (%)	RSD (%)
0	0.1	0.0859	85	81	6.4	0.0993	98	94	5.8
0	0.1	0.0850	84			0.0983	97		
0	0.1	0.0757	75			0.0875	87		
35	0.1	0.0790	78	79	1.5	0.1075	106	107	1.7
35	0.1	0.0789	78			0.1073	106		
35	0.1	0.0807	80			0.1098	109		
61	0.1	0.0717	71	73	2.7	0.0996	99	101	2.6
61	0.1	0.0759	75			0.1054	104		
61	0.1	0.0733	72			0.1018	100		
82	0.1	0.0765	76	80	5.9	0.0922	92	96	5.5
82	0.1	0.0853	85			0.1028	102		
82	0.1	0.0794	79			0.0957	95		
181	0.1	0.0713	71	77	6.4	0.0914	91	99	6.6
181	0.1	0.0782	78			0.1003	100		
181	0.1	0.0821	82			0.1053	105		
305	0.1	0.0712	70	71	2.9	0.0879	86	88	3.2
305	0.1	0.0760	74			0.0938	91		
305	0.1	0.0713	70			0.0880	86		

Table B.7.1.1.2-4 Results of frozen storage stability samples for clopyralid residues – olive fruit

Days of storage	Spike level mg/kg	Uncorrected mg/kg found	Uncorrected Recovery (%)	Mean (%)	SD (%)	Corrected mg/kg found	Recovery (%)	Mean (%)	RSD (%)
0	0.1	0.0941	91	94	3.5	0.1006	97	101	3.8
0	0.1	0.0971	94			0.1039	101		
0	0.1	0.1006	98			0.1076	105		
35	0.1	0.1004	97	94	2.6	0.1109	107	104	2.5
35	0.1	0.0966	93			0.1067	103		
35	0.1	0.0958	92			0.1059	102		
61	0.1	0.0933	92	90	2.1	0.1060	105	102	2.6
61	0.1	0.0896	89			0.1018	101		
61	0.1	0.0884	88			0.1005	100		
82	0.1	0.0931	93	96	2.5	0.0980	98	101	2.7
82	0.1	0.0964	96			0.1015	101		
82	0.1	0.0987	98			0.1039	103		
181	0.1	0.0858	85	80	7.6	0.1073	106	100	8.7
181	0.1	0.0836	83			0.1045	104		
181	0.1	0.0715	71			0.0894	89		
305	0.1	0.0910	89	91	4.4	0.0948	93	95	4.8
305	0.1	0.0895	88			0.0932	92		
305	0.1	0.0972	96			0.1013	100		

Table B.7.1.1.2-5 Results of frozen storage stability samples for clopyralid residues in olive oil.

Days of storage	Spike level mg/kg	Uncorrected mg/kg found	Uncorrected Recovery (%)	Mean (%)	RSD (%)	Corrected mg/kg found	Recovery (%)	Mean (%)	RSD (%)
0	0.1	0.0951	95	92	4.2	0.1017	102	98	3.8
0	0.1	0.0932	93			0.0997	99		
0	0.1	0.0891	88			0.0953	94		
35	0.1	0.0678	67	73	8.9	0.0869	86	93	8.4
35	0.1	0.0804	80			0.1031	103		
35	0.1	0.0719	71			0.0922	91		
61	0.1	0.0809	80	84	4.1	0.0889	88	92	3.5
61	0.1	0.0872	86			0.0958	95		
61	0.1	0.0857	85			0.0942	93		
82	0.1	0.0932	93	82	15.5	0.1158	116	102	17.4
82	0.1	0.0694	69			0.0862	84		
82	0.1	0.0847	84			0.1052	104		
181	0.1	0.0896	89	89	6.9	0.1042	103	103	6.1
181	0.1	0.0836	83			0.0972	97		
181	0.1	0.0952	95			0.1107	110		
305	0.1	0.0799	78	86	9.0	0.0868	85	93	8.5
305	0.1	0.0897	88			0.0975	96		
305	0.1	0.0935	92			0.1016	100		

B.7.1.2 Products of animal origin

Data to address this point were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. However, a data gap was identified for the frozen storage stability in fat tissue. An additional clopyralid frozen storage stability study in bovine fat was conducted to address this gap and is summarized below.

B.7.1.2.1 Frozen Storage Stability of Clopyralid in Bovine Fat

REFERENCE	[redacted] 2015; Frozen Storage Stability of Clopyralid in Bovine Fat; [redacted] Lab Study No. 69209; DAS Study No. 120602; 15 January 2015; Unpublished
Guideline(s):	OECD 506, EC Guideline 1607/VI/97 rev.2, Appendix H 7032/VI/95 rev.5
US EPA Guideline(s):	OPPTS 860.1380
Deviations:	None
Test Item and purity	99.9%
Analytical method	Dow AgroSciences Method 120483
Matrixes tested	Bovine fat
Dates of work:	15 November 2012 to 26 November 2014
GLP status:	Yes

Materials and Methods

Method Scope

The objective of this study is to provide frozen storage stability data for the determination of clopyralid in bovine fat. The samples used for the storage stability study were stored frozen at approximately –20 °C under conditions which resemble the storage conditions of the residue study samples. The analytical method used for the determination of clopyralid was Dow AgroSciences Method 120483, "Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection." Control bovine fat sample replicates were fortified with clopyralid at 0.10 mg/kg. Samples were stored frozen (approximately –20°C) and analyzed using procedures validated in Dow AgroSciences Method 120483, "Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection," at various time points representative of the long-term frozen storage. The method principle and method evaluateion have been presented in the Section B.5, and is not repeated here.

Test Procedure

Control bovine fat sample replicates were fortified with clopyralid at 0.10 mg/kg. One-gram aliquots of bovine fat samples were weighed into an appropriate number of containers to provide sufficient samples for eight sampling intervals, and an additional two contingency intervals. For the Day 0 sampling point, duplicate fresh fortified samples were prepared for analysis. For all sampling intervals beyond Day 0 (approximately 1, 4, 8, 12, 16, 20, and 24

months), two appropriately aged fortified samples (AF) were analyzed concurrently with two freshly fortified samples (FF) and one untreated control sample (C).

Results and Discussion

Method Performance

The validated method performance was further demonstrated by virtue of concurrent recovery samples analysed in this study. Mean concurrent recovery values were 70-120%; RSD ≤ 20%. Sample recoveries from stored fortifications were normalized for the residues found in the current analytical set based on the mean of the recoveries found in the fresh fortified samples. The stored sample recovery was divided by the average of the recoveries found in the two fresh fortified samples, then multiplied by 100 to attain a normalized percentage result.

Stability

Storage stability of clopyralid was demonstrated for up to 24 months in bovine fat.

Table B.7.1.2-1 Summary of clopyralid residues in bovine fat at 0.10 mg/kg and stored at approximately -20°C

Matrix	Fort level in mg/kg	Storage interval (Months)	Rep	Recovery					
				Recovered Residues (mg/kg)		Recovery (%)		Normalized Recoverya	
				Stored (-20°C) Forts	Fresh Forts	Stored	Fresh	Individual	Average
Bovine Fat	0.10	0	1	--	0.0940	--	94	--	-
Bovine Fat	0.10	0	2	--	0.0863	--	86	--	
Bovine Fat	0.10	1	1	0.0854	0.0804	85	80	104	114
Bovine Fat	0.10	1	2	0.1014	0.0836	101	84	124b	
Bovine Fat	0.10	4	1	0.0729	0.0758	73	76	97	100
Bovine Fat	0.10	4	2	0.0773	0.0750	77	75	102	
Bovine Fat	0.10	8	1	0.0852	0.0799	85	80	103	102
Bovine Fat	0.10	8	2	0.0826	0.0848	83	85	100	
Bovine Fat	0.10	12	1	0.0809	0.0802	81	80	97	98
Bovine Fat	0.10	12	2	0.0819	0.0868	82	87	98	
Bovine Fat	0.10	16	1	0.0974	0.0800	97	80	116	112
Bovine Fat	0.10	15	2	0.0903	0.0873	90	87	108	
Bovine Fat	0.10	20	1	0.0857	0.0974	86	97	91	97
Bovine Fat	0.10	20	2	0.0978	0.0910	98	91	104	
Bovine Fat	0.10	24	1	0.0909	0.0830	91	83	104	103
Bovine Fat	0.10	24	2	0.0884	0.0915	88	92	101	

[a] Normalized recovery for stored fortifications is based on the average of the two fresh fortifications for the analyte at the time point.

Note: All percent recovery values are based on unrounded values. Hand calculations may vary from reported values because of rounding

Example Calculation: Clopyralid, 8 month, Replicate 2

Mean % Fresh Fortifications of Replicates 1 and 2: (80 + 85)/2 = 82%

Stored Fortification: 0.0826 mg/kg = 83%

Normalized Recovery: 83/82 = 1.00 x 100 = 100%

[b] Outside acceptable criteria range (70-120%) NA = Not yet available

B.7.1.2.2 Frozen Storage Stability of Clopyralid in Bovine Muscle, Bovine Liver, Kidney, Milk and Chicken Egg

REFERENCE	[redacted] 2004; Frozen Storage Stability of Clopyralid in Beef Muscle, Liver, Kidney, Milk and Chicken Egg; [redacted] DAS Study No. 020120.01; 28 September 2004; Unpublished
Guideline(s):	EPA OPPTS 860.1380
Deviations:	None
Test Item	Clopyralid 99.8%
Analytical method	Dow AgroSciences Method 120483 Dow AgroSciences Method GRM 02.14 GC –MS with negative ion chemical ionisation.
Matrixes tested	Bovine muscle, kidney, liver, whole milk, and chicken egg
GLP status:	Yes

Materials and Methods

Method Scope

The purpose of this study was to determine the stability of clopyralid residues in beef muscle,liver, kidney, milk, and chicken egg when stored under frozen conditions for a duration of eighteen months. This study was designed to fulfill requirements under the following guidelines: EPA OPPTS 860.1380, Amending Council Directive *911414IEEC* and EC Commission Directive 96/68/EC.

The samples used for the storage stability study were stored frozen at approximately –20 °C under conditions which resemble the storage conditions of the residue study samples. Control animal tissue matrix sample replicates were fortified with clopyralid at 0.10 mg/kg. Samples were stored frozen (approximately –20°C) and analyzed using procedures validated in Dow AgroSciences Method GRM 02.14, "Determination of Residues of Clopyralid in Animal Tissues by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry," at various time points representative of the long-term frozen storage.

Method Principle

Residues of clopyralid were extracted from each sample by adding 20 mL of 2.5N NaOH. Each sample was firmly capped and placed on a horizontal shaker for 1 minute. After shaking, all samples were placed in an oven set at 105°C for 2 hours. After 2 hours, the samples were removed from the oven and allowed to equilibrate to ambient temperature. After cooling, lids were sealed and each sample centrifuged for 5 minutes at 2000 rpm. A 1.0 mL aliquot was then taken from each sample and placed into clean, separate culture tubes,

diluted with 5 mL of IN HCl, and vortex mixed in. Residues of clopyralid were concentrated and purified using solid-phase extraction (SPE) cartridge. Following sample clean-up with the SPE cartridges, residues of clopyralid were eluted into clean, separate screw-top vials with two 2-mL aliquots of dichloromethane.

The eluates were evaporated to incipient dryness using a concentrator set at 40°C with approximately 10 psi of nitrogen. Upon evaporation, 0.5 mL of propylation reagent was added to each sample vial.

Each vial was firmly capped and placed in a 105°C oven for 30 minutes. After 30 minutes, the samples were removed from the oven and allowed to equilibrate to ambient temperature. The samples were then placed in a concentrator set at 50°C with nitrogen for evaporative removal of the l-propanol until about 50 µL of the propylation reagent remained. Then, 1 mL of an aqueous NaCl solution, 10% w/v, and internal standard, clopyralid butyl ester, was added to each vial. Each vial was capped, placed on a horizontal shaker at approximately for 2 minutes and centrifuged for 5 minutes. An aliquot of the internal standard from each sample was transferred to clean, separate GC vials, firmly capped, and analyzed by gas chromatography with mass selective detection (GC/MSD).

The derivatisation is accomplished with with n-propanol/sulphuric acid to form the propyl ester of clopyralid. The internal standard has been added after the derivatisation step.

Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by power regression analysis.

A calibration curve resulting from the injection of 8 standard concentrations is included in the method and demonstrates linearity with a correlation coefficient (r^2) of 0.9996.

However, the calibration curve reflects the chromatographic step only, i.e. demonstrates the instrumental performance, but does not give any information of the method performance as such.

Test Procedure

Control animal tissue sample replicates were fortified with clopyralid at 0.10 mg/kg. One-gram aliquots of bovine tissue samples were weighed into an appropriate number of containers to provide sufficient samples for the planned sampling intervals, and an additional two contingency intervals. For the Day 0 sampling point, duplicate fresh fortified samples were prepared for analysis For all sampling intervals beyond Day 0 (approximately 1, 3, 8, 13, and 18 months), two appropriately aged fortified samples were analyzed concurrently with two freshly fortified samples and one untreated control sample.

Results and Discussion

Method Performance

The validated method performance was further demonstrated by virtue of concurrent recovery samples analysed in this study. Concurrent recovery values were within 70-120%; RSD ≤ 20%.

Sample recoveries from stored fortifications were normalized for the residues found in the current analytical set based on the mean of the recoveries found in the fresh fortified samples. The stored sample found concentration was divided by the average of the recoveries found in the two fresh fortified samples, then multiplied by 100 to attain a normalized percentage result.

This data normalization process is not considered approbiate by the RMS, and all the normalized data in the Tables have painted with gray tone and have been ignored.

The method has not been tested with clopyralid conjugates. As found in 7.6.1.2. extraction with methanol/NaOH does not break the conjugates.

Stability

Storage stability of clopyralid was demonstrated for up to 18 months in bovine muscle, bovine liver, bovine kidney, milk and chicken egg.

B.7.1.2.2-1 Summary of clopyralid residues in bovine muscle at 0.10 mg/kg and stored at approximately -20°C

Fort level in mg/kg	Storage interval (Months)	Rep	Recovered Residues (mg/kg)		Recovery (%)		Normalized Recovery	
			Stored (-20°C) Forts	Fresh Forts	Stored	Fresh	Individual	Average
0.10	0	1	--	0.0893	--	89	--	--
0.10	0	2	--	0.0867	--	87	--	
0.10	0	3	--	0.0851	--	85	--	
0.10	3	1	0.0736	0.0888	74	88	86	84
0.10	3	2	0.0733	0.0826	73	83	86	
0.10	3	3	0.0689	--	69	--	80	
0.10	8	1	0.0765	0.0814	77	81	92	95
0.10	8	2	0.0798	0.0849	80	85	96	
0.10	8	3	0.0814	--	81	--	98	
0.10	13	1	0.0748	0.0728	75	73	102	106
0.10	13	2	0.0788	0.0743	79	74	107	
0.10	13	3	0.0799	--	80	--	109	
0.10	18	1	0.1144	0.1022	114	102	105	92
0.10	18	2	0.0852	0.1160	85	116	78	
0.10	18	3	0.1008	--	101	--	92	

Note: All percent recovery values are based on unrounded values. Hand calculations may vary from reported values because of rounding.

The grey tone indicates data that has not been taken into account in the evaluation, because normalised recovery is not considered informative.

B.7.1.2.2 -2 Summary of clopyralid residues in bovine liver at 0.10 mg/kg and stored at approximately -20°C

Fort level in mg/kg	Storage interval (Months)	Rep	Recovered Residues (mg/kg)		Recovery (%)		Normalized Recovery[a]	
			Stored (-20°C) Forts	Fresh Forts	Stored	Fresh	Individual	Average
0.10	0	1	--	0.0937	--	94	--	--
0.10	0	2	--	0.0876	--	88	--	
0.10	0	3	--	0.0837	--	84	--	
0.10	3	1	0.0857	0.0801	86	80	106	103
0.10	3	2	DNU	0.0818	NC	82	NC	
0.10	3	3	0.0809	--	81	--	100	
0.10	8	1	0.0785	0.0698	79	70	107	101
0.10	8	2	0.0688	0.0771	69	77	94	
0.10	8	3	0.0753	--	75	--	103	
0.10	13	1	0.0956	0.0867	96	87	115	109
0.10	13	2	0.0929	0.0801	93	80	111	
0.10	13	3	0.0835	--	84	--	100	
0.10	18	1	0.1119	0.0972	112	97	113	108
0.10	18	2	0.1033	0.1009	103	101	104	
0.10	18	3	0.1060	--	106	--	107	

Note: All percent recovery values are based on unrounded values. Hand calculations may vary from reported values because of rounding.

The grey tone indicates data that has not been taken into account in the evaluation, because normalised recovery is not considered informative.

DNU – Data Not Used – the internal standard response was 50% of the value of all other samples
NC – Not Calculated

B.7.1.2.2 -3 Summary of clopyralid residues in bovine kidney at 0.10 mg/kg and stored at approximately -20°C

Fort level in mg/kg	Storage interval (Months)	Rep	Recovery					
			Recovered Residues (mg/kg)		Recovery (%)		Normalized Recovery[a]	
			Stored (-20°C) Forts	Fresh Forts	Stored	Fresh	Individual	Average
0.10	0	1	--	0.0941	--	94	--	--
0.10	0	2	--	0.0870	--	87	--	
0.10	0	3	--	0.0837	--	84	--	
0.10	3	1	0.0806	0.0815	81	82	93	92
0.10	3	2	0.0797	0.0925	80	93	92	
0.10	3	3	0.0800	--	80	--	92	
0.10	8	1	0.0692	0.0851	69	85	89	94
0.10	8	2	0.0776	0.0707	78	71	100	
0.10	8	3	0.0719	--	72	--	92	
0.10	13	1	0.0786	0.0798	79	80	95	97
0.10	13	2	0.0806	0.0864	81	86	97	
0.10	13	3	0.0817	--	82	--	98	
0.10	18	1	0.1168	0.1034	117	103	118	110
0.10	18	2	0.1080	0.0946	108	95	109	
0.10	18	3	0.1027	--	103	--	104	

Note: All percent recovery values are based on unrounded values. Hand calculations may vary from reported values because of rounding.

The grey tone indicates data that has not been taken into account in the evaluation, because normalised recovery is not considered informative.

B.7.1.2.2 -4 Summary of clopyralid residues in bovine milk at 0.10 mg/kg and stored at approximately -20°C

Fort level in mg/kg	Storage interval (Months)	Rep	Recovery					
			Recovered Residues (mg/kg)		Recovery (%)		Normalized Recovery[a]	
			Stored (-20°C) Forts	Fresh Forts	Stored	Fresh	Individual	Average
0.10	0	1	--	0.0801	--	80	--	--
0.10	0	2	--	0.0872	--	87	--	
0.10	0	3	--	0.0780	--	78	--	
0.10	3	1	0.0691	0.0755	69	75	94	92
0.10	3	2	0.0651	0.0723	65	72	88	
0.10	3	3	0.0695	--	70	--	94	
0.10	8	1	0.0774	0.0762	77	76	104	94
0.10	8	2	0.0639	0.0723	64	72	86	
0.10	8	3	0.0673	--	67	--	91	
0.10	13	1	0.0714	0.0715	71	71	101	95
0.10	13	2	0.0662	0.0705	66	70	93	
0.10	13	3	0.0654	--	65	--	92	
0.10	18	1	0.0914	0.0913	91	91	102	97
0.10	18	2	0.0736	0.0873	74	87	82	
0.10	18	3	0.0954	--	95	--	107	

Note: All percent recovery values are based on unrounded values. Hand calculations may vary from reported values because of rounding.

The grey tone indicates data that has not been taken into account in the evaluation, because normalised recovery is not considered informative.

B.7.1.2.2 -5 Summary of clopyralid residues in chicken egg matrix at 0.10 mg/kg and stored at approximately -20°C

Fort level in mg/kg	Storage interval (Months)	Rep	Recovered Residues (mg/kg)		Recovery (%)		Normalized Recovery[a]	
			Stored (-20°C) Forts	Fresh Forts	Stored	Fresh	Individual	Average
0.10	0	1	--	0.0858	--	86	--	--
0.10	0	2	--	0.0845	--	85	--	
0.10	0	3	--	0.0839	--	84	--	
0.10	3	1	0.0895	0.0747	90	75	115	110
0.10	3	2	0.0819	0.0804	82	80	106	
0.10	3	3	0.0848	--	85	--	109	
0.10	8	1	0.0717	0.0797	72	80	86	84
0.10	8	2	0.0736	0.0867	74	87	88	
0.10	8	3	0.0634	--	63	--	79	
0.10	13	1	0.0780	0.0764	78	76	103	104
0.10	13	2	0.0759	0.0751	76	75	100	
0.10	13	3	0.0830	--	83	--	110	
0.10	18	1	0.0903	0.0942	90	94	92	96
0.10	18	2	0.0963	0.1015	96	102	98	
0.10	18	3	0.0952	--	95	--	97	

Note: All percent recovery values are based on unrounded values. Hand calculations may vary from reported values because of rounding.

The grey tone indicates data that has not been taken into account in the evaluation, because normalised recovery is not considered informative.

B.7.2 Metabolism, Distribution and Expression of Residues

B.7.2.1 Metabolism in plants

The metabolism of clopyralid residues in primary crops has been investigated in leafy vegetables (cabbage), root and tuber vegetables (sugar beet), oilseeds (rapeseed). The studies were conducted using radioactive labels at two different positions at foliar application rates, which were compliant with intended GAPs. Consequently there are sufficient data concerning three crop groups allowing derivation of universal residue definitions.

B.7.2.1.1 Previously submitted plant metabolism studies

B.7.2.1.1.1 Previously submitted **sugar beet** metabolism studies

Sugar beet metabolism of clopyralid included in the DAR which was prepared by MS Finland.

Report	Pillar, I. Chapleo, S. and Caley, C.Y. (2002) The Metabolism of [^{14}C]-Clopyralid in Sugar Beet. Inveresk Research study number 397619
DAS Study number	GHE-P-9939
Guidelines	EC Directive 91/414/EEC
Test material	[^{14}C]-Clopyralid; 4.1 mCi
Test system/test conditions:	The ground used for this study was prepared for rotary cultivation. Two areas each 4x4 m and ca 33 m apart were prepared, one for test plot and one as a control plot.
Application rate and timing	Application of radioactive formulation corresponds to 300 g as/ha and foliar sprays were done when the sugar beet leaf rosettes were at ca 60% final size (growth stage 36)
Formulation	Lontrel 100 (EF 1136, 100 g/L SL)
Plant species	sugar beet (*Beta vulgaris* cv. Wildcat).
Site	EU
GLP	Yes

The nature of the radioactivity was characterised by HPLC and TLC. All of the radioacticity in the surface water wash, the acetonitrile/water extract and ether fraction on the day 0 and day 28 was determined by HPLC to be clopyralid (97.4 % and 84.8 % of TRR, respectively), unextracted residues accounted for 0.5 – 0.7 % of TRR. At maturity in shoots and roots clopyralid was detected in acetonitrile/water ectracts and the ether fractions (51.3 % and 57.8 % of TRR, respectively). A polar component was characterised in the acetonitrile/water fractions (37.3 % - 39.1 % of TRR). When more polar HPLC conditions or acid hydrolysis (6 M HCl, 60 °C, 1h) were used, it was concluded that this polar fraction represents free clopyralid. Characterisation of radioacticity is presented in the table B.7.2.1-2

Table B.7.2.1-1 Distribution and characterisation of radioactivity to sugar beet extracts

Fraction and components	Day 0		Day 28		Maturity			
					Shoots		Roots	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Identified components						(89.5)		(96.9)
Clopyralid	11.465	97.4	1.097	84. 8	0.218	51.3	0.212	57.8
Polar form of clopyralid	-	-	-	-	0.159	37.3	0.144	39.1
Clopyralid conjugates	-	-	-	-	0.004	0.9	-	-
Uncharacterised fractions								
Aqueous surface wash		(0.9)		(16.3)		(11.0)		(13.9)
Dichloromethane surface wash	(9.579)*	(81.4)*	(0.148)*	(11.5)*	0.001	0.3	0.004	1.2
Aqueous residue	0.012	0.1	0.003	0.2	0.002	0.5	0.000	0.0
6M HCl ectract	0.039	0.3	0.091	7.1	0.023	5.5	0.025	6.7
Non-extractable	-	-	0.107	8.3	0.014	3.3	0.020	5.4
	0.055	0.5	0.009	0.7	0.006	1.4	0.002	0.6
Unextractable (URR)	0.106	1.8	0.21	32.6	0.046	22	0.051	27.8
Recovery	11.571	98.3	1.307	101.1	0.427	100.5	0.407	110.8

*characterised on day 0 and day 28

No positive identification was made for any of the components. The identification of the compounds was based on co-chromatography and presence of the radioactivity.

Peak identification as clopyralid for the polar peak, found at a level of 37 - 39% TRR in sugar beet shoots and toos and roots, is uncertain.

At maturity 22-27.8 %TRR in shoots and roots was uncharacterised. Though each fraction was < 10 % of TRR some of them exceeded 0.05 mg/kg.

Based on the supportive study on pasture, the metabolism of clopyralid in grass is also very limited and the reduction of residue levels (from 13 mg/kg to 0.16 mg/kg) is due to the growth dilution.

B.7.2.1.1.2 Previously submitted **oilseed rape** metabolism studies

Oilseed rape metabolism of clopyralid included in the DAR which was prepared by MS Finland.

Report	Chapleo, S. and Caley, C.Y. (2002) The Metabolism of [^{14}C]-Clopyralid in Oilseed Rape. Inveresk Research study number 397624
DAS Study number	GHE-P-9938
Guidelines	draft Commission guidance document, 7028/VI/95 EN rev. 3. 22/7/97
Test material	[^{14}C]-Clopyralid; 4.1 mCi
Test system/test conditions:	^{14}C-clopyralid (radiochemical purity >97%, specific acitivity 30.9 mCi/mmol)
Application rate and timing	Application rate was 300 g as/ha and foliar sprays were done when the crop had reached ca 60% of final size (growth stage 36 28 days after application and at maturity. PHI 28 days.
Formulation	Lontrel 100 (EF 1136, 100 g/L SL
Plant species	*Brassica napa* cv. Mascot
Site	EU
GLP	Yes

Methods:

^{14}C-clopyralid (radiochemical purity >97%, specific acitivity 30.9 mCi/mmol) formulated as Lontrel 100 (EF-1136, 100 g/L SL) was applied on oilseed rape (*Brassica napa* cv. Mascot). Application rate was 300 g as/ha and foliar sprays were done when the crop had reached ca 60% of final size (growth stage 36, 14.6.2000). Study was conducted outdoors using plants grown in field plots. Whole plants were harvested on the day of application, at 28 days after application and at maturity (30.8.2000). Immature plants were analysed whole. Plants at maturity were separated into straw (including pods) and seeds prior to analysis. Whole plants and straw were surface washed with water and dichloromethane on the day of sampling. The tissue and seeds were subsequently milled in cardice, which was allowed to sublime prior to further analysis.

Liquid samples were analysed by LSC. Solid samples were analysed by oxidative combustion followed by LSC. Aliquots of milled tissue were extracted with acetonitrile:water (1:1, v/v) followed by 0.125M sodium hydroxide:methanol (1:1, v/v). Radioactivity in the sodium hydroxide:methanol extracts was extracted using ether. The remaining unextracted residues at maturity were further extracted with 6M HCl (90 ºC, 5h). The nature of polar components in selected extracts was investigated by re-analysis following acid hydrolysis (6M HCl, 60 ºC, 1h) and following incubation with β-glucosidase activity. Analysis were performed by HPLC, the gradient was modified to characterise polar radioactive components in selected extracts. TLC was used for confirmation of the identification of radioactive components in selected extracts.

Table B.7.2.1.1.2-1 Distribution of total radioactive residue (TRR) in treated oilseed rape, mg/kg expressed as clopyralid equivalents

Harvest Point	Day 0		Day 28		Maturity			
					Straw		Seeds	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Water wash	3.947	88.2	0.010	0.7	0.009	1.2	Na	na
DCM wash	0.009	0.2	0.003	0.2	nd	nd	na	na
Tissue	0.521	11.6	1.275	99.0	0.746	98.8	0.067	100.0
Whole sample	4.477	100	1.288	100	0.755	100	0.067	100

Table B.7.2.1.1.2-2 Distribution and characterisation of radioactivity in oilseed rape extracts

	Day 0		Day 28		Maturity			
					Straw		Seeds	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Identified components				(84.6)		(94.1)		(89.0)
Clopyralid	4.47	99.9	0.811	62.9	0.245	32.4	0.029	43.4
Polar form of clopyralid	-	-	-	-	0.244	32.3	0.018	27.5
Clopyralid conjugates	-	-	0.280	21.7	0.222	29.4	0.012	18.1
Uncharacterised fractions		(1.0)		(16.2)		(9.9)		(11.8)
Aqueous surface wash	(3.95)*	(88.2)*	0.010	0.7	0.009	1.2	-	-
Dichloromethane surface wash	0.009	0.2	0.003	0.2	0.000	0.0	-	-
Aqueous residue	0.139	0.3	0.075	5.8	0.011	1.5	0.002	2.4
6M HCl ectract	-	-	-	-	0.045	6.0	0.002	3.8
Unextractable (URR)	0.023	0.5	0.122	9.5	0.009	1.2	0.004	5.6
Recovery	4.513	100.9	1.301	100.8	0.785	104.0	0.067	110.8

At day 28, 62.9 % of recovered radioactivity was clopyralid in acetonitrile and ether fraction from tissue extraction, in acetonitrile extract also another, maybe conjugated clopyralid, was found (21.7 % of TRR). Non-extractable radioactivity at day 28 was quite high, 9.5 % of TRR.

Most of the recovered radioactivity was removed from the rape plants by surface washing on the day of application. At day 28 and at maturity most of the radioactivity was taken up into plants. Less than 10 % of TRR was unextractable by solvents. At maturity the major radioactive compound was unchanged parent compound and others presumably polar and conjugated forms of clopyralid, together these fractions accounted for 89 -94 % of TRR. No significant metabolites were detected, but at maturity 9.9 -11.8 % of TRR was uncharacterised, though each fraction was < 10 % of TRR.

No positive identification, such aa HPLC MS/MS was made for any of the components. The identification of the compounds was based on co-chromatography and presence of the radio label.

B.7.2.1.1.3 Previously submitted **cabbage** metabolism studies

to reflect the requirements of 96/68/EC amending EC Directive 91/414/EEC.

Report	Guo, C (1996). Metabolism of 14C-Clopyralid and Cabbage. Dow AgroSciences unpublished report RES95095 (GH-C 4289), 6th December 1996.
DAS Study number	RES95095 (GH-C 4289)
Guidelines	Commission guidance document, 7028/VI/95 EN rev. 3. 22/7/97 US EPA Pesticide Assessment Guidelines (Subdivision O, Section 171-4) The requirements of 96/68/EC amending EC Directive 91/414/EEC.
Test material	^{14}C-clopyralid (radiochemical purity 99.5%, specific radioactivity 31 mCi/mmol)
Test system/test conditions:	
Application rate and timing	Application at at the 8 – 10 leaf stage at an application rate of 0.375 lb a.s./acre, equivalent to 0.4203 kg as/ha (ca 1.5x the commercial use rate). Foliar spray 46 days after planting. PHI 38 DALA.
Formulation	Not given.
Plant species	Cabbage plants (‘Copenhagen Market’)
Site	outside EU
GLP	Yes Good Laboratory Practice Standards set forth in 40 CFR, Part 160; Final Rule, and Nuclear Regulatory Commission (NRC) regulations.

Almost all (103 % in cabbage head and 104% in cabbage wrapper leaf) of total radioactive residue (TRR) found in the tissues was extracted with alkaline methanol (0.125M $NaOH/CH_3OH$). Bound residues were 2.3% and 14% of TRR in cabbage head and wrapper leaf, respectively. No further characterization was conducted for the bound residues as only <0.03 ppm was present.

The extractable residues in cabbage heads and cabbage wrapper leaves were characterized separately by HPLC and TLC analyses. Ciopyralid (parent) represented 92% of the TRR in both tissues by two chromatographic procedures. The identity of clopyralid was confirmed

via co-chromatography with "C-clopyralid using HPLC and TLC analyses. Other minor unknown components were detected but each represented less than 0.02 ppm residue and were not further characterized. The TRR and its characterization in cabbage heads and wrapper leaves is summarized in the **Table B.7.2.1.1.3-1.**

Table B.7.2.1.1.3-1 Summary of distribution of radioactivity (TRR) in extracts of cabbage at maturity following application of clopyralid at a rate of 0.42 kg as/ha

	Head		Wrapper Leaf	
	% TRR	mg/kg	% TRR	mg/kg
Total radioactivity	100	0.351	100	1.22
Extractable				
- 0.125 M NaOH/CH_3OH	103	0.361	104	1.27
- Ether	96.9	0.340	102	1.24
Non -extractable	2.28	0.008	2.38	0.029
HPLC Components of ether fraction:				
Clopyralid	91.5	0.321	99.2	1.21
Unknown 1 (3 min)	nd	nd	0.41	0.005
Unknown 2 (11-12)	4.27	0.015	0.9	0.011
Unknown 3 (14)	nd	nd	0.33	0.004
Unknown 4 (18-19)	1.14	0.004	0.16	0.002
Unknown 5 (20-20.5)	nd	nd	0.57	0.007

The application rate was 0.42 kg as/ha (ca 1.5x the commercial use rate).

B.7.2.1.1.4 **Pasture grass** metabolism

REFERENCE	Bauriedel WR, Miller JL 1981: A field metabolism study 3,6-Dichloropicolinic acid applied to pasture grass Unpublished Study report GH-C-1424
Guideline(s):	None
Test material	
GLP status:	N

The first study by Bauriedel and Miller (1981) has not been conducted following OECD GLP guidelines published in 1993, and thus is currently regarded as supportive suty only. It was very briefly reported and most of the necessary raw data values were not included. Thus the thorough evaluation of the study is not possible.

Methods

In the Bauriedel et Miller study (1981) An established plot of pasture grass was treated in the spring of 1980 with 3,6-dichloropicolinic acid-2,6 ^{14}C, at the rate of 1 lb a.e. per acre. Samples of the grass were removed at 1, 21 4, 8 and 18 weeks after treatment to determine the amount and identity of the residue.

^{14}C-clopyralid (radiochemical purity >99%, specific activity 1.52 mCi/mmol) formulated as Lontrel was applied to three plots (A, B, C) of established pasture grass at an application rate of 1.121 kg as/ha. Grass samples were collected from separate plots 1 (A), 2 (B), 4 (A, B), 8 (C) and 18 (A, B,C) weeks after application by cutting the entire plot 5 cm above ground level to simulate grazing. The application rate according to present critical GAP for pasture is approximately 240 g ai/ha and PHI 7 days.

At harvest, samples were air-dried, ground to a fine powder prior to determination of radioactive residue by combustion and LSC. Tissues were extracted with 0.125M NaOH/methanol and partitioned into ether. Residues were characterised by HPLC or GC-MS and TLC by comparison against known reference compounds.

Results

The residue decreased in time from a 1-week concentration of 100 ppm to an 18-week concentration of 0.6 ppm (both values dry-weight basis). The data indicated a half-life of about 2.3 weeks. The residue in all samples consisted of only unchanged 3,6 dichloropicolinic acid.

B.7.2.1.2 Plant metabolism studies submitted for the present application renewal

B.7.2.1.2.1 Plant uptake of ^{14}C-labelled clopyralid in wheat and oilseed rape under greenhouse conditions

REFERENCE	Gourlay, V.; 2015; Plant uptake of ^{14}C-labelled clopyralid in wheat and oilseed rape under greenhouse conditions; RLP AgroScience GmbH, 67435 Neustadt a.d. Weinstraße, Germany; Lab Study No. AS421; DAS Study No. 150297; 25.06.2015; Unpublished
Guideline(s):	Non-guideline study
US EPA Guideline(s):	-
Deviations:	-
Dates of work:	16 January 2015 to 25 June 2015
Test material	^{14}C -label at two sites of the pyridine ring. Radiochemical purity: 98.0% (by HPLC, TLC) Specific radioactivity: 32.8 mCi.mmol^{-1}
GLP status:	Yes

Biodistribution is one of the issues, which is to be studied to clarify metabolism. The study by Gourlay (2015) was the only new plant metabolism study submitted. The study gives information on uptake, but metabolism as such is not covered by this type of study.

Materials and Methods

Theory

The plant uptake factor is defined as the ratio of the concentration of a compound in the solution consumed by the plant (C_{uptake}) to the concentration of that compound in the soil solution ($C_{solution}$), given in Equation 1 (Sweeney et al., 2013. A Simple Method for Measuring the Uptake of Chemicals from Solution into Plant, submitted for publication).

$$PUF = \frac{C_{solution}}{C_{solution}} \ [-] \qquad \text{Equation 1}$$

with

$$C_{uptake} = \frac{m_{uptake}}{V_{uptake}} \ [\mu g.L^{-1}] \qquad \text{Equation 2}$$

and

m_{uptake}	mass of compound taken up by plant	[µg]
V_{uptake}	volume of soil solution taken up by plant	[L]

$C_{solution}$	concentration of compound in soil solution	[µg.L^{-1}]

When Equation 1 and Equation 2 are combined, they can be transformed into a differential equation, given in Equation 3.

$$\frac{dm_{uptake}}{dV_{uptake}} = PUF \times \frac{m_{solution\text{-}0} - m_{uptake}}{V_{solution\text{-}0} - V_{uptake}} \quad [\mu g.L^{-1}] \qquad \text{Equation 3}$$

with

$m_{solution\text{-}0}$	initial mass of test item in test solution	[µg]
$V_{solution\text{-}0}$	initial volume of test solution	[L]
PUF	plant uptake factor	[-]

The general solution to that differential equation resulting in the PUF can be written as Equation 4:

$$PUF = \frac{\ln\left(\frac{m_{solution\text{-}f}}{m_{solution\text{-}i}}\right)}{\ln\left(\frac{V_{solution\text{-}f}}{V_{solution\text{-}i}}\right)} \quad [-] \qquad \text{Equation 4}$$

with

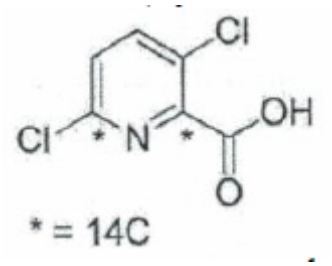
PUF	plant uptake factor	[-]
$m_{solution\text{-}f}$	mass of test item remaining in test solution on termination	[µg]
$V_{solution\text{-}f}$	remaining volume of test solution after eight days	[L]
$m_{solution\text{-}i}$	Initial or pseudo-initial mass of test item	[µg]
$V_{solution\text{-}i}$	initial or pseudo-initial volume of test solution	[L]

Test Item(s)

Reference item

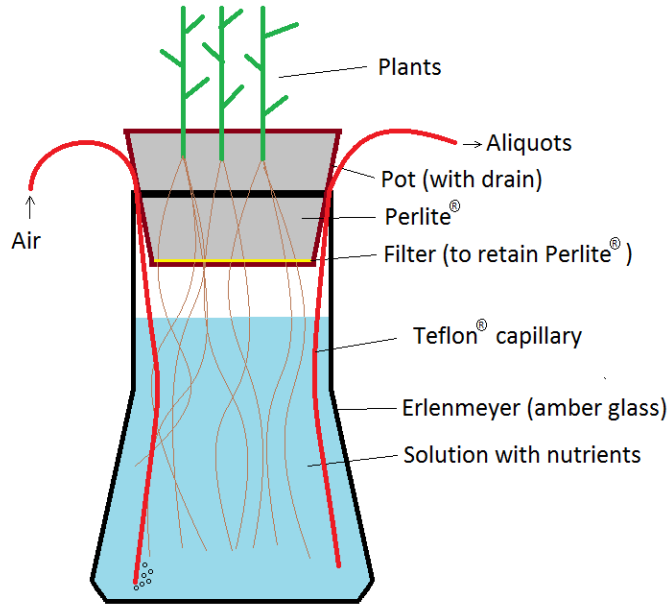
ISO Common name: Clopyralid

Radiolabelled test item #1

Name:	Clopyralid-2,6-^{14}C
Test item (chemical/other name):	3,6-Dichloropicolinic acid-2,6-^{14}C
Position of labelling (*)	

Test System

Figure B.7.2.1.1.1-1: Schematic view of the hydroponic test system



The test system consists of 1 L brown glass vessel filled with 800 mL nutrient solution. Test plants were raised Perlite filled pots. These pots were put onto the test vessels. Roots of test plants could grow through the bottom of the pots.

Experimental Conditions

Test plants

Wheat (variety "Taifun") and winter oilseed rape (variety "Palma BS") were chosen to represent monocotyledonous and dicotyledonous crops. Plants were raised in 10 cm diameter pots filled with Perlite®.

Plants were grown in nutrient solution in open trays until growth stage BBCH 20 was reached for wheat and oilseed rape priot to transfer to the hydroponic test system, which. was aerated on a regular basis.

The concentration of the test item in the stock solution was 0.68 mg in 10 mL.

Application of test item

Each test vessel was filled with little nutrient solution and stock solution was applied and mixed in Aliquots were taken and measured for radioactivity by LSC to determine the test item concentration.

The mean concentrations over all applied vessels were 75.69 µg.L-1 (CV = 0.6%) for wheat plants and 75.47 µg.L-1 (CV = 0.7%) for oilseed rape plants.

Controls

Untreated plant control was employed to check impact of test item on volume uptake of solution and on variation in biomass during the test period.

Cold-treated plant control was used monitor impact of test item on test solution and on biomass during the test period

Stability control without plants Purpose: monitor stability of test item under actual test conditions.

Vessel was filled with nutrient solution and spiked with test item. The vessel was covered with a dry Perlite filled pot.

The following table summarizes the experimental design.

Table B.7.2.1.1.1-1: Experimental setting

Test item	Solution	No plants	Wheat	Oilseed rape	Sum
Clopyralid (^{14}C)	Test solutions (^{14}C)	-	4	4	9
	Stability control (^{14}C)	1	-	-	
Clopyralid (cold)	Cold control	-	1	1	2
Non-treated	Plant controls	-	4	4	9
	Evaporation control	1	-	-	
Sum of test vessels		2	9	9	20

Incubation conditions

The daily cultivation conditions in the artificially illuminated greenhouse were set up to achieve a mean daily temperature of 20-25°C during day period and 16-18°C during night period and a daily mean air humidity of 50% ± 25%.

Sampling

Intermediate samplings were performed after 0.25, 1, 2, 5 and 6 days.

The intermediate biomass was estimated via the linear variation of the mass of planted pots, and from which was subtracted the mass of pot with substrate. The value taken for the filled pot was the mean value over all mass measurements taken for systems without plants.

At each sampling sub-aliquots were measured for radioactivity by LSC to determine the concentration.

At the end of the incubation period, the plants were carefully removed from the test vessels. The test solution that retained at the root system was collected by raising the planted pot over the vessel and letting the excess solution drip back for about two minutes.

The amount of test item potentially adsorbed onto the root system in contact with the solution was removed and measured by rinsing the free roots and the rinsing solution was then

filtrated and analysed by LSC to determine the radioactivity that is adsorbed on the root system.

The volume of the remaining test solutions was determined by weighting the test vessel with solution alone. Aliquots were measured for radioactivity by LSC to determine the concentration of the test item. In one replicate solution and the stability solution, one further aliquot (2 mL) was used to determine the radiochemical purity of the test item in the nutrient solution by means of radio-HPLC analysis.

In order to prepare a radioactive mass balance, the plant material was fractionated into free roots (in contact with the test solution), pot roots (in contact with the Perlite®) and stem/leaves system. Perlite® was removed from pot roots and kept for further analysis. Fresh weights were determined for each plant fraction and each was separately deep frozen (i.e. ≤ -18°C). The deep frozen plant material was then frozen dry and homogenized when enough material was obtained. Full mass or aliquots of the powdered plant material were combusted in a sample oxidizer and were analysed for radioactivity via LSC.

For one replicate (OSR, b), the Perlite® fraction was air dried and then homogenized using a mortar. Aliquots of the powdered Perlite® were combusted in a sample oxidizer and then analysed for radioactivity via LSC measurement.

The radioactive mass balance was calculated as the sum of the radioactivity recovered from the test solution, the root rinsing solution, the plant material and the Perlite. With the exception of the replicate “OSR, b”, the radioactive mass recovery was over 90% of applied radioactivity.

Identical measurement procedures were followed for test solution volume of the controls.

Additionally, pH level and oxygen saturation of the nutrient solutions were measured on sampling days 0, 0.25, 1, 2, 5, and end of experiment. Throughout the experimental phase plant development and health, as well as root system condition were documented with photos.

Analytical Methodology

Liquid Scintillation Counting (LSC)

Liquid specimens were measured using a liquid scintillation counter.

High Performance Liquid Chromatography (HPLC)

The following gradient reversed phase radio-HPLC method was used for the identification of clopyralid by co-chromatography with the reference item and to determine the radiochemical purity of the test item in the stock solution and in the applied vessels at the end of the incubation.

Data evaluation

PUF values were calculated for each test vessel according to the equations presented in the Theory section. A statistical evaluation was performed on the PUF values and volume uptake

values in order to investigate a potential impact of test item or crop type. All statistical tests were conducted with R statistical system and Excel.

Results and Discussion

Test Chamber Conditions Daily conditions in the greenhouse are presented below.

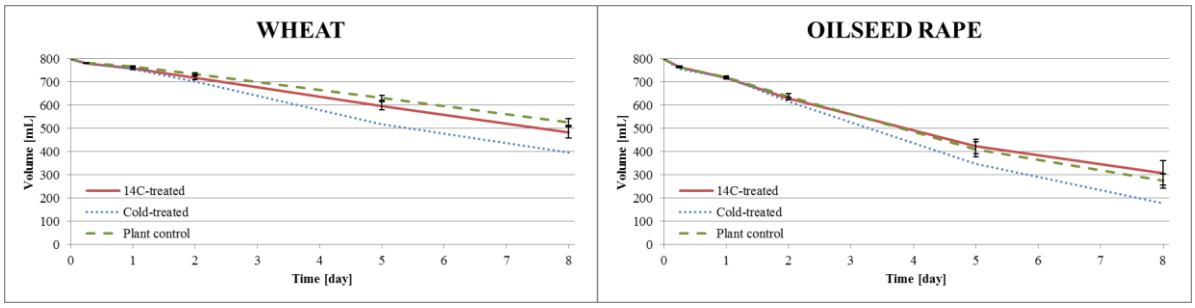
Plant health

The exact fresh biomass of the test plants was determined at the onset and at the end of the test period and estimated. An analysis of variance showed there was a significant difference between crop types, which could be expected, but no significant difference between treatments, therefore indicating no influence of the test item on the biomass variation. The plants showed constant or decreasing biomass over time. The visual assessment nevertheless confirmed the health of the plants throughout the incubation period.

Water consumption

An important requirement for the determination of a reliable PUF is a sufficient volume of water used as transport media for the test item from the artificial soil solution into the plants. Concentration changes are the basis for determining the plant uptake factor.

Figure B.7.2.1.1.1-2: Test solution volume over eight days



Error bars: ± SE, n = 4

Table B.7.2.1.1.1-3: Cumulative test solution uptake

Pre-incubation	Replicate	Wheat		Oilseed rape	
		Cumulative test solution volume uptake[1]			
		[mL]	% of initial vol.	[mL]	% of initial vol.
None	Mean	312.4	39.10	487.1	60.90
	CV (%)	14.90	15.00	21.70	21.70
6h	Mean	294.4	37.70	451.9	59.10
	CV (%)	15.10	15.50	23.20	23.30
1 day	Mean	271.8	35.90	406.3	56.60
	CV (%)	14.50	15.50	25.40	25.60
2 days	Mean	233.5	32.60	317.6	50.70
	CV (%)	13.60	15.70	31.10	31.90

1 corrected for volume of aliquots

The cumulative solution loss without plants (i.e. evaporation) was under 1.5% of the initial volume indicating that the variation in volume observed with plants could be attributed solely to water consumption by the plants.

Overall, the water consumption was over 30% of the initial volume, thus insuring the accuracy of the concentration measurements.

Analysis of variance showed that there was a significant difference between crop types. There was not a significant difference between treatments, confirming the lack of effect of the test item on the water consumption.

Water consumption rate was constant until the last sampling day for wheat plants. With oilseed rape plants, the rate slightly decreased, which corresponds to the apparition of little phytotoxicity symptoms on the older leaves. The water uptake was nevertheless still consequent.

<u>Parameters pH, oxygen saturation and internal temperature in non-treated controls (plant and evaporation controls)</u>

pH level	The pH values measured in the evaporation control solution without plant showed a relatively constant behaviour with pH 5.63 (CV = 3.3%). T The non-treated and cold-treated solutions with wheat plants showed a mean decrease over time from pH 5.45 to pH 3.81 (CV ≤ 6.8%) and pH 5.37 to pH 3.65 respectively. As the pH level depends on the nutrients concentration, the consumption by plants may be the

	reason for this decrease. The pKa of the test item is 2.01. At the lowest pH value recorded (i.e. pH 3.51 on Day 8); more than 95% of compound remained in its ionic form .
Oxygen saturation	With wheat plants oxygen levels were always above 85% of saturation, ensuring optimal oxygen content. With oilseed rape plants, there was a stronger variation over time and between replicates.
Internal temperature	Internal temperature of the test system ranged between 18.0 °C and 20.3°C. This is the same range as observed for air temperature.

Radioactive mass balance

A radioactive mass balance was established at the end of the study to validate the experiment. On termination, the sum of all processed fractions and solutions yielded in the following mean recoveries expressed in fraction of applied radioactivity: 96.3% without plants, 95.1% (CV = 1.5%) with wheat plants and 94.8% (CV = 4.4%) with oilseed rape plants.

The main proportion of the radioactivity was detected in the test solutions and in the shoots. The comparison of the different plant fraction showed a strong translocation to the leaves, with 87.2% (CV = 1.8%) and 75.8% (CV = 5.3%) of the taken radioactivity located in leaves for wheat and oilseed rape plants respectively.

Test item stability in test solutions

The radiochemical purity of the test item was assessed before the application by radio-HPLC analysis of the stock solution. To confirm the stability of the test item in the nutrient solutions during the experiment, the radiochemical purity of the test item in solution was determined just after application and at the end of the incubation period for representative solutions. The purity of the test item in solution remained at 100% for the whole study, independently from crop and nutrients.

Test item glass adsorption (stability controls)

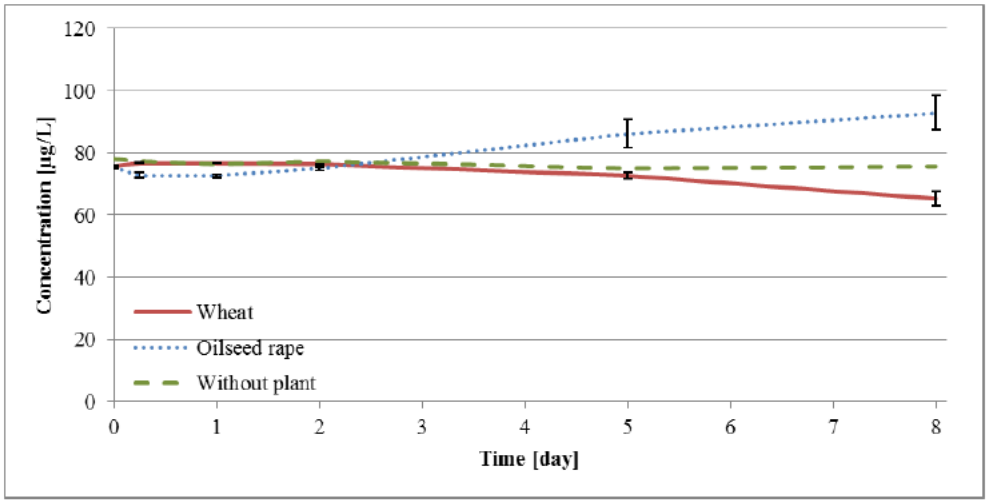
. There was negligible loss of test item by glass adsorption or volatilization (i.e. < 4% of applied amount).

Plant uptake factors (PUF) – calculation over 8 days

PUF values were calculated from the final concentrations of test item and the cumulative volume plant uptake of test solution after eight days.

The mean plant uptake factors after eight days were 1.07 (CV = 12.3%) for wheat plants and 0.68 (CV = 18.3%) for oilseed rape plants.

Figure B.7.2.1.1.1-3: Test item concentration over seven days



Error bars: ± SE, n = 4

Plant Uptake Factor (PUF) – Calculation Including Pre-incubation Phase

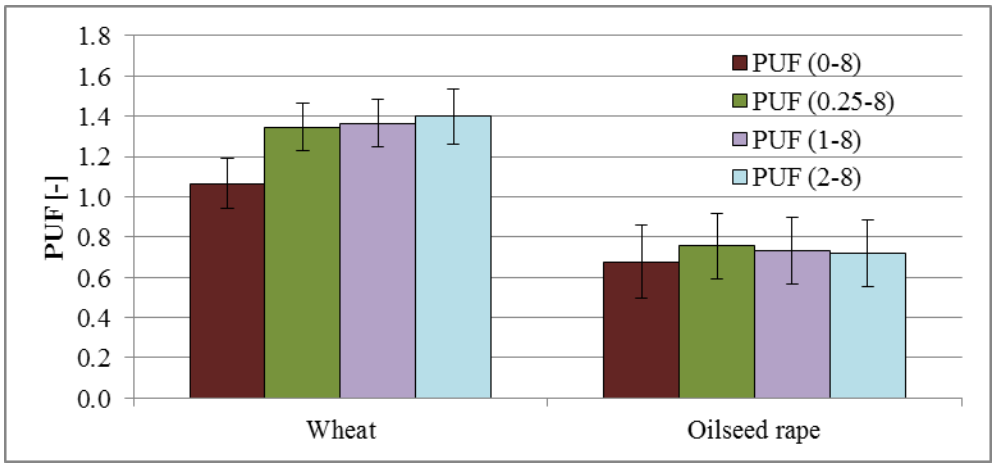
Taking into account the discussions at the York Workshop (2013) on the relevance of including the pre-incubations phase into the calculation of PUF, a second set of calculations is provided.

A pre-incubation phase allows letting aside any possible adsorption on the roots material, as the pseudo-initial time point is taken after the adsorption process occurs. Three different pre-incubation phases were considered for the calculation: 6 hours, 1 day and 2 days. Results are presented below. Analysis of variance showed a significant difference between crop types but none between different pre-incubation phases. However, PUF values were all within a narrow range.

Table 1: Plant uptake factors with consideration of pre-incubation phases

Pre-incubation	Wheat		Oilseed rape	
	Mean	CV	Mean	CV
6 hours	1.34	11.8%	0.75	16.5%
1 day	1.37	12.0%	0.73	16.7%
2 days	1.40	13.6%	0.72	17.1%

Figure B.7.2.1.1.1-4: Comparison of different calculation methods



Error bars: ± SE, n = 4 /Legend: PUF(X-8) with X the initial calculation day

B.7.2.2 Metabolism in livestock

Data to address this point were presented in the dossier submitted in April 2002 for the Active Approval and was found to be insufficient in quality following evaluation and peer review at EU level. A new study was conducted according to the current guidelines and complete characterization of the tissues.

It was a specific provision of the inclucion in Annex I to Directive 91/414/EEC that the applicant was required to submit to the European Commission further studies to confirm the results on animal metabolism by 1 May 2009. In accordance with the specific provision, the applicant, Dow AgroSciences, submitted an updated dossier in September 2005 and February 2006, which was evaluated by the designated RMS, Finland, in the form of an Addendum to the Draft Assessment Report.

As summarized by EFSA, (EFSA Scientific Report (2005) 50, 1-65, Conclusions on the peer review of clopyralid, 14 December 2005):

"In laying hens, radio-labelled clopyralid was orally administered. It is noted that the study was conducted from the 70's and the early 80's and show partially significant deviations from the requirements of current applicable guidelines. The studies indicated that the majority of administered radioactivity was excreted as unchanged clopyralid. The residues in tissues and eggs of hens were identified as unchanged clopyralid. Hence, it was concluded that, apart from conjugation with glycine, clopyralid was not metabolised in livestock."

B.7.2.2.1 Poultry

Previously submitted data

Fate of ^{14}C -DOWCO 290 in laying hens ([redacted], 1974)

Fate of DOWCO 290 in sheep ([redacted], 1974)

The previous dossier, which was evaluated uner directive 91/414/EEC, was comprised of rather old studies dating back to 70s. These studies have neither been performed using any official guideline nor been conducted under GLP nor according to any official guidline. Deficiencies exists concerning to sample storage.

It was a specific provision of the inclucion in Annex I to Directive 91/414/EEC that the applicant was required to submit to the European Commission further studies to confirm the results on animal metabolism by 1 May 2009. In accordance with the specific provision, the applicant, Dow AgroSciences, submitted an updated dossier in September 2005 and February 2006, which was evaluated by the designated RMS, Finland, in the form of an Addendum to the Draft Assessment Report.

REFERENCE	The Fate of ^{14}C -labelled DOWCO 290 Fed as a single oral dose to broiler chickens [redacted], 1974). Report number 29074.
Guideline(s):	None
GLP status:	No

In the study by [redacted] (1974) ^{14}C -labeled DOWCO 2390, i.e. 3,6 dichloropicolinic acid, was fed as a single oral dose as both the free compound and as "grown in" residue in wheat to broiler chickens. These birds had received feed containing 100 ppm added DOWCO 290 for over two weeks before test. Droppings were collected for 24 hours and were found to contain essentially all of the administered ^{14}C activity in its original form.

No detectable (less than 0.01 ppm DOWCO 290 equivalent) ^{14}C activity was found in the tissues 24 hours after treatment.

How samples stored has not been stated in the orginal study. A benzene solution has been used for oral dosing of the clopyralid.

The results indicate that TRR in all hen tissues is below 0.01 ppm. The radioactivity is excreted within 8 hrs almost quantitatively.

The authors concluded that DOWCO 290 is not metabolized by the chicken, and that the compound passes rapidly through the animal without accumulation in the tissues.

REFERENCE	[redacted] (1983) The metabolic fate of ^{14}C-3,6-dichloropicolinic acid (Dowco 290) fed to lactating goats Report number GH-C 1600
Guideline(s):	None
GLP status:	No

Two lactating goats were fed 14C-ring labelled 3,6-dichloropicolinic acid (Dowco 290), labelled in the 2,6- position, at the rate of 230 and 69 ppm in the feed for 7 days. The radiochemical purity of the test substance was > 99% with a specific activity of 1.32 Ci/mole.

Each goat was milked twice daily. Milk was analysed whole and following separation by centrifugation into milk fat and skimmed milk phases. Blood samples were taken immediately prior the evening milking. These were assayed as whole and after centrifugation as plasma and red cell fractions. Urine and faeces were also collected on a daily basis. Radioactivity in expired air was monitored in one goat in an indirect calorimetric chamber. Animals were sacrificed within 15 hours of the last dose and tissue samples (comprising blood, liver, kidney, heart, skeletal muscle and composite fat samples (visceral and subcutaneous)) were taken. Gastrointestinal contents (GI-tract) were also collected.

Total ^{14}C-residue levels in liquid samples were determined by liquid scintillation counting (LSC). Solid samples were analysed by oxidative combustion followed by liquid scintillation counting. To quantify and characterise the nature of the radioactive residue, samples of milk, liver, kidney, muscle and urine were processed and extracted into an appropriate solvent, prior to chromatographic analysis by HPLC or GC-MS. Confirmation of identity was

obtained by comparison with known reference compounds and GC-MS. The lower limit of detection was determined to be 0.015 mg/kg.

Results

Recovery of administered radioactivity is presented in Table 7.2-1. More than 95% of the administered radioactivity was recovered, with the majority being found in urine (>93%, of the recovered radioactivity). Most of the balance was recovered from faeces or gastrointestinal tract.

Daily average levels in milk reached a plateau of clopyralid in 4 to 5 days. Radioactivity in milk fat was below the limit of detection, indicating no tendency to accumulate in milk fat. Blood levels indicated that the rate of assimilation was essentially equivalent to the rate of elimination, and no radioactivity was determined in the expired carbon dioxide, indicating no total degradation of the active substance.

HPLC examination of the urine samples indicated the major component (>97%) as unchanged clopyralid. The remainder of the radioactivity was determined to be a glycine conjugate of clopyralid. ^{14}C residues in milk consisted of two components, unchanged clopyralid and the same glycine conjugate observed in urine. In milk, the two residues were present in about equal amounts. Under alkaline hydrolysis, the conjugate in milk was hydrolysed to clopyralid. Residues in liver and kidney comprised unchanged clopyralid only.

Table 7.2-1 Total recovered radioactivity from lactating goats fed with clopyralid

SAMPLE	% TOTAL RADIOACTIVITY	
	Animal 3 (230 ppm)	Animal 4 (69 ppm)
Total	95.5	107.8
Urine	93.3	96.1
Faeces	0.7	9.4
GI Tract	1.4	2.2
Muscle	0.09	0.03
Liver		
Kidney		
Fat		
Milk	0.03	0.04

Conclusion

Orally administered clopyralid was rapidly excreted in the urine, primarily unchanged. Milk and tissue residues are low and consist of about equal amounts of clopyralid and the glycine conjugate of clopyralid. The study is acceptable.

Present submission

The following information has not been previously reviewed for Annex I inclusion:

A new poultry nature of residue (NOR) study was conducted according to the current guideline requirements and also performed complete characterization on the tissues. Findings in this study for the tissue residues and the excretion and metabolite profiles were consistent with findings in a prior goat nature of residue study. Unchanged clopyralid was the major residue with a minor amount of X36538 in both studies.

B.7.2.2.1.1 Nature of the Residue Study in the Laying Hen with [^{14}C]-Clopyralid

REFERENCE	[redacted] 2014; A Nature of the Residue Study in the Laying Hen with [^{14}C]-Clopyralid; [redacted] Lab Study No. 130906; DAS Study No. 130906; 20 November 2014; Unpublished
Guideline(s):	OECD Guidance Document 503 for Metabolism in Livestock (Issued 8 January 2007)
US EPA Guideline(s):	None
Deviations:	Water analyses conducted at [redacted] were not conducted as per GLPs Paperwork generated from the supplier of the test animals, [redacted], was not collected per GLPs. Company does not claim GLP compliance. HPLC solvents were prepared on 03-Oct-2013 for this study; however the preparation of the HPLC solvents was not documented until 15-Jan-2014. The documentation of the HPLC preparation was written by the analyst who had previously prepared them.
Sample storage conditions	Tissues were extracted and characterized within 4 months of sacrifice. Samples were extracted with neutral solvent and analyzed by HPLC within 2 weeks of extraction. Samples were stored at approximately -20 °C when not being analyzed.
GLP status:	Yes

Background Information

Results from a previous laying hens metabolism study conducted using ^{14}C -Clopyralid at dose rates of 100 mg eq/kg in the feed for 5 and 6 days indicated that clopyralid was not significantly metabolized. The only radioactive residue in kidney, liver, and leg muscle was clopyralid. Residues in egg white and yolk rapidly reached plateaus of 0.15 ppm and 0.02 ppm, respectively. All radioactive residue recovered in the excreta was unchanged clopyralid. The current study was conducted because the prior study was judged to be insufficient in quality. This was communicated in an EFSA Reasoned Opinion, Modification of the existing MRLs for clopyralid in various commodities.

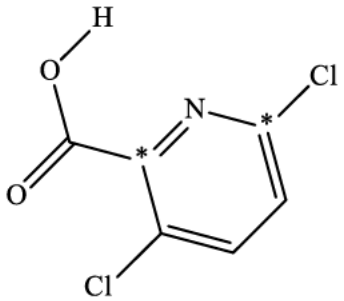
Materials and Methods

Test Item(s)

Non-radiolabelled test item #1

ISO Common name:	Clopyralid
Purity:	99.9%

Radiolabelled test item #1

Name:	Clopyralid-2,6-^{14}C
Test item (chemical/other name):	^{14}C-clopyralid, X755015 3,6-dichloropicolinic acid-2,6-^{14}C
Structural formula: Position of labelling (*)	
Radiochemical purity:	98.0%
Specific radioactivity:	32.8 mCi/mmol (379,250 dpm/µg)

Methods

Test Site Information

The in-life phase of this study was conducted at [redacted]

Table B.7.2.2.1.1- 1: General test animal information

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Gallus domesticus	Hy-line browns laying hen	27 weeks at receipt	1.781 to 2.058	Healthy	Metabolism cages, 20 inches in length x 15 inches in width x 20 inches in height, With wire mesh flooring, no bedding, and easy ad libitum access to water and feed.

Table B.7.2.2.1.1- 2: Test animal dietary regime

Composition of diet	Feed consumption (kg/day)	Water	Acclimation period	Pre-dosing
39 Layer A-18, a custom-mixed layer ration	0.112	ad libitum	14 days	none

Table B.7.2.2.1.1- 3: Test animal dosing regime

Treatment type	Feeding level (ppm test material in food on a dry weight basis)	Vehicle	Timing/duration
Oral	11.4 ppm	Size 0 gelatin capsules with no absorbent, within a size 00 gelatin capsule	A single capsule was administered once a day orally by pet piller to each animal for 7 consecutive days.

Dose Solution Preparation

The ^{14}C-Clopyralid, pyridine ring radiolabeled test substance of the technical product was obtained from the Dow AgroSciences Process Chemistry Group. The ^{14}C-Clopyralid was prepared by bringing 42.704 mg (7.30 mCi) of radiolabeled clopyralid in about 20mL of 50:50 acetone/ethanol to volume in a 25 mL volumetric flask, using acetonitrile. The radiolabeled clopyralid was diluted with non-radiolabeled clopyralid. This was done by weighting 46.254 mg of non-radiolabeled clopyralid into a 10 mL reaction vial. The

remaining solution of ^{14}C-Clopyralid was subsequently transferred using a Pasteur pipette to the 10 mL reaction vial containing the non-radiolabeled clopyralid. This was done in fourths (about 6.25 mL aliquots) and brought to dryness under a gentle stream of nitrogen. This process was continued until the entire remaining solution of ^{14}C-Clopyralid was taken to dryness. The original specific activity of radiolabeled clopyralid was 32.8 mCi/mmol or 379,250 dpm/µg. After accounting for radioactivity removed for analysis, the diluted specific activity was 15.7 mCi/mmol or 181,775 dpm/µg. This prepared and dried test substance was shipped to the contract laboratory. Before shipping, the purity of the ^{14}C-Clopyralid was confirmed by HPLC analysis.

Table B.7.2.2.1.1- 4: Sample collection information

Eggs Collected	Excreta and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analysed
Eggs were collected twice daily (morning and evening). The evening egg collection was then pooled with the eggs collected in the morning, except for the Day 7 pooled eggs. Day 7 was not a complete collection day, it was a partial laying day for the hens, and no a.m. eggs were collected.	Starting on the final day of the acclimation phase and continuing through sacrifice excreta was collected at approximately 24-hour intervals. A cage rinse was performed the day before dosing and on the last day of dosing.	All animals sacrificed within 8 hours	Eggs (whole pooled), liver, leg muscle, breast muscle, fat skin with fat, (subcutaneous), excreta, cage rinse, (for mass balance only).

Sample Handling and Preparation

Whole Pooled Eggs

Egg production was recorded daily for each hen throughout both the acclimation and dosing phases of the study. Eggs were collected twice daily (morning and evening). The evening egg collection was refrigerated overnight. The evening egg collection was then pooled with the eggs collected in the morning, except for the Day 7 pooled eggs. Day 7 was not a complete collection day, it was a partial laying day for the hens, and no a.m. eggs were collected. The evening eggs were pooled with the morning eggs to provide a single composite sample for each day. The whole eggs (whites and yolks) were broken into tared vessels, weighed, and homogenized using a hand-held mixer. Egg shells were discarded. After blending, the homogenized whole eggs were stored frozen at approximately -20 °C prior to analysis and shipment to the sponsor.

Excreta

Starting on the final day of the acclimation phase and continuing through sacrifice excreta was collected at approximately 24-hour intervals. On the day of collection, samples were weighed and stored frozen at approximately -20 °C. Day 7 excreta consisted of a partial collection day, since the hens were sacrificed within 8 hours of the final dose. On the processing day the pooled excreta samples were homogenized with deionized water, using a Robot Coupe Processer. The ratio of water to excreta was 1.5:1. The homogenized samples were stored frozen at approximately -20 °C prior to analysis and shipment to the sponsor.

Sacrifice

The animals were sacrificed within 8 hours of the final dose. Samples of liver, leg muscle, breast muscle, fat, and skin with subcutaneous fat were collected. Each sample type was pooled within the respective treatment or control group, weighed, and stored frozen at approximately -20 °C, prior to sample preparation.

All tissue samples (liver, leg muscle, breast muscle, fat, and skin with subcutaneous fat) were homogenized with dry ice to a fine powder using a Robot Coupe Processer. All homogenized samples were stored at approximately -20 °C prior to analysis and shipment to the sponsor.

A cage rinse was performed the day before dosing and on the last day of dosing. The cage rinse consisted of an 80/20 water/methanol solution, v/v. The cage rinse from each cage was pooled and weighted. It was stored at approximately -20 °C, when not being analyzed.

Gastrointestinal tract with contents, blood, and the carcasses were not processed or analyzed, since above 90% of the dosed radioactivity was recovered without including those samples.

Identification/ Characterisation of Residues

TRR

Determination of TRR was conducted at [redacted] Aliquots (5 or 10 replicates approximately 0.2 or 0.5 g) of the homogenized tissue, egg, and excreta samples were analyzed by oxidative combustion to determine the radioactive residues in the samples . Fat was analyzed by direct LSC of 0.2 g aliquots. The fat aliquots were mixed with scintillation cocktail and allowed to sit at least overnight at room temperature before analysis.

Day 7 Whole Pooled Eggs

The Day 7 egg sample was allowed to come to room temperature, once at room temperature the sample was stirred well. Approximately 20.0 g of the pooled eggs was transferred, in duplicate, to a tared and labeled 250 mL Nalgene™ tube. Extraction solvent was added to the tube - 75 mL of 80/20 acetonitrile/water, v/v - the tube was shaken for 30 minutes on a horizontal shaker set at high speed. The sample was centrifuged, and then decanted into a tared jar. The extraction was performed a total of three times. The extract was analyzed by taking three 1.0 mL aliquots, which were analyzed by LSC.

All remaining extract was concentrated using an evaporator. Fat was partitioned once, from the sample using 10 mL of hexane. Hexane were analyzed by LSC, three 250 µL aliquots. Once fat was removed from extract, the sample was concentrated and purified using two SPE cartridges. Three 1.0 mL aliquots of the load/wash and three 50.0 µL aliquots of the elution were analyzed by LSC.

Parameter		**Description**
Sampling intervals		Day 7 of dosing
Neutral Extraction	**Solvent**	80/20 Acetonitrile/Water (v/v)
	Procedure	20.0 gram of sample shook for 30 min with 75 mL of extraction solvent, in triplicate then centrifuged. Extracted was analyzed by LSC. Extract was concentrated using a Rocket Evaporator set at 40 °C, vacuum of 0 mBar, and chiller at -10 °C. After 1.5 hours the Rocket chiller temp was switched to 4 °C. Once the sample was concentrated to all aqueous 10 mL of 1.5N HCl was added to the sample. Fat was partitioned from the sample using 10 mL of Hexanes. Hexanes were analyzed by LSC. Once fat was removed from extract, the sample was concentrated and purified using two SPE (Oasis Max, 6cc (150mg), Part # 186000369, Waters). Load and Wash were analyzed by LSC, elution was analyzed by LSC. (SPE procedure explained in detail in Section 3.5.2) The flasks the samples were concentrated in were rinsed with 10 mL of ethyl acetate/trifluoroacetic acid solution (99:1). The rinse was analyzed by LSC. The elution was concentrated using a Turbovap at 7 psi nitrogen and 35 °C Concentrated extract was reconstituted in 86/14 Water w/0.1% Formic acid/Acetonitrile w/0.1% Formic acid, and then analyzed by LSC and HPLC.
	Method of analyses	LSC and HPLC
Extracted Tissue Combustion	**Procedure**	Obtained weight of remaining air-dried tissue sample by oxidative combustion of three aliquots.

Excreta Analysis

The Days 1, 4, and 7 excreta samples were allowed to come to room temperature, once at room temperature the samples were stirred well. Approximately 5.0 g of the excreta was transferred in duplicate to a tared and labeled 35 mL Nalgene™ Conical Oak Ridge Tube. Extraction solvent was added to the tube - 25 mL of 80/20 acetonitrile/water, v/v - the tube was shaken for 30 minutes on a horizontal shaker set at high speed. The sample was

centrifuged at 2500 rpm for 15 minutes, and then decanted into a tared 125 mL jar. The extraction was performed a total of three times. The pooled extract was analyzed by taking three 250 µL aliquots for LSC. One mL of the extract was transferred to a 45 mL glass vial. The extract was concentrated to approximately 100 µL of volume using a Turbovap® set at 7 psi nitrogen and 35 °C. The concentrated sample was reconstituted in 0.9 mL of HPLC grade water with 0.1% formic acid and 0.1 mL of acetonitrile with 0.1% formic acid. Once reconstituted, the sample was weighed and three 25 µL aliquots were analyzed using LSC. The samples were then analyzed via HPLC.

Table B.7.2.2.1.1- 5: Summary for the analysis of ^{14}C-Clopyralid residues in excreta

Parameter		Description
Sampling intervals		Day 1, 4, and 7 of dosing
Neutral Extraction	Solvent	80/20 Acetonitrile/Water (v/v)
	Procedure	5.0 gram of sample shaken for 30min with 25 mL of extraction solvent, in triplicate, then centrifuged. Extract was analyzed by LSC. Extract was concentrated using a Turbovap® at 7 psi Nitrogen and 35 °C. Concentrated extract was reconstituted in 90/10 Water w/0.1% Formic acid/Acetonitrile w/0.1% Formic acid, and then analyzed by LSC and HPLC.
	Method of analyses	LSC and HPLC
Extracted Tissue Combustion	Procedure	Obtained weight of remaining air-dried tissue sample.
	Method of analyses	Oxidative combustion of three aliquots.

Extraction and Clean-up Procedures for Metabolite Identification

The Day 1, 4, and 7 excreta and the Day 7 pooled egg samples were analyzed directly by HPLC. After HPLC analysis one of the replicates of the Day 7 excreta and one of the replicates of the Day 7 eggs were analyzed directly by Liquid Chromatography/Tandem Mass Spectrometry.

Analytical Methodology

Total ^{14}C measurement

Liquid scintillation counters automatically convert the radioactivity counting rate in counts per minute (cpm) to disintegrations per minute (dpm). This is done using an external standard

to correct for sample quenching. The instrument was calibrated at least every six months with a set of ten quenched standards. Each day of use, the instrument was normalized and its performance was checked. Performance was checked with respect to the background cpm value, unquenched standard cpm value, and quenched standard dpm value for a range of quenched standards. The quenched standards are certified by comparison to Standard Reference Material 4222C, (National Institute of Standards and Technology. Gaithersburg, MD). The dpm value for an extraction sample was determined by LSC. An appropriate aliquot of the sample was diluted with scintillation cocktail. Then the sample was counted on the LSC for at least five minutes. Samples that were expected to have low amounts of radioactivity were often counted for 10 minutes or longer.

Solid phase extraction (SPE)

For the Day 7 egg sample only, all remaining extract (approximately 230 mL) was concentrated using a Rocket Evaporator set at 40 °C, vacuum of 0 mBar, and chiller at -10 °C. Keeper (100 µL of 80/20 methanol/glycerol) was added to each sample, before evaporation. After 1.5 hours the chiller temp of the evaporator was switched to 4 °C. Once the sample was concentrated to all aqueous, 10 mL of 1.5 N HCl was added to the sample. Fat was partitioned once, from the sample using 10 mL of hexanes. Hexanes were analyzed by LSC, three 250 µL aliquots. Once fat was removed from extract, the sample was concentrated and purified using two 150 mg SPE cartridges. First, 45-mL vials were tared for the load/wash and elution. Next, 1 cm depth of Empore Filter Aid 400 was added to SPE cartridges per replicate. The cartridge was conditioned 2 times with 2.5 mL methanol followed by 2 times 2.5 mL of water. The acidic sample solution was added to the SPE reservoir. It was drawn through the SPE cartridge using house vacuum. The eluted solution was collected into the tared load/wash vial. The sample flask was rinsed with 5 mL of 1.0 N hydrochloric acid (HCl) and drawn through the cartridge with vacuum and collected. This was done a second time with 5.0 mL of water. For both replicates, during the third rinse with 5.0 mL water the cartridge stopped flowing under vacuum. A second SPE cartridge was conditioned following the procedure above. The remaining rinse was then added to the 2nd SPE cartridge. The rinse was drawn through with vacuum and collected in the load/wash vial. Both SPE cartridges in-line with one another were then rinsed with 5.0 mL of 10:90:0.1 methanol/water/acetic acid, v/v/v. For both replicates, during the rinse the SPE cartridge flow stopped again. The frit of the top SPE cartridge was gently punctured with a dissection needle and flow resumed. Next, the collection vial was switch to the tared elution vial. The SPE cartridge was dried for approximately 15 minutes under full vacuum. The SPE cartridge was eluted 3 times with 5.0 mL aliquots of 99:1 ethyl acetate/trifluoroacetic acid solution. The elution was collected. Three 1.0 mL aliquots of the load/wash and three 50.0 µL aliquots of the elution were analyzed by LSC. The elution was concentrated using an evaporator at 7 psi nitrogen and 35 °C. Concentrated extract was reconstituted in HPLC grade water w/0.1% formic acid/acetonitrile w/0.1% formic acid, and then analyzed by LSC. Lastly, the flasks that the samples were concentrated in were rinsed with 10-mL of ethyl acetate/trifluoroacetic acid solution (99:1, v:v). Three 0.5 mL aliquots of the rinse were analyzed by LSC. The samples were then analyzed with HPLC

High performance liquid chromatography (HPLC) for quantitation

HPLC analyses of all sample extracts following SPE clean-up, if applicaible, were accomplished using a Phenomenex Hydro-RP column (150 x 4.6 mm i.d., 4.0 µm; 1.0 mL/min; UV detection at 254 nm) and a four step, non-linear gradient.

Detection Limits

Using the method of Currie, the limit of detection can be calculated from the expression:

$$LOD = \frac{2.71 + 4.65\sqrt{dpm_B \times T}}{T}$$

and the limit of quantitation can be calculated:

$$LOQ = \frac{50\left(1 + \sqrt{1 + \frac{dpm_B \times T}{12.5}}\right)}{T}$$

where LOD is the limit of detection (dpm), LOQ is the limit of quantitation (dpm), dpm_B is the typical background (dpm) and T is the counting time (minutes). Samples were normally counted for 5 minutes while the blank was counted for 10 minutes. Typical background levels were 20 dpm. The resulting LOD was 10 dpm above background and LOQ was 40 dpm above background.

For HPLC radiochemical flow-through detection the LOD formula is modified slightly from that used for LSC counting:

Limit of Detection (LOD) dpm =

$$\frac{2.71 + \left(4.65\sqrt{\text{bkg cpm x } \left(\frac{\text{efficiency}}{100}\right) \text{ x count time}}\right)}{\text{count time}}$$

Where efficiency equals the radioactive detector efficiency (in percent), and count time equals the cell volume (in mL) total flow rate (eluate plus scintillant in mL/min). For stop-flow mode, the count time is the nominal setting, for example, if a 30-second stop-flow method is chosen, the count time is 30 seconds.

For HPLC analyses conducted using a radio-LC system on-line radioactivity detector. A typical background for stop-flow mode (a mode used for samples containing low amounts of radioactivity) was 5 cpm, the stop–flow time was 30 seconds, and a static cell efficiency of 94.0% was used. Using the above equation, this resulted in an LOD of 20 dpm over background. The amount of dpm injected ranged from approximately 5,400 to 27,120 dpm. The least amount of radioactivity injected for a sample was 5,400 dpm. The LOD was approximately 0.4% of the injected radioactivity for the least radioactive sample.

Mass spectral analysis (LC/MS) for identification of transformation products

Whenever possible, initial metabolite identification was accomplished by co-chromatography with available reference standards using HPLC. Structure confirmation of any such tentatively identified components as well as the identification of any significant fraction that did not co-elute with a standard was accomplished by LC/MS and/or LC/MS/MS.

Results and Discussion

Dosing and in-life summary

The target dose rate of 10.0 mg/kg dry feed per day was based on the average feed consumption during the acclimation period. However, the hens consumed less than expected, resulting in an actual dose of 11.4 mg a.i./kg dry feed. The feed consumption, egg production, and animal weights, combined with the observations by veterinary personnel suggest that the animals remained healthy during the course of the study.

All HPLC analyses were conducted along with analysis of a clopyralid reference standard.

HPLC purity analysis of pre-dose and post-dose aliquots indicated that clopyralid was stable.

Eggs, Tissue and Excreta TRR Levels

As summarized below, 90.14% of the dose was recovered from the ^{14}C-Clopyralid dosed animals. The majority of the dose was excreted, with 88.35% of the dose accounted for in the excreta.

Pooled eggs contained less than 0.03% of the dose. During the dosing period the residue level in pooled eggs reached a plateau of 0.007 mg/kg at Day 2 of the study. The Day 7 pooled eggs was excluded, since the Day 7 pooled eggs was not an equivalent sample to Day 1 through Day 6 pooled eggs. This is due to the Day 7 pooled eggs consisting only of eggs laid within 8 hours of the Day 7 dose, p.m. collection only.

It can also be hypothesized that rapid uptake and excretion of ^{14}C-Clopyralid caused the Day 7 pooled eggs sample to have a concentration spike. Rapid uptake and excretion of ^{14}C-Clopyralid was observed in the milk collected in the nature of the residue study in the ruminant. This is due to the Day 7 pooled eggs consisting only of eggs laid within 8 hours of the Day 7 dose. Day 7 was not a complete collection day. It was a partial laying day for the hens. No a.m. eggs were collected, on the day following Day 7 dose, for the Day 7 pooled eggs.

Low residue levels resulted in all tissues collected at sacrifice; less than 0.02% of the dose was recovered in the edible tissues. Residues in liver, leg and breast muscle, fat, and skin with subcutaneous fat were 0.006, 0.003, 0.001, 0.002, and 0.005 mg eq./kg, respectively. Liver, leg and breast muscle, fat, and skin with subcutaneous fat were not characterized further due to the low residue levels.

Table B.7.2.2.1.1- 6: Total radioactive residues of ^{14}C - Clopyralid in eggs, tissue and excreta

Matrix	Collection Timing	^{14}C- Clopyralid	
		(% dose)	(mg eq./kg)
Eggs	Day 1	0.003	0.004
	Day 2	0.005	0.007
	Day 3	0.004	0.006
	Day 4	0.004	0.006
	Day 5	0.006	0.009
	Day 6	0.005	0.007
	Day 7[a]	0.002	0.012
Total		0.029	Not applicable
Excreta	Day 1	11.516	9.264
	Day 2	12.465	9.526
	Day 3	12.976	10.479
	Day 4	12.424	10.227
	Day 5	12.249	10.093
	Day 6	13.902	11.432
	Day 7[b]	12.816	20.061
Total		88.348	Not applicable
Liver	Sacrifice	0.003	0.006
Leg Muscle	Sacrifice	0.007	0.003
Breast Muscle	Sacrifice	0.002	0.001
Fat	Sacrifice	0.001	0.002
Skin with Fat	Sacrifice	0.005	0.005
Total		0.018	Not applicable
Cage Rinse	Sacrifice	1.744	2.166
Total (Mass Balance)		90.139	Not applicable

aNot a complete sampling day, hens sacrificed with 8 hours of Day 7 dose. No a.m. eggs or excreta collect from the following day for Day 7 sample. Sample consisted of eggs and excreta from a p.m. collection only.

Figure B.7.2.2.1.1-1: Accumulation of clopyralid residues in eggs. Correlation coefficient = 0.66, p< 0.05, linear regression)

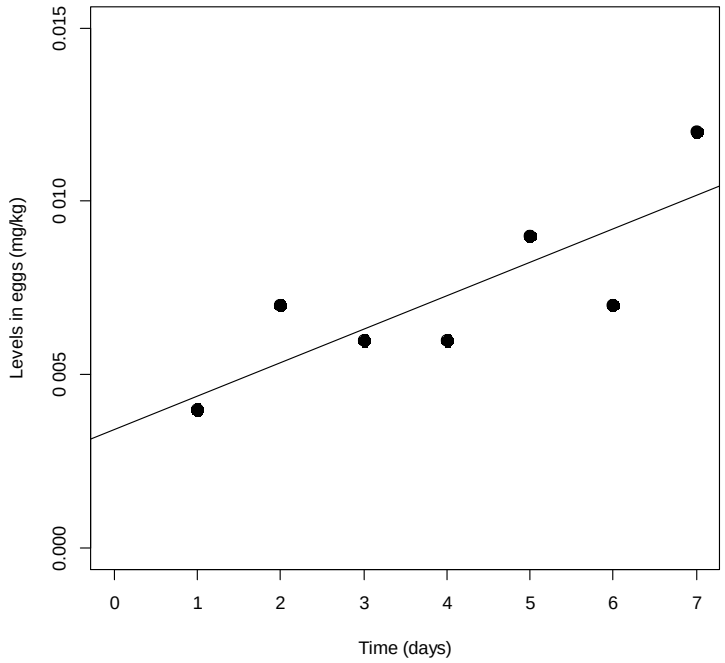
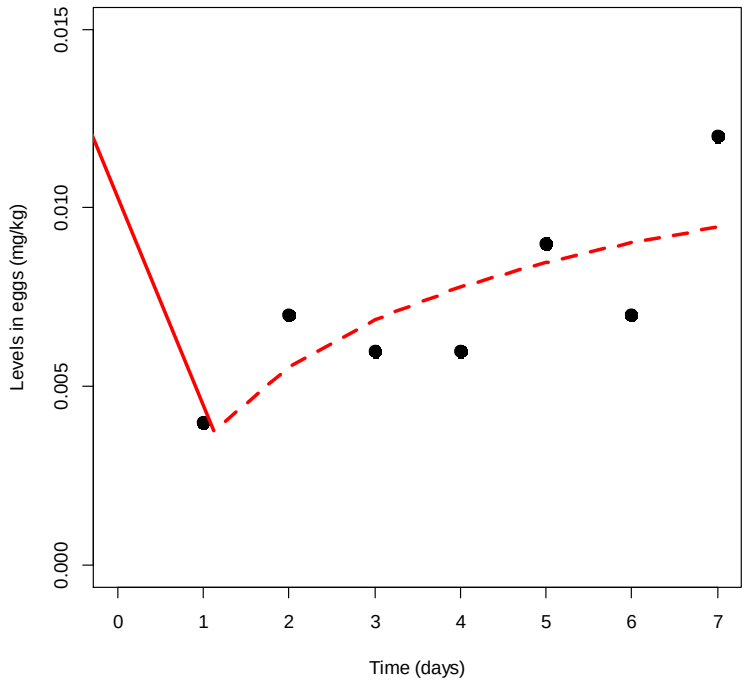


Figure B.7.2.2.1.1-2: Accumulation of clopyralid residues in eggs. The same data as above fitted with non-linear method r = 0.77, p< 0.05.



Characterisation of Residues

Characterisation of residues in eggs

The Day 7 pooled eggs sample was extracted with 80/20 acetonitrile/water this yielded approximately 95% of the TRR. The extract was concentrated and partitioned with hexanes, and approximately 11% of the TRR partitioned into the hexanes. SPE was performed on the fat-partitioned concentrated extract. Approximately 56% to 71% of the TRR was accounted for in the eluate, while 0% to 14% of the TRR was accounted for in the load/wash. Since the amount of TRR accounted for in the hexane and load/wash combine for both replicated was less than 0.003 mg eq./kg, only the eluate was analyzed by HPLC. The eluate from both replicates was concentrated, reconstituted, and analyzed by HPLC. HPLC analysis showed two components in the TRR, clopyralid at 72.0% and 52.2% TRR (0.009 and 0.006 mg eq./kg) and metabolite 1, RT:14.2 min at 2.0% TRR (0.0002 mg eq./kg). Clopyralid was confirmed by LC/MS/MS. Metabolite 1 was well below 0.010 mg eq./kg, thus was not further analyzed.

Characterisation of residues in excreta

The 1, 4, and Day 7 excreta samples were extracted with 80/20 acetonitrile/water this yielded 105.5% to 108.5% of the TRR. From each sample, 1 mL was concentrated, reconstituted, and analyzed by HPLC. HPLC analysis showed one component, clopyralid, at 102.9 to 112.1% TRR (20.652 and 11.260 mg eq./kg). Clopyralid in the excreta was confirmed by LC/MS/MS.

Table B.7.2.2.1.1- 7: Distribution of Radioactivity in Day 7 Pooled Eggs When Dosed with ^{14}C -Clopyralid at 11.4 mg a.i./kg/day for 7 days

	Excreta, Day 1, Rep 1		Excreta, Day 1, Rep 2		Excreta, Day 4, Rep 1	
Procedure **Fraction**	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg
TRR[a]	100.0	9.264	100.0	9.264	100.0	10.227
Extraction **80/20 ACN/Water**[b]	106.4	9.860	108.5	10.049	107.8	11.026
Concentration forHPLC **Concentratec** **Recovery**[d]	110.6 103.9	10.244	112.1 103.3	11.260	106.1 98.4	10.847

	Excreta, Day 4, Rep 2		Excreta, Day 7, Rep 1		Excreta, Day 7, Rep 2	
Procedure Fraction	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg
TRR[a]	100.0	10.227	100.0	20.061	100.0	20.061
Extraction						
80/20 ACN/Water[b]	107.7	11.012	107.3	21.528	105.5	21.171
Concentration forHPLC						
Concentrate[c]	110.5	11.301	108.3	21.718	103.5	20.760
Recovery[d]	102.6		100.9		98.1	

a The mg eq./kg value is from the LSC counting of collected excreta.

b This is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

c This total is the recovery compared to the LSC counting of collected excreta.

*d These are recoveries compared to the radioactivity in the fraction taken from the previous procedure. Recovery is determined by comparing the resulting %TRR (underlined value) for a procedure to the previous procedure's %TRR (underlined value). Example for Excreta, Day 1, Rep 1: Recovery for Concentration for HPLC is 103.9% = (110.6%/106.4%)*100%.*

Table B.7.2.2.1.1- 8: Distribution of Radioactivity in Day 7 Pooled Eggs When Dosed with ^{14}C -Clopyralid at 11.4 mg a.i./kg/day for 7 days

	Pooled Eggs, Day 7, Rep 1		Pooled Eggs, Day 7, Rep 2	
Procedure **Fraction**	**%TRR**	**mg eq./kg**	**%TRR**	**mg eq./kg**
TRR[a]	100.0	0.012	100.0	0.012
Extraction				
80/20 ACN/Water[b]	95.1	0.011	95.8	0.012
SPE				
Hexane Phase	10.9	0.001	11.4	0.001
Load & Washes	ND[c]	ND	13.8	0.002
Eluate[b]	70.8	0.009	56.2	0.007
Rinse of Flasks	ND	ND	1.0	<0.001
Total[d]	81.8	0.010	82.3	0.010
Recovery[e]	86.0		85.9	
Concentration for HPLC				
Concentrate[b]	74.0	0.009	54.2	0.007
Recovery[e]	103.8		94.9	

a The mg eq/kg value is from the LSC counting of collected eggs.

b This is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

c ND, non detect, below instrument limit of detection

d This total is the recovery compared to the LSC counting of collected eggs.

*e These are recoveries compared to the radioactivity in the fraction taken from the previous procedure. Recovery is determined by comparing the resulting %TRR (underlined value) for a procedure to the previous procedure's %TRR (underlined value). Example for Pooled Eggs, Day 7, Rep 1: Recovery for SPE is 86.0% = (81.8%/95.1%)*100%.*

Table B.7.2.2.1.1- 9: Distribution of Radioactivity in Day 1, 4, and 7 Excreta When Dosed with ^{14}C -Clopyralid at 11.4 mg a.i./kg/day for 1, 4, and 7 days

	Excreta, Day 1, Rep 1		Excreta, Day 1, Rep 2		Excreta, Day 4, Rep 1	
Procedure **Fraction**	**%TRR**	**mg eq./kg**	**%TRR**	**mg eq./kg**	**%TRR**	**mg eq./kg**
TRR[a]	100.0	9.264	100.0	9.264	100.0	10.227
Extraction						
80/20 ACN/Water[b]	106.4	9.860	108.5	10.049	107.8	11.026
Concentration forHPLC						
Concentrate[c]	110.6	10.244	112.1	11.260	106.1	10.847
Recovery[d]	103.9		103.3		98.4	

	Excreta, Day 4, Rep 2		Excreta, Day 7, Rep 1		Excreta, Day 7, Rep 2	
Procedure **Fraction**	**%TRR**	**mg eq./kg**	**%TRR**	**mg eq./kg**	**%TRR**	**mg eq./kg**
TRR[a]	100.0	10.227	100.0	20.061	100.0	20.061
Extraction						
80/20 ACN/Water[b]	107.7	11.012	107.3	21.528	105.5	21.171
Concentration forHPLC						
Concentrate[c]	110.5	11.301	108.3	21.718	103.5	20.760
Recovery[d]	102.6		100.9		98.1	

a The mg eq./kg value is from the LSC counting of collected excreta.

b This is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

c This total is the recovery compared to the LSC counting of collected excreta.

*d These are recoveries compared to the radioactivity in the fraction taken from the previous procedure. Recovery is determined by comparing the resulting %TRR (underlined value) for a procedure to the previous procedure's %TRR (underlined value). Example for Excreta, Day 1, Rep 1: Recovery for Concentration for HPLC is 103.9% = (110.6%/106.4%)*100%.*

Table B.7.2.2.1.1- 10: Summary of characterisation and identification of radioactive residues in livestock matrices following application of radiolabeled ^{14}C -Clopyralid at 11.4 mg a.i./kg/day for 7 days

	^{14}C -Clopyralid Pooled Eggs, Day 7, Rep-1 TRR = 0.012 mg e.q./kg		^{14}C -Clopyralid Pooled Eggs, Day 7, Rep-2 TRR = 0.012 mg e.q./kg		^{14}C -Clopyralid Excreta, Day 1, Rep-1 TRR = 9.264 mg e.q./kg		^{14}C -Clopyralid Excreta, Day 1, Rep-2 TRR = 9.264 mg e.q./kg	
Compound	**% TRR**	**mg e.q./kg**	**% TRR**	**mg e.q./kg**	**% TRR**	**mg e.q./kg**	**% TRR**	**mg e.q./kg**
Clopyralid	72.0	0.009	52.2	0.006	110.6	10.244	112.1	11.260
Metabolite 1, RT:14.2min	2.0	0.0002	2.0	0.0002	ND[a]	ND	ND	ND
Total Identified	72.0	0.009	52.2	0.006	110.6	10.244	112.1	11.260
Total characterized[b]	10.9	0.001	26.2	0.003	N/A	N/A	N/A	N/A
Total extractable	95.1	0.011	95.8	0.012	106.4	9.860	108.5	10.049
Total unextractable	2.5	0.0003	2.3	0.0003	0.25	0.024	0.27	0.025
Accountability[c]	97.6	0.012	98.1	0.012	106.7	9.884	108.7	10.074
	^{14}C-Clopyralid Excreta, Day 4, Rep-1 TRR = 10.227 mg e.q./kg		^{14}C -Clopyralid Excreta, Day 4, Rep-2 TRR = 10.227 mg e.q./kg		^{14}C -Clopyralid Excreta, Day 7, Rep-1 TRR = 20.061 mg e.q./kg		^{14}C -Clopyralid Excreta, Day 7, Rep-2 TRR = 20.061 mg e.q./kg	
Compound	**% TRR**	**mg e.q./kg**	**% TRR**	**mg e.q./kg**	**% TRR**	**mg e.q./kg**	**% TRR**	**mg e.q./kg**
Clopyralid	106.1	10.847	110.5	11.301	107.8	21.621	102.9	20.652
Metabolite 1, RT:14.2min	ND	ND	ND	ND	ND	ND	ND	ND
Total Identified	106.1	10.847	110.5	11.301	107.8	21.621	102.9	20.652
Total characterized[b]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total extractable	107.8	11.026	107.7	11.012	107.3	21.528	105.5	21.171
Total unextractable	0.23	0.023	0.21	0.021	0.18	0.035	0.18	0.036
Accountability[c]	108.0	11.049	107.9	11.033	107.5	21.563	105.7	21.207

a Not Detected

b Characterized, radioactivity extracted with 80/20 Acetonitrile/Water and not identified by Mass Spectrometry. At least 100% TRR in excreta was identified as clopyralid,

c Accountability, %TRR Extracted + %TRR Unextracted

Identification of Residues Species

Representative samples "^{14}C-Clopyralid-Excreta-7DAT-HPLC2" and "^{14}C -Clopyralid-Eggs-7DAT-EXT1" were analyzed by the mass spectrometer.

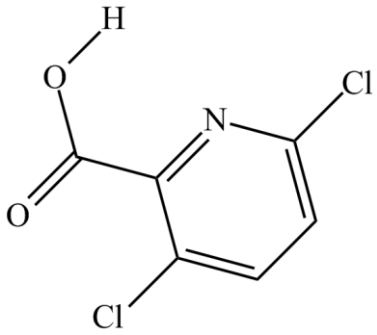
Two peaks of interest - Peak A and Peak B - were detected and identified in the analyzed "^{14}C -Clopyralid-Excreta-7DAT-HPLC2" and "^{14}C -Clopyralid-Eggs-7DAT-EXT1" samples, respectively.

Peak A in the "^{14}C-Clopyralid-Excreta-7DAT-HPLC2" sample, which eluted at approximately 10.03 minutes (RAM trace), was compared to the standard of X159934 (clopyralid). Peak A was identified as X159934 based upon relative retention time and mass spectral match with the reference standard.

Peak B in the "^{14}C-Clopyralid-Eggs-7DAT-EXT1" sample, which eluted at approximately 9.70 minutes (RAM trace), was compared to the standard of X159934 (clopyralid). Peak B was identified as X159934 based upon relative retention time and mass spectral match with the reference standard.

Names and structures of the identified metabolites are below.

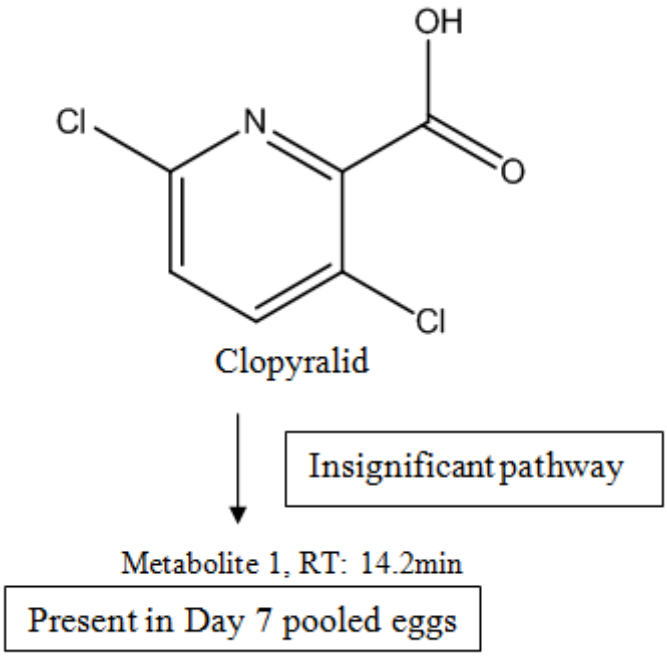
Table B.7.2.2.1.1- 11: Identification of compounds from metabolism study

Common name/code ID no.	Compound names	Chemical structure
X159934	3,6-dichloropicolinic acid Clopyralid	

Metabolic Pathway

A metabolic pathway is proposed below and metabolites are identified above. Clopyralid is minimally metabolized in laying hens.

Figure B.7.2.2.1- 7: Proposed Metabolic Pathway for ^{14}C -Clopyralid in Laying Hens



B.7.2.2.2 Lactating ruminants

As summarized by EFSA, (EFSA Scientific Report (2005) 50, 1-65, Conclusions on the peer review of clopyralid, 14 December 2005):

"radio-labelled clopyralid was orally administered to lactating goats. In addition the excretion of unlabelled clopyralid was investigated in lambs. The goat studies are lacking of the detailed experimental report. Thus, this report should be provided in order to assess the validity of the study.

The studies indicated that the majority of administered radioactivity was excreted as unchanged clopyralid. Residues found in milk from goats consisted of about equal amounts of clopyralid and the glycine conjugate of clopyralid, which could be hydrolysed to clopyralid under alkaline conditions. In goat tissues conjugate levels were low or undetectable."

The following study has not been previously reviewed for Annex I inclusion:

B.7.2.2.2.1 Nature of the Residue in the Ruminant fed with [^{14}C]Clopyralid labelled at two sites of the molecule

Data to address metabolism in ruminants were presented in the dossier submitted in April 2002 for the Active Approval was found to be insufficient in quality following evaluation and peer review at EU level. A new study was conducted according to the current guidelines and complete characterization of the tissues.

REFERENCE	[redacted] 2015; A Nature of the Residue Study in the Ruminant with [^{14}C]Clopyralid; [redacted] Lab Study No. 130202; DAS Study No. 130202; 16 January 2015; Unpublished
Guideline(s):	OECD Guidance Document 503 for Metabolism in Livestock (Adopted 8 January 2007)
US EPA Guideline(s):	None
Deviations:	Water analyses conducted at [redacted] and [redacted] were not conducted as per GLPs Data collected prior to protocol approval – includes everything from Study Day (-16) through (-8) and covers – Facility Preparation, Facilities, Receipt and ID of Animals (-16 & -9), Physical exam (-15), Daily Observations, 2x mortality and morbidity, Feed and Milk Collections (-15 to -7pm), Body weights (-14), Feed Sample (-11), Feed Moisture Content (-10) prior to protocol approval. The collection of the data was done per GLPs and has no negative impact on the study
Sample Storage Conditions	Samples and extracts at DAS were stored at ca. -20 °C. Milk, kidney, liver, renal fat and flank and loin muscle were re-extracted and analyzed by HPLC, with similar results, demonstrating stability of the tissues for the storage interval noted. Extracts of milk, tissues and urine and feces were analyzed by HPLC and then by LC/MS, with similar results, demonstrating stability of the extracts. Tissue stability was demonstrated for as long as 251 days (renal fat) and extract stability was demonstrated for as long as 240 days (urine).
GLP status:	Yes

Background Information

Results from a previous animal metabolism conducted using ^{14}C-clopyralid at dose rates of 230 and 69 mg eq/kg in the feed for 7 days indicated that clopyralid was not significantly

metabolized. The only radioactive residue in liver and kidney was clopyralid, and in milk both clopyralid and a clopyralid-glycine conjugate (X36538) were identified. The current study was conducted because the prior study was judged as incomplete information as communicated in an EFSA Reasoned Opinion, *Modification of the existing MRLs for clopyralid in various commodities.*

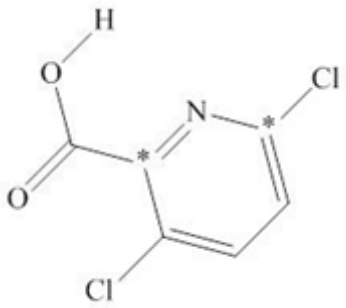
Materials and Methods

Test Item(s)

Non-radiolabelled test item #1

ISO Common name:	Clopyralid
Purity:	99.9%

Radiolabelled test item #1

Name:	Clopyralid-2,6-^{14}C
Test item (chemical/other name):	3,6-dichloropicolinic acid-2,6-^{14}C
Structural formula:	
Position of labelling (*)	
Radiochemical purity:	99.9%
Specific radioactivity:	51.2 mCi/mmol (592,000 dpm/µg)

Methods

Test Site Information

The in-life phase of this study was conducted at [redacted] The address of the contract lab is: [redacted].

Livestock

Table B.7.2.2.2.1- 1: General test animal information

Species	**Breed**	**Age**	**Weight at study initiation (kg)**	**Health Status**	**Description of housing/holding area**
Caprine	Nubian	5	105 pounds	Healthy	Initially placed into study room pens, moved to individual metabolism cages (47 inches long, 22 inches wide, and 34 inches high) on study DAY (-2).

Table B.7.2.2.2.1- 2: Test animal dietary regime

Composition of diet	Feed consumption (kg/day)	Water	Acclimation period	Pre-dosing
50/50 alfalfa/grass, plus dairy goat pellets and sweet grain ad libitum throughout the study	**1.852 kg/day (dosing period)**	**ad libitum**	**7 days**	**None**

Table B.7.2.2.2.1- 3: Test animal dosing regime

Treatment type	**Feeding level (ppm test material in food on a dry weight basis)**	**Vehicle**	**Timing/duration**
Oral bolus (by balling gun)	**50.9 mg a.i./kg/day**	**Clear gelatin capsules**	**5 days (12-16 April 2013, ≈9-10 a.m.)**

Dose Solution Preparation

The ^{14}C-clopyralid dose was prepared by dissolving the 9.5 mCi (35.6 mg) radiolabeled test substance and 502.5 mg (500.0 mg when corrected for 99.5% purity) non-radiolabeled test substance in methanol (5.0 mL) for a final specific activity of 3.40 mCi/mmol (39.367 dpm/µg). The solvent was removed under a gentle stream of nitrogen. The dried test substance was shipped to the contract laboratory.

Sample Handling and Preparation

Table B.7.2.2.2.1- 4: Sample collection information

Milk/eggs Collected	Urine, faeces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analysed
Milk was collected twice daily (morning and evening, maintained separately) daily throughout both the acclimation and dosing phases of the study, except for Day 5 of dosing, the day of sacrifice, when only one milk sample was collected in the morning.	Urine and feces were collected separately via collection devices suspended under the metabolism cage. Urine and feces from the treated animal was collected and weighed once daily for Days (-1) through 5. A cage rinse using a mixture of water and methanol was conducted after the final urine and feces collection on Day 5.	6-7 hours	muscle (loin) muscle (flank) liver kidney fat (subcutaneous) fat (omental) fat (renal) GI tract and contents (for mass balance only)

Milk

Milk was collected twice daily (morning and evening, maintained separately) daily throughout both the acclimation and dosing phases of the study, except for Day 5 of dosing, the day of sacrifice, when only one milk sample was collected in the morning. Whole milk was stored frozen at approximately ≤-10 °C prior to analysis and shipment to the sponsor.

Urine and feces

Urine and feces were collected separately via collection devices suspended under the metabolism cage. Urine and feces from the treated animal was collected and weighed once daily for Days (-1) through 5. A cage rinse using a mixture of water and methanol was conducted after the final urine and feces collection on Day 5. Urine, feces and cage rinse were stored frozen at approximately ≤-10 °C prior to analysis and shipment to the sponsor.

Sacrifice

The animal was sacrificed at 4:01 pm on April 16, 2013 (Day 5), which was approximately 6 to 7 hours after the final dose administration. The following samples were collected from the treated animal: the entire liver less gall bladder; both kidneys (combined); all available loin and flank muscle, maintained separately; all available subcutaneous, renal, and omental fat, maintained separately; gastrointestinal (GI) tract, GI contents, and blood. All samples were stored frozen at approximately ≤-10 °C prior to analysis and shipment to the sponsor.

Identification/ Characterization of Residues

TRR

Determination of TRR was conducted at SBL. Aliquots (5 x approximately 0.2 g each) of the homogenized liver, kidney, fat, muscle, GI tract and contents and feces samples were analyzed by oxidative combustion to determine the radioactive residues in the samples. Milk, urine and cage rinse were analyzed by direct LSC 5 x approximately 0.2 to 0.5 g aliquots. The collected blood was not analyzed because it was not required to determine mass balance.

Milk

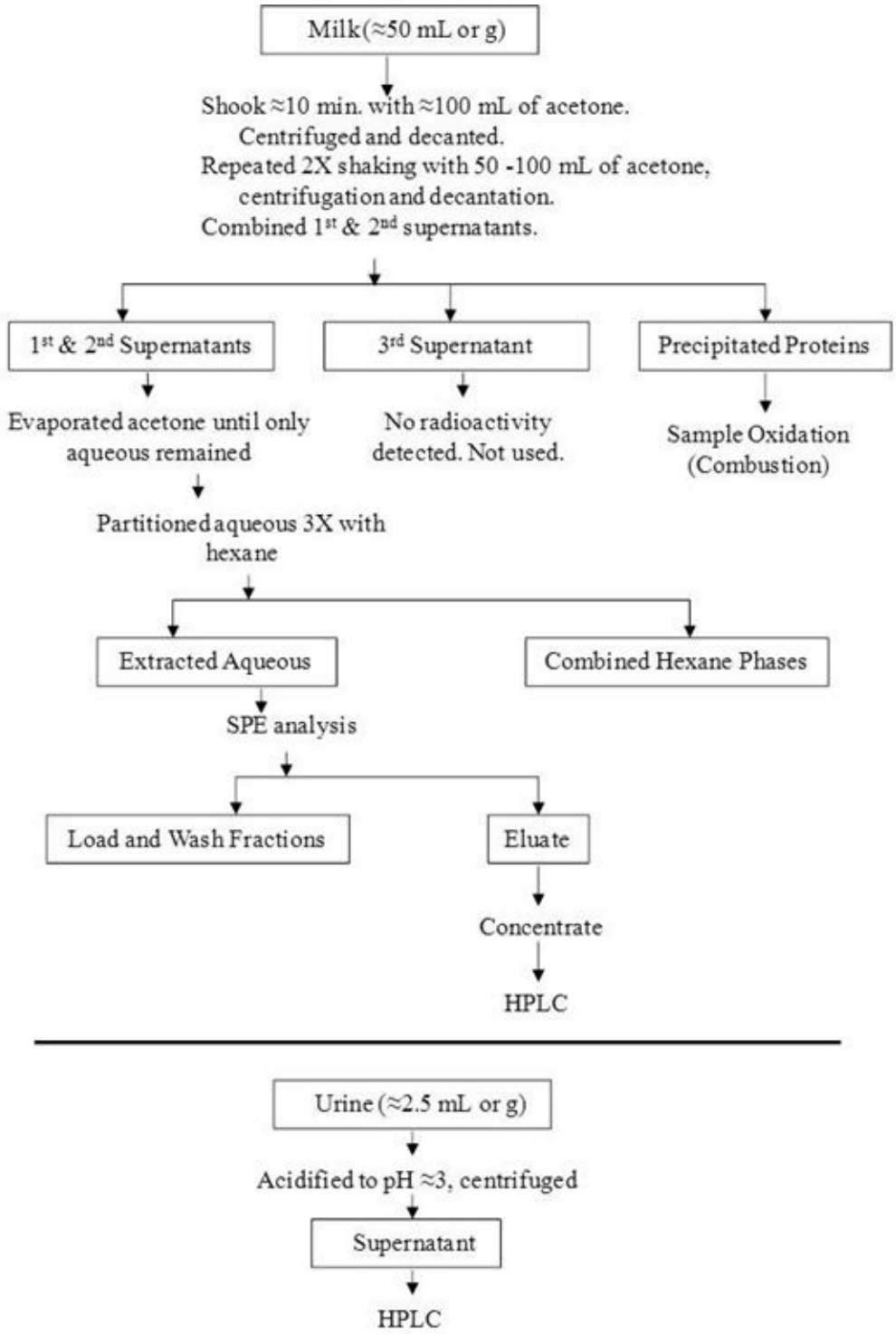
Samples of milk (approximately 50 mL or g each) were shaken for approximately 10 minutes with approximately 2 volumes (approximately 100 mL) of acetone, then centrifuged (approximately 10 minutes at approximately 2500 rpm) and the supernatant decanted. The remaining precipitate was shaken for approximately 10 minutes with approximately 1 volume (approximately 50 mL) of acetone, then centrifuged as previously described and the supernatant decanted. The remaining precipitate was shaken a third time for approximately 10 minutes with approximately 2 volumes (approximately 100 mL) of acetone, then centrifuged as previously described and the supernatant decanted. Weights of each extract were determined and aliquots were analyzed by LSC. The first two supernatants were combined. The acetone was vacuum evaporated using a Rocket evaporator (Rocket Evaporation System, Model Chemi, Genevac Limited) at approximately 40 °C until approximately 50 mL of aqueous remained. The remaining aqueous phases were partitioned 3 x approximately 50 mL using hexane. All three hexane phases were combined, and aliquots of the hexane and aqueous phases were analyzed by LSC. The aqueous phases were vacuum concentrated to less than 20 mL using a Rocket evaporator at approximately 40 °C. Then aqueous phases were prepared for solid phase extraction (SPE). Selected SPE fractions were prepared for HPLC by concentrating to dryness or near dryness and then dissolving in mixtures of water and acetonitrile containing 0.1 % formic acid.

For initial analyses of the 2 DAY PM and 5 DAY PM milk samples, hexane partition was conducted first and then protein precipitation. From this probe work, it appeared that conducting the protein precipitation first and then partitioning with hexane, as described above, was simpler and yielded samples that were easier to analyze.

Urine

Aliquots of selected urine samples were checked for pH, acidified using concentrated HCl to approximately pH 3 and centrifuged for ≈5 min. at ≈14,000 rpm using an Eppendorf 5415C. Aliquots of the supernatants were analyzed by LSC and chromatographed by direct injection onto HPLC.

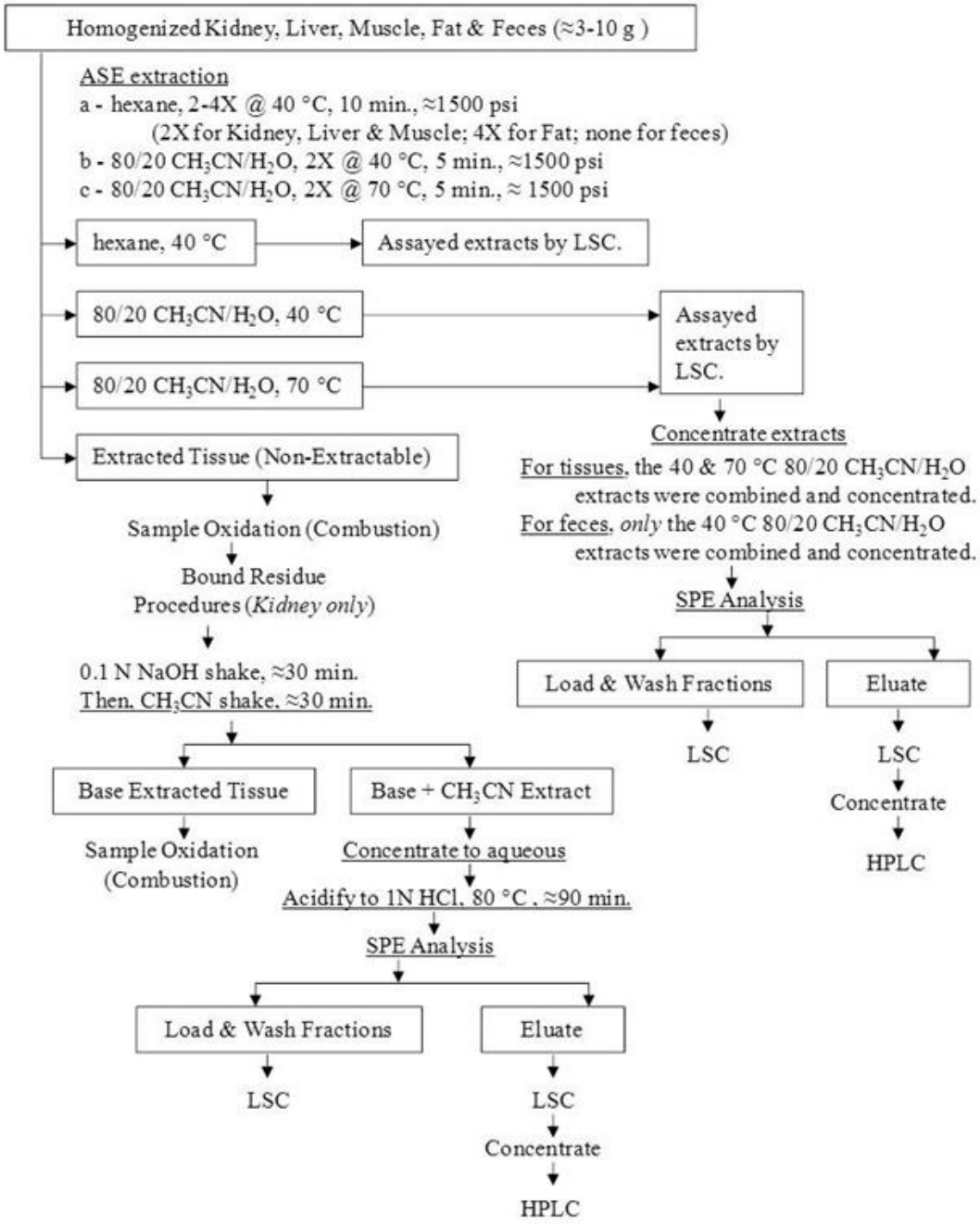
Figure B.7.2.2.2.1- 1: Schematic flowchart for the analysis of ^{14}C -Clopyralid residues in milk and urine



Kidney, Liver, Muscle, Fat and Faeces

An accelerated solvent extractor (Dionex model ASE 350) was used for all accelerated solvent extractions. Diatomaceous earth (typically 3-4 mL) was added to the bottom of the extraction cell prior to adding the sample. Kidney, liver, muscle, fat and feces samples (approximately 3 to 10 g each) were weighed into a separate container containing diatomaceous earth (typically 6-20 mL), mixed thoroughly and then transferred to prepared extraction cells. If necessary, diatomaceous earth was added to fill any remaining space in the extraction cell. Extraction cells were composed of stainless steel with frits on entrance and exit ports to contain the sample. For fat, the initial extraction solvent used was hexane, and four extractions were conducted at 40 °C. For kidney, liver and muscle only two initial hexane extractions at 40 °C were conducted. After hexane extraction, the next two extractions for all tissues were conducted using 80/20 acetonitrile/water at 40 °C, and the last two extractions were conducted using 80/20 acetonitrile/water at 70 °C . For feces, no initial hexane extractions were conducted, and extractions with 80/20 acetonitrile/water at 40 and 70 °C were conducted as described for the tissues. All extractions were conducted with a heating step and a static step. During the heating step for all extractions, the ASE extractor filled each cell with solvent, pressurized the cell to approximately 1,500 pounds per square inch (psi), heated the cell for 5 minutes to achieve thermal equilibrium (at approximately at 40 or 70 °C) and then held between approximately 1,500 and 1700 psi . For the hexane extractions, the static step (≈40 °C) lasted approximately 10 minutes, and the pressure was held at approximately 1,400 psi with solvent continually flowing through the cell and with the extract being collected . For the 80/20 acetonitrile/water extractions, the static step (≈40 or 70 °C) lasted approximately 5 minutes, the pressure in the cell was held between approximately 1,500 and 1,700 psi, cell pressure was relieved upon reaching approximately 1,700 psi, a small amount of solvent was added to the cell, a small amount of extract was collected and the cell was again pressurize to approximately 1,500 psi. Each of the extracts per sample (two to four 40 °C hexane extracts, two 40 °C 80/20 acetonitrile/water extracts and the two 80/20 acetonitrile/water 70 °C extracts) was collected separately. The volume of each of the extracts was determined (approximately 30 to 50 mL for each extract), and triplicate aliquots (0.25-1.0 mL) were analyzed by LSC.

Figure B.7.2.2.2.1- 2: Schematic flowchart for the analysis of ^{14}C -Clopyralid residues in liver, kidney, and faeces



Extraction and Clean-up Procedures for Metabolite Identification

A reference standard was available for clopyralid. Retention time comparisons were made between this reference standard and the retention time of radioactive peaks in the chromatograms to provide tentative identifications using HPLC. Identities of the individual peaks of radioactivity in the samples analysed in this study were confirmed as clopyralid on

the basis of the mass spectral results. X36538 was proposed on the basis of mass spectral results and on its observance in a prior study.

Analytical Methods

Total ^{14}C measurement

At SBL, the pre-application initial activity check of the Dose Solution was counted on a Beckman Coulter Liquid Scintillation Counter (LSC), Model LS 6500, SN7071503 set for full 1 minute counts. The dose solution radioactivity checks for capsule production results were counted on the same LSC set for full 5 minute counts. All TRR determination (direct count and oxidized) samples were counted on the Beckman Coulter Liquid Scintillation Counter (LSC), Model LS 6500, SN7071503 set to count for 5 minutes or to a 2 sigma level of 0.5% . For additional mass balance data, the gastrointestinal tract and gastrointestinal contents (oxidized) were counted on the Beckman Coulter Liquid Scintillation Counter (LSC), Model LS 6500, SN 7071503 set to count for 5 minutes or to a 2 sigma level of 0.5%.

The liquid scintillation counters used at DAS automatically converted the radioactivity counting rate in counts per minute (cpm) to disintegrations per minute (dpm) using an external standard to correct for sample quenching. The instrument was calibrated at least every six months with a set of ten quenched standards. Each day of use, the instrument was normalized and its performance was checked with respect to background cpm value, unquenched standard cpm value, and quenched standard dpm value for a range of quenched standards. The dpm value for a sample extract was determined by LSC after diluting an appropriate aliquot of the sample extract with ScintiSafe Plus 50% scintillation cocktail (Fisher Scientific, Fair Lawn, NJ) and counting for at least two minutes.

Solid phase extraction (SPE)

The general clean-up procedure for extracts was with a 150 mg SPE cartridge. SPE cartridges were conditioned with 5 mL of methanol followed by 5 mL of water. Each sample concentrate (dryness, near dryness or only aqueous remaining) was diluted with water, if necessary, and acidified to a concentration of approximately 1 N HCl, applied to the conditioned SPE and eluted at approximately 1 mL/min, collecting the load. The SPE cartridge was dried just long enough to evacuate most of the liquid. The sample vial was rinsed with 5 mL of 1 N HCl, the rinse was transferred to the SPE cartridge, and the sample was eluted at approx. 1 mL/min and pooled with the load. The SPE cartridge was dried as before, rinsed with four sequential 5-mL aliquots of HPLC grade water, and then with 5 mL of methanol/water/acetic acid 10/90/0.1, eluting at approx. 1 mL/min and all rinses were combined. The SPE cartridge was dried under full vacuum for approximately 15 minutes. The SPE cartridge was eluted with three 5 mL aliquots of ethyl acetate/trifluoroacetic acid 99/1 and the eluates were combined. Triplicate aliquots of each load, rinse and eluate fraction were analyzed by LSC. The eluate fraction for each sample was concentrated to dryness or near dryness using a Turbovap (40 ºC water bath and 10 psi nitrogen) or a Rocket evaporator at approximately 40 °C. Selected SPE fractions were prepared for HPLC by concentrating to dryness or near dryness and then dissolving in mixtures of water and acetonitrile containing 0.1 % formic acid.

High performance liquid chromatography (HPLC) for quantitation

The extracts were analyzed using reverse-phase HPLC column (Phenomenex Synergi Hydro-RP, 4 µm, 150 x 4.6 mm) and eluted with a gradient of water and acetonitrile containing 0.1 % formic acid. An HPLC was connected to a Radio LC-System Detector from LabLogic Systems, Inc., Brandon, FL. The flow-through radioactivity monitor (RAM) was used to quantify the amount of radioactivity present in each peak. Laura™ software (IN/US Systems, Inc.) was used to control the HPLC, collect UV and RAM data, and process the data. A direct spike of each sample analyzed by HPLC was assayed by LSC and compared to the sum of the radioactivity eluted from the column to determine chromatographic recovery. A UV detector at 254 nm wavelength was used to determine the retention time of a non-radiolabeled standard.

Mass spectral analysis (LC/MS) for identification of transformation products

In general, liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) were used to analyze the samples and the standards. A Berthold radioactivity monitor (RAM) was used to assist in location of the metabolite peaks.

Detection Limits

Using the method of Currie, the limit of detection can be calculated from the expression:

$$LOD = \frac{2.71 + 4.65\sqrt{dpm_B \times T}}{T}$$ ¶

and the limit of quantitation can be calculated:

$$LOQ = \frac{50\left(1 + \sqrt{1 + \frac{dpm_B \times T}{12.5}}\right)}{T}$$ ¶

where LOD is the limit of detection (dpm), LOQ is the limit of quantitation (dpm), dpmB is the typical background (dpm) and T is the counting time (minutes). Samples were normally counted for 5 minutes. Typical background levels were 45 dpm. The resulting LOD was 14 dpm above background and LOQ was 54 dpm above background.

For HPLC radiochemical flow-through detection the LOD the formula is modified slightly from that used for LSC counting:

$$\text{Limit of Detection (LOD) (dpm)} = \frac{2.71 + \left(4.65\sqrt{bkg\ cpm\ x\ \left(\frac{efficiency}{100}\right)\ x\ count\ time}\right)}{count\ time}$$ ¶

Where efficiency = radioactive detector efficiency (in %), and count time = cell volume (in

mL) total flow rate (eluate plus scintillant in mL/min) . For stop-flow mode, the count time is the nominal setting, for example, if a 30-second stop-flow method is chosen, the count time is 30 seconds.

For HPLC analyses conducted using the INUS/LabLogic Radio-LC System on-line radioactivity detector, a typical background for stop-flow mode (a mode used for samples containing low amounts of radioactivity) was 8 cpm, the stop–flow time was 30 seconds, and a static cell efficiency of 94.0% was used. Using the above equation, this resulted in an LOD of 23 dpm over background. The amount of dpm injected ranged from approximately 1,143 to 57,990 dpm. The average sample injected was greater than 11,428 dpm. Therefore, the LOD ranged from approximately 0.04 to 2.05% of the injected amount of radioactivity and was approximately 0.21% for the average sample.

Results and Discussion

Dosing and in-life summary

The purity value of the ^{14}C- clopyralid used for specific activity calculations was 99.9%. Purity checks of the ^{14}C- clopyralid prior to specific activity adjustment averaged 99.1%. The target dose rate for the study was 50 mg/kg feed. Each capsule contained 94.2 mg clopyralid. A dose rate of 48.8 mg/kg feed was calculated based on the average feed consumption during the acclimation period, however, the goats consumed slightly less than expected during dosing, resulting in an actual dose of 50.9 mg/kg for the ^{14}C-clopyralid and dosed animal. The feed consumption, milk production, and animal weights, combined with the observations by veterinary personnel suggest that the animals remained healthy during the course of the study. Aliquots of the dose solutions were collected before and after dosing, and shipped to DAS for analysis. HPLC analysis demonstrated that the ^{14}C-clopyralid radiopurity was 98.38% before dosing and was 98.31% after dosing. Therefore, the test substance did not degrade during the dosing period.

Milk, Tissue and Excreta TRR Levels

To summarize, 90.300% of the dose was recovered from the ^{14}C-clopyralid dosed animal. The majority of the dose was excreted with 65.554% and 13.689% in the urine and feces, respectively. Overall, there was good absorption and rapid elimination of the ^{14}C- clopyralid. Urine and feces samples from days 2 and 5 were extracted and further characterized.

Milk contained 0.014% of the dose. During the dosing period the residue level in milk was considered to reach a plateau almost immediately because concentrations varied each day from low concentrations of 0.003 to 0.004 mg eq/kg at AM sample times to higher concentrations of 0.010 to 0.014 mg eq/kg at PM sample times. This phenomenon demonstrated how rapidly clopyralid was absorbed and excreted. Since dosing took place in the AM, the pattern of residues indicated that clopyralid was well absorbed, had increased by the PM sampling the same day and then rapidly declined by the next day's AM sampling. Milk samples collected in the PM on days 1, 2, 3, 4 and 5 were extracted and further characterized.

Low to moderate residue levels were found in all edible tissues collected at sacrifice, except for high residue levels in kidney, and less than 0.057% of the dose was recovered in the edible tissues. Residues in muscle, liver and fat ranged from 0.022 to 0.057 mg eq/kg, and

kidney residues were considerably higher at 0.863 mg eq/kg. Due to the fact that most of the ^{14}C-clopyralid dose was excreted in the urine and that urine residues were very high (over 34 mg eq/kg), it was likely the high residues in kidney were due to radioactivity in any residual urine that had not been completely emptied from the kidneys. Aliquots of muscle, fat, liver and kidney were extracted and further characterized.

Table B.7.2.2.2.1- 5: Total radioactive residues (TRRs) of ^{14}C -Clopyralid in milk, tissue and excreta

Matrix	Collection timing	^{14}C-clopyralid	
		(% dose)	(mg eq/kg)
Milk	Day 1 pm	0.001%	0.011
Milk	Day 1 am	0.001%	0.004
Milk	Day 2 pm	0.002%	0.013
Milk	Day 2 am	0.001%	0.004
Milk	Day 3 pm	0.002%	0.010
Milk	Day 3 am	0.001%	0.003
Milk	Day 4 pm	0.002%	0.010
Milk	Day 4 am	0.001%	0.003
Milk	Day 5 pm	0.002%	0.014
Feces	Day 1	1.891%	5.219
Feces	Day 2	4.015%	10.117
Feces	Day 3	3.648%	9.135
Feces	Day 4	3.004%	7.697
Feces	Day 5	1.132%	6.922
Urine	Day 1	11.335%	40.721
Urine	Day 2	12.724%	54.880
Urine	Day 3	19.356%	45.085
Urine	Day 4	16.629%	34.887
Urine	Day 5	5.509%	50.288
Cage rinse	Sacrifice	0.232%	1.984
Liver	Sacrifice	0.011%	0.057
Kidney	Sacrifice	0.033%	0.863
Muscle, loin	Sacrifice	0.003%	0.022
Muscle, flank	Sacrifice	0.007%	0.031
Fat, omental	Sacrifice	0.001%	0.024
Fat, subcutaneous	Sacrifice	0.002%	0.054
Fat, renal	Sacrifice	0.002%	0.054
GI tract	Sacrifice	1.182%	1.363

Matrix	Collection timing	^{14}C-clopyralid	
		(% dose)	(mg eq/kg)
GI Contents	Sacrifice	9.572%	3.468
Total		90.300%	Not applicable

Characterisation of Residues

Milk

Aliquots of the DAY 1 through DAY 5 PM milk samples were extracted. Over 91% of the TRR (0.010 to 0.013 mg eq/kg) remained in the aqueous phase after protein precipitation and hexane partition. Less than 9% of the TRR (0.001 mg eq/kg) remained in the precipitate and less than 1% of the TRR (less than 0.001 mg eq/kg) remained in the hexane phase. When the radioactivity from the aqueous phases was analyzed by SPE and concentrated for HPLC, greater than 86% of the TRR (0.009 to 0.013 mg eq/kg) remained in the samples that were analyzed by HPLC. Recoveries for SPE and concentration were greater than 92%.

Over 63% of the radioactivity analyzed by HPLC was found as parent clopyralid and over 21% was found as X36538. The results in the chromatograms for the DAY 1, 3, 4 and 5 PM samples were very similar in appearance . From 54 to 70% of the TRR or 0.006 to 0.010 mg eq/kg was found as clopyralid, and from 11 to over 21% of the TRR or 0.002 mg eq/kg was found as X36538. No other component exceeded 9% of the TRR or 0.001 mg eq/kg. Overall, from 73 to over 82% of the TRR (0.008 to 0.011 mg eq/kg) in milk was identified.

Characterisation of residues in liver

An aliquot of liver was extracted. Less than 0.3% of the TRR (<0.001 mg eq/kg) was extracted by hexane, over 83% of the TRR (0.048 mg eq/kg) was extracted by 80/20 acetonitrile/water and less than 23% of the TRR (0.013 mg eq/kg) remained in the post-extracted tissue. When the radioactivity from the combined 80/20 acetonitrile/water extracts was analyzed by SPE and concentrated, greater than 71% of the TRR (0.041 mg eq/kg) remained in the sample that was analyzed by HPLC. Recoveries for SPE and concentration were greater than 92% . For liver, 100% of the radioactivity analyzed by HPLC was found as parent clopyralid. The results in the chromatogram for liver are very similar in appearance to those of fat and muscle samples. Over 71% of the TRR or 0.041 mg eq/kg was found as clopyralid. No components other than clopyralid were found in liver, and over 71% of the TRR or 0.041 mg eq/kg in liver was identified.

Characterisation of residues in fat

Aliquots of renal, omental and subcutaneous fat samples were extracted . For all three fat types, less than 14% of the TRR (0.007 mg eq/kg or less) was extracted by hexane, over 99% of the TRR (0.024 to 0.060 mg eq/kg) was extracted by 80/20 acetonitrile/water and less than 8% of the TRR (0.002 mg eq/kg or less) remained in the post-extracted tissue. When the radioactivity from the combined 80/20 acetonitrile/water extracts was analyzed by SPE and concentrated, greater than 94% of the TRR (0.023 to 0.058 mg eq/kg) remained in the samples that were analyzed by HPLC. Recoveries for SPE and concentration were greater

than 85% . For the renal and omental fat, 100% of the radioactivity analyzed by HPLC was found as parent clopyralid . For the subcutaneous fat, over 96% of the radioactivity analyzed by HPLC was found as parent clopyralid and less than 4% was found as X36538. The results in the chromatograms for the fat samples are very similar in appearance . From 95 to 104% of the TRR or 0.023 to 0.056 mg eq/kg was found as clopyralid, and X36538 (less than 4% of the TRR or 0.002 mg eq/kg) was found only in the subcutaneous fat. No components other than clopyralid and X36538 were found in fat. Overall, from 94 to over 107% of the TRR (0.023 to 0.058 mg eq/kg) in fat was identified.

Characterisation of residues in muscle

Aliquots of flank and loin muscle samples were extracted . For both muscle types, less than 8% of the TRR (0.002 mg eq/kg) was extracted by hexane, over 111% of the TRR (0.024 to 0.034 mg eq/kg) was extracted by 80/20 acetonitrile/water and less than 9% of the TRR (0.002 mg eq/kg) remained in the post-extracted tissue. When the radioactivity from the combined 80/20 acetonitrile/water extracts was analyzed by SPE and concentrated, greater than 106% of the TRR (0.023 to 0.035 mg eq/kg) remained in the samples that were analyzed by HPLC. Recoveries for SPE and concentration were greater than 91% . For both loin and flank muscle, 100% of the radioactivity analyzed by HPLC was found as parent clopyralid. The results in the chromatograms for the muscle samples are very similar in appearance . From 106 to 113% of the TRR or 0.023 to 0.035 mg eq/kg was found as clopyralid. No components other than clopyralid were found in muscle. Overall, from 106 to over 112% of the TRR or 0.023 to 0.035 mg eq/kg in muscle was identified.

Characterisation of residues in kidney

An aliquot of kidney was extracted. No radioactivity above background was extracted by hexane, over 95% of the TRR (0.825 mg eq/kg) was extracted by 80/20 acetonitrile/water and less than 7% of the TRR (0.053 mg eq/kg) remained in the post-extracted tissue. When the radioactivity from the combined 80/20 acetonitrile/water extracts was analyzed by SPE and concentrated, greater than 85% of the TRR (0.742 mg eq/kg) remained in the sample that was analyzed by HPLC. Recoveries for SPE and concentration were greater than 93% . For this 80/20 acetonitrile/water extract of kidney, 100% of the radioactivity analyzed by HPLC was found as parent clopyralid.

The post-extracted kidney tissue contained 6.2% of the TRR or 0.053 mg eq/kg. According to OECD guidance, the trigger for further characterization was 10% of the TRR or 0.050 mg/kg, whichever was greater. In this case, 10% of the TRR for kidney was 0.086 mg eq/kg, which was greater than 0.050 mg/kg, and therefore no further characterization was required. However, because the residue in kidney was relatively high compared to the other tissues, it was feasible to attempt characterization of the bound residue. An aliquot of post-extracted kidney was extracted. After base extraction 5.4% of the TRR (0.047 mg eq/kg) was extracted and less than 0.7% of the TRR (0.006 mg eq/kg) remained in the base-extracted tissue. After acid hydrolysis of the base extract, SPE analysis of the acid hydrolyzate and concentration, 3.9% of the TRR (0.034 mg eq/kg) remained in the sample that was analyzed by HPLC. Recoveries for base and acid hydrolysis and for SPE and ranged from 79 to103% . For this base extract of kidney, 100% of the radioactivity analyzed by HPLC was found as parent clopyralid.

The results in the chromatograms of the 80/20 acetonitrile/water extract of kidney and the extract of the hydrolyzed post-extracted kidney are very similar in appearance to those of fat, muscle and liver samples. When the results from kidney extraction and hydrolysis of the extracted tissue were considered together, over 89% of the TRR or 0.776 mg eq/kg was found as clopyralid. No components other than clopyralid were found in kidney, and over 89% of the TRR or 0.776 mg eq/kg in kidney was identified.

Characterisation of residues in urine

Aliquots of only the 2 and 5 DAY samples were chosen for analysis because they represented samples from early and late in the dosing period. After the radioactivity from the samples was prepared for HPLC by acidification and centrifugation, over 96 and 101% of the TRR (52.966 and 50.915 mg eq/kg) remained in the 2 and 5 DAY samples, respectively, that were analyzed by HPLC. Recoveries for the acidification and centrifugation were greater than 96%. Over 99% of the radioactivity analyzed by HPLC was found as parent clopyralid, less than 0.5% was found as X36538 and less than 0.3% was found as an unknown. The results in the chromatogram for the 5 DAY urine sample were very similar in appearance to the 2 DAY urine sample. Over 95 and 100% of the TRR (52.527 and 50.538 mg eq/kg) was found as clopyralid for the 2 and 5 DAY samples, respectively. X36538 was found in both samples, but comprised less than 0.8% of the TRR or 0.440 mg eq/kg. The only component other than clopyralid and X36538 was found in the 5 DAY sample at less than 0.3% of the TRR or 0.132 mg eq/kg. Overall, from 96 to over 101% of the TRR (50.783 to 52.966 mg eq/kg) in urine was identified.

Characterisation of residues in feces

Aliquots of only the 2 and 5 DAY samples were chosen for analysis because they represented samples from early and late in the dosing period. Over 95 and 96% of the TRR (9.701 and 6.687 mg eq/kg) was extracted by 80/20 acetonitrile/water from the 2 and 5 DAY samples, respectively, and less than 0.4% of the TRR (0.026 mg eq/kg) remained in the post-extracted tissue. When the radioactivity from the combined 80/20 acetonitrile/water extracts was analyzed by SPE and then concentrated for HPLC, over 103 and 106% of the TRR (10.504 and 7.346 mg eq/kg) remained in the 2 and 5 DAY samples, respectively, that were analyzed by HPLC. Recoveries for SPE and concentration were 100% or greater. Over 98% of the radioactivity analyzed by HPLC was found as parent clopyralid, less than 1.1% was found as X36538 and less than 0.6% was found as an unknown. The results in the chromatogram for the 5 DAY feces sample are very similar in appearance to the 2 DAY feces sample. Over 101 and 104% of the TRR (10.293and 7.230 mg eq/kg) was found as clopyralid for the 2 and 5 DAY samples, respectively. X36538 was found in both samples, but comprised less than 1.7% of the TRR or 0.168 mg eq/kg. An unknown component was found in the 5 DAY sample at less than 0.6% of the TRR or 0.043 mg eq/kg. Overall, from 103 to over 105% of the TRR (10.461 to 7.307 mg eq/kg) in feces was identified.

Table B.7.2.2.2.1- 6: Distribution of Radioactivity in Milk, Day 1-3, when dosed with ^{14}C - Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure / Fraction	Milk, 1 DAY PM		Milk, 2 DAY PM		Milk, 3 DAY PM	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	0.011	100.0	0.013	100.0	0.010
Precipitation/Extraction						
Acetone Extracts 1 & 2[b]	92.0	0.010	90.4	0.012	93.8	0.010
Acetone Extract 3	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]
Precipitate	6.2	0.001	8.2	0.001	6.7	0.001
Total[d]	98.2	0.010	98.6	0.013	100.6	0.010
Hexane Partition						
Hexane Phase	0.2	<0.001	0.4	<0.001	0.8	<0.001
Aqueous Phase[b]	96.6	0.010	91.7	0.012	97.2	0.010
Total	96.8	0.010	92.1	0.012	98.0	0.010
Recovery[e]	105.2		101.9	104.4		
SPE						
Load & Washes	2.4	<0.001	2.4	<0.001	3.0	<0.001
Eluate[b]	91.7	0.010	87.5	0.012	90.3	0.009
Total	94.1	0.010	89.8	0.012	93.3	0.009
Recovery[e]	97.4		97.9		95.9	
Concentration for HPLC						
Concentrate[b]	92.3	0.010	86.3	0.012	92.3	0.009

Procedure Fraction	Milk, 1 DAY PM		Milk, 2 DAY PM		Milk, 3 DAY PM	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Recovery[e]	**100.6**		**98.7**		**102.3**	

aThe mg eq/kg value is from the results of LSC counting of collected milk.

bThis is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

cNo radioactivity above background detected.

dThis total is the recovery compared to the results of LSC counting of collected milk.

eThese are recoveries compared to the radioactivity in the fraction taken from the previous procedure.

Table B.7.2.2.2.1- 7: Distribution of Radioactivity in milk, Day 4 and 5, when dosed with ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure Fraction	Milk, 4 DAY PM		Milk, 5 DAY PM	
	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	0.010	100.0	0.014
Precipitation/Extraction				
Acetone Extracts 1 & 2[b]	91.5	0.009	96.0	0.013
Acetone Extract 3	-[c]	-[c]	-[c]	-[c]
Precipitate	6.5	0.001	6.3	0.001
Total[d]	98.0	0.010	102.3	0.014
Hexane Partition				
Hexane Phase	0.8	<0.001	1.1	<0.001
Aqueous Phase[b]	95.2	0.010	96.2	0.013
Total	96.0	0.010	97.3	0.013
Recovery[e]	104.9		101.4	
SPE				
Load & Washes	2.3	<0.001	2.4	<0.001
Eluate[b]	85.9	0.009	91.1	0.013

Procedure Fraction	Milk, 4 DAY PM		Milk, 5 DAY PM	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Total	**88.2**	**0.009**	**93.5**	**0.013**
Recovery[e]	**92.6**		**97.2**	
Concentration for HPLC				
Concentrate[b]	**86.5**	**0.009**	**92.1**	**0.013**
Recovery[e]	**100.6**		**101.1**	

aThe mg eq/kg value is from the results of LSC counting of collected milk.

bThis is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

cNo radioactivity above background detected.

dThis total is the recovery compared to the results of LSC counting of collected milk.

eThese are recoveries compared to the radioactivity in the fraction taken from the previous procedure.

Table B.7.2.2.2.1- 8: Distribution of Radioactivity in Fat, when dosed with ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure / Fraction	Fat, Renal		Fat, Omental		Fat, Subcutaneous	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	0.054	100.0	0.024	100.0	0.054
Extraction						
Hexane Extracts	13.2	0.007	7.6	0.002	1.7	0.001
80/20 Ext. 1 & 2, 40 °C	104.6	0.056	98.8	0.023	111.3	0.060
80/20 Ext. 3 & 4, 70 °C	5.6	0.003	1.1	<0.001	1.1	0.001
Total 80/20 Ext. 1-4[b]	110.2	0.059	99.8	0.024	112.4	0.060
Total Extractable	123.4	0.067	107.4	0.026	114.1	0.061
Post-extracted Tissue	0.8	<0.001	7.4	0.002	1.4	0.001
Total[c]	124.2	0.067	114.8	0.027	115.5	0.062
SPE						
Load & Washes	0.5	<0.001	2.0	<0.001	0.9	<0.001
Eluate[b]	91.9	0.050	103.6	0.025	104.3	0.056
Total	92.5	0.050	105.7	0.025	105.2	0.056
Recovery[d]	85.0		105.8		93.6	
Concentration for HPLC						
Concentrate[b]	97.0	0.052	94.8	0.023	107.5	0.058
Recovery[d]	105.5		91.5		103.1	

aThe mg eq/kg value is from the results of combustion and LSC counting of muscle tissue.

bThis is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

cThis total is the recovery compared to the results of combustion and LSC counting of muscle tissue.

dThese are recoveries compared to the radioactivity in the fraction taken from the previous procedure

Table B.7.2.2.2.1- 9: Distribution of Radioactivity in Muscle when dosed with ^{14}C - Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure / Fraction	Muscle, Flank		Muscle, Loin	
	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	0.031	100.0	0.022
Extraction				
Hexane Extracts	0.5	<0.001	7.8	0.002
80/20 Ext. 1 & 2, 40 °C	106.9	0.033	99.4	0.021
80/20 Ext. 3 & 4, 70 °C	4.4	0.001	14.1	0.003
Total 80/20 Ext. 1-4[b]	111.4	0.034	113.4	0.024
Total Extractable	111.9	0.035	121.3	0.026
Post-extracted Tissue	7.2	0.002	8.6	0.002
Total[c]	119.0	0.037	129.8	0.028
SPE				
Load & Washes	1.1	<0.001	1.4	<0.001
Eluate[b]	111.1	0.034	102.7	0.022
Total	112.2	0.035	104.2	0.022
Recovery[d]	100.7		91.8	
Concentration for HPLC				
Concentrate[b]	112.9	0.035	106.8	0.023
Recovery[d]	101.6		103.9	

aThe mg eq/kg value is from the results of combustion and LSC counting of muscle tissue.

bThis is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

cThis total is the recovery compared to the results of combustion and LSC counting of muscle tissue.

dThese are recoveries compared to the radioactivity in the fraction taken from the previous procedure

Table B.7.2.2.2.1- 10: Distribution of Radioactivity in Liver and Kidney when dosed with ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure Fraction	Liver		Kidney	
	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	0.057	100.0	0.863
Extraction				
Hexane Extracts	0.3	<0.001	<0.1	<0.001
80/20 Ext. 1 & 2, 40 °C	52.0	0.030	71.7	0.619
80/20 Ext. 3 & 4, 70 °C	31.3	0.018	23.8	0.205
Total 80/20 Ext. 1-4[b]	83.4	0.048	95.5	0.825
Total Extractable	83.6	0.048	95.5	0.825
Post-extracted Tissue	22.1	0.013	6.2	0.053
Total[c]	105.8	0.061	101.7	0.878
SPE				
Load & Washes	0.8	<0.001	0.2	0.002
Eluate[b]	76.6	0.044	92.3	0.796
Total	77.5	0.044	92.5	0.798
Recovery[d]	92.9		96.8	
Concentration for HPLC				
Concentrate[b]	71.4	0.041	85.9	0.742
Recovery[d]	93.2		93.1	
Base Hydrolysis of Extracted Tissue				
Base Extract[b]	NA	NA	5.4	0.047
Base Extracted Tissue	NA	NA	0.7	0.006
Total	NA	NA	6.1	0.053
Recovery[d]	NA	NA	99.1	
Acid Hydrolysis of Base Extract				
Acid Hydrolyzate[b]	NA	NA	4.9	0.042

Procedure Fraction	Liver		Kidney	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Recovery[d]	NA	NA	90.0	
SPE of Acid Hydrolyzate				
Load & Washes	NA	NA	0.1	0.001
Eluate[b]	NA	NA	3.8	0.033
Total	NA	NA	3.9	0.034
Recovery[d]	NA	NA	79.9	
Concentration forHPLC				
Concentrate[b]	NA	NA	3.9	0.034
Recovery[d]			103.1	

[a]*The mg eq/kg value is from the results of combustion and LSC counting of liver and kidney tissue.*

[b]*This is the fraction (underlined value) from each procedure that was taken to the next step of analysis.*

[c]*This total is the recovery compared to the results of combustion and LSC counting of liver and kidney tissue.*

[d]*These are recoveries compared to the radioactivity in the fraction taken from the previous procedure.*

Table B.7.2.2.2.1- 11: Distribution of Radioactivity in Urine when dosed with ^{14}C - Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure / Fraction	Urine, 2 DAY		Urine 5 DAY	
	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	54.880	100.0	50.288
Acidified Centrifuged[b,c]	96.5	52.966	101.2	50.915

[a]*The mg eq/kg value is from the results of LSC counting of urine.*

[b]*The sample was acidified and centrifuged in one step and then assayed for radioactivity; consequently, the underlined values were the recoveries compared to the results of LSC counting of urine.*

[c]*Also, the underlined values were the amounts expressed as %TRR and mg eq/kg analyzed by HPLC.*

Table B.7.2.2.2.1- 12: Distribution of Radioactivity in Feces when dosed with ^{14}C - Clopyralid at 50.9 mg/kg/day for 5 Days

Procedure Fraction	Feces, 2 DAY		Feces, 5 DAY	
	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR[a]	100.0	10.117	100.0	6.922
Extraction				
80/20 Ext. 1 & 2, 40 °C	95.8	9.696	96.5	6.679
80/20 Ext. 3 & 4, 70 °C	0.0	0.005	0.1	0.009
Total Extractable[b]	95.9	9.701	96.6	6.687
Post-extracted Feces	0.2	0.023	0.4	0.026
Total[c]	96.1	9.725	97.0	6.713
SPE				
Load & All Washes	1.0	0.099	0.1	0.006
Eluate[b]	95.0	9.607	96.7	6.692
Total	95.9	9.706	96.8	6.698
Recovery[d]	100.1		100.3	
Concentration for HPLC				
Concentrate[b]	103.8	10.504	106.1	7.346
Recovery[d]	109.3		109.8	

aThe mg eq/kg value is from the results of combustion and LSC counting of feces.

bThis is the fraction (underlined value) from each procedure that was taken to the next step of analysis.

cThis total is the recovery compared to the results from combustion of LSC counting of feces.

dThese are recoveries compared to the radioactivity in the fraction taken from the previous procedure.

Table B.7.2.2.2.1- 13: Summary of characterisation and identification of radioactive residues in goat milk, day 1 – 3, following application of radiolabeled ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Milk, 1 DAY PM		Milk, 2 DAY PM		Milk, 3 DAY PM	
	RT (min)	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		100.0	0.011	100.0	0.013	100.0	0.010
analyzed by HPLC[a]		92.3	0.010	86.3	0.012	92.3	0.009
clopyralid (X159934)	12.7	65.4	0.007	54.7	0.007	61.4	0.006
unknown ≈13.0 min	13.0	6.1	0.001	8.5	0.001	7.1	0.001
X36538	13.7	16.8	0.002	18.5	0.002	21.5	0.002
unknown ≈15.5 min	15.5	3.9	<0.001	4.5	0.001	2.3	<0.001
unknowns ≈11-19 min	11-19	_[b]	_[b]	_[b]	_[b]	_[b]	_[b]
identified[c]		82.2	0.009	73.3	0.010	82.9	0.008
characterized[d]		9.8	0.001	17.1	0.002	11.0	0.001
extractable		92.0	0.010	90.4	0.012	93.8	0.010
unextractable		6.2	0.001	8.2	0.001	6.7	0.001
total accountability[e]		98.2	0.010	98.6	0.013	100.6	0.010

a This was the amount concentrated and analyzed by HPLC.

b No peak was found at this retention time (RT).

cidentified = clopyralid + X36538 (clopyralid-glycine conjugate).

dcharacterized = extractable – identified.

etotal accountability = extractable + unextractable.

Table B.7.2.2.2.1- 14: Summary of characterisation and identification of radioactive residues in goat milk, day 4 and 5, following application of radiolabeled ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Milk, 4 DAY PM		Milk, 5 DAY PM	
	RT (min)	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		100.0	0.010	100.0	0.014
analyzed by HPLC[a]		86.5	0.009	92.1	0.013
clopyralid (X159934)	12.7	58.3	0.006	70.1	0.010
unknown ≈13.0 min	13.0	6.0	0.001	6.3	0.001
X36538	13.7	18.2	0.002	11.6	0.002
unknown ≈15.5 min	15.5	4.0	<0.001	4.2	0.001
unknowns ≈11-19 min	11-19	-[b]	-[b]	-[b]	-[b]
identified[c]		76.5	0.008	81.7	0.011
characterized[d]		15.0	0.002	14.3	0.002
extractable		91.5	0.009	96.0	0.013
unextractable		6.5	0.001	6.3	0.001
Total accountability[e]		98.0	0.010	102.3	0.014

a This was the amount concentrated and analyzed by HPLC.

b No peak was found at this retention time (RT).

cidentified = clopyralid + X36538 (clopyralid-glycine conjugate).

dcharacterized = extractable – identified.

etotal accountability = extractable + unextractable.

Table B.7.2.2.2.1- 15: Summary of characterisation and identification of radioactive residues in goat fat following application of radiolabeled ^{14}C - Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Fat, Renal		Fat, Omental		Fat, Subcutaneous	
	RT (min)	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		100.0	0.054	100.0	0.024	100.0	0.054
analyzed by HPLC[a]		97.0	0.052	94.8	0.023	107.5	0.058
clopyralid (X159934)	12.7	97.0	0.052	94.8	0.023	104.0	0.056
unknown ≈13.0 min	13.0	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
X36538	13.7	-[b]	-[b]	-[b]	-[b]	3.5	0.002
unknown ≈15.5 min	15.5	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
unknowns ≈11-19 min	11-19	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
identified[c]		97.0	0.052	94.8	0.023	107.5	0.058
characterized[d]		26.4	0.014	12.6	0.003	6.6	0.004
extractable		123.4	0.067	107.4	0.026	114.1	0.061
unextractable		0.8	<0.001	7.4	0.002	1.4	0.001
total accountability[e]		124.2	0.067	114.8	0.027	115.5	0.062

[a]This was the amount concentrated and analyzed by HPLC.

[b]No peak was found at this retention time (RT).

cidentified = clopyralid + X36538 (clopyralid-glycine conjugate).

[d]characterized = extractable – identified.

etotal accountability = extractable + unextractable.

[f]Because of variation in liquid scintillation counting, 'identified' values were slightly higher than 'extractable' values, and the difference would have been negative. It did not make physical sense to have negative radioactivity, so the values were recorded as 0.0 and 0.000.

Table B.7.2.2.2.1- 16: Summary of characterisation and identification of radioactive residues in goat muscle and the liver following application of radiolabeled ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Muscle, Flank		Muscle, Loin		Liver	
	RT (min)	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		100.0	0.031	100.0	0.022	100.0	0.057
analyzed by HPLC[a]		112.9	0.035	106.8	0.023	71.4	0.041
clopyralid (X159934)	12.7	112.9	0.035	106.8	0.023	71.4	0.041
unknown ≈13.0 min	13.0	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
X36538	13.7	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
unknown ≈15.5 min	15.5	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
unknowns ≈11-19 min	11-19	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]
identified[c]		112.9	0.035	106.8	0.023	71.4	0.041
characterized[d]		0.0[f]	0.000[f]	14.5	0.003	12.2	0.007
extractable		111.9	0.035	121.3	0.026	83.6	0.048
unextractable		7.2	0.002	8.6	0.002	22.1	0.013
total accountability[e]		119.0	0.037	129.8	0.028	105.8	0.061

aThis was the amount concentrated and analyzed by HPLC.

bNo peak was found at this retention time (RT).

cidentified = clopyralid + X36538 (clopyralid-glycine conjugate).

dcharacterized = extractable – identified.

etotal accountability = extractable + unextractable.

fBecause of variation in liquid scintillation counting, 'identified' values were slightly higher than 'extractable' values, and the difference would have been negative. It did not make physical sense to have negative radioactivity, so the values were recorded as 0.0 and 0.000.

Table B.7.2.2.2.1- 17: Summary of characterisation and identification of radioactive residues in goat kidney following application of radiolabeled ^{14}C - Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Kidney	
	RT (min)	% TRR	mg eq/kg
TRR		100.0	0.863
analyzed by HPLC[a]		89.8	0.776
clopyralid (X159934)[b]	12.7	89.8	0.776
unknown ≈13.0 min	13.0	-[c]	-[c]
X36538	13.7	-[c]	-[c]
unknown ≈15.5 min	15.5	-[c]	-[c]
unknowns ≈11-19 min	11-19	-[c]	-[c]
identified[c]		89.8	0.776
characterized[d]		11.1	0.096
extractable		100.9	0.871
unextractable		0.7	0.006
total accountability[e]		101.6	0.877

aThis was the sum of the concentrates of the extract and acid hydrolyzate that were to be analyzed by HPLC.

bThis was the sum from the HPLC analyses of concentrates of the extract and acid hydrolyzate..

cNo peak was found at this retention time (RT).

didentified = clopyralid + X36538 (clopyralid-glycine conjugate).

echaracterized = extractable – identified.

ftotal accountability = extractable + unextractable.

Table B.7.2.2.2.1- 18: Summary of characterisation and identification of radioactive residues in goat urine, day 2 and 5, following application of radiolabeled ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Urine, 2 DAY		Urine, 5 DAY	
	RT (min)	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		100.0	54.880	100.0	50.288
analyzed by HPLC[a]		96.5	52.966	101.2	50.915
clopyralid (X159934)	12.7	95.7	52.527	100.5	50.538
unknown ≈13.0 min	13.0	-[b]	-[b]	-[b]	-[b]
X36538	13.7	0.8	0.440	0.5	0.244
unknown ≈15.5 min	15.5	-[b]	-[b]	-[b]	-[b]
unknowns ≈11-19 min	11-19	-[b]	-[b]	0.3	0.132
identified[c]		96.5	52.966	101.0	50.783
characterized[d]		0.0[e]	0.000[e]	0.3	0.132
extractable		96.5	52.966	101.2	50.915
unextractable		-[f]	-[f]	-[f]	-[f]
total accountability[g]		96.5	52.966	101.2	50.915

aThis was the amount remaining after acidification and centrifugation and analyzed by HPLC.

bNo peak was found at this retention time (RT).

cidentified = clopyralid + X36538 (clopyralid-glycine conjugate).

dcharacterized = extractable – identified.

eIn this case the identified and the extractable were the same value, so nothing remained as characterized.

fNo tissue remained after preparation of sample for HPLC, so there was no unextractable.

gtotal accountability = extractable + unextractable.

Table B.7.2.2.2.1- 19: Summary of characterisation and identification of radioactive residues in goat feces day 2 and 5 following application of radiolabeled ^{14}C -Clopyralid at 50.9 mg/kg/day for 5 Days

Metabolite fraction		Feces, 2 DAY		Feces, 5 DAY	
	RT (min)	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR		100.0	10.117	100.0	6.922
analyzed by HPLC[a]		103.8	10.504	106.1	7.346
clopyralid (X159934)	12.7	101.7	10.293	104.5	7.230
unknown ≈13.0 min	13.0	-[b]	-[b]	-[b]	-[b]
X36538	13.7	1.7	0.168	1.1	0.077
unknown ≈15.5 min	15.5	-[b]	-[b]	-[b]	-[b]
unknowns ≈11-19 min	11-19	0.4	0.043	0.6	0.040
identified[c]		103.4	10.461	105.6	7.307
characterized[d]		0.0[e]	0.000[e]	0.0[e]	0.000[e]
Extractable		95.9	9.701	96.6	6.687
Unextractable		0.2	0.023	0.4	0.026
total accountability[f]		96.1	9.725	97.0	6.713

aThis was the amount concentrated and analyzed by HPLC.

bNo peak was found at this retention time (RT).

cidentified = clopyralid + X36538 (clopyralid-glycine conjugate).

dcharacterized = extractable – identified.

eBecause of variation in liquid scintillation counting, 'identified' values were slightly higher than 'extractable' values, and the difference would have been negative. It did not make physical sense to have negative radioactivity, so the values were recorded as 0.0 and 0.000 . From a practical standpoint, the low levels in the row listed as 'unknowns ≈11-19 min' could be considered to be characterized.

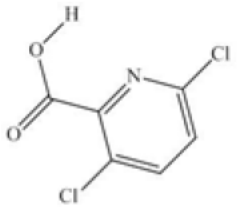
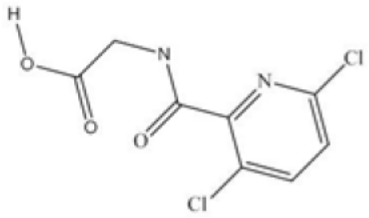
ftotal accountability = extractable + unextractable.

Identification of Residues

Initially tentative assignments of clopyralid in milk, all tissues, urine and feces were made based upon HPLC retention time comparisons with clopyralid reference standard and then confirmed by mass spectral analysis of extracts or preparations of the samples. In addition, the proposed glycine conjugate (X36538) of clopyralid was detected by mass spectral analysis in the milk samples only. Presence of the X36538 in the subcutaneous fat, urine and feces was proposed from observance of retention times similar to the X36538 peak in the milk samples. There was no reference standard available to confirm the structure of the proposed X36538 but its identity in a prior study was consistent with the structural assignment.

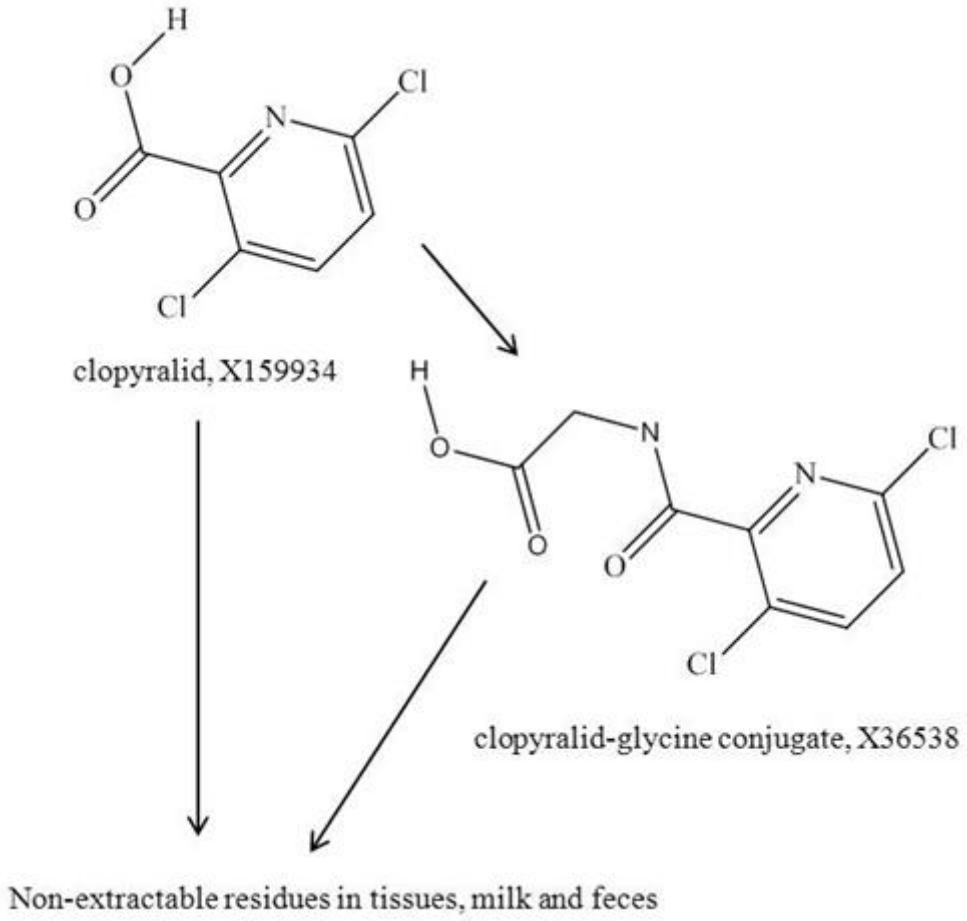
Names and structures of the identified metabolites are below.

Table B.7.2.2.2.1- 20: Identification of compounds from metabolism study

Common name/code number.	Compound name	Chemical structure
clopyralid, X159934	**3,6-dichloro-2-pyridinecarboxylic acid (CAS name)**	
clopyralid-glycine conjugate, X36538	**2-(3,6-dichloropicolinamido)acetic acid (proposed name)**	

Metabolic Pathway has been depicted below:

Figure B.7.2.2.2.1- 3: Proposed metabolic profile of active substance in domestic animals



B.7.2.2.2.2 Pigs

Metabolism in animals has been thoroughly characterised in rats, poultry and lactating ruminants. Metabolism is similar in the animals tested, and no further studies are required.

B.7.2.2.2.3 Fish

Data for metabolism in fish are available in the bioconcentration in fish (BCF) study (CA 8.2.2.3) which satisfies this requirement.

B.7.3 Magnitude of Residues in Supervised Residue Trials

Overall Conclusions: B.7.3 Magnitude of residue trials in plants

In the Active approval under directive 91/414, the use of spring/summer application of clopyralid was intended for oilseed rape, sugar beet, cereals and pasture. Product type was a liquid concentrate EF-1136 (Lontrel 100* or Matrigon*) containing 100 g/L clopyralid.

The representative formulation for Annex I Renewal (AIR) is GF-1374, an emulsifiable concentrate containing the active substance clopyralid at 80 g a.i./L and 2 mixing partners namely fluroxypyr mepthyl 144 g/l (100 g as/L) and florasulam 2.5 g/l.

All residue trials conducted with formulation Bofix* BP (EF-1403), which contains 27 g ae/L clopyralid were performed at 3 L/ha, which means about 80 g a.i./ha. This application rate of 80 g ae/ha is the same as the cGAP used for cereals.

Pasture grass / Established pasture:

The critical GAP supported for clopyralid use in pasture grass for Annex I Renewal (AIR) has changed and is less critical compared to the critical GAP accepted during Active Approval. The critical GAP for pasture grass proposed for clopyralid AIR is based on an application of clopyralid at a maximum rate of 0.12 kg a.i./ha with a 7-day PHI in both the N-EU and S-EU (although for the representative formulation, GF-1374, there is a 14-day PHI in the S-EU due to limitation of the PHI for fluroxypyr). For Active Approval the critical GAP in the S-EU was 0.24 kg ae/ha, 7-day PHI and in the N-EU the critical GAP was 0.12 kg ae/ha, 7-day PHI. For the N-EU, trials available during Active Approval had been carried out using rates above the critical GAP (carried out at 0.2 – 0.24 kg ae/ha), but were accepted to provide a conservative, worst case evaluation of potential livestock dietary burden from treated grass.

Results from new pasture residue trials are presented in this dossier which provide a full set of 8 trials in the S-EU that are compliant with the critical GAP proposed for clopyralid AIR and it is proposed that these trials are accepted for use to support clopyralid AIR in the S-EU. The new S-EU trials were treated with clopyralid at a nominal rate of 105 g ae/ha (actual rate ranged from 104 g ae/ha to 112 g ae/ha). Clopyralid residue in grass in the S-EU trials at a 7-day PHI ranged from 1.66 mg/kg to 4.09 mg/kg.

The additional 4 trials for the N-EU available from the new study were carried out at 0.2 kg a.i./ha and were therefore at a rate, twice as high as proposed for clopyralid AIR. However, it was proposed that results from these additional 4 trials be combined with the 6 trials accepted durig Active Approval to provide a conservative, worst case evaluation of potential livestock dietary burden for the N-EU. The clopyralid residues in grass in the combined group of 10 N-EU trials ranged from 2.49 mg/kg to 6.95 mg/kg.

It is proposed that results from pasture grass trials accepted during Active Approval together with trials from a new study included in this submission are adequate to provide a conservative, worst case evaluation of potential dietary burden for livestock consuming treated grass.

Cereals (Wheat and Barley):

The critical GAP supported for clopyralid use in cereals for Annex I Renewal (AIR) has changed and is less critical compared to the critical GAP accepted during Active Approval. The critical GAP for cereals proposed for clopyralid AIR is based on an application of clopyralid at a maximum rate of 0.08 kg a.i./ha with application at a latest growth stage of BBCH 39 in both the N-EU and S-EU.

For Active Approval, the critical GAP that was supported was application at a maximum rate of 0.127 kg ae/ha in both the N-EU and S-EU with application at a latest growth stage of BBCH 39 and BBCH 45 in the N-EU and S-EU, respectively. Since the critical GAP supported for Active Approval and upon which current EU MRLs in cereals are based is more critical than the GAP proposed for Annex I Renewal, the residue trial data supporting the Active Approval and the EU MRLs also covers the less critical GAP proposed for Annex I Renewal.

Although residue data for cereals from the more critical GAPs accepted for Active Approval are expected to equal or exceed residue levels from the less critical GAP proposed for Annex

I Renewal, results from a limited number of available barley and wheat trials having GAPs considered equivalent to the critical GAP proposed for Annex I Renewal (e.g. ± 25% of the cGAP rate) have been summarized and presented in this document as confirmatory information. Although the application rates are within 25% of the cGAP for Annex I Renewal, most of the available trials had application at a somewhat earlier stage of growth (BBCH 32) rather than BBCH 39, but these were considered comparable since the plants in these growth stages are in similar stages of development and occur well before development of the grain / consumable part of the plant.

As expected, residues from trials considered to be in compliance with the GAP proposed for clopyralid Annex I Renewal are considered to be within the range of residue values from trials accepted for Active Approval, which were conducted under a more critical GAP. Therefore, it is proposed that the exiting residue data for cereals that was accepted during Active Approval and upon which EU MRLs are based is adequate to also support the less critical GAP proposed for Annex I Renewal.

A summary of the critical GAP for residues is presented in **Table B.7.3-1**.

Table B.7.3-1 Summary of the critical GAPs proposed for clopyralid Annex I Renewal

Crop	Maximum rate g a.i./ha	Number of applications (minimum interval in days)	PHI (days)	Growth stage at last application (BBCH)
Pasture grass / Established pasture	**120**	**1**	**7 days** **(Note: for the formulated product GF-1374, a PHI of 14 days is used due to requirements associated with fluroxypyr).**	**N/A – Minimum interval between application and harvest (cutting or grazing) is determined by PHI rather than on maximum growth stage.**
Cereals: wheat (including triticale and spelt), barley, oat and rye	**80**	**1**	**N/A - latest timing for application to be based on maximum growth stage rather than PHI.**	**BBCH 39**

Residue trials in pasture and cereals previously evaluated during the Active Approval for clopyralid supported GAPs more critical than the current critical GAPs proposed in this submission. Additionally, EU MRLs for clopyralid are established based on the residue data associated with the pasture and cereal trials evaluated during the Active Approval. Therefore, the previously evaluated studies that support the current EU MRLs in cereals and commodities of animal origin (based on dietary burden from treated pasture grass and cereals) cover the less critical GAPs currently proposed. Where new / additional cereal trials

are available at a GAP considered equivalent to the less critical GAPs supported for clopyralid AIR, results are presented to demonstrate that the residue levels associated with the GAPs supported for clopyralid AIR are within the range of or lower than the residues from trials accepted for the Active Approval.

For pasture grass there is a full set of 8 new trials for the S-EU that are compliant with the critical GAP proposed for clopyralid AIR and it is proposed that these trials be used in place of the S-EU pasture trials accepted for Active Approval at the higher rate of 0.24 kg ae/ha. Also, for the N-EU there are 4 new pasture trials available that were carried out at a GAP similar to the existing 6 trials for the N-EU accepted during Active Approval. Although all the N-EU trials were carried aout at a rate higher than the critical GAP, it is proposed to use the data from these trials as a conservative, worst-case evaluation of residues in grass and associated intake of residues in livestock. Combining the original 6 N-EU trials accepted for Active Approval with 4 new trials provides a total of 10 pasture trials for the N-EU.

A summary of the number of new / additional trials submitted to support clopyralid AIR and the associated proposed critical GAPs is presented in Table B.7.3-2.

Table B.7.3-2 Summary of the number of residue trials with active substance clopyralid

Year	Crop	Zone	Study Type		Total Number of Trials
			Decline	At-Harvest	
2014	Pasture grass	N-EU	4	0	4
2014	Pasture grass	S-EU	8	0	8
1996	Barley	N-EU	0	1	1
1997	Barley	N-EU	0	1	1
2004	Barley	N-EU	0	3	3
2014	Barley	S-EU	2	2	4
1996	Wheat	N-EU	0	1	1
1997	Wheat	N-EU	0	1	1
2003	Wheat	N-EU	0	3	3
2004	Wheat	N-EU	0	1	1
2012	Wheat	N-EU	2	2	4
1996	Wheat	S-EU	0	2	2
1997	Wheat	S-EU	0	2	2
2003	Wheat	S-EU	0	3	3
2012	Wheat	S-EU	2	2	4

The following table provides a justification for the use of additional studies to provide supplemental information to support magnitude of residues in plants along with that evaluated for the Active Approval.

Data point/Study	Rationale
(Pasture grass) 6.3.1/1	Provides trials in the S-EU compliant with the critical GAP for clopyralid AIR. Also, provides additional results from 4 trials in the N-EU, but at a GAP similar to that supported for Active Approval, which uses a higher rate than the critical GAP proposed for clopyralid AIR. The results from the additional N-EU trials are combined with the 6 trials previously accepted for Active Approval to provide a conservative, worst-case evaluation of residue in pasture grass and associated livestock dietary burden.
(Barley) 6.3.2/1, 6.3.2/2, 6.3.2/3, 6.3.2/4, 6.3.2/5	Provides a limited number of N-EU and S-EU trials conducted at a GAP considered equivalent to the critical GAP proposed for clopyralid AIR, which is less critical than the critical GAP and associated trials accepted during Active Approval and which were used in setting current EU MRLs. The data presented is intended to further demonstrate that residues from the less critical GAP proposed for clopyralid AIR are fully covered with use of trials and associated residue data accepted during Active Approval.
(Wheat) 6.3.3/1, 6.3.3/2, 6.3.3/3, 6.3.3/4, 6.3.3/5, 6.3.3/6	Provides a limited number of N-EU and S-EU trials conducted at a GAP considered equivalent to the critical GAP proposed for clopyralid AIR, which is less critical than the critical GAP and associated trials accepted during Active Approval and which were used in setting current EU MRLs. The data presented is intended to further demonstrate that residues from the less critical GAP proposed for clopyralid AIR are fully covered with use of trials and associated residue data accepted during Active Approval.

B.7.3.1 Pasture grass

Data to address this point were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. However, the critical GAP supported for clopyralid Annex I Renewal has changed such that the maximum application rate is proposed at 120 g ae/ha rather than the higher rate of 0.24 kg ae/ha (240 g ae/ha) that was supported for the S-EU in the critical GAP for the original Annex I / Active Substance approval. Additionally, in the N-EU the critical GAP for Active Approval was 0.120 kg ae/ha, although the available trial data was from trials conducted using clopyralid at rates of 0.2 kg ae/ha to 0.24 kg ae/ha. These higher rate (1.7x to 2x) trials for the N-EU were accepted as a conservative, worst case evaluation of potential residue levels in grass for evaluating livestock dietary burden since MRLs are not set on pasture grass.

A new study is presented in B.7.3.1.11 that provides 8 trials in the S-EU that are compliant with the GAP supported for clopyralid AIR (0.120 kg ae/ha, PHI 7 days – although for the

particular representative formulation the PHI of 14 days is in place in the S-EU due to restrictions from fluroxypyr; a PHI of 7 days is needed for other clopyralid formulations). It is proposed that the 8 new S-EU trials be accepted in place of the previously evaluated trials that were based on a nominal application rate of 0.24 kg ae/ha. With regard to the N-EU, as mentioned previously, the trials supporting the Active Approval were conducted at 0.2 kg ae/ha to 0.24 kg ae/ha, although the critical GAP in the N-EU at Active Approval was for 0.120 kg ae/ha. The new study that is presented in 6.3.1/1 also has pasture grass residue trials for the N-EU that were conducted only at the higher rate of 0.2 kg ae/ha. Therefore, it is proposed that as a conservative, worst case evaluation that the N-EU trials previously accepted during Active Approval also be accepted for this evaluation and that in addition the 4 trials from the new study presented in 6.3.1/1 also be included since they were conducted at a similar rate (0.2 kg ae/ha).

The references for the previously accepted pasture trials for the N-EU are listed below (6.3.1/2 to 6.3.1/5). Additionally, although not relied upon for this evaluation, the references for the S-EU trials used in the Active Approval are listed in 6.3.1/6 and 6.3.1/7.

A summary of the new study is presented in 6.3.1/1 along with a summary of trial results, which are presented in Table B.7.3.1-2.

Shown below in Table B.7.3.1-1 is a brief summary of critical residue trial results for clopyralid in pasture grass that are proposed as adequate to support the use of clopyralid in pasture grass for clopyralid AIR. Values shown in italics are from a new study (**Table B.7.3.1-1**), while the other listed values from the Active Approval were taken from the 'Conclusion regarding the peer review of the pesticide risk assessment of the active substance clopyralid' (EFSA Scientific Report (2005) 50, 1-65).

Table B.7.3.1-1: Summary of critical pasture grass residue trial results for clopyralid

EU Residue / Climate Zone	Critical residue trial data – Clopyralid (mg/kg)	Comments
N-EU	2.6, 2.8, 3.0, 4.4, 5.0, 5.4 *2.49, 3.48, 3.73, 6.95*	Residue results accepted in the Active Approval are listed in the first row, while additional results from new trials are listed in the second row in italics. Both previously accepted and new trial results are based on a higher rate than the critical GAP, but it is proposed that the data is acceptable since MRLs are not currently set on grass and the results are unlikely to underestimate residues in grass and associated livestock dietary burden.
S-EU	*1.66, 2.49, 2.91, 3.06, 3.21, 3.31, 3.93, 4.09*	Residue results displayed are from a new study (6.3.1/1) with trials conducted at rates in compliance with the critical GAP proposed for clopyralid AIR. It is proposed that these residue values be accepted in place of the previously evaluated residue trials which were

		conducted at approximately 0.24 kg ae/ha.

B.7.3.1.1 Magnitude of the residues of clopyralid in grassland pasture (RAC fresh grass, hay and silage), following one application of GF-1966, Northern and Southern Europe – 2014.

Report	Delmotte, R., 2015 Magnitude of the residues of clopyralid in grassland pasture (RAC fresh grass, hay and silage), following one application of GF-1966, Northern and Southern Europe – 2014. 140653; Lab study No. CES-14-18931
Guidelines	Commission Regulations (EU) No. 283/2013 and 284/2013, implementing Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, "Commission Working Document 7029/VI/95 Rev. 5, General Recommendations for the Design, Preparation and Realization of Residue Trials, July 22, 1997"
Analytical Method	Dow ID 120610 LC-MS/MS
Storage conditions	Grass, hay and silage samples were stored frozen at ≤ -18°C for a maximum period of 308 days grass for clopyralid, before extraction
GLP	Yes

Twelve trials, four in northern Europe and eight in southern Europe were conducted in 2014-2015 with clopyralid in pasture grass. A summary of the trials in this study is provided in Table B.7.3-1. All residue results regarding clopyralid from the treatment that includes data from 7 days before commercial cut timing is summarized in this dossier. Residue decline data is presented for fresh grass in all trials in northern and in southern Europe and residue at harvest data for hay and silage. Two plots were established in each trial: U plot was left untreated. In Southern Europe the T plots were treated once with GF-1966 at the target rate of 0.1458 kg fp/ha, representing 105 g ae/ha of clopyralid.

The actual application rate ranged from 104 to 112 g ae/ha. In Northern Europe the T plots were treated once with GF-1966 at the rate of 0.2778 kg fp/ha, representing 200 g ae/ha of clopyralid. The actual application rate ranged from 201 to 218 g ae/ha. Fresh grass specimens were taken at 0, 3, 6/7, 13/15 and 20/22 DALA. For hay and silage processes, fresh grass was cut at 6/7 DALA on every trials. Processes were made accordingly to the study plan. Hay specimens were collected at 9/14 DALA. Silage specimens were collected at 35/48 DALA.

Samples were analysed by adapting the Dow Study ID 120610 for LC-MS/MS determination. The limit of quantification (LOQ) and the limit of determination (LOD) for parent clopyralid in grassland pasture (fresh grass, hay and silage) were 0.01 mg/kg and 0.003 mg/kg, respectively. Grass, hay and silage samples were stored frozen at ≤ -18°C for a maximum period of 308 days grass for clopyralid, before extraction and analysis for the samples considered in this dossier. Recoveries for parent compound clopyralid, in fresh grass averaged 83%, in hay 97% and 85% in silage. In treated specimens, clopyralid (parent)

residue in fresh grass at 6 – 7 DALA ranged from 0.67 mg/kg to 4.67 mg/kg. As shown in data presented in the study report, there were 3 trials in which residues in fresh grass were higher at 13–15 DALA than at the 7 DALA sampling (Trials FR01, ES10, and IT12 with residues in fresh grass at 6.95 mg/kg, 1.66 mg/kg and 3.06 mg/kg, respectively). In silage, clopyralid residues ranged from 0.96 mg/kg to 5.8 mg/kg. In hay, clopyralid resideus ranged from 1.1 mg/kg – 18.8 mg/kg.

No data has been made available of the analysis of conjugates.

As previously discussed, the study presented in 6.3.1/1 provides a complete set of data for the S-EU that is compliant with the new critical GAP proposed for the clopyralid AIR. However, the trial data for the N-EU from that study was based on a GAP more critical than the GAP proposed for clopyralid AIR. The N-EU pasture trials in the dossier submitted in April, 2002 supporting the Active Substance approval that were previously peer reviewed and accepted at the EU level also support a GAP more critical than specified during the Active Approval as well as the currently proposed critical GAP for clopyralid AIR in northern Europe. In those N-EU trials clopyralid was applied at a nominal rate of 0.240 kg ae/ha. The currently available trials were conducted at a GAP more critical than is being supported for clopyralid AIR with an application rate of approximately 1.7x to 2x greater than the currently supported critical GAP (i.e. 0.2 kg ae/ha to 0.24 kg ae/ha vs. 0.120 kg ae/ha). However, since MRLs are not currently set on grass and since the residues from these trials are not substantially different than those from the S-EU trials at the 120 g ae/ha rate, it is proposed that the available N-EU trials at 200 to 240 g ae/ha be used as a worst case evaluation of grass residue and livestock dietary burden. A list of the previously peer reviewed studies supporting the Active Substance evaluation for pasture in the N-EU are presented below (B.7.3.1/2 to 6.3.1/5). Although the trial data for the S-EU from the Active Approval is not relied upon for this evaluation since the data from study 6.3.1/1 is being used, the references for the studies from which the S-EU trials in the Active Approval were taken are listed in 6.3.1/6 and 6.3.1/7.

B.7.3.1.2 Determination of residues of 3,6-dichloropicolinic acid (DOWCO* 290) in grass & vegetable crops treated with FORMAT ** U.K. 1981

Report	IIA 6.3/24 Freeman, J.M.H., Almond, R.H., MCDonald, I.A., Dawson, J., Ellis, S.E., Green, S.L., 1982
Report title	Determination of residues of 3,6-dichloropicolinic acid (DOWCO* 290) in grass & vegetable crops treated with FORMAT ** U.K. 1981
DAS Study number / report number	Report No. GHE-P-912; Trial nos. RT/32/81, RT/53/81
Guidelines	Not available
GLP	No

This study was evaluated during the Active Substance evaluation.

Analytical

Residues of clopyralid are extracted from wheat grain and straw with a caustic methanol solution. An aliquot of the extract is acidified and purified by partitioning clopyralid into diethyl ether and then into aqueous sodium bicarbonate solution. Sodium bicarbonate solution

is acidified and treated with potassium permanganate. After destroying excess potassium permanganate clopyralid is again extracted into diethyl ether. The ether phase is evaporated to dryness and derivatised with sulphuric acid/n-butanol mixture to form the butyl ester of clopyralid. Aqueous potassium sulphate solution is added, and the butyl ester is partitioned into hexane. The extract is then purified using a Florisil mini-column prior to analysis by packed column gas chromatography with ECD. Validated limit of quantitation was 0.05 mg/kg for wheat grain and 0.10 mg/kg for wheat straw (O25, ERC 83.23, Freeman and Smith 1983).

The study does not meet GLP requirements and is considered as supplementary information.

B.7.3.1.3 Clopyralid residues in grass following application of SHIELD – UK 1987

Report	IIA 6.3/25 Osborne, K.A (S Flatt – Student)., 1988
Report title	Clopyralid residues in grass following application of SHIELD – UK 1987
DAS Study number / report number	Report No. GHE-P-1881; Trial No. RT 51/87
Guidelines	Not available
GLP	N

This study was evaluated during the Active Substance evaluation.

B.7.3.1.4 Residues of clopyralid in grass at intervals following a single application of DOW SHIELD (EF-584), UK – 1994

Report	IIA 6.3/26 Wood, S.,1995
Report title	Residues of clopyralid in grass at intervals following a single application of DOW SHIELD (EF-584), UK – 1994
DAS Study number / report number	R94-09; Report No. GHE-P-3918
Analytical method	O47, RV94.08, Wood 1994
Guidelines	OECD Principles of Good Laboratory Practice
GLP	Y

This study was evaluated during the Active Substance evaluation.

Analytical method

Residues of clopyralid are extracted from grass with a caustic methanol solution. After acidification clopyralid is partitioned into dichloromethane and then into aqueous sodium bicarbonate solution. Potassium permanganate is added, followed by sodium metabisulphite to destroy the excess potassium permanganate. After acidification clopyralid is partitioned into diethyl ether. The ether is evaporated, and clopyralid is derivatised with n-butanol/sulphuric acid to form the butyl ester. Water is added, and the butyl ester is partitioned into hexane for purification using silica solid-phase extraction prior to analysis by

fused silica capillary column gas chromatography with electron capture detector (ECD). Validated limit of quantitation is 0.05 mg/kg.

B.7.3.1.5 Residues of clopyralid in established grassland at intervals following application of DOW SHIELD (EF 584), UK – 1993

Report	IIA 6.3/27 Wood, S.,1995
Report title	Residues of clopyralid in established grassland at intervals following application of DOW SHIELD (EF 584), UK – 1993
DAS Study number / report number	R93-63; Report No. GHE-P-3919
Analytical method	O47, RV94.08, Wood 1994
Guidelines	OECD Principles of Good Laboratory Practice
GLP	Y

This study was evaluated during the Active Substance evaluation.

Analytical method is the same as in Wood (1994) *suppra*.

B.7.3.1.6 Residues of clopyralid in pasture at intervals under open field conditions following a single application of LONTREL 100 (EF 1136), Southern France and Spain – 2000

Report	IIA 6.3/28 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in pasture at intervals under open field conditions following a single application of LONTREL 100 (EF 1136), Southern France and Spain – 2000
DAS Study number / report number	CEMS-1298; Report No GHE-P-9369;
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17]
GLP	Y

This study was evaluated during the Active Substance evaluation.

B.7.3.1.7 Residues of clopyralid in pasture at intervals following a single application of LONTREL 100 (EF 1136), EU Southern Zone – 2001

Report	IIA 6.3/29 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in pasture at intervals following a single application of LONTREL 100 (EF 1136), EU Southern Zone – 2001
DAS Study number / report number	CEMS-1545; Report No. GHE-P-9386
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17]
Analytical	O95, GHE-P-9567 (Rawle 2002)
GLP	Yes

This study was evaluated during the Active Substance evaluation.

Analytical method

Cereals, grass. Residues of clopyralid are extracted from crop samples with a caustic methanol solution. An aliquot of the extract is diluted with 1 N hydrochloric acid and purified using solid-phase extraction (SPE) column with dichloromethane as the eluent. The eluate is evaporated to dryness and derivatised with n-propanol/sulphuric acid solution to form clopyralid propyl ester. The derivatising reagent is evaporated, and clopyralid propyl ester is partitioned into hexane containing clopyralid butyl ester as an internal standard. The hexane extract is then analysed by fused silica capillary column gas chromatography with mass selective detector (GC/MSD). Monitoring of two characteristic ions were used, m/z 233 for quantitation and 235 for confirmation. Validated limit of quantitation is 0.01 mg/kg. The method has undergone an independent laboratory validation (O95, GHE-P-9567, Rawle 2002). (O97, GH-C 5439, Hastings 2002a).

The independent laboratory validation of the method Hastings 2002a (O95, GHE-P-9567, Rawle 2002) was also evaluated under Directive 91/414 EEC.

The method has not been optimized for conjugates.

Table B.7.3.1-2 Supervised residue trials in grassland pasture

Trial ID Study ID	Zone Location	Formulation No.	Application Details					GS at Last Appl	PHI (days)	Portion Analysed	Residue (mg/Kg)	% Recovery
			No.	Appl Rate (g a.i./ha)	Spray Vol (L/ha)	Appl Conc (g a.i./hL)	Appl Date				Clopyralid	
FR01 CES-14-18931	France NZ	GF-1966	1	205.7	201	102.3	11-Aug-2014	BBCH.39	0 3 7 <u>14</u> 21 7 7	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass Hay Silage	18.9 6.30 4.67 <u>6.95</u> 6.25 13.6 4.98	83 (Fresh grass) 97 (Hay) 85 (Silage)
GB02 CES-14-18931	United Kingdom NZ	GF-1966	1	218.3	214	102.0	14-May-2014	BBCH.37	0 3 <u>7</u> 14 21 7 7	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass Hay Silage	5.85 3.37 <u>3.48</u> 1.37 1.47 UT: 0.0209 T: 12.05 UT: 0.0156 T: 4.14	83 (Fresh grass) 97 (Hay) 85 (Silage)
DE03 CES-14-18931	Germany NZ	GF-1966	1	217.8	320	68.1	21-Jul-2014	BBCH.21-34	0 3 <u>7</u> 14 21 7 7	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass Hay Silage	20.7 3.05 <u>2.49</u> 1.60 1.04 5.60 3.69	83 (Fresh grass) 97 (Hay) 85 (Silage)

Table B.7.3.1-2 (Cont'd.): Supervised residue trials in grassland pasture

Trial ID Study ID	Zone Location	Formulation No.	Application Details					GS at Last Appl	PHI (days)	Portion Analysed	Residue (mg/Kg)	% Recovery
			No.	Appl Rate (g ae/ha)	Spray Vol (L/ha)	Appl Conc (g ae/hL)	Appl Date				Clopyralid	
PL04 CES-14-18931	Poland NZ	GF-1966	1	201.3	296	68.0	23-May-2014	BBCH.37	0 3 6 14 21 7 7	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass Hay Silage	7.45 3.92 3.73 2.75 2.53 7.35 5.80	83 (Fresh grass) 97 (Hay) 85 (Silage)
FR05 CES-14-18931	France SZ	GF-1966	1	107.3	252	107.3	15-Jul-2014	BBCH.61	0 3 7 14 21 7 7	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass Hay Silage	8.90 5.65 4.09 3.76 3.04 1.10 4.09	83 (Fresh grass) 97 (Hay) 85 (Silage)
FR06 CES-14-18931	France SZ	GF-1966	1	106.6	198	53.8	17-Jul-2014	BBCH.51-57	0 3 7 14 21 7 7	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass Hay Silage	7.35 5.50 3.31 2.55 2.25 10.3 5.15	83 (Fresh grass) 97 (Hay) 85 (Silage)
ES07 CES-14-18931	Spain SZ	GF-1966	1	111.0	258	43.0	06-Jun-2014	BBCH.37	0 3 7 14 21	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass	2.95 4.43 3.93 2.16 1.40	83 (Fresh grass)

Table B.7.3.1-2 (Cont'd.): Supervised residue trials in grassland pasture

Trial ID Study ID	Zone Location	Formulation No.	Application Details					GS at Last Appl	PHI (days)	Portion Analysed	Residue (mg/Kg)	% Recovery
			No.	Appl Rate (g ae/ha)	Spray Vol (L/ha)	Appl Conc (g ae/hL)	Appl Date				Clopyralid	
									7	Hay	18.8	97 (Hay)
									7	Silage	2.51	85 (Silage)
ES08 CES-14-18931	Spain SZ	GF-1966	1	112.5	262	42.9	12-Jun-2014	BBCH.37	0 3 7 14 21	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass	7.30 5.60 2.91 1.68 1.59	83 (Fresh grass)
									7	Hay	6.10	97 (Hay)
									7	Silage	1.76	85 (Silage)
ES09 CES-14-18931	Spain SZ	GF-1966	1	104.4	292	35.8	29-Jul-2014	BBCH.32	0 3 7 13 20	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass	5.85 3.03 2.49 0.74 1.21	83 (Fresh grass)
									7	Hay	UT: 0.272 T: 3.02	97 (Hay)
									7	Silage	1.91	85 (Silage)
ES10 CES-14-18931	Spain SZ	GF-1966	1	111.0	310	35.8	31-Jul-2014	BBCH.32	0 3 7 13 21	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass	5.50 1.60 0.67 1.66 1.26	83 (Fresh grass)
									7	Hay	UT: 0.083	97 (Hay)

Table B.7.3.1-2 (Cont'd.): Supervised residue trials in grassland pasture

Trial ID Study ID	Zone Location	Formulation No.	Application Details					GS at Last Appl	PHI (days)	Portion Analysed	Residue (mg/Kg)	% Recovery
			No.	Appl Rate (g ae/ha)	Spray Vol (L/ha)	Appl Conc (g ae/hL)	Appl Date				Clopyralid	
									7	Silage	T: 3.56 0.96	85 (Silage)
IT11 CES-14-18931	Italy SZ	GF-1966	1	108.1	202	53.5	20-May-2014	BBCH.35	0 3 7 13 21	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass	4.56 1.77 3.21 3.06 2.67	83 (Fresh grass)
									7	Hay	UT: 0.027 T: 6.60	97 (Hay)
									7	Silage	4.27	85 (Silage)
IT12 CES-14-18931	Italy SZ	GF-1966	1	110.3	257	42.9	22-Jul-2014	BBCH.37	0 3 7 15 22	Fresh grass Fresh grass Fresh grass Fresh grass Fresh grass	7.05 3.13 3.00 3.06 1.18	83 (Fresh grass)
									7	Hay	10.4	97 (Hay)
									7	Silage	3.57	85 (Silage)

B.7.3.2 Barley

Data to address this point were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. However, the critical GAP supported for clopyralid Annex I Renewal has changed such that the maximum application rate in both the N-EU and S-EU is proposed at 80 g ae/ha rather than the higher rate of 0.127 kg ae/ha that was included in the critical GAP for the original Annex I / Active Substance approval. A comparison of the critical GAPs supported for Active Approval to the critical GAP proposed for Annex I Renewal is shown in Table B.7.3.2-1.

Table B.7.3.2-1: Comparison of critical GAP for clopyralid use in cereals in Active Substance Approval and proposed for Annex I Renewal (AIR)

GAP supported	Residue Zone	Maximum rate (kg ae/ha)	Maximum number of applications	Latest growth stage at application
Active Approval*	N-EU	0.127	1	BBCH 39
Active Approval*	S-EU	0.127	1	BBCH 45
Annex I Renewal	N-EU and S-EU	0.08	1	BBCH 39

*GAP taken from the 'Conclusion regarding the peer review of the pesticide risk assessment of the active substance clopyralid' (EFSA Scientific Report (2005) 50, 1-65).

Since the critical GAP supported for Active Approval and upon which current EU MRLs in cereals are based is more critical than the GAP proposed for Annex I Renewal, the residue trial data supporting the Active Approval and the EU MRLs also covers the less critical GAP proposed for Annex I Renewal.

Although residue data from the more critical GAPs supported for Active Approval are expected to equal or exceed residue levels from the less critical GAP proposed for Annex I Renewal, results from available barley trials having GAPs considered equivalent to the critical GAP proposed for Annex I Renewal (e.g. ± 25% of the cGAP rate) are summarized in 6.3.2/1 to 6.3.2/5 and the associated trial results are presented in Table B.7.3.2-4. Although the application rates are within 25% of the cGAP for Annex I Renewal, trials available for the N-EU had application at a somewhat earlier stage of growth (BBCH 32) rather than BBCH 39, but these were considered comparable since the plants in these growth stages are in similar stages of development and occur well before development of the grain / consumable part of the plant.

An overview / summary of the residue trial data from the available trials carried out based on a GAP considered equivalent to the critical GAP proposed for Annex I Renewal is presented in Table B.7.3.2-2 and a more detailed summary of these trials is presented in Table B.7.3.2-4. For comparison, the residue trial data accepted for Active Approval and relied upon for setting of current MRLs is presented in Table B.7.3.2-3. As expected, residues from trials considered to be in compliance with the GAP proposed for clopyralid Annex I Renewal are within the range of residue values from trials conducted according to the GAPs that supported Active Approval. Therefore, together with the results from wheat trials supporting Active Approval, it is proposed that the exiting residue data for barley accepted during Active Approval and upon which EU MRLs are based is adequate to also support the less critical GAP proposed for Annex I Renewal.

Table B.7.3.2-2: Summary of Clopyralid Residue Results in Barley from Trials with GAP Considered Equivalent to the Proposed Critical GAP for Annex I Renewal

Residue Zone	Commodity	Clopyralid (mg/kg)
N-EU	Grain	< 0.15 x 3, 0.14, 0.24
S-EU		0.193, 0.325, 0.840, 0.870
N-EU	Straw	0.48, 1.58
S-EU		0.445, 0.488, 0.713, 1.57

Table B.7.3.2-3: Summary of Clopyralid Residue Results in Barley from Trials Accepted for Active Approval and Supporting MRLs in Cereals *

Residue Zone	Commodity	Clopyralid (mg/kg)
N-EU	Grain	0.14, 0.24, 0.34, 0.37, 0.38, 0.47, 0.61, 0.82, 0.95
S-EU		0.13, 0.68, 1.16, 1.34
N-EU	Straw	0.17, 0.28, 0.31, 0.40, 0.50, 0.87, 1.05, 1.08
S-EU		0.59, 0.84, 1.16, 1.20

* Results taken from 'Conclusion regarding the peer review of the pesticide risk assessment of the active substance clopyralid' (EFSA Scientific Report (2005) 50, 1-65).

B.7.3.2.1 Individual residue trials on barley

B.7.3.2.1.1 Boissinot 2015

Report	Boissinot, J.C., 2015 Magnitude of the residues of clopyralid in spring barley (RAC whole plant, grain and straw), following one application of GF-1966, Northern and Southern Europe – 2014.
Report title	Magnitude of the residues of clopyralid in spring barley (RAC whole plant, grain and straw), following one application of GF-1966, Northern and Southern Europe – 2014.
DAS Study number	140655
Guidelines	OECD Principles of Good Laboratory Practices as revised in 1997 (ENV/MC/CHEM(98)17); The application of GLP principles to field studies (ENV/JM/MONO(99)22) and the application of the OECD principles of GLP to the organisation and management of multi-site studies (ENV/JM/MONO(2002)9) -Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. Document SANCO 3029/99, 2000. -Guidance document on residue analytical methods. Document SANCO 825/00, 2004. -Method validation and quality control procedures for pesticide residues analysis in food and feed. Document SANCO/10684/2009, 2010.
Analytical method	According to Dow Study ID 120610 for LC-MS/MS determination
Storage conditions	The maximum period of frozen storage of samples prior to extraction for analysis was 385 days.
GLP	Yes

Ten trials, six in northern Europe and four in southern Europe were conducted in 2014-2015 with clopyralid in spring barley. A summary of the trials conducted in southern Europe in this study is provided in Table B.7.3.2-4. Residue decline data is presented for whole plant in one trial in northern and in two trials in the southern Europe and residue at harvest data for grain and straw. Two plots were established in each trial: U plot was left untreated. In Southern Europe the T plots were treated once with GF-1966 at the rate of 0.1389 kg fp/ha, representing 100 g ae/ha of clopyralid. In Northern Europe the T plots were treated once with GF-1966 at the rate of 0.1667 kg fp/ha, representing 120 g a.i./ha of clopyralid. Results from the Northern Europe trials were not included in this summary since they were treated at a rate greater than 25% above the proposed critical GAP. Whole plants specimens were taken at 0, 7, 13/14 and 26/28 DALA. Grain and straw specimens were taken at commercial harvest depending on each trial, between 40 and 78 DALA.

Residues of clopyralid were determined by adapting the Dow Study ID 120610 for LC-MS/MS determination. The limit of quantification (LOQ) and the limit of determination (LOD) for clopyralid in spring barley (whole plant, grain and straw) were 0.01 mg/kg and 0.003 mg/kg, respectively. Whole plant, grain and straw samples were stored frozen at ≤ -18°C for a maximum period of 385 days grass for clopyralid, before extraction and analysis for the samples considered in this dossier. Overall average recovery was 93% in whole plant, 88% in grain and 93% in straw. In treated specimens from the Southern Europe trials, clopyralid residue in whole plant ranged from 0.512 mg/kg to 1.78 mg/kg. In grain, clopyralid residues ranged from 0.193 mg/kg to 0.870 mg/kg. In straw, clopyralid residues ranged from 0.445 mg/kg to 1.57 mg/kg.

B.7.3.2.1.2 Clements 1997

Report	Clements, B., 1997
Report title	Residues of fluroxypyr-BPE, clopyralid and MCPA in cereals at harvest following a single application of BOFIX (NEW) EF-1403, France (North and South) – 1996
DAS Study number	R96-138
Guidelines	OECD Principles of Good Laboratory Practice. The method has not been submitted and evaluated under Directive 91/414 EEC.
Storage time	218 days at -20C
Analytical method	ERC 97.10
GLP	Yes

Four at harvest trials on cereals, of which one on winter barley in the Indre-et-Loire region of Northern France and two trials on winter wheat in the Haute Garonne region of Southern France were carried out during 1996. The other three trials are on wheat and described under B.7.3.3.

In each trial, a single application of BOFIX·(NEW) EF-1403, containing 267 g a.i./L MCPA, 27 g a.i./L clopyralid and 54 g a.i./L of fluroxypyr was applied at a nominal rate of 160/80/800 g ae/ha (Fluroxypyr/Clopyralid/ MCPA) when the cereals were at growth stage BBCH 32 – 33.

Samples of winter barley grain and straw were collected at normal harvest, 70 days after treatment (BBCH 89). Samples of winter wheat grain and straw were collected at normal harvest, 85 - 89 days after treatment (BBCH 89).

A single untreated plot was also included in each trial and corresponding cereal grain and straw samples were taken at the corresponding sampling harvest.

The samples were analysed for residues of MCPA, clopyralid and fluroxypyr using DowElanco Analytical Method ERC 97.10 which has a lowest validated level of 0.05 mg/kg for grain and 0.20 mg/kg for straw. The mean procedural recovery for clopyralid was 96% (range 94%-97%) in grain and 91% (range 87% - 96%) in straw. Residues of clopyralid were detected in the treated winter barley grain sample at a concentration of 0.24 mg/kg.

Clopyralid residues were found in the treated winter barley straw sample at a concentration of 1.58 mg/kg.

B.7.3.2.1.3 Butler 1997

Report	Butler, R.E., 1998
Report title	Residues of clopyralid, fluroxypyr and MCPA in Winter Barley at harvest following a single application of BOFIX* BP (EF-1403), UK, 1997
DAS Study number	R97-104
Guidelines	OECD Principles of Good Laboratory Practice
GLP	Y

One trial was conducted to determine the residues of clopyralid, fluroxypyr and MCPA in winter barley at harvest following a single application of Bofix* BP (EF-1403). This formulation contains 27 g as/L clopyralid, 54 g a.i./L fluroxypyr present as the butoxypropyl ester and 267 g as/L MCPA. The trial was conducted in the UK during 1997 and consisted of one untreated and one treated plot.

The treated plot was sprayed at a nominal application rate of 3 L/ha when the crop was at growth stage BBCH 32. Samples of grain and straw were taken at normal harvest, 81 days after application. Residues in grain and straw were determined using DowElanco Analytical Method ERC 97.10 which has a lowest validated level of 0.05 mg/kg for grain and 0.20 mg/kg for straw for each active ingredient. The mean procedural recoveries of clopyralid for grain and straw were 92% and 92%, respectively. Residues in treated grain were: 0.14 mg/kg clopyralid. Residues in treated straw were 0.48 mg/kg for clopyralid. No residues were detected in any of the untreated grain or straw samples.

B.7.3.2.1.4 Garbay 2005

Report	Garbay, M., 2005
Report title	Determination of residue in Barley for malt and brewery in spring barley in France
DAS Study number	S04DAR.BREGG39.JL34
Guidelines	none
Storage	Samples kept at -20C for 214 days
Analytical method	The samples of grains were analysed for clopyralid with the method R-T-M-76-1, by GC-MS
GLP	OECD Principles on GLP, France GLP: Décret 98-1312 dú 31Décembre 1998 and OECD Principles of GLP; FAO guidelines and Good Agricultural Practice (GAP).

During the 2004 growing season two field trials were carried out on spring barley and located in champaing (trial S04DAR.BREGG39) and in Touraine (S04DAR.BREJl34). Each trial was composed of one untreated plot named control plot and six treated plots.

Plot 1 received one application of Chardex at rate of 2 l/ha at stage 32;
plot 2 received one application of Chardex at rate of 1.5 l/ha at stage 39;
plot 3 received one application of Chardex at rate of 1.5 l/ha at stage 69;
plot 4 received one application of Lonpar at 2 l/ha at stage 32;
plot 5 received one application of Ariane at rate of 3 l/ha at stage 32;
plot 6 received one application of Lontrel 100 at rate of 0.75 l/ha in mixture with Actirob B at rate of 1 L/ha, applied at stage 69.

The formulation Bofix Herbicide (SPI498) has been stated in the report to contain on one hand 20 g/L of clopyralid and 20 g/L of clopyralid olamine in the other. In the latter case the concentration of clopyralid in the formulation is 15 g/L. This value has been selected as it was stated in a Table describing all characteristics of the test substance in the original study report.

Olamine can be regarded as a penetration enhancer. It is not clear whether a formulation with olamine can be considered as equal to e.g. the one used in the DAR, Lontrel 100.

In the study specimens of grains were collected at harvest, stored and shipped to the analytical laboratory in deep freezer conditions. The samples of grains were analysed for clopyralid with the method R-T-M-76-1, by GC-MS. No residues of clopyralid were found above the limit of quantification in samples of barley treated with Chardex, Lonpar and Ariane from the two trials. Samples of barley treated with Lontrel: level of c1opyralid residue observed was 0.391 mg/kg in trial S04DAR.BREGG39 and 0.300 mg/kg in trial S04DAR.BREJl34, that is below the MRL. The limit of detection was 0.050 mg/kg and the limit of quantification was 0.150 mg/kg for clopyralid.

B.7.3.2.1 Determination of residue in barley for malt and brewery in winter barley in France

Report	Garbay, M., 2005
Report title	Determination of residue in barley for malt and brewery in winter barley in France
DAS Study number	S04DAR.BREGG40,JL35
Guidelines	none
Storage	Samples kept at -20C for 179 – 214 days
Analytical method	The samples of grains were analysed for clopyralid with the method R-T-M-76-1, by GC-MS Limit of quantification: 0.150 mg/kg Limit of detection: 0.050 mg/kg
GLP	Y, France GLP: Décret 98-1312 dú 31Décembre 1998 and OECD Principles of GLP FAO quldelines and Good Agricultural Practice (GAP)

Two field trials were conducted on winter barley. Trials were carried out on winter barley located in champaing (trial S040AR.BREGG40) and in Touraine (S04DAR.BREJL3S). Each trial was composed of one untreated plot named control plot and six treated plots.
Plot 1 received one application of Chardex at rate of 2 L/ha at stage 32;
plot 2 received one application of Chardex at rate of 1.5 L/ha at stage 39;
plot 3 received one application of Chardex at rate of 1.5 L/ha at stage 69;
plot 4 received one application of Lonpar at 2 L/ha rate at stage 32;
plot 5 received one application of Ariane at rate of 3 L/ha at stage 32;
plot 6 received one application of Lontrel 100 at rate of 0.75 L/ha plus Actirob B at rate of 1 L/ha applied at stage 69. Specimens of grains were collected at harvest, stored and shipped to the analytical laboratory in deep freezer conditions. The samples of grains were analysed for clopyralid with the method R-T-M-76-1, by GC-MS. Samples of barley treated with Chardex: no residues of clopyralid were found above the limit of quantification in samples treated at stage 32 and 39, whereas residues were observed in the two trials when the applications were made at stage 69. Samples treated with Lonpar: no residues of clopyralid were found above the limit of quantification. Samples treated with Ariane: there was no residue of clopyralid above the limit of quantification. Samples treated with Lontrel: residues of clopyralid of 0.539 mg/kg and 0.265 mg/kg were found in the two trials, where compounds were applied 43 and 47 days before harvest, respectively. The limit of detection was 0.050 mg/kg and the limit of quantification was 0.150 mg/kg for clopyralid.

The following points list the references for previously peer reviewed studies providing residue data relied upon for Active Approval.

B.7.3.2.1.5 Freeman 1982

Report	IIA 6.3/19 Freeman, JMH et al, 1982
Report title	Effect of Length of Period Between Application of CYRONAL* and Harvest on Residues of 3,6-dichloropicolinic Acid (DOWCO 290**) in Winter Wheat, Winter Barley and Maize - Belgium 1981
DAS Study number	RT/140-141/81; Report No. GHE-P-943
Guidelines	Not Available
Storage	Analysis date missing
GLP	No

As noted yhis study was evaluated during the Active Substance evaluation. The data have been ignored in the present evaluation given the fact that GLP requirements were not met.

B.7.3.2.1.6 Rawle and Koshab 2012 abcde

Report a	IIA 6.3/20 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in barley at intervals and at harvest following a single application of LONTREL 100 (EF-1136), EU Northern Zone – 2011
DAS Study number	CEMS-1542; DAS Report No. GHE-P-9383
Guidelines	EU guideline (ANONYMOUS (Directorate General for Agriculture), 1997), IVA guideline for residue trials (BEUTEL 1992), the BBA guidelines part IV, 3-3 (HOHGARDT 1990)
Storage	Deep-frozen (not more than 246 days) until extraction and analysis
Analytical method	GRM 01.16
GLP	Yes

This study was evaluated during the Active Substance evaluation.

Report b	IIA 6.3/21 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in barley at intervals under open field conditions following a single application of LONTREL (EF-1136), UK – 2000
DAS Study number	CEMS-1289; DAS Report No. GHE-P-9360
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17] FAO "Guidelines on Producing Residue Data from Supervised Trials", 1990
Storage	Deep-frozen (not more than 574 days) until extraction and analysis
Analytical method	GRM 01.16
GLP	Yes

This study was evaluated during the Active Substance evaluation.

Report c	IIA 6.3/31 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in barley at harvest under open field conditions following a single application of LONTREL (EF-1136), UK – 2000
DAS Study number	CEMS-1288; DAS Report No. GHE-P-9359
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17] FAO "Guidelines on Producing Residue Data from Supervised Trials", 1990
Storage	Deep-frozen (not more than 449 days) until extraction and analysis
Analytical method	GRM 01.16
GLP	Yes

This study was evaluated during the Active Substance evaluation.

Report d	IIA 6.3/22 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in barley at harvest following a single application of LONTREL 100 (EF-1136), EU Southern Zone – 2001
DAS Study number	CEMS-1543; DAS Report No. GHE-P-9384
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17] FAO "Guidelines on Producing Residue Data from Supervised Trials", 1990
Storage	Deep-frozen (not more than 180 days) until extraction and analysis.
Analytical method	GRM 01.16
GLP	Y

This study was evaluated during the Active Substance evaluation.

Report e	IIA 6.3/23 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in barley at intervals under open field conditions following a single application of LONTREL 100 (EF-1136), Southern France and Italy – 2000
DAS Study number	CEMS-1292; DAS Report No. GHE-P-9363
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17] FAO "Guidelines on Producing Residue Data from Supervised Trials", 1990
Storage	Deep-frozen (not more than 595 days) until extraction and analysis
Analytical method	GRM 01.16
GLP	Y

This study was evaluated during the Active Substance evaluation.

Table B.7.3.2-4: Supervised residue trials in barley

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g ae/ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/Kg)	% Recovery clopyralid
R96-138B R96-138 GHE-P-6502 Y 1996	Winter Barley Clarine	France NZ Outdoor (field)	EF-1403	1	77.1	288.8	--	23-Apr-1996	BBCH.32	70 70	Grain Straw	0.24 1.58	96 91
R97-104A R97-104 GHE-P-6807 Y 1997	Winter Barley Pastoral	United Kingdom NZ Outdoor (field)	EF-1403	1	81	200	--	02-May-1997	BBCH.32	81 81	Grain Straw	0.14 0.48	92 92
S04DAR.BREGG39 S04DAR_BREGG39_JL34 Y 2004	Barley Prestige	France NZ Outdoor (field)	EF-685	1	67.9	193.75	--	11-May-2004	BBCH.32	75	Grain	<0.15	94
S04DAR.BREJL34 S04DAR_BREGG39_JL34 Y 2004	Barley Scarlett	France NZ Outdoor (field)	EF-685	1	72.1	206.25	--	24-May-2004	BBCH.32	57	Grain	<0.15	94
S04DAR.BREJL35 S04DAR_BREGG40_JL35 ACC 240810 Y 2004	Winter Barley Esterel	France NZ Outdoor (field)	EF-685	1	67.2	191.7	--	21-Apr-2004	BBCH.32	70	Grain	<0.15	94

Table B.7.3.2-4 (Cont'd.): Supervised residue trials in barley

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g ae/ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/Kg)	% Recovery clopyralid
IT05 140655 EGL-14-18930 Y 2015	Spring Barley Concerto	Italy SZ Outdoor (field)	GF-1966	1	101.3	298	--	19-May-2014	BBCH.39	0 7 14 28 43 43	Whole Plants Whole Plants Whole Plants Whole Plants Grain Straw	1.03 0.775 0.790 0.930 0.840 0.713	93 88 93
ES06 140655 EGL-14-18930 Y 2015	Spring barley Shakira	Spain SZ Outdoor (field)	GF-1966	1	105.1	308	--	30-May-2014	BBCH.39	61 61	Grain Straw	0.325 1.57	88 93
ES07 140655 EGL-14-18930 Y 2015	Spring Barley Unknown	Spain SZ Outdoor (field)	GF-1966	1	101.4	298	--	06-Jun-2014	BBCH.39	0 7 14 28 60 60	Whole Plants Whole Plants Whole Plants Whole Plants Grain Straw	1.78 0.388 0.535 0.512 0.193 0.488	93 88 93
IT08 140655 EGL-14-18930 Y 2015	Spring Barley Loreta	Italy SZ Outdoor (field)	GF-1966	1	101.2	297	--	22-May-2014	BBCH.39	40 40	Grain Straw	0.870 0.445	88 93

B.7.3.3 Wheat

Data to address this point were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. However, the critical GAP supported for clopyralid Annex I Renewal has changed such that the maximum application rate in both the N-EU and S-EU is proposed at 80 g ae/ha rather than the higher rate of 0.127 kg ae/ha that was included in the critical GAP for the original Annex I / Active Substance approval. A comparison of the critical GAPs supported for Active Approval to the critical GAP proposed for Annex I Renewal is shown in Table B.7.3.3-1.

Table B.7.3.3-1: Comparison of critical GAP for clopyralid use in cereals in Active Substance Approval and proposed for Annex I Renewal (AIR)

GAP supported	Residue Zone	Maximum rate (kg ae/ha)	Maximum number of applications	Latest growth stage at application
Active Approval*	N-EU	0.127	1	BBCH 39
Active Approval*	S-EU	0.127	1	BBCH 45
Annex I Renewal	N-EU and S-EU	0.08	1	BBCH 39

*GAP taken from the 'Conclusion regarding the peer review of the pesticide risk assessment of the active substance clopyralid' (EFSA Scientific Report (2005) 50, 1-65).

Since the critical GAP supported for Active Approval and upon which current EU MRLs in cereals are based is more critical than the GAP proposed for Annex I Renewal, the residue trial data supporting the Active Approval and the EU MRLs also covers the less critical GAP proposed for Annex I Renewal.

Although residue data from the more critical GAPs supported for Active Approval are expected to equal or exceed residue levels from the less critical GAP proposed for Annex I Renewal, results from available wheat trials having GAPs considered equivalent to the critical GAP proposed for Annex I Renewal (e.g. ± 25% of the cGAP rate) are summarized in Tables B.7.3.3-1 to B.7.3.3-6 and the associated trial results are presented in Table B.7.3.3-4. Although the application rates are within 25% of the cGAP for Annex I Renewal, these trials had application at a somewhat earlier stage of growth (BBCH 32) rather than BBCH 39, but these were considered comparable since the plants in these growth stages are in similar stages of development and occur well before development of the grain / consumable part of the plant.

The study report references for trials previously reviewed during the Active Approval and used in supporting current EU MRLs in cereals are listed below in 6.3.3/7 to 6.3.3/12.

An overview / summary of the residue trial data from the available trials carried out based on a GAP considered equivalent to critical GAP proposed for Annex I Renewal is presented in Table B.7.3.3-2 and a more detailed summary of these trial results is presented in Table B.7.3.3-4. For comparison, the residue trial data accepted for Active Approval and relied upon for setting of current MRLs is presented in Table B.7.3.3-3. As expected, residues from trials considered to be in compliance with the GAP proposed for clopyralid Annex I Renewal

are within the range of residue values from trials conducted according to the GAPs that supported Active Approval. Therefore, together with the results from barley trials supporting Active Approval, it is proposed that the exiting residue data for wheat accepted during Active Approval and upon which EU MRLs are based is adequate to also support the less critical GAP proposed for Annex I Renewal.

Table B.7.3.3-2: Summary of Clopyralid Residue Results in Wheat from Trials with GAP Considered Equivalent to the Proposed Critical GAP for Annex I Renewal

Residue Zone	Commodity	Clopyralid (mg/kg)
N-EU	Grain	ND/<0.01, 0.11, <0.15, 0.22, 0.24, 0.25, 0.40, 0.41, 0.43, 0.542
S-EU		0.029, 0.17, 0.218, 0.26, 0.28 x 2, 0.29, 0.369, 0.467, 0.48, 0.62
N-EU	Straw	0.12, 0.34, 0.52, 0.88, 1.0, 1.3
S-EU		0.037, 0.075, 0.36, 0.41, 0.44, 0.54, 0.79, 0.92

Table B.7.3.3-3: Summary of Clopyralid Residue Results in Wheat from Trials Accepted for Active Approval and Supporting MRLs in Cereals *

Residue Zone	Commodity	Clopyralid (mg/kg)
N-EU	Grain	0.07, 0.23, 0.73, 0.79, 0.93, 1.06, 1.11, 1.26
S-EU		0.26, 0.68, 1.16, 1.42
N-EU	Straw	0.26, 0.59, 0.79, 0.93, 1.06, 1.11, 1.26
S-EU		0.39, 0.63, 0.99, 1.18

* Results taken from 'Conclusion regarding the peer review of the pesticide risk assessment of the active substance clopyralid' (EFSA Scientific Report (2005) 50, 1-65).

B.7.3.3.1.1 Pronier 2013

Report	Pronier, I., 2013
Report title	Residues of fluroxypyr-meptyl, clopyralid MCPA-2-ethylhexyl in wheat at intervals and at harvest following a single application of GF-1681. Northern and Southern Zone – 2012
DAS Study number	14SRFR12R03
Guidelines	U. S. EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 (I), the European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7 (2) and SANCO/3029/99 rev.4 (3), and PMRA Residue Chemistry Guidelines as Regulatory Directive Dir98-02 (4).
Formulation	Type EC: GF-1681: fluroxypyr-meptyl (60 g a.i./L), clopyralid (23.34 g a.i./L), MCPA-2-ethylexyl ester (266.72 g a.i./L)
Application rate	70.02 g a.i./L; from 66.41 to 73.45 g a.i./ha depending on plot.
Analytical	Adaption of the Dow AgroSciences analytical method GRM 01.16, with a limit of quantification of 0.01 mg/kg.
Max. frozen storage period prior to analysis:	216 - 249 - 304 days depending on trial
GLP	Yes: OECD Principles of Good Laboratory Practice, N°1, as revised in 1997 [ENV/MC/CHEM (98) 17]; GLP Principles to field studies, N°6, as revised in 1999 [ENV/JM/MONO (99) 22] and OECD Principles of GLP to the Organisation and Management of Multi-site Studies, N°13, 2002 [ENV/JM/MONO (2002) 9]

Eight trials were conducted in 2012, four in Northern EU zone, one in Northern France, one in Hungary, one in UK, one in Germany and four in Southern EU zone, one in Southern France, one in Spain, one in Italy and one in Greece.

The data comprise of two decline studies and one normal residue trial.

A single application of the formulated product GF-1681 was applied at BBCH 32, at a rate of 3 L fp /ha. GF-1681 is an EC formulation that contains fluroxypyr-meptyl (60 g a.i./L), clopyralid (23.34 g a.i./L) and MCPA-2-ethylhexyl (266.72 g a.i./L). Specimens of whole plants were collected at 0, 10, 30 and 60 days after application for decline trials only; grain and straw were collected at normal commercial harvest in all trials. Residues of clopyralid were determined by adapting Dow AgroSciences analytical method GRM 01.16, with the limit of quantification of 0.01 mg/kg and the limit of detection of 0.002 mg/kg. Maximum frozen storage period prior to analysis was 328 days for clopyralid.

The method extracts 5-g aliquots of the homogenized wheat material by homogenizing and shaking with a methanol/10 N sodium hydroxide solution (100/1). Following a clean-up procedure by polymeric sorbent solid phase extraction (SPE), derivatisation and partition with aqueous sodium chloride solution into n-hexane, the analyte is determined by GC/MS.

Any data exist neither on conjugate recoveries nor whether the method is able to determine conjugates. At least extraction with methanolic sodium hydroxide does not yield clopyralid in its acid form from clopyralid conjugates as demonstrated by studies on rotational crops where radiolabelled clopyralid was used (Section B.7.6 residues in rotational crops; Hall 2015).

B.7.3.3.1.2 Devine 2006

Report	Devine, H.C., 2006
Report title	Residues of clopyralid in wheat and process fractions at harvest following a single application of EF-1498, Northern France – 2005
DAS Study number	CEMS-2711; DAS Report No. GHE-P-11274
Guidelines	OECD Principles of Good Laboratory Practice [ENVIMCICHEM(98)17] FAO "Guidelines on Producing Pesticide Residue Data from Supervised Trials", Rome 1990.
Species	Wheat
Analytical method	GRM 01.16
Storage time	Maximum frozen storage period prior to analysis was 191 days for clopyralid.
GLP	Y

A trial was conducted in Northern France during 2005 to determine the residues of clopyralid in wheat grain, straw and process fractions (whiteflour, wholemeal flour, wheat germ, bran, white bread and wholemeal bread) at harvest. A single application of EF-1498 an EW formulation containing 20gas/L clopyralid were made. The first application at a nominal rate of 60gas/ha was made in April at growth stage 30, 31 or 32 (BBCH). For all plots samples of grain and straw were taken at harvest. For the plots where the application occurred at BBCH 30 or 32 additional samples of grain were also taken at harvest for processing into white flour, wholemeal flour, wheat germ, bran, white bread and wholemeal bread. Residues were determined using Dow AgroSciences Analytical Method GRM 01.16, with a limit of quantification (LOQ) of 0.01mg/kg. Maximum frozen storage period prior to analysis was 191 days for clopyralid.

B.7.3.3.1.3 Clements 1997

Report	Clements, B., 1997, Residues of fluroxypyr-BPE, clopyralid and MCPA in cereals at harvest following a single application of BOFIX (NEW) EF-1403, France (North and South) – 1996 DAS Study number R96-138
Guidelines	OECD Principles of Good Laboratory Practice FAO "Guidelines on Producing Pesticide Residues Data from Supervised Trials", Rome 1990
Species	Winter wheat and winter barley
Storage time	Trial A - 197 days at -20C Trials B,C et D 218 days at -20C
Analytical method	DowElanco Analytical Method ERC 97.10 which has a lowest validated level of 0.05 mg/kg for grain and 0.20 mg/kg for straw. All residue values equivalent to less than 20% of lowest validated level are classified as "not detected" (ND).
GLP	Y

Four at harvest trials, one on winter wheat (trial A) and one on winter barley (trial B) in the Indre-et-Loire region of Northern France and two trials on winter wheat in the Haute Garonne region of Southern France (tials C and D) were carried out during 1996. In each trial, a single application of BOFIX* (NEW) EF-1403, containing 267 g a.i./L MCPA, 27 g a.i./L clopyralid and 54 g a.i./L of fluroxypyr was applied at a nominal rate of 160/80/800 g ae/ha (Fluroxypyr/Clopyralid/ MCPA) when the cereals were at growth stage BBCH 32 - 33. Samples of winter barley grain and straw were collected at normal harvest, 70 days after treatment (BBCH 89). Samples of winter wheat grain and straw were collected at normal harvest, 85 - 89 days after treatment (BBCH 89). A single untreated plot was also included in each trial and corresponding cereal grain and straw samples were taken at the corresponding sampling harvest. The samples were analysed for residues of clopyralid using DowElanco Analytical Method ERC 97.10 which has a lowest validated level of 0.05 mg/kg for grain and 0.20 mg/kg for straw.

Results

Residue trials on barley are dealt under B.7.3.2

Results have been compiled with other studies in the Table B.7.3.3-4: Supervised residue trials in wheat.

Table B.7.3.3.1-1 Clopyralid residue levels in different cereal crops (Clements 1997)

Trial Number	Substrate	Formulation	Treatment Rate (g ae/ha)	Growth Stage (BBCH) (at	Days After Last Application	Mean Residue Residue Found

				last application)		(mg/kg)
R96-138A Wheat	Grain	Untreated	-	32	88	ND
	Straw	Untreated	-	32	88	ND
	Grain	EF-1403	77.5	32	88	0.43
	Straw	EF-1403	77.5	32	88	1.30
R96-138B Barley	Grain	Untreated	-	32	70	ND
	Straw	Untreated	-	32	70	ND
	Grain	EF-1403	77.1	32	70	0.24
	Straw	EF-1403	77.1	32	70	1.58
R96-138C Winter wheat	Grain	Untreated	-	32	89	ND
	Straw	Untreated	-	32	89	ND
	Grain	EF-1403	81.9	32	89	0.28
	Straw	EF-1403	81.9	32	89	0.36
R96-138D Wheat	Grain	Untreated	-	32 - 33	85	ND
	Straw	Untreated	-	32 - 33	85	ND
	Grain	EF-1403	82.1	32 - 33	85	0.26
	Straw	EF-1403	82.1	32 - 33	85	0.54

B.7.3.3.1.4 Butler 1998

Report	Butler, R.E., 1998, Residues of clopyralid, fluroxypyr and MCPA in winter wheat at harvest following a single application of BOFIX* BP (EF-1403), Belgium, 1997, DAS Study number: R97-103
Guidelines	OECD Principles of Good Laboratory Practice Guidelines on Producing Pesticide Residues Data from Supervised Trials. - FAO Rome 1990
Analytical	DowElanco Analytical Method ERC 97
Storage	Samples of grain and straw were taken at normal harvest and placed in a deep freeze within 24 hours. Samples kept at -20C not more than 84 days.
GLP	Y

One trial was conducted to determine the residues of clopyralid, fluroxypyr and MCPA in winter wheat at harvest following a single application of Bofix* BP (EF -1403). This formulation contains 27 g as/L clopyralid, 54 g a.i./L fluroxypyr and 267 g as/L MCPA.

The trial was conducted in Belgium during 1997 and consisted of one untreated and one treated plot. The treated plot was sprayed at a nominal application rate of 3 L/ha when the crop was at growth stage BBCH 32. Principal Investigator for the trial was P Reynens, Redebel.

The trial consisted of one untreated and one treated plots. The treated plot was sprayed at a nominal application rate of 3 Llha when the crop was at growth stage BBCR 32. Samples of grain and straw were taken at normal harvest and placed in a deep freeze within 24 hours and transported frozen to the Letcombe Laboratory. Samples for residue analysis were despatched on 15 Oct 1997 and arrived in Letcombe on 16 Oct 1997 in good condition.

Samples of grain and straw were taken at normal harvest, 86 days after application. Residues in grain and straw were determined using DowElanco Analytical Method ERC 97.10 which has a lowest validated level of 0.05 mg/kg for grain and 0.20 mg/kg for straw for clopyralid.

Clopyralid, fluroxypyr and MCP A were extracted from grain and straw by macerating and shaking with caustic methanol. An aliquot was acidified and the analytes were partitioned into methyl-tertiary-butyl ether (MTBE) then into aqueous sodium bicarbonate, which was acidified and the analytes were extracted back into MTBE. The organic phase was evaporated to dryness and the residuum treated with 4% v/v concentrated sulphuric acid / n-butanol to form the butyl esters of clopyralid, fluroxypyr and MCP A. Following the addition of water clopyralid, fluroxypyr and MCP A butyl esters were partitioned into hexane. The hexane extract was then analysed by capillary gas chromatography using mass selective detection.

Recoveries for parent compound copyralid were within acceptable limits.

The method involves transesterification and in principle can be expected to be able to determine also clopyralid conjugates. Conjugates are not even mentioned in the orginal studies. There are no validation data on clopyralid conjugates available. The method starts with an extraction, but no data is available whether this extraction is able to extract clopyralid conjugates and to which extent.

B.7.3.3.1.5 Garbay 2005

Report	Garbay, M., 2005 Residue Study with Fluroxypyr and Clopyralid and 2,4-MCPA (Bofix = EF-1498) in or on Wheat in France (North and South); (Analyse de Residus de Florasulam, Fluroxypyr, 2,4-MCPA et Clopyralid dans l'Orge, le Ble, la Farine et les Produits Transformes [Analysis of Residues of Florasulam, Fluroxypyr, 2,4-MCPA and Clopyralid in Barley, Corn, Flour and the Processed Products] DAS
Study number	S03DAHBOFIX; 03/123-E257
Guidelines	France GLP: Décret 98-1312 dú 31Décembre 1998 and OECD Principles of GLP
Storage conditions	73 days at -20C
Analytical method	Clopyralid method R-T-M76-0 : extraction by methanol, purification by dividing liquid/liquid, esterification, analysis by GCMS
GLP	Y

During the 2003 growing season 6 field trials were conducted on winter wheat and one trial on hard wheat. The trials carried out on winter wheat were located in north of France(3 trials named S03DAH.BOFV027, S03DAH.BOFV028, S03DAH.BOFJL17), and in south of France (2 trials named S03DAH.BOFGL13 and S03DAH.BOFPR07). The trial conducted on hard wheat was located in south of France and named S03DAH.BOFPR08. Each trial was composed of one untreated plot named control plot and two treated plots named plot 1, plot 2, which received one application of Bofix at the respective rates of 4 l/ha (plot 1), and 8 l/ha (plot 2). The application was made at crop stage 32 BBCH. Specimens of grains were collected at harvest, stored and shipped to the analytical laboratory in deep freezer conditions. The grain's samples were analysed for clopyralid with the method R-T-M76-0, analysis by GC-MS.

Specimens of grains from two trials (S03DAH. BOFV028, S03DAH.BOFGL13) in which the clopyralid residue level was the highest were subject to processing. Theis part of the report has been evaluated in Chapter B.7.5.3.1

Table 7.4.3.3-1. Individual trials on wheat and parameters employed.

N° of trial	Actual spray volume L/ha	BBCH crop stage at application	Rate of Bofix	Rate as clopyralid min (plot T1) g/ha	Rate as clopyralid max (plot T2) g/ha
S03DAH.BOFV027	202-217	32	4/8.7	80.6	173.4
S03DAH.BOFY028	182 -192	32	3.6/7.7	72.75	153.4
S03DAH.BOFJLI7	198•168	32	4/6.7	80	134.6

S03DAH.BOFGL 13	400	32	4/8	64.0	160
S03DAH.BOFPR07	242-210	31-32	4.8/8.4	96.6	168
S03 DAH.BOFPR08	213-217	32	4.3/8.7	85.4	173.4

a) underdosed

B.7.3.3.1.6 Butler 1998

Report	Butler, R.E., 1998 Residues of Clopyralid, Fluroxypyr and MCPA in Winter Wheat and Durum Wheat at Harvest Following a Single Application of BOFIX* BP (EF-1403), Southern France, 1997 DAS Study number R97-105
Guidelines	the FAO "Guidelines on Producing Residue Data from Supervised Trials", 1990.
Analytical method	DowElanco Analytical Method ERC 97.10
Storage conditions	224 days at -20C
GLP	Yes, OECD Principles of Good Laboratory Practice

Two trials were conducted to determine the residues of clopyralid, fluroxypyr and MCPA in winter wheat and durum wheat at harvest following a single application of Bofix* BP (EF-1403). This formulation contains 27 g as/L clopyralid, 54 g a.i./L fluroxypyr present as the butoxypropyl ester and 267 g a.i./L MCPA.

The trials were conducted in Southern France during 1997 and consisted of one untreated and one treated plot. The treated plot was sprayed at a nominal application rate of 3 L/ha when the crop was at growth stage BBCH 32. Samples of grain and straw in winter wheat and durum wheat were taken at normal harvest, 91 and 76 days after application respectively. Residues in grain and straw were determined using DowElanco Analytical Method ERC 97.10 which has a lowest validated level of 0.05 mg/kg for grain and 0.20 mg/kg for straw for clopyralid.

The following points list the references for previously peer reviewed studies providing residue data relied upon for Active Approval.

B.7.3.3.1.7 Freeman et al. 1982

Report	IIA 6.3/13 Freeman, JMH et al, 1982 Effect of Length of Period Between Application of CYRONAL* and Harvest on Residues of 3,6-dichloropicolinic Acid (DOWCO 290**) in Winter Wheat, Winter Barley and Maize – Belgium 1981, DAS Report No.GHE-P-943; Trial Nos. RT/142-143/81
Guidelines	Not Available
GLP	No

This study was evaluated during the Active Substance evaluation, but not included in the present evaluation, because the study does not meet GLP-requirements.

B.7.3.3.1 Clopyralid residues in wheat grain and straw treated with either LONPAR* or LONTREL* 100 from French trials, 1983

B.7.3.3.1.8 Freeman et al. 1984

Report	IIA 6.3/14 Freeman, JMH et al, 1984, Clopyralid residues in wheat grain and straw treated with either LONPAR* or LONTREL* 100 from French trials, 1983 DAS Report No. GHE-P-1258; Trial Nos RT-176-177/83, RT-172-173/83
Guidelines	EC working document 1608/VI/97 “The Lundehn document.
Formulation	EF-1136 (Lontrel 100* or Matrigon*) containing 100 g/L clopyralid
Analytical method	ERC 83.23
Storage	Not more than 595 at -20C
GLP	N

This study was evaluated during the Active Substance evaluation, but not included in the present evaluation, because the study does not meet GLP-requirements.

B.7.3.3.1.9 Rawle and Khoshab 2002 abcd

Report a	IIA 6.3/15 Rawle, N.W., Khoshab, A., 2002, Residues of clopyralid in wheat at intervals under open field conditions following a single application of LONTREL (EF-1136), UK and Germany – 2000, CEMS-1287; DAS Report No. GHE-P-9358
Guidelines	
Formulation	EF-1136 (Lontrel 100* or Matrigon*) containing 100 g/L clopyralid
Analytical method	GRM 01.16
Storage	Not more than 530 at -20C
GLP	Yes: OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17]

This study was evaluated during the Active Substance evaluation.

Report b	IIA 6.3/16 Rawle, N.W., Khoshab, A., 2002, Residues of clopyralid in wheat at intervals following a single application of LONTREL 100 (EF-1136), EU Northern Zone – 2001, CEMS-1544; DAS Report No. GHE-P-9385
Guidelines	None indicated
Formulation	EF-1136 (Lontrel 100* or Matrigon*) containing 100 g/L clopyralid
Analytical method	GRM 01.16
Storage	Not more than 530 at -20C
GLP	Yes: OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17]

This study was evaluated during the Active Substance evaluation.

Report c	IIA 6.3/17 Rawle, N.W., Khoshab, A., 2002
Report title	Residues of clopyralid in wheat at intervals under open field conditions following a single application of LONTREL 100 (EF-1136), Southern France and Italy – 2000, DAS Study number: CEMS-1242; DAS Report No. 9351
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17]
Analytical method	GRM 01.16
GLP	Yes

This study was evaluated during the Active Substance evaluation.

Report d	IIA 6.3/18 Rawle, N.W., Khoshab, A., 2002, Residues of clopyralid in wheat at harvest under open field conditions following a single application of LONTREL 100 (EF-1136), Greece – 2000, DAS Study number: CEMS-1290; DAS Report No. GHE-P-9361
Guidelines	OECD Principles of Good Laboratory Practice [ENV /MC/CHEM(98) 17]
Formulation	LONTREL 100 (EF-1136),
Analytical method	GRM 01.16
Storage	Not more than 530 at -20C
GLP	Yes

This study was evaluated during the Active Substance evaluation.

Table B.7.3.3-4: Supervised residue trials in wheat

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g a.i./ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/kg)	% Recovery clopyralid
SRAT12-013 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Impression	Germany NZ Outdoor (field)	GF-1681	1	69.1	296	23	04-May-2012	BBCH.32	75 75 0 10 31 59	Grain Straw Whole plant Whole plant Whole plant Whole plant	0.24 0.88 1.3 0.54 0.47 0.43	97 93 89 89 89 89
SRFR12-016 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Courtot	France NZ Outdoor (field)	GF-1681	1	71.3	305.3	23	18-Apr-2012	BBCH.32	85 85	Grain Straw	0.11 0.34	97 93
SRHU12-031 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Esperia	Hungary NZ Outdoor (field)	GF-1681	1	71.5	306.5	23	27-Apr-2012	BBCH.32	70 70 0 10 30 60	Grain Straw Whole plant Whole plant Whole plant Whole plant	0.22 1 1.5 0.62 0.89 0.83	97 93 89 89 89 89
SRUK12-004 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat cv. Consort	United Kingdom NZ Outdoor (field)	GF-1681	1	68.9	196.7	35	16-Apr-2012	BBCH.32	126 126	Grain Straw	0.25 0.12	97 93
CEMS-2711A CEMS-2711 GHE-P-11274 Y 2004	Winter Wheat Not reported	France NZ Outdoor (field)	EF-1498	1	60.2	201	--	28-Apr-2005	BBCH.32	75	Grain	0.41	89

Table B.7.3.3-4 (Cont'd.): Supervised residue trials in wheat

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g ae/ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/Kg)	% Recovery clopyralid
R96-138A R96-138 GHE-P-6502 Y 1996	Winter Wheat Rossini	France NZ Outdoor (field)	EF-1403	1	77.5	294.4	--	26-Apr-1996	BBCH.32	88 88	Grain Straw	0.43 1.3	96 87
R97-103A R97-103 GHE-P-6806 Y 1997	Winter Wheat Pajero	Belgium NZ Outdoor (field)	EF-1403	1	85.39	214	--	13-May-1997	BBCH.32	86 86	Grain Straw	0.4 0.52	87 90
S03DAH.BIFV028 S03DAHBOFIX Sponsor Study Code: BOFIXKARTY 2003	Winter Wheat Apache	France NZ Outdoor (field)	BOFIX* Herbicide (EF-1498) ()	1	72.6	181.7	--	28-Apr-2003	BBCH.32	82	Grain	0.542	94
S03DAH.BOFJL17 S03DAHBOFIX Sponsor Study Code: BOFIXKARTY 2003	Winter Wheat Apache	France NZ Outdoor (field)	BOFIX* Herbicide (EF-1498) ()	1	79.4	198.3	--	09-Apr-2003	BBCH.32	91	Grain	<0.15	94
S03DAH.BOF	Winter	France	BOFI	1	80.6	201.7	--	28-Apr-2003	BBCH.32	82	Grain	ND	94

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g a.i./ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/kg)	% Recovery clopyralid
V027 S03DAHBOFIX Sponsor Study Code: BOFIXKARTY 2003	Wheat Orvantis	NZ Outdoor (field)	X* Herbicide (EF-1498) ()										
14SRFR12R03/1 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Simeto	Greece SZ Outdoor (field)	GF-1681	1	70	300	23	18-Apr-2012	BBCH.32	64 64	Grain Straw	0.62 0.037	97 93
SRES12-137 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Marion	Spain SZ Outdoor (field)	GF-1681	1	66.4	237.3	28	13-Apr-2012	BBCH.32	76 76 0 10 31 60	Grain Straw Whole plant Whole plant Whole plant Whole plant	0.48 0.4.1 4.3 0.91 1.4 1.7	97 93 89 89 89 89
SRFR12-017 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Accor	France SZ Outdoor (field)	GF-1681	1	73.5	262.2	28	29-Mar-2012	BBCH.32	82 82	Grain Straw	0.17 0.44	97 93
SRIT12-1021 14SRFR12R03 GHE-P-12974 Y 2012	Winter wheat Augustus	Italy SZ Outdoor (field)	GF-1681	1	69.2	296.7	23	03-Apr-2012	BBCH.32	105 105 0 9 30 59	Grain Straw Whole plant Whole plant Whole plant Whole plant	0.029 0.075 3.7 0.43 0.11 0.05	97 93 89 89 89 89

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g a.i./ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/kg)	% Recovery clopyralid
R96-138C R96-138 GHE-P-6502 Y 1996	Winter Wheat Soinon	France SZ Outdoor (field)	EF- 1403	1	81.9	258.4	--	11-Apr-1996	BBCH.32	89 89	Grain Straw	0.28 0.36	96 87
R96-138D R96-138 GHE-P-6502 Y 1996	Durum Wheat Neodur	France SZ Outdoor (field)	EF- 1403	1	82.1	259.3	--	10-Apr-1996	BBCH.32 to 33	85 85	Grain Straw	0.26 0.54	96 95
R97-105A R97-105 GHE-P-6808 Y 1997	Winter Wheat Soissons	France SZ Outdoor (field)	EF- 1403	1	85.6	317	--	26-Mar-1997	BBCH.32	91 91	Grain Straw	0.29 0.79	85 97
R97-105B R97-105 GHE-P-6808 Y 1997	Durum Wheat Neodur	France SZ Outdoor (field)	EF- 1403	1	81	300	--	10-Apr-1997	BBCH.32	76 76	Grain Straw	0.28 0.92	92 97
S03DAH.BOF GL13 S03DAHBOFI X Sponsor Study Code: BOFIXKART Y 2003	Winter Wheat Apache	France SZ Outdoor (field)	BOFI X* Herbic ide (EF- 1498) ()	1	80	400	--	22-Apr-2003	BBCH.32	69	Grain	0.218	94
S03DAH.BOF PR07 S03DAHBOFI X	Winter Wheat Aztec	France SZ Outdoor (field)	BOFI X* Herbic ide	1	96.6	241.7	--	18-Apr-2003	BBCH.31 to 32	73	Grain	0.369	94

Trial ID Study ID Report No. GLP(Y/N) Trial Year	Crop Variety	Country Zone Location	Form No.	No. of Appls	Appl Rate (g a.i./ha)	Spray Vol (L/ha)	Appl Conc ()	Appl Date	GS at Last Appl	PHI (days)	Portion Analysed	Clopyralid (mg/kg)	% Recovery clopyralid
Sponsor Study Code: BOFIXKARTY 2003			(EF-1498) ()										
S03DAH.BOFPR08 S03DAHBOFIX Sponsor Study Code: BOFIXKARTY 2003	Durum Wheat Not Recorded	France SZ Outdoor (field)	BOFIX* Herbicide (EF-1498) ()	1	85.4	213.3	--	23-Apr-2003	BBCH.32	86	Grain	0.467	94

B.7.4 Feeding Studies

Food Commodities of Animal Origin:

The livestock dietary burden was determined based on guidance given for the EU on livestock diets along with dry matter intake and livestock body weight in the OECD Guidance Document on Residues in Livestock (Series on Pesticides No. 73, ENV/JM/MONO(2013)8, 10-Jul-2013). In this document the values used for dry matter content of fresh grass forage, grass silage and grass hay are 25%, 40% and 88%, respectively.

The input values for calculation of livestock dietary burden are presented in Table B.7.4-1. Since the HR and STMR values for clopyralid in grass were higher for trials conducted in the N-EU than in the S-EU, the HR and STMR used for calculation of livestock dietary burden was based on the N-EU trials. As mentioned previously, forage / pasture grass residue was used in the livestock dietary burden calculations since the HR value of 6.95 mg/kg when expressed on a dry matter basis (25% DM) resulted in a higher residue level than for available residue data for grass silage 940% DM) or grass hay (88% DM).

For bran, the residue value was calculated based on the STMR for grain and the average processing factor of 6.1 for bran: grain STMR 0.92 mg/kg x 6.1 = 5.61 mg/kg.

Table B.7.4-1: Input values for livestock dietary burden calculation

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment [a]	Input value (mg/kg)	Comment [a]
Residue definition: Clopyralid, its salts and conjugates, expressed as clopyralid				
Grass forage	3.61	STMR - NEU	6.95	HR - NEU
Wheat grain	0.92	STMR - N-EU, wheat and barley	0.92	STMR - N-EU, wheat and barley
Barley grain	0.92	STMR - N-EU, wheat and barley	0.92	STMR - N-EU, wheat and barley
Wheat bran / milled by-products	5.61	PF x STMR - N-EU, wheat and barley	5.61	PF x STMR - N-EU, wheat and barley
Barley bran	5.61	PF x STMR - N-EU, wheat and barley	5.61	PF x STMR - N-EU, wheat and barley
Wheat straw	0.93	STMR -S-EU, wheat and barley	1.26	HR - NEU, wheat and barley
Barley straw	0.93	STMR - S-EU, wheat and barley	1.26	HR - NEU, wheat and barley

[a] Residue value for bran calculated using the mean processing factor of 6.1 multiplied by the grain STMR value of 0.92: 6.1 x 0.92 mg/kg = 5.61 mg/kg.

Based on the input values in Table B.7.4-2, livestock dietary burden was calculated based on guidance given for the EU on dry matter content of feed commodities, composition of livestock diets, dry matter intake and livestock body weight in the OECD Guidance

Document on Residues in Livestock (Series on Pesticides No. 73, ENV/JM/MONO(2013)8, 10-Jul-2013). Dietary burdens were calculated for beef cattle, dairy cattle, sheep - rams / ewes, sheep - lambs, swine - breeding, swine - finishing, poultry - broiler, poultry - laying hens and poultry - turkey and results are presented in **Tables B.7.4-2 - 10**

Results are presented based on both the median and maximum dietary burdens and are expressed both as concentration in the diet on a dry matter basis (mg/kg dry feed (DM)) and as residue intake per unit of livestock body weight (mg/kg bw/day).

Table B.7.4-2: Clopyralid dietary burden for beef cattle

Beef Cattle – EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Beef Cattle - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Grass	forage (fresh)	(HR) 6.95	Forages/ Fodders	25	50	(HR) 13.90	(HR) 0.3336
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	30	(STMR) 1.913	(STMR) 0.0459
Barley	grain	(STMR) 0.920	Cereal Grains	88	20	(STMR) 0.209	(STMR) 0.0050
					Maximum Dietary Intake (mg/kg feed DM):	**16.02**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.385**
Grass	forage (fresh)	(STMR) 3.610	Forages/ Fodders	25	50	(STMR) 7.220	(STMR) 0.1733
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	30	(STMR) 1.913	(STMR) 0.0459
Barley	grain	(STMR) 0.920	Cereal Grains	88	20	(STMR) 0.209	(STMR) 0.0050
					Median Dietary Intake (mg/kg feed DM):	**9.34**	
						Median Dietary Burden (mg/kg bw/d):	**0.224**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-3: Clopyralid dietary burden for dairy cattle

Dairy Cattle – EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Dairy Cattle - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Grass	forage (fresh)	(HR) 6.95	Forages/ Fodders	25	60	(HR) 16.680	(HR) 0.6415
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	30	(STMR) 1.913	(STMR) 0.0736
Barley	grain	(STMR) 0.920	Cereal Grains	88	10	(STMR) 0.105	(STMR) 0.0040
					Maximum Dietary Intake (mg/kg feed DM):	**18.70**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.719**
Grass	forage (fresh)	(STMR) 3.61	Forages/ Fodders	25	50	(STMR) 8.664	0.3332
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	30	(STMR) 1.913	0.0736
Barley	grain	(STMR) 0.920	Cereal Grains	88	20	(STMR) 0.105	(STMR) 0.0040
					Median Dietary Intake (mg/kg feed DM):	**10.68**	
						Median Dietary Burden (mg/kg bw/d):	**0.411**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-4: Clopyralid dietary burden for sheep – rams / ewes

Sheep - Ram/Ewe – EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Sheep - Ram/Ewe - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Grass	forage (fresh)	(HR) 6.95	Forages/ Fodders	25	95	26.410	0.8803
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	5	0.319	0.0106
					Maximum Dietary Intake (mg/kg feed DM):	**26.73**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.891**
Grass	forage (fresh)	(STMR) 3.61	Forages/ Fodders	25	95	13.718	0.4573
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	5	0.319	0.0106
					Median Dietary Intake (mg/kg feed DM):	**14.04**	
						Median Dietary Burden (mg/kg bw/d):	**0.468**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-5: Clopyralid dietary burden for sheep - lambs

Sheep - Lamb - EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Sheep - Lamb - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Grass	forage (fresh)	(HR) 6.95	Forages/ Fodders	25	50	13.900	0.5908
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	50	3.188	0.1355
					Maximum Dietary Intake (mg/kg feed DM):	**17.09**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.726**
Grass	forage (fresh)	(STMR) 3.61	Forages/ Fodders	25	50	7.220	0.3068
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	50	3.188	0.1355
					Median Dietary Intake (mg/kg feed DM):	**10.41**	
						Median Dietary Burden (mg/kg bw/d):	**0.442**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-6: Clopyralid dietary burden for swine / pigs - breeding

Swine - Breeding - EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Swine - Breeding - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Grass	forage (fresh)	(HR) 6.95	Forages/ Fodders	25	20	5.560	0.1283
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	50	3.188	0.0736
Barley	grain	(STMR) 0.920	Cereal Grains	88	30	0.314	0.0072
					Maximum Dietary Intake (mg/kg feed DM):	**9.06**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.209**
Grass	forage (fresh)	(STMR) 3.61	Forages/ Fodders	25	20	2.888	0.0666
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	50	3.188	0.0736
Barley	grain	(STMR) 0.920	Cereal Grains	88	30	0.314	0.0072
					Median Dietary Intake (mg/kg feed DM):	**6.39**	
						Median Dietary Burden (mg/kg bw/d):	**0.147**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-7: Clopyralid dietary burden for swine - finishing

Swine - Finishing - EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Swine - Finishing - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	50	3.188	0.0956
Barley	grain	(STMR) 0.920	Cereal Grains	88	50	0.523	0.0157
			Maximum Dietary Intake (mg/kg feed DM):			**3.71**	
					Maximum Dietary Burden (mg/kg bw/d):		**0.111**
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	50	3.188	0.0956
Barley	grain	(STMR) 0.920	Cereal Grains	88	50	0.523	0.0157
			Median Dietary Intake (mg/kg feed DM):			**3.71**	
					Median Dietary Burden (mg/kg bw/d):		**0.111**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-8: Clopyralid dietary burden for poultry - broiler

Poultry - Broiler - EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Poultry - Broiler - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	20	1.275	0.0900
Barley	grain	(STMR) 0.920	Cereal Grains	88	70	0.732	0.0517
			Maximum Dietary Intake (mg/kg feed DM):			**2.01**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.142**
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	20	1.275	0.0900
Barley	grain	(STMR) 0.920	Cereal Grains	88	70	0.732	0.0517
			Median Dietary Intake (mg/kg feed DM):			**2.01**	
						Median Dietary Burden (mg/kg bw/d):	**0.142**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-9: Clopyralid dietary burden for poultry – laying hens

Poultry – Laying hens - EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Poultry – Layer - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	20	1.275	0.0872
Wheat	straw	(HR) 1.26	Forages / Fodders	88	10	0.143	0.0098
Barley	grain	(STMR) 0.920	Cereal Grains	88	70	0.732	0.0501
					Maximum Dietary Intake (mg/kg feed DM):	**2.15**	
						Maximum Dietary Burden (mg/kg bw/d):	**0.147**
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	20	1.275	0.0872
Wheat	straw	(STMR) 0.93	Forages / Fodders	88	10	0.106	0.0073
Barley	grain	(STMR) 0.920	Cereal Grains	88	70	0.732	0.0501
					Median Dietary Intake (mg/kg feed DM):	**2.11**	
						Median Dietary Burden (mg/kg bw/d):	**0.145**

Intakes >0.004 mg/kg bw/d are highlighted

Table B.7.4-10: Clopyralid dietary burden for poultry - turkey

Poultry - Turkey - EU							
Crop	Commodity	Residue (mg/kg)	RWCF Classification	DM (%)	Poultry - Turkey - EU - Diet (%)	Dietary Intake (mg/kg feed DM)	Dietary Burden (mg/kg bw/d)
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	20	1.275	0.0911
Barley	grain	(STMR) 0.920	Cereal Grains	88	60	0.627	0.0448
			Maximum Dietary Intake (mg/kg feed DM):			**1.90**	
					Maximum Dietary Burden (mg/kg bw/d):		**0.140**
Wheat	milled bypdts / bran	(STMR) 5.610	By-Products	88	20	1.275	0.0911
Barley	grain	(STMR) 0.920	Cereal Grains	88	60	0.627	0.0448
			Median Dietary Intake (mg/kg feed DM):			**1.90**	
					Median Dietary Burden (mg/kg bw/d):		**0.140**

Intakes >0.004 mg/kg bw/d are highlighted

For purposes of calculating anticipated livestock dietary burden, results from the new cattle feeding study and the new poultry feeding study were used rather than results from the earlier studies since residue levels were generally higher in the new than in the old studies.

In the case of tissues, it is thought that the short interval (< 6 hours) between final dosing and collection of samples along with the rapid depuration of clopyralid may be responsible, at least in part, for the higher residue values in the new studies.

Additionally, since there is no guidance on livestock diets, dry matter intake, or body weight for goats in the OECD guidance, it is proposed to extrapolate the results for sheep to goats.

Similarly, since there is no OECD guidance on equine, it is proposed to extrapolate the results from cattle to equine. For poultry (broiler, laying hens and turkey), the greatest dietary burden was calculated for laying hens. Therefore, it is proposed to use the dietary burden for laying hens to calculate anticipated residues across the poultry group.

Based on the dietary burden values calculated above together with results from the new cattle feeding study, anticipated clopyralid residues in cattle, sheep and swine were calculated and are presented in **Tables B.7.4-11, B.7.4-12, and B.7.4-13**, respectively.

As previously discussed, in the new cattle feeding study residue values were obtained separately for fat from each of three different sources based on their location in the body (subcutaneous, mesenteric, and perirenal fat). Additionally, a composite residue value for fat was calculated based on an average of the residue values for fat from each of the three sources. While the composite / average fat value could be used for calculation of anticipated residues and proposal of an MRL, the residue values for perirenal fat were used instead since the residues in fat from this source were generally greater than in fat from the two other sources. Use of perirenal fat for calculation of anticipated residues and proposal of MRLs in fat helps ensure that residues will not be underestimated and the MRLs will not be set too low.

The dietary burdens for cattle, sheep and swine were found to be near or below the lowest dose level (0.3x dose group, 0.451 mg/kg bw/day) and transfer factors based on residues at this dose level along with the calculated dietary burden were used to calculate anticipated residues for all commodities except meat (muscle) and milk. For muscle and milk, transfer factors were calculated based on residues from the 1x dose group (1.67 mg/kg bw/day) since residue were quantifiable at this dose level, but were <0.01 mg/kg at the 0.3x dose level.

STMR anticipated residues in livestock commodities were calculated based on median dietary burden values and mean residue levels from the cattle or poultry feeding study, while HR (maximum) anticipated residue values in livestock commodities were calculated based on maximum dietary burden values and maximum residue values from the cattle or poultry feeding study. Results from calculation of anticipated clopyralid residues in cattle, sheep, swine and poultry are presented in Tables **B.7.7.2-11, B.7.7.2-12, B.7.7.2-13, and B.7.7.2-14,** respectively.

Clopyralid MRLs were proposed taking into consideration the HR anticipated residue values in livestock commodities. Increased MRLs were proposed where HR values exceed the current EU MRLs, but in the instances where the HR values were below the current EU MRLs, it is proposed to retain the current MRL. A summary of results of anticipated residue calculation along with existing and proposed MRLs for clopyralid in livestock commodities is presented in **Table B.7.4-15**.

Table B.7.4-11: Summary of anticipated clopyralid residues inedible commodities derived from cattle.

Commodity	Dietary burden		Results of clopyralid cattle feeding study (2015)				STMR [b] (mg/kg)	HR [c] (mg/kg)	MRL proposal (mg/kg)
	Median (mg/kg bw/d)	Max. (mg/kg bw/d)	Dose Level [a] (mg/kg bw/d)	N	Clopyralid residues				
					Mean (mg/kg)	Max. (mg/kg)			
Residue definition for enforcement and risk assessment: clopyralid , its salts and conjugates, expressed as clopyralid									
Meat (muscle)	0.411	0.719	0.451	4	<0.01(0.007)	<0.01(0.007)	0.006	0.012	0.08 [d]
			1.670	4	0.023	0.029			
			8.571	4	0.104	0.113			
Fat (perirenal)	0.411	0.719	0.451	4	0.023	0.041	0.021	0.065	0.07
			1.670	4	0.109	0.264			
			8.517	4	0.519	1.048			
Liver	0.411	0.719	0.451	4	0.032	0.036	0.029	0.057	0.06 [d]
			1.670	4	0.112	0.145			
			8.517	4	0.502	0.560			
Kidney	0.411	0.719	0.451	4	0.429	0.606	0.391	0.966	1
			1.670	4	1.460	1.559			
			8.517	4	5.100	6.030			
Milk	0.411	0.719	0.451	36	ND	N/A	0.002	0.003	0.05 [d]
			1.670	36	<0.01 (0.008)	N/A			
			8.517	36	0.040	N/A			

[a] Dose levels from feeding study are displayed in units of mg/kg bw/d and shown here are the corresponding dose levels from the study when expressed as mg/kg dry feed (DM): 0.451 mg/kg bw/d corresponds to 16.7 mg/kg feed DM; 1.670 mg/kg bw to 56.6 mg/kg feed DM; and 8.571 mg/kg bw/d corresponds to 309.8 mg/kg feed DM.

[b] STMR calculated with use of a transfer factor based on median dietary burden and mean values from the feeding study using the lowest dose level (0.451 mg/kg bw/d) as follows: STMR residue = (Median dietary burden / Dose level in feeding study) x Mean residue value from feeding study. Note; for meat / muscle and milk residue values from the 1.670 mg/kg bw/day dose level were used for calculation since residues were <0.01 mg/kg at the 0.451 mg/kg bw/day dose level.

[c] HR calculated with use of a transfer factor based on maximum dietary burden and maximum values from the feeding study using the lowest dose level (1.646 mg/kg bw/d) as follows: HR residue = (Maximum dietary burden / Dose level in feeding study) x Maximum residue value from feeding study. Note; for meat / muscle and milk residue values from the 1.670 mg/kg bw/day dose level were used for calculation since residues were <0.01 mg/kg at the 0.451 mg/kg bw/day dose level.

[d] Propose to retain the current EU MRL, which is the value displayed, since the data presented here did not indicate an increase in the current EU MRL is needed

Table B.7.4-12: Summary of anticipated clopyralid residues in edible commodities derived from sheep.

Commodity	Dietary burden		Results of clopyralid cattle feeding study (2015)				STMR [b] (mg/kg)	HR [c] (mg/kg)	MRL proposal (mg/kg)
	Median (mg/kg bw/d)	Max. (mg/kg bw/d)	Dose Level [a] (mg/kg bw/d)	N	Clopyralid residues				
					Mean (mg/kg)	Max. (mg/kg)			
Residue definition for enforcement and risk assessment: clopyralid , its salts and conjugates, expressed as clopyralid									
Meat (muscle)	0.468	0.891	0.451	4	<0.01(0.007)	<0.01(0.007)	0.006	0.015	0.08 [d]
			1.670	4	0.023	0.029			
			8.571	4	0.104	0.113			
Fat (perirenal)	0.468	0.891	0.451	4	0.023	0.041	0.024	0.081	0.09
			1.670	4	0.109	0.264			
			8.517	4	0.519	1.048			
Liver	0.468	0.891	0.451	4	0.032	0.036	0.033	0.071	0.08
			1.670	4	0.112	0.145			
			8.517	4	0.502	0.560			
Kidney	0.468	0.891	0.451	4	0.429	0.606	0.445	1.197	1.5
			1.670	4	1.460	1.559			
			8.517	4	5.100	6.030			
Milk	0.468	0.891	0.451	36	ND	N/A	0.002	0.004	0.05 [d]
			1.670	36	<0.01 (0.008)	N/A			
			8.517	36	0.040	N/A			

[a] Dose levels from feeding study are displayed in units of mg/kg bw/d and shown here are the corresponding dose levels from the study when expressed as mg/kg dry feed (DM): 0.451 mg/kg bw/d corresponds to 16.7 mg/kg feed DM; 1.670 mg/kg bw corresponds to 56.6 mg/kg feed DM; 8.571 mg/kg bw/d corresponds to 309.8 mg/kg feed DM.

[b] STMR calculated with use of a transfer factor based on median dietary burden and mean values from the feeding study using the lowest dose level (1.646 mg/kg bw/d) as follows: STMR residue = (Median dietary burden / Dose level in feeding study) x Mean residue value from feeding study. Note; for meat / muscle and milk residue values from the 1.670 mg/kg bw/day dose level were used for calculation since residues were <0.01 mg/kg at the 0.451 mg/kg bw/day dose level.

[c] HR calculated with use of a transfer factor based on maximum dietary burden and maximum values from the feeding study using the lowest dose level (1.646 mg/kg bw/d) as follows: HR residue = (Maximum dietary burden / Dose level in feeding study) x Maximum residue value from feeding study. Note; for meat / muscle and milk residue values from the 1.670 mg/kg bw/day dose level were used for calculation since residues were <0.01 mg/kg at the 0.451 mg/kg bw/day dose level.

[d] Propose to retain the current EU MRL, which is the value displayed, since the data presented here did not indicate an increase in the current EU MRL is needed

Table B.7.4-13: Summary of anticipated clopyralid residues in swine edible commodities

Commodity	Dietary burden		Results of clopyralid cattle feeding study (2015)				STMR [b] (mg/kg)	HR [c] (mg/kg)	MRL proposal (mg/kg)
	Median (mg/kg bw/d)	Max. (mg/kg bw/d)	Dose Level [a] (mg/kg bw/d)	n	Clopyralid residues				
					Mean (mg/kg)	Max. (mg/kg)			
Residue definition for enforcement and risk assessment: clopyralid , its salts and conjugates, expressed as clopyralid									
Meat (muscle)	0.147	0.209	0.451	4	<0.01(0.007)	<0.01(0.007)	0.002	0.004	0.05 [d]
			1.670	4	0.023	0.029			
			8.571	4	0.104	0.113			
Fat (perirenal)	0.147	0.209	0.451	4	0.023	0.041	0.007	0.019	0.05 [d]
			1.670	4	0.109	0.264			
			8.517	4	0.519	1.048			
Liver	0.147	0.209	0.451	4	0.032	0.036	0.010	0.017	0.05 [d]
			1.670	4	0.112	0.145			
			8.517	4	0.502	0.560			
Kidney	0.147	0.209	0.451	4	0.429	0.606	0.140	0.281	0.3
			1.670	4	1.460	1.559			
			8.517	4	5.100	6.030			
Milk	---	---	0.451	36	ND	N/A	---	---	---
			1.670	36	<0.01 (0.008)	N/A			
			8.517	36	0.040	N/A			

[a] Dose levels from feeding study are displayed in units of mg/kg bw/d and shown here are the corresponding dose levels from the study when expressed as mg/kg dry feed (DM): 0.451 mg/kg bw/d corresponds to 16.7 mg/kg feed DM; 1.670 mg/kg bw to 56.6 mg/kg feed DM; and 8.571 mg/kg bw/d corresponds to 309.8 mg/kg feed DM.

[b] STMR calculated with use of a transfer factor based on median dietary burden and mean values from the feeding study using the lowest dose level (1.646 mg/kg bw/d) as follows: STMR residue = (Median dietary burden / Dose level in feeding study) x Mean residue value from feeding study. Note; for meat / muscle residue values from the 1.670 mg/kg bw/day dose level were used for calculation since residues were <0.01 mg/kg at the 0.451 mg/kg bw/day dose level.

[c] HR calculated with use of a transfer factor based on maximum dietary burden and maximum values from the feeding study using the lowest dose level (1.646 mg/kg bw/d) as follows: HR residue = (Maximum dietary burden / Dose level in feeding study) x Maximum residue value from feeding study. Note; for meat / muscle residue values from the 1.670 mg/kg bw/day dose level were used for calculation since residues were <0.01 mg/kg at the 0.451 mg/kg bw/day dose level.

[d] Propose to retain the current EU MRL, which is the value displayed, since the data presented here did not indicate an increase in the current EU MRL is needed

Table B.7.4-14: Summary of anticipated clopyralid residues in poultry commodities

Commodity	Dietary burden		Results of clopyralid cattle feeding study (2015)				STMR [b] (mg/kg)	HR [c] (mg/kg)	MRL proposal (mg/kg)
	Median (mg/kg bw/d)	Max. (mg/kg bw/d)	Dose Level [a] (mg/kg bw/d)	n	Clopyralid residues				
					Mean (mg/kg)	Max. (mg/kg)			
Residue definition for enforcement and risk assessment: clopyralid , its salts and conjugates, expressed as clopyralid									
Meat (muscle)	0.145	0.147	0.280	3	<0.01 (0.005)	0.011	0.003	0.006	0.05 [d]
			0.571	3	<0.01 (0.009)	0.011			
			1.086	3	<0.01 (0.005)	<0.01 (0.005)			
Fat	0.145	0.147	0.280	3	ND (<0.003)	ND (<0.003)	0.002	0.002	0.05 [d]
			0.571	3	ND (<0.003)	<0.01 (0.006)			
			1.086	3	ND (<0.003)	ND (<0.003)			
Liver	0.145	0.147	0.280	3	0.019	0.032	0.010	0.017	0.05 [d]
			0.571	3	0.023	0.033			
			1.086	3	0.017	0.027			
Eggs	0.145	0.147	0.280	30	ND (<0.003)	<0.01 (0.005)	0.002	0.003	0.05 [d]
			0.571	30	<0.01 (0.004)	0.011			
			1.086	30	0.011	0.018			

[a] Dose levels from feeding study are displayed in units of mg/kg bw/d and shown here are the corresponding dose levels from the study when expressed as mg/kg dry feed (DM): 0.28046 mg/kg bw/d corresponds to 4.90 mg/kg feed DM; 0.571 mg/kg bw corresponds to 10.26 mg/kg feed DM; 1.086 mg/kg bw/d corresponds to 19.82 mg/kg feed DM.

[b] STMR calculated with use of a transfer factor based on median dietary burden and mean values from the feeding study using the lowest dose level (0.280 mg/kg bw/d) as follows: STMR residue = (Median dietary burden / Dose level in feeding study) x Mean residue value from feeding study

[c] HR calculated with use of a transfer factor based on maximum dietary burden and maximum values from the feeding study using the lowest dose level (0.280 mg/kg bw/d) as follows: HR residue = (Maximum dietary burden / Dose level in feeding study) x Maximum residue value from feeding study

[d] Propose to retain the current EU MRL, which is the value displayed, since the data presented here did not indicate an increase in the current EU MRL is needed

Table B.7.4-15: Summary of clopyralid residues and proposed MRLs in livestock commodities

Livestock	Commodity	Clopyralid (mg/kg)			
		STMR	HR	Existing MRL#	Proposed MRL [a]
Bovine	Muscle	<0.01	0.012	0.08	0.08
	Fat	0.021	0.065	0.05*	**0.07**
	Liver	0.029	0.057	0.06	0.06
	Kidney	0.391	0.966	0.4	**1**
	Edible offal (except liver/kidney)	0.029	0.057	0.05*	**0.06**
	Others	0.029	0.057	0.05*	**0.06**
	Milk	<0.01	---	0.05*	0.05
Sheep	Muscle	<0.01	0.015	0.08	0.08
	Fat	0.024	0.081	0.05*	**0.09**
	Liver	0.033	0.071	0.06	**0.08**
	Kidney	0.445	1.197	0.4	**1.5**
	Edible offal (except liver/kidney)	0.033	0.071	0.05*	**0.08**
	Others	0.033	0.071	0.05*	**0.08**
	Milk	<0.01	---	0.05*	0.05
Goat	Muscle	<0.01	0.015	0.08	0.08
	Fat	0.024	0.081	0.05*	**0.09**
	Liver	0.033	0.071	0.06	**0.08**
	Kidney	0.445	1.197	0.4	**1.5**
	Edible offal (except liver/kidney)	0.033	0.071	0.05*	**0.08**
	Others	0.033	0.071	0.05*	**0.08**
	Milk	<0.01	---	0.05*	0.05
Swine	Muscle	<0.01	<0.01	0.05*	0.05
	Fat	<0.01	0.019	0.05*	0.05
	Liver	0.010	0.017	0.05*	0.05
	Kidney	0.140	0.281	0.05*	**0.3**
	Edible offal (except liver/kidney)	0.010	0.017	0.05*	0.05
	Others	0.010	0.017	0.05*	0.05
Equine	Muscle	<0.01	0.012	0.08	0.08
	Fat	0.021	0.065	0.05*	**0.07**
	Liver	0.029	0.057	0.06	0.06
	Kidney	0.391	0.966	0.4	**1**
	Edible offal (except liver/kidney)	0.029	0.057	0.05*	**0.06**
	Others	0.029	0.057	0.05*	**0.06**
	Milk	<0.01	---	0.05*	0.05
Poultry	Muscle	<0.01	<0.01	0.05*	0.05
	Fat	<0.01	<0.01	0.05*	0.05
	Liver	0.010	0.017	0.05*	0.05
	Kidney	0.010	0.017	0.05*	0.05
	Edible offal (except liver/kidney)	0.010	0.017	0.05*	0.05
	Others	0.010	0.017	0.05*	0.05
	Eggs	<0.01	<0.01	0.05*	0.05

[a] Proposed changes in MRLs are shown in bold # Reg. (EU) No 322/2012

B.7.4.1 Poultry

Results from a poultry feeding study with clopyralid were previously evaluated during Annex I inclusion / Active Approval (Clopyralid Draft Assessment Report, Vol.3, B7.8, February, 2005) and were considered acceptable following evaluation and peer review at the EU level. As indicated below, a new poultry feeding study was conducted to provide additional, updated information since the previous study (documented in two reports) was not conducted fully in compliance with current guidelines and was not conducted under GLP since the work was completed prior to implementation of GLP requirements. A summary concerning the justification for use of a different study to the study evaluated for the Active Approval / original Annex I inclusion follows:

Data point/Study	Rationale
6.4.1/3	The poultry feeding study (documented in two study reports) evaluated for the Active Approval is not a GLP study and was not conducted according to current guidelines. A new GLP study was conducted according to current guidelines to provide additional information for determination of the transfer of clopyralid residues from the diet to poultry eggs and tissues. Residue levels observed in the new study were generally similar to or greater than those observed in the original study. Considering the new study meets current guideline requirements, is GLP compliant and indicates residues at approximately equal or greater levels compared to the original study, it is proposed to use the new poultry study rather than the original study for purposes of determining the transfer of residues from the diet to eggs and poultry tissues in the Annex I Renewal evaluation.

Although a summary of the new poultry feeding study is presented in B.7.4.1-1, reference to the previous study evaluated during the Active Approval along with a brief presentation of key results are presented below for comparison with the new study.

Results from a poultry feeding study have previously been evaluated during Annex I inclusion / Active Approval (Clopyralid Draft Assessment Report, Vol.3, B7.8, February, 2005). These data were considered valid for decision making, but there were some unresolved questions on certain experimental details. A new poulty feeding study was conducted since some experimental details were not well documented in the earlier study and since the study did not meet GLP and did not follow current study guidelines because the study was conducted prior to initation of GLP requirements and development of study guidelines. Additionally, residues were generally found at equal ot greater levels in the new study than in the previous study. Therefore, it is proposed that the new poultry feeding study will be used to evaluate transfer of clopyralid residues from the diet to eggs and tissues.

In this new poultry feeding study, laying hens were dosed orally for 28 or 29 consecutive days via gelatine capsules containing clopyralid. Based on actual feed consumption during the period of dosing, the average following dose levels of were chosen to reflect clopyralid concentration in the diet (DM feed basis) 4.90 mg/kg (1x), 10.26 mg/kg (2x), 19.82 mg/kg (4x) and 50.50 mg/kg (10x). If the daily dosage of clopyralid is expressed on the basis of

bodyweight of the individual hens (mg/kg bw/day), the average dosage over the four weeks of dosing was 0.280 mg/kg bw/day, 0.571 mg/kg bw/day, 1.086 mg/kg bw/day and 2.779 mg/kg bw/day, for the 1x, 2x, 4x and 10x treatment groups, respectively.

No adverse treatment-related effects were observed on body weight, feed consumption or egg production. No treatment-related behavioural reactions or systemic signs of toxicity were noted and gross necropsies showed no treatment-related effects.

Residues of clopyralid in eggs and tissues were measured using an analytical method based on LC-MS/MS with a limit of detection (LOD) and limit of quantitation (LOQ) in each of the sample matrices of 0.003 mg/kg and 0.01 mg/kg, respectively. Overall average procedural recovery for clopyralid in all matrices ranged from 75% to 87%.

Results showed transfer of clopyralid residues, in aamounts exceeding the LOQ, into eggs in hens in groups receiving 2x, 4x and 10x doses. Residues of clopyralid in eggs appeared to reach a plateau within the first 7 days of dosing. Residues of clopyralid above the LOQ were found in muscle in hens from the 1x, 2x and 10x dose groups and in liver in hens from the 1x, 2x, 4x and 10x dose groups. Residues of clopyralid were below the LOQ in fat in hens from all dose groups.

Regression analysis shows a generally linear relationship between dosing level and residue in eggs, muscle and liver, although in liver and to a lesser degree in muscle variability in residue levels among replicates in the 1x, 2x and 4x dose levels affected the analysis. Regression analysis has not been performed in fat, as in all cases the residues were below the LOQ.

Depuration data generated using hens in the 10x dose level showed that residues of clopyralid declined rapidly following withdrawal of the test item from the hens' diet. All residues were below the LOQ by 3 days of depuration (i.e. 3 days after the end of the dosing period).

B.7.4.1.1 Dowco 290 and 2,4-D Chicken Feeding Study

Report	[redacted] 1974
Report title	Dowco 290 and 2,4-D Chicken Feeding Study
DAS Study / Report number	Study report no. TA-517
Guidelines	N/A
GLP	Non-GLP. This study was conducted prior to the effective date of the final rule, 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

This report provides the live-phase information for generation of samples that are analyzed and reported in report no. GH-C 819, which is listed below in B.7.4.1.2.

B.7.4.1.2 Residues of Dowco 290 (3,6-dichloropicolinic acid) in Tissues of Chickens Fed the Herbicide

Report	IIA 6.4/03 [redacted] 1975. Residues of Dowco 290 (3,6-dichloropicolinic acid) in Tissues of Chickens Fed the Herbicide. DAS Study / Report number: Study report no. GH-C 819
Guidelines	N/A
GLP	Non-GLP. This study was conducted prior to the effective date of the final rule, 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

A summary of residue results in eggs and tissues for clopyralid is provided in Table B.7.4.1/2-1

Table B.7.4.1.2-1: Residues of clopyralid in poultry from the livestock feeding study*

Dose Level (mg/kg bw/d) [mg/kg feed DM]	**Results in Poultry meat, fat, liver; eggs**	
	Mean (mg/kg)	**Max. (mg/kg)**
0.065 (1)	n.a	n.a
0.19 (3)	n.a	n.a
0.67 (10)	<0.05	<0.05

n.a Not analysed

***** Taken from: EFSA Journal 2011; 9(10): 2418. 'Reasoned Opinion. Modification of the existing MRLs for clopyralid in various commodities.'

B.7.4.1.3 AIR3 Clopyralid Livestock Feeding Study: Magnitude of Residue in Eggs, Muscle, Liver and Fat of Laying Hens

REFERENCE	[redacted] 2015; Summary of Clopyralid Livestock Feeding Study: Magnitude of Residue in Eggs, Muscle, Liver and Fat of Laying Hens; [redacted], UK; Lab Study No. CEMS-6921; DAS Study No. 150031; 09 September 2015; Unpublished
Guideline(s):	EC Council Directive 91/414/EEC – Working Document 7031/VI/95 rev. 4, OECD Guidance Document: Overview for Residue Chemistry Studies (As Revised in 2009), OECD Guidelines for the Testing of Chemicals, No. 505: Residues in Livestock (2007), EPA Residue Chemistry Test Guideline OPPTS 860.1480 – Meat/Milk/Poultry/Eggs, APVMA Residue Guideline No. 1 – Animal Transfer Studies
US EPA Guideline(s):	EPA Residue Chemistry Test Guideline OPPTS 860.1480 – Meat/Milk/Poultry/Eggs
Deviations:	None
Dates of work:	05 February 2015 to 03 July 2015
GLP status:	Yes

BACKGROUND INFORMATION

Proposed uses of clopyralid may result in residues in feed commodities that are consumed by laying hens. The purpose of this study is to determine the magnitude of residues of clopyralid in eggs and tissues (muscle, liver and fat) from laying hens dosed with clopyralid, for a minimum of 28 days. Information generated by this study on the transfer of residues from dietary intake to eggs, muscle, liver and fat is intended for use in supporting MRLs (tolerances) in these commodities, if needed.

Materials and Methods

Test Item(s)

ISO Common name:	Clopyralid
IUPAC chemical name	3,6-dichloropyridine-2-carboxylic acid
Test item (chemical/other name)	Clopyralid (technical grade)
Purity:	96.5% (w/w)

Methods

In-life Phase

Ninety six Hy Line laying hens were assigned for use in this study and were individually identified with unique leg bands. The animals were divided into 5 separate treatment groups. The birds were weighed prior to the start of dosing and were allocated to their treatment groups on the basis of bodyweight.

Hens were randomly allocated to treatment on the basis of bodyweight. Twelve hens (4 hens in each of 3 replicates) were allocated to each of the five treatment groups and a further 9 hens (3 hens in each of 3 replicates) to each of the four depuration slaughter dates in the 10x group. Although randomly assigned to a treatment, the hens were allocated to treatment groups to produce groups of birds with similar average hen weight and egg production at the start of dosing.

One treatment group of 12 hens was an untreated control group, which was dosed by adding acetone only to the feed in the gelatine capsules. The remaining groups were treated with clopyralid, targeted at a nominal dose equivalent to a concentration in the animals' diet (on a dry matter (DM) basis) of:

5.0 mg/kg clopyralid (1x, 12 hens)

10.0 mg/kg clopyralid (2x, 12 hens)

20.0 mg/kg clopyralid (4x, 12 hens)

50.0 mg/kg clopyralid (10x, 48 hens)

The animals were dosed for 28 or 29 consecutive days. Thirty six of the hens in the 10x treatment group were used to generate depuration data. At the end of the dosing period, they were transferred to the control diet to measure the decline in residues following withdrawal of the test item from the diet.

Table B.7.4.1.3-1: Description of livestock used in the feeding study

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Laying hens	Hy Line	Approx. 33 weeks on arrival.	Average body weight by dose group prior to the start of dosing was 1.826 – 1.847	All hens selected for use in the study were examined and identified as fit, healthy and suitable for use in the study prior to dosing.	Animals were housed in environmentally-controlled rooms in triangular pens under artificial lighting, with ventilation provided by computer-controlled extractor fans. Animals within each treatment group were housed adjacent to one another with beak-to-beak contact possible. Where hens from different treatment groups were housed next to each other, contact was prevented by a barrier.

All hens were offered a layer mash diet to appetite. The layer mash was composed primarily of ground wheat, ground barley and protein concentrate from soya meal and also contained minerals and vitamins. Any uneaten mash from the previous day was weighed and discarded. Fresh mash was weighed into the feeder, which was then returned to the pen. A sample of the layer mash was taken each week and used for dry-matter analysis.

Dosage Rates

Potential dietary intake of residues for poultry used for selection of the 1x and 2x dose levels (Group B and Group C, respectively) took into consideration relevant crop residue data for the EU and US. Dietary burden for poultry in the EU was calculated by EFSA when evaluating the modification of MRLs for clopyralid in various commodities. In this evaluation median and maximum dietary burden values for poultry were calculated at 0.377 mg/kg bw/day and 0.439 mg/kg bw/day, respectively. Based on a standard body weight of 1.9 kg and daily feed consumption (dry feed / DM basis) of 120 g/day, the median and maximum dietary burden expressed in dry feed (DM) is 5.97 mg/kg and 6.98 mg/kg, respectively.

Dietary burden for clopyralid in poultry in the US was calculated using Maximum Reasonably Balanced Diet (MRBD) methodology as documented by US EPA in the revised OPPTS 860.1000 "Table 1 Feedstuffs, June, 2008", except that the results were expressed on a dry feed (DM) basis rather than an 'as fed' basis in order to provide greater consistency with dietary burden calculations in other regions of the world that are determined on a dry feed (DM) basis. Based on the US tolerances for clopyralid and the MRBD methodology, except with use of dry matter content, a dietary burden for poultry of 9.37 mg/kg feed was calculated. Feed commodities and their dietary contributions that result in this dietary burden are summarized in Table B.7.4.1/3-3.

Table B.7.4.1.3-2: Test animal dietary regimen

Composition of diet (fresh weight or as-fed basis)	Feed consumption (kg/day) – Average consumption of feed (dry weight basis) during the dosing period	Water	Acclimation, dosing and withdrawal period
Layer mash fed to appetite. The layer mash was composed primarily of ground wheat, ground barley and protein concentrate from soya meal and also contained minerals and vitamins. Average dry matter content of the feed was 87.9% during dosing period.	0x (A) Control group: 0.1027 1x (Dose Group B): 0.1073 2x (Dose Group C): 0.1039 4x (Dose Group D): 0.1023 10x (Dose Group E): 0.1039	Water offered *ad libitum*.	A 20-day acclimation period was used in this study. Dietary regime was the same through all phases of the study.

Dose Capsule Preparation and Administration

Solutions containing the test item were prepared in acetone at CEMAS on a weekly basis at a range of specified concentrations. The dosing solutions were prepared by dissolving the appropriate amount of the test substance in acetone. The prepared dosing solutions were transferred to colour-coded glass bottles and were stored in a refrigerator set to 4°C. Samples of each week's dosing solutions were analysed to confirm the concentrations of the test substance. In addition, samples of the second week's dosing solutions were reanalysed after storage at ca. 4°C for approximately 7 and 14 days to confirm stability. The volume of dosing solution added to a given hen's dosing capsules was adjusted individually based on that animal's level of feed consumption on a DM (dry matter) basis during the acclimatisation period, in order to provide a closer match to the target dose level in that animal's diet. The calculated dry matter intake (based on measured feed intake during a four-day period in the week prior to beginning dosing) was the mean measured dry matter intake plus 10%, or the maximum dry matter intake, whichever was smaller. The treatment doses were administered to the hens once daily in their individual pens. For each hen, the appropriate volume of test item solution was added to a size 5 gelatine capsule containing ground diet. The capsules were filled using a Profill 100 capsule-making machine and a calibrated positive-displacement pipette was used to add the correct amount of dosing solution to the capsule. Once spiked, each capsule was sealed and placed in a plastic bag containing the hen's identification number.

Each hen was removed from its pen. The capsule was placed in a capsule dosing applicator and ejected down the bird's throat. A check was made to ensure that the capsule had passed the hen's throat before returning the bird to its pen.

Table B.7.4.1.3-3: Estimated dietary intake of residues – used for determining 1x dose level in feeding study Clopyralid Dietary Burden in Poultry – US

Category /Feed Commodity	US Tolerance (mg/kg)	% DM (Dry Matter)	% Poultry Diet (maximum)	% Poultry Diet (used)	Dietary burden (mg/kg feed (DM))
Carbohydrate Conc.	---	---	**75**	---	---
Grain, barley	**3**	**88**	**75**	**25**	**0.85**
Milled by-products, wheat	**12**	**88**	**50**	**50**	**6.82**
Protein Conc.	---	---	**25**	---	---
Flax, meal	**6**	**88**	**20**	**20**	**1.36**
Rapeseed (canola), meal	**6**	**88**	**15**	**5**	**0.34**
Total:				**100**	**9.37**

Table B.7.4.1.3-4 Test dosing regime oft he birds

Treatment group	Treatment Type	Average clopyralid dose expressed as level in diet – dry feed basis (mg/kg) [a, b]	Average clopyralid dose expressed as mg/kg bw/day [a, c]	N rate	Vehicle [c]	Timing/ duration
Untreated control (Dose Group A)	**Oral – once per day**	**0**	**0**		**Gelatine capsule**	**30 days feed only added**
Dose Group B (1x; 4.9 mg/kg bw/d)	Oral – once per day	4.90	0.280	1.9	Gelatine capsule	29 days
Dose Group C (2x; 10.26 mg/kg bw/d)	Oral – once per day	10.26	0.571	3.9	Gelatine capsule	28 days
Dose Group D (4x; 19.82 mg/kg bw/d)	Oral – once per day	19.82	1.086	7.4	Gelatine capsule	29 days
Dose Group E (10x; 50.5 mg/kg bw/d)	Oral – once per day	50.50	2.779	18.9	Gelatine capsule	28 days

a *Average for treatment group over dosing period*

b *Calculated based on average daily dry matter consumption of 102.7 g, 107.3 g, 103.9 g, 102.3 g and 106.2 g for Dose Groups A, B, C, D and E, respectively.*

c *Average clopyralid dose expressed as mg/kg body weight / day based on amount of clopyralid administered to each animal and the associated body weights. Test substance added to gelatine capsule containing ground diet and administered once per day.*

On specified days, samples of eggs were collected for analysis to quantify residues of clopyralid.

Eggs were collected from each bird on Days 0, 1, 3, 5, 7, 10, 14, 16, 18, 20, 22, 24, 27, 28, 29, 30, 31, 32, 34, 36, 39, 41, 45, 47 and 48.

For each replicate, eggs were collected from each bird where available and pooled. The eggs from each treatment replicate collected on a given Study Day were cracked into a clean screw-top bottle and the bottle was shaken vigorously to mix the contents. Two sub-samples (R1 and R2) were taken in screw-cap HDPE bottles and transferred to a freezer set to maintain a temperature of less than -18°C.

All hens were sacrificed within 6 hours of administration of the final dose, with the exception of the hens used to collect depuration data. Tissue samples were collected from each hen immediately after slaughter. For all tissues, two replicate specimens (R1 and R2) of approximately 500 g each (or as available) were taken. Skeletal muscle specimens comprised approximately equal pieces of leg and breast muscle. The whole liver with the gall bladder removed was collected from each bird. Abdominal fat samples were collected from each bird when available and pooled to produce two replicate samples (R1 and R2) of approximately 50 g each. Immediately upon collection, the samples were stored in a polystyrene box containing ice packs to reduce the temperature and then transferred to a freezer set to maintain a temperature of less than -18°C within three hours of collection.

Table B.7.4.1.3-5 Sample collection

Eggs collected	**Amount of eggs produced during normal production**	**Interval from last dose to sacrifice (days)**	**Tissues harvested and analysed**
<u>Dosing period:</u> Day before dosing (study day 0) and dosing (study) days 1, 3, 5, 7, 10, 14, 16, 18, 20, 22, 24, 27, 28 and 29 <u>Depuration period:</u> Eggs collected on study days 28, 29, 30, 31, 32, 34, 36, 39, 41, 45, 47 and 48	Average daily egg production was close to one egg per day per hen throughout the study. (Weekly average across Dose Groups during the dosing period was 92% to 100% where 100% equates to one egg per hen per day).	Less than 6 hours Depuration of residues in tissues also evaluated at 3, 7, 14 and 21 days after administration of final dose.	Muscle – approx. 0.5 kg Liver – approx. 0.05 kg Fat – approx. 0.05 kg

Sampling, Handling and Preparation

All tissue samples were double-wrapped in polythene bags. Immediately upon collection, the samples were stored in a polystyrene box containing ice packs to reduce the temperature and then transferred to a freezer set to maintain a temperature of less than -18°C within four hours of collection. All samples remained frozen during shipment and were stored in temperature-monitored freezers at ≤-18°C on arrival.

Complete samples of muscle, liver and fat were homogenised in a Robot Coupe processor, in the presence of dry ice in the case of fat samples. After appropriate mixing of each sample, samples were transferred to HDPE plastic containers. No preparation was required for egg samples.

Analytical Phase

Residues of clopyralid were determined according to the validated analytical method described in Dow AgroSciences Study Number 120483 (Shaffer, S., 2012. ABC Laboratories, Inc. Study Number 68447). The method principle is listed below.

Residues of clopyralid were extracted from egg and tissue samples with 2.5 N NaOH, with heating at approximately 105°C for a minimum of 2 hours. An additional clean-up for poultry liver was effected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract was acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean up and elution with DCM. After removal of the DCM using nitrogen blow down, the sample was reconstituted in acetonitrile:water 10:90 (v/v) containing 0.1% formic acid. Residues of clopyralid were measured by LC-MS/MS with negative-ion electrospray ionization.

The limit of detection (LOD) and limit of quantitation (LOQ) of clopyralid in all matrices were 0.003 mg/kg and 0.01 mg/kg, respectively.

Results and Discussion

Animal Health

Treatment did not appear to affect body weights, feed intake or egg production. There were no clinical observations made during the study that were attributed to treatment.

Storage Stability

The maximum intervals of frozen storage for samples between collection and extraction for analysis along with supporting frozen storage stability study references are presented in Table B.7.4.13-6. The maximum interval between collection of samples and extraction for analysis of clopyralid in eggs, muscle, liver and fat was 119, 40, 38 and 39 days, respectively. In an earlier study, residues of clopyralid in chicken eggs

were found to remain stable in frozen storage at approximately -20°C for a period of at least 569 days, which was the maximum storage interval evaluated ([redacted] 2004. Dow AgroSciences Study ID 020120.01). Frozen storage stability of clopyralid in chicken muscle, liver and fat was evaluated as part of this poultry feeding study. Results of this evaluation are presented in Table B.7.4.1.3-7 and show that clopyralid residues remain stable in frozen storage for a period of at least 43 days, which was the maximum period of storage evaluated in this study. Therefore, results of the frozen storage stability evaluations confirm stability of clopyralid in poultry matrices for the maximum periods of frozen storage encountered in this study.

Table B.7.4.1.3-6: Summary of sample frozen storage conditions

Matrix	Storage temperature (°C)	Maximum storage duration (days)	Interval of demonstrated frozen storage stability (days)
Eggs	**≤-18**	**119**	**569** [a]
Muscle	**≤-18**	**40**	**43** [b]
Liver	**≤-18**	**38**	**43** [b]
Fat	**≤-18**	**39**	**43** [b]

a [redacted] 2004. "Frozen Storage Stability of Clopyralid in Beef Muscle, Liver, Kidney, Milk and Chicken Egg". Dow AgroSciences Study ID 020120.01, an unpublished report of Dow AgroSciences LLC.

b Data from this poultry feeding study (DAS Study ID 150031, summarized below in Table B.7.4.1/3-7).

Table B.7.4.1.3-7: Frozen Storage Stability of Clopyralid in Chicken Muscle, Liver and Fat

Matrix	Storage Interval	Storage Recovery (%)*
Muscle	0 days	90, 81, 84
	43 days	92, 89, 91
Liver	0 days	80, 75, 82
	43 days	74, 74, 70
Fat	0 days	73, 75, 75
	43 days	77, 78, 76

* Storage recoveries are shown as a percentage of the target value. Storage recoveries are not corrected for the procedural recovery of the batch.

Analytical Method Performance

The efficiency of the analytical method was determined at the time of analysis of each set of samples by fortifying aliquots of the appropriate control matrix with analyte and analysing according to the method. Each analytical batch contained at least three procedural recovery samples fortified at the LOQ of 0.01 mg/kg and at least two procedural recovery samples fortified at a higher level. An unfortified control matrix, reagent blank, and control matrix fortified at the limit of detection (LOD) were included in each set as well. The LOD recovery samples were analysed only to demonstrate observable peaks at the LOD level. Therefore, the percent recovery of analyte is not reported for these samples. Recoveries are corrected for any apparent residue in the corresponding control sample. A summary of procedural recovery results by matrix and by fortification level is presented in Table B.7.4.1.3-8. Overall average procedural recovery for clopyralid in all matrices (eggs, muscle, liver and fat) ranged from 75% to 87%.

Residue Results – Eggs and Tissues

Residue results in eggs and tissues are discussed briefly below. Additionally, data from this poultry feeding study are presented in several Tables and Figures that follow. Residues of clopyralid in eggs during the period when the test material was being administered to the hens are presented in Table B.7.4.1/3-9, while residues of clopyralid in eggs during the depuration period when dosing with the test material has ended are presented in Table B.7.4.1/3-10. Residues of clopyralid in tissues are presented in Table B.7.4.1/3-11. A summary of residue results for clopyralid in eggs and tissues is presented in Table B.7.4.1/3-12. In Table B.7.4.1/3-12, the period over which egg residue data is summarized is Study Day 6 to the end of the dosing period (Study Day 28 or 29) since this is the period of time during which residues in eggs have reached a plateau level. Residues of clopyralid in eggs during the dosing period are shown in Figure B.7.4.1/3-1. Residues of clopyralid in eggs and tissues during the depuration period are shown in Figure B.7.4.1/3-2 to Figure B.7.4.1/3-4. Linear regression results for clopyralid residues in eggs, muscle and liver by dose level of clopyralid are presented in Figure B.7.4.1/3-5, Figure B.7.4.1/3-6 and Figure B.7.4.1/3-7, respectively.

Residues in Eggs

Residues of clopyralid eggs during the dosing period were below the LOD (<0.003 mg/kg) or LOQ (<0.010 mg/kg) in the 1x dosing group and were below or above the LOQ in the 2x and 4x dosing groups. Residues above the LOQ were found in the eggs from the 10x dosing group and appeared to reach a plateau in within the first 7 days of dosing. There was a generally linear relationship between the residue and the level of clopyralid test item in the hens' diet. In the depuration animals, average residues of clopyralid in the 10x treatment group fell to below the LOQ by Day 30 of the study (3 days after withdrawal of the dose from the hens' diet).

Residues in Muscle

Residues of clopyralid above the LOQ were found in the muscle of one replicate in the 1x and 2x dosing groups and in all replicates in the 10x dosing group. There was a generally linear relationship between the residue and the level of clopyralid test item in the hens' diet. Residues of clopyralid in the muscle of the depuration animals fell to below the LOQ by 3 days after withdrawal of the test item from the hens' diet

Residues in Liver

Residues of clopyralid were found in the liver of treated hens in the 1x, 2x, 4x and 10x treatment groups. There was a linear relationship between the residue and the level of clopyralid test item in the hens' diet. Residues of clopyralid in the liver of the depuration animals fell to below the LOQ by 3 days after withdrawal of the test item from the hens' diet

Residues in Fat

No residues of clopyralid above the LOQ were found in the fat from birds in any treatment group.

Table B.7.4.1.3-8: Summary of procedural recoveries for clopyralid

Matrix	Fortification level (mg/kg)	n	Recovery (%)	Mean recovery (%)	RSD (%)
Eggs	0.01	54	77, 81, 82, 89, 89, 89, 88, 92, 88, 77, 84, 78, 79, 81, 75, 73, 79, 80, 75, 73, 77, 81, 76, 69, 75, 75, 72, 80, 82, 80, 78, 77, 79, 76, 74, 71, 81, 86, 97, 84, 78, 80, 82, 77, 78, 76, 71, 79, 72, 73, 73, 71, 74, 72	79	7.5
	0.10	30	77, 65, 77, 78, 74, 75, 78, 80, 74, 77, 84, 84, 88, 82, 79, 79, 76, 78, 80, 85, 80, 82, 83, 77, 75, 74, 76, 74, 80, 70	78	5.9
	1.0	6	73, 71, 78, 76, 81, 77	76	4.7
Muscle	0.01	6	83, 87, 82, 83, 83, 89	85	3.3
	0.10	10	88, 85, 85, 91, 89, 89, 89, 86, 89, 87	88	2.3
Liver	0.01	6	77, 74, 82, 74, 74, 77	76	4.1
	0.10	10	70, 70, 72, 75, 77, 72, 75, 78, 72, 78	74	4.2
Fat	0.01	6	76, 68, 68, 82, 78, 80	75	8.0
	0.10	10	68, 71, 73, 75, 74, 83, 82, 72, 79, 81	76	6.8

Table B.7.4.1.3-9: Residue data for clopyralid in eggs from hen feeding study with clopyralid during dosing

Dose group *mg/kg*	Rep	Depur -atino Days	Days of Dosing													
			0	2	4	6	8	11	15	17	19	21	23	25	28	29	30
0	1	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	ND
0	2	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.012	ND	ND	-	-	ND
0	3	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	ND
4.90	1	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	ND	-
4.90	2	-	ND	ND	ND	ND	ND	ND	(0.004)	ND	ND	ND	ND	(0.004)	-	ND	-
4.90	3	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	(0.005)	-	(0.003)	-
10.26	1	-	ND	ND	ND	(0.004)	(0.004)	(0.005)	(0.004)	(0.004)	(0.004)	(0.009)	(0.004)	(0.005)	(0.004)	-	-
10.26	2	-	ND	ND	(0.004)	(0.003)	(0.005)	(0.004)	ND	ND	ND	(0.006)	(0.003)	(0.004)	(0.004)	-	-
10.26	3	-	N7D	ND	(0.009)	(0.004)	(0.005)	(0.005)	(0.004)	(0.004)	(0.005)	(0.007)	(0.005)	(0.006)	0.011	-	-
19.82	1	-	ND	(0.005)	(0.006)	0.010	0.012	(0.009)	(0.005)	(0.009)	(0.007)	0.015	(0.008)	0.012	-	(0.009)	-
19.82	2	-	ND	(0.004)	(0.006)	(0.007)	0.010	(0.008)	(0.004)	(0.009)	(0.007)	0.017	(0.005)	(0.009)	-	0.010	-
19.82	3	-	ND	(0.008)	0.013	0.014	0.015	0.012	0.011	0.017	0.012	0.015	0.011	0.012	-	0.018	-
50.50	1	-	ND	0.012	0.017	0.029	0.014	0.024	0.014	0.019	0.020	0.015	0.025	0.024	0.042	-	-
50.50	2	-	ND	0.011	0.019	0.020	0.013	0.017	0.013	0.015	0.015	0.011	0.013	0.023	0.017	-	-

Dose group *mg/kg*	Rep	Depur -atino Days	Days of Dosing														
			0	2	4	6	8	11	15	17	19	21	23	25	28	29	30
50.50	3	-	ND	0.017	0.019	0.035	0.024	0.023	0.016	0.028	0.017	0.016	0.027	0.025	0.046	-	-
50.50	1	3	ND	-	-	-	-	-	0.010	-	-	-	0.021	-	-	0.021	
50.50	2	3	ND	-	-	-	-	-	0.017	-	-	-	0.021	-	-	0.044	
50.50	3	3	ND	-	-	-	-	-	0.012	-	-	-	0.014	-	-	0.021	
50.50	1	7	ND	-	-	-	-	-	0.015	-	-	-	0.021	-	-	0.033	
50.50	2	7	ND	-	-	-	-	-	0.017	-	-	-	0.023	-	-	0.017	
50.50	3	7	ND	-	-	-	-	-	0.031	-	-	-	0.020	-	-	0.037	
50.50	1	14	ND	-	-	-	-	-	(0.010)	-	-	-	0.011	-	-	0.017	
50.50	2	14	ND	-	-	-	-	-	0.022	-	-	-	0.033	-	-	0.033	
50.50	3	14	ND	-	-	-	-	-	0.021	-	-	-	0.030	-	-	0.031	
50.50	1	21	ND	-	-	-	-	-	0.023	-	-	-	0.023	-	-	0.024	
50.50	2	21	ND	-	-	-	-	-	0.016	-	-	-	0.030	-	-	0.032	
50.50	3	21	ND	-	-	-	-	-	0.019	-	-	-	0.022	-	-	0.027	

a Average clopyralid concentration in the diet (dry feed (DM) basis) for Dose Groups A, B, C, D and E was: 0 mg/kg, 4.90 mg/kg, 10.26 mg/kg, 19.82 mg/kg and 50.50 mg/kg, respectively. If expressed on the basis of bodyweight, the average dosage for Dose Groups A, B, C, D and E was: 0.000 mg/kg bw/day, 0.280 mg/kg bw/day, 0.571 mg/kg bw/day, 1.086 mg/kg bw/day and 2.779 mg/kg bw/day, respectively.

Table B.7.4.1.3-10: Residue data for clopyralid in eggs from hen feeding study with clopyralid during depuration

Dose	Rep	Depur-	Days after final dose (study day)											
			0 (27)	1 (28)	3 (30)	4 (31)	5 (32)	7 (34)	9 (36)	12 (39)	14 (41)	18 (45)	20 (47)	21 (48)
E	1	3	0.021	0.013	(0.005)	-	-	-	-	-	-	-	-	-
E	2	3	0.044	0.014	ND	-	-	-	-	-	-	-	-	-
E	3	3	0.021	(0.007)	ND	-	-	-	-	-	-	-	-	-
E	1	7	0.033	-	(0.009)	(0.003)	ND	(0.004)	-	-	-	-	-	-
E	2	7	0.017	-	ND	ND	ND	ND	-	-	-	-	-	-
E	3	7	0.037	-	(0.006)	(0.004)	ND	ND	-	-	-	-	-	-
E	1	14	0.017	-	ND	ND	ND	ND	ND	ND	ND	-	-	-
E	2	14	0.033	-	(0.008)	(0.003)	(0.003)	ND	ND	ND	ND	-	-	-
E	3	14	0.031	-	(0.008)	(0.010)	(0.006)	(0.005)	(0.006)	(0.007)	(0.004)	-	-	-
E	1	21	0.024	-	(0.004)	ND	ND	ND	ND	ND	ND	ND	ND	ND
E	2	21	0.032	-	(0.003)	ND	(0.004)	ND	ND	ND	ND	ND	ND	ND

Dose	Rep	Depur-	Days after final dose (study day)											
			0 (27)	1 (28)	3 (30)	4 (31)	5 (32)	7 (34)	9 (36)	12 (39)	14 (41)	18 (45)	20 (47)	21 (48)
E	3	21	0.027	-	(0.009)	(0.003)	ND	(0.004)	(0.006)	ND	(0.004)	ND	ND	ND

[a] Average clopyralid concentration in the diet (dry feed (DM) basis) for Dose Group E was 50.50 mg/kg. If expressed on the basis of bodyweight, the average dosage for Dose Group E was 2.779 mg/kg bw/day.

Table B.7.4.1.3-11: Residue data for clopyralid in tissues from hen feeding study with clopyralid upon completion of dosing and during depuration

Dose group [a]	Replicate	Days of Dosing	Days after last dose	Residues(mg/kg)		
				Muscle	Liver	Fat
A	1	30	-	ND	ND	ND
A	2	30	-	ND	ND	ND
A	3	30	-	ND	ND	ND
B	1	29	-	0.011	0.032	ND
B	2	29	-	(0.004)	0.013	ND
B	3	29	-	ND	0.011	ND
C	1	28	-	0.011	0.033	(0.006)
C	2	28	-	(0.006)	0.017	ND
C	3	28	-	(0.009)	0.018	ND
D	1	29	-	(0.008)	0.027	ND
D	2	29	-	(0.004)	0.012	ND
D	3	29	-	(0.004)	0.011	ND
E	1	28	-	0.013	0.032	(0.005)
E	2	28	-	0.016	0.032	ND
E	3	28	-	0.017	0.034	(0.005)
E	1	28	3	ND	ND	ND
E	2	28	3	ND	ND	ND
E	3	28	3	ND	ND	ND

Dose group [a]	Replicate	Days of Dosing	Days after last dose	Residues(mg/kg)		
				Muscle	Liver	Fat
E	1	28	7	ND	ND	ND
E	2	28	7	ND	ND	ND
E	3	28	7	ND	(0.004)	ND
E	1	28	14	ND	ND	ND
E	2	28	14	ND	(0.005)	(0.004)
E	3	28	14	ND	(0.004)	ND
E	1	28	21	ND	ND	ND
E	2	28	21	ND	ND	ND
E	3	28	21	ND	ND	ND

[a] Average clopyralid concentration in the diet (dry feed (DM) basis) for Dose Groups A, B, C, D and E was: 0 mg/kg, 4,90 mg/kg, 10.26 mg/kg, 19.82 mg/kg and 50.50 mg/kg, respectively. If expressed on the basis of bodyweight, the average dosage for Dose Groups A, B, C, D and E was: 0.000 mg/kg bw/day, 0.280 mg/kg bw/day, 0.571 mg/kg bw/day, 1.086 mg/kg bw/day and 2.779 mg/kg bw/day, respectively.

Table B.7.4.1.3-12: Summary of residue data for clopyralid from hen feeding study with clopyralid

Matrix	Feeding level - dry feed (mg/kg) [a]	Residue levels (mg/kg)					
		n	Min.	Max.	Median (STMdR)	Mean (STMR)	Std. Dev.
Eggs Days 6 - 29	4.90	30	ND	(0.005)	ND	ND	0.001
	10.26	30	ND	0.011	(0.004)	(0.004)	0.002
	19.82	30	(0.004)	0.018	0.010	0.011	0.004
	50.50	54	0.010	0.046	0.020	0.021	0.008
Muscle	4.90	3	ND	0.011	(0.004)	(0.005)	0.006
	10.26	3	(0.006)	0.011	(0.009)	(0.009)	0.003
	19.82	3	(0.004)	(0.008)	(0.004)	(0.005)	0.002
	50.50	3	0.013	0.017	0.016	0.015	0.002
Liver	4.90	3	0.011	0.032	0.013	0.019	0.012
	10.26	3	0.017	0.033	0.018	0.023	0.009
	19.82	3	0.011	0.027	0.012	0.017	0.009
	50.50	3	0.032	0.034	0.032	0.033	0.001

Matrix	Feeding level - dry feed (mg/kg) [a]	Residue levels (mg/kg)					
		n	Min.	Max.	Median (STMdR)	Mean (STMR)	Std. Dev.
Fat	4.90	3	ND	ND	ND	ND	0.000
	10.26	3	ND	(0.006)	ND	ND	0.003
	19.82	3	ND	ND	ND	ND	0.000
	50.50	3	ND	(0.005)	(0.005)	(0.003)	0.003

[a] Average clopyralid concentration in the diet expressed on a dry feed (DM) basis.

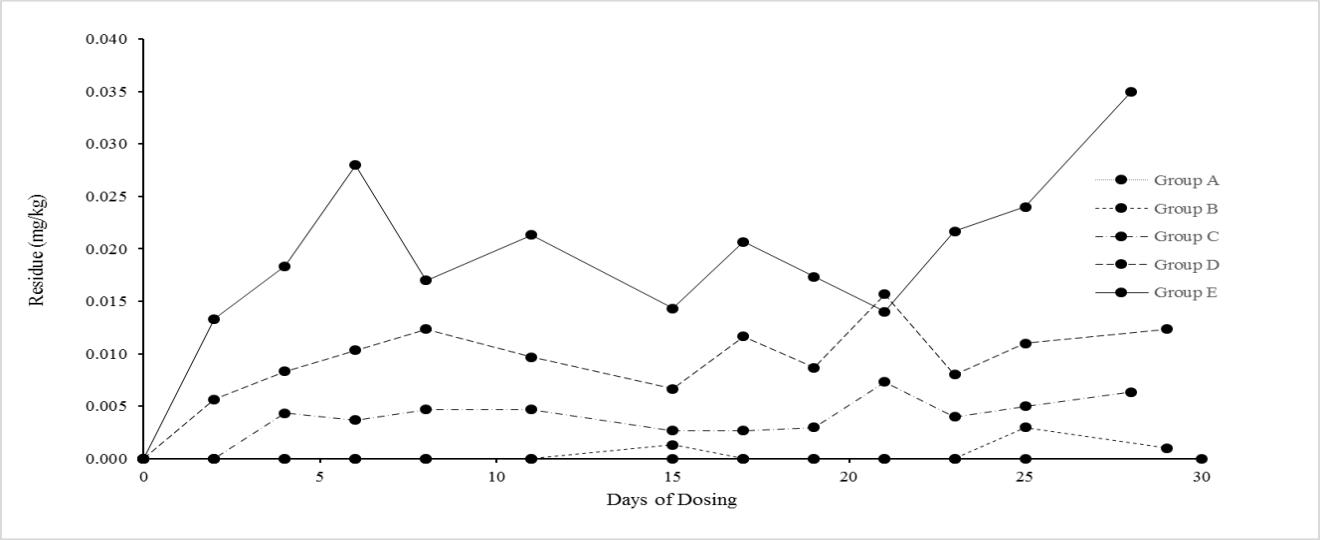


Figure B.7.4.1.3-1: Average residues of clopyralid in eggs during 28- or 29-day dosing period

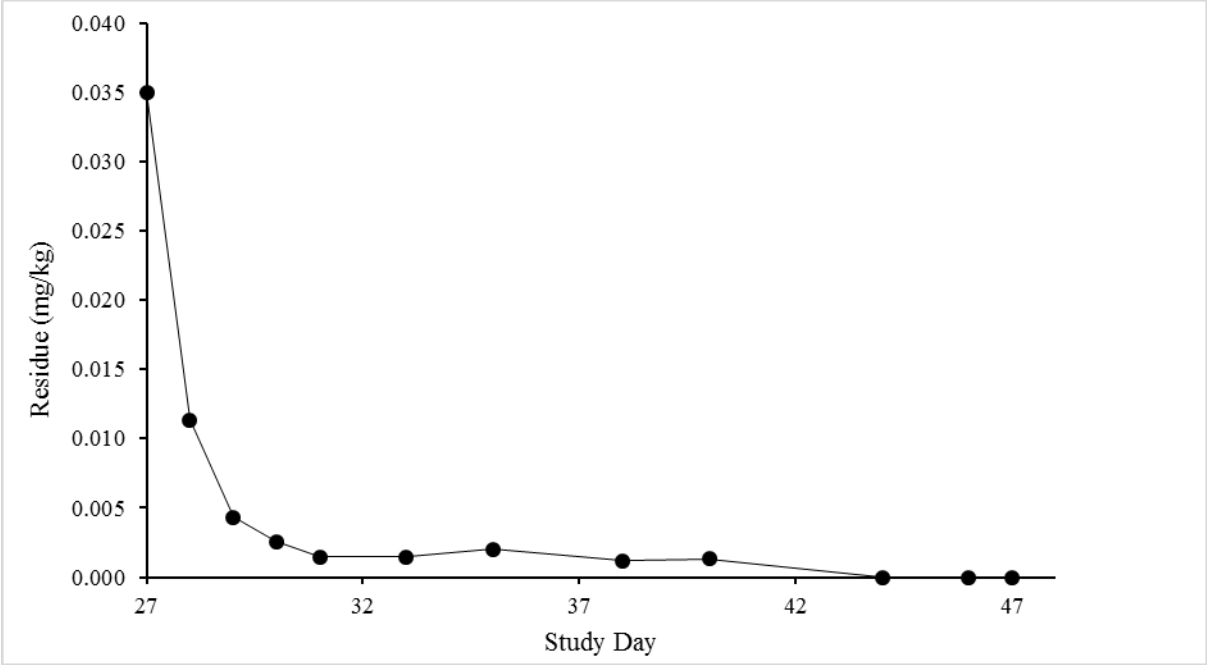


Figure B.7.4.1.3-2: Average residues of clopyralid in eggs during depuration period

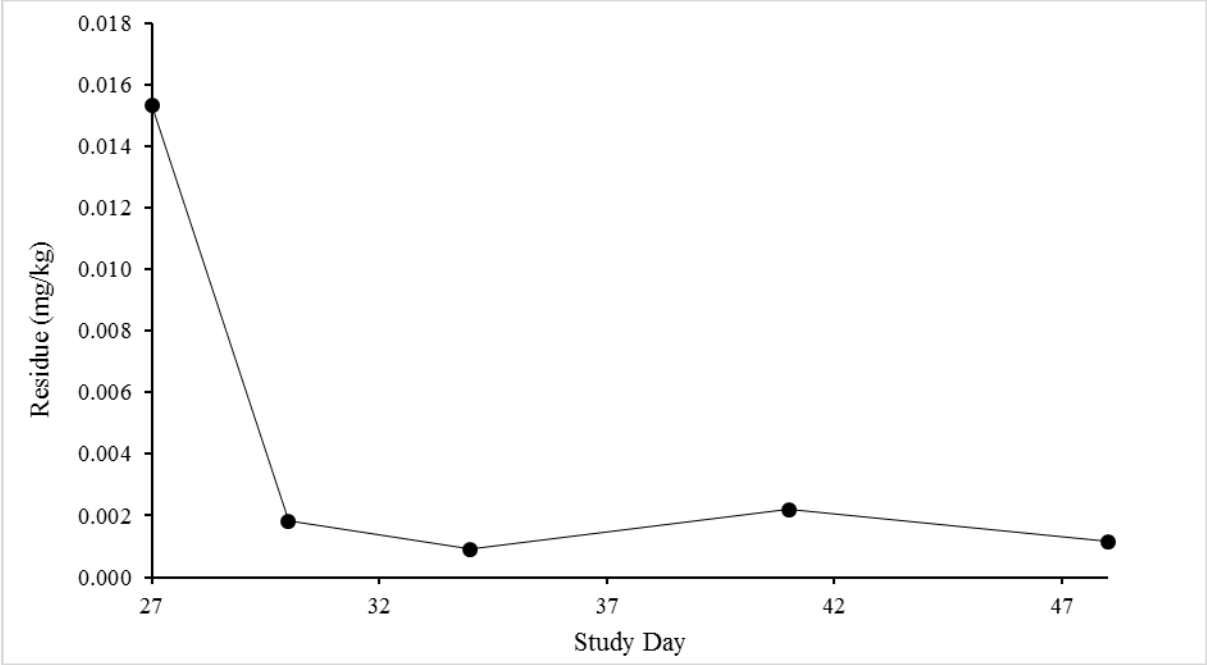


Figure B.7.4.1.3-3: Average residues of clopyralid in muscle during depuration period

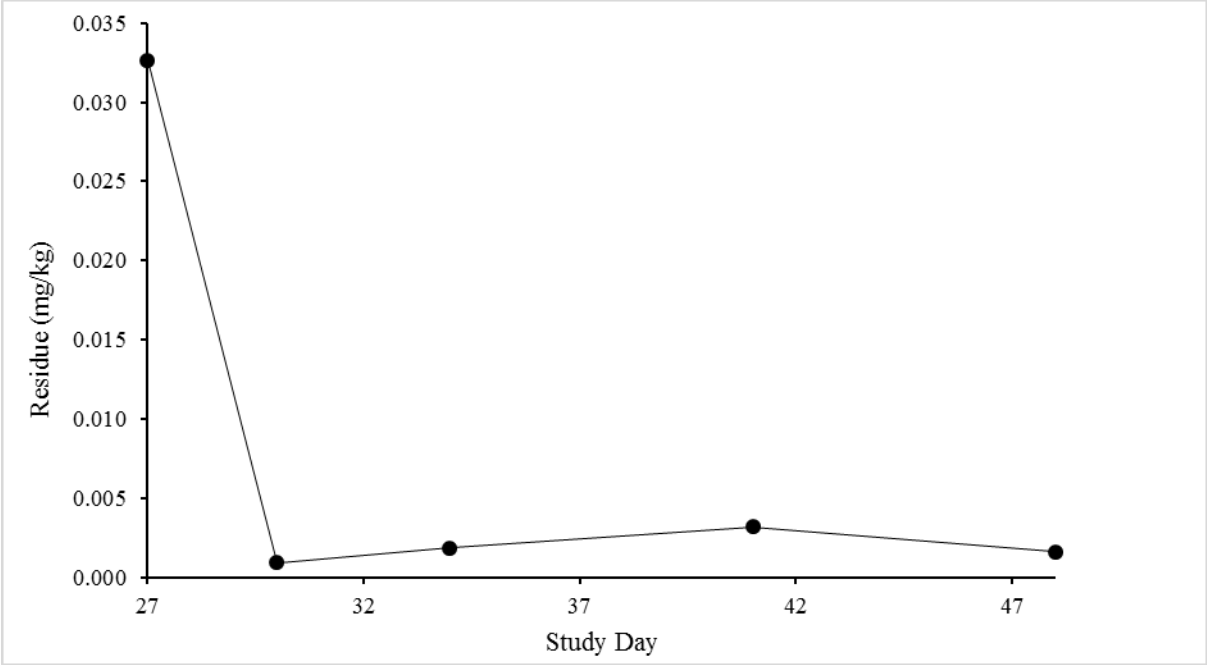


Figure B.7.4.1.3-4: Average residues of clopyralid in liver during depuration period

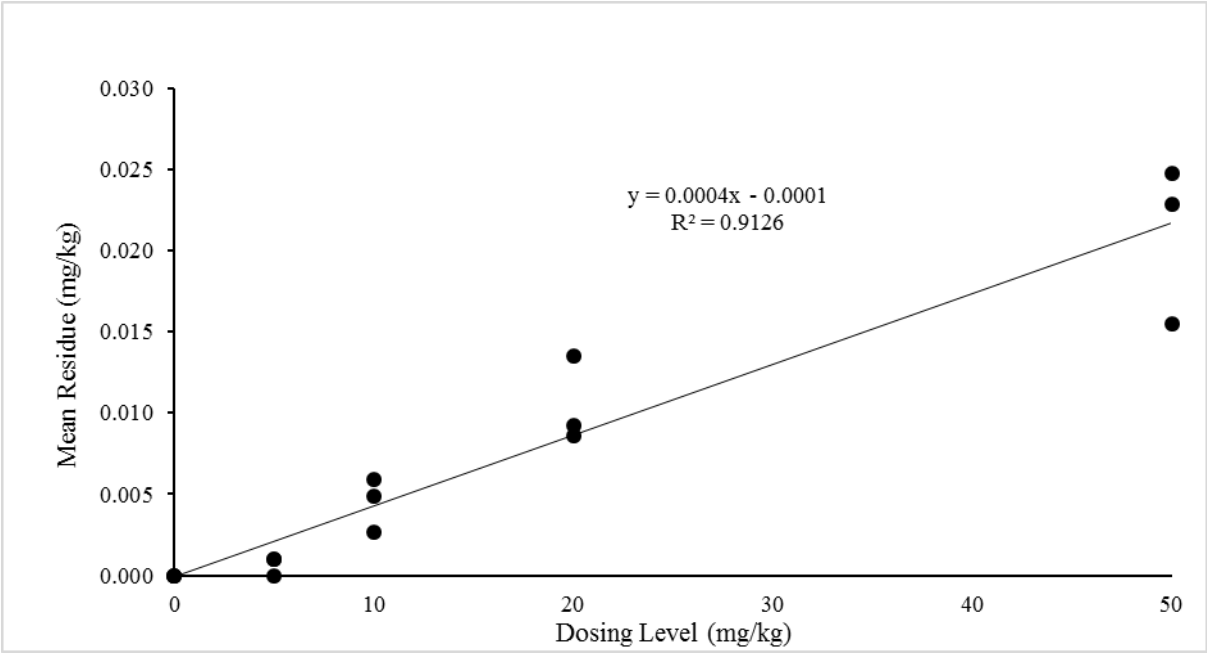


Figure B.7.4.1.3-5: Linear regression of clopyralid residue concentration (mg/kg) in eggs (Days 14 to 9) vs. feeding level (mg/kg)

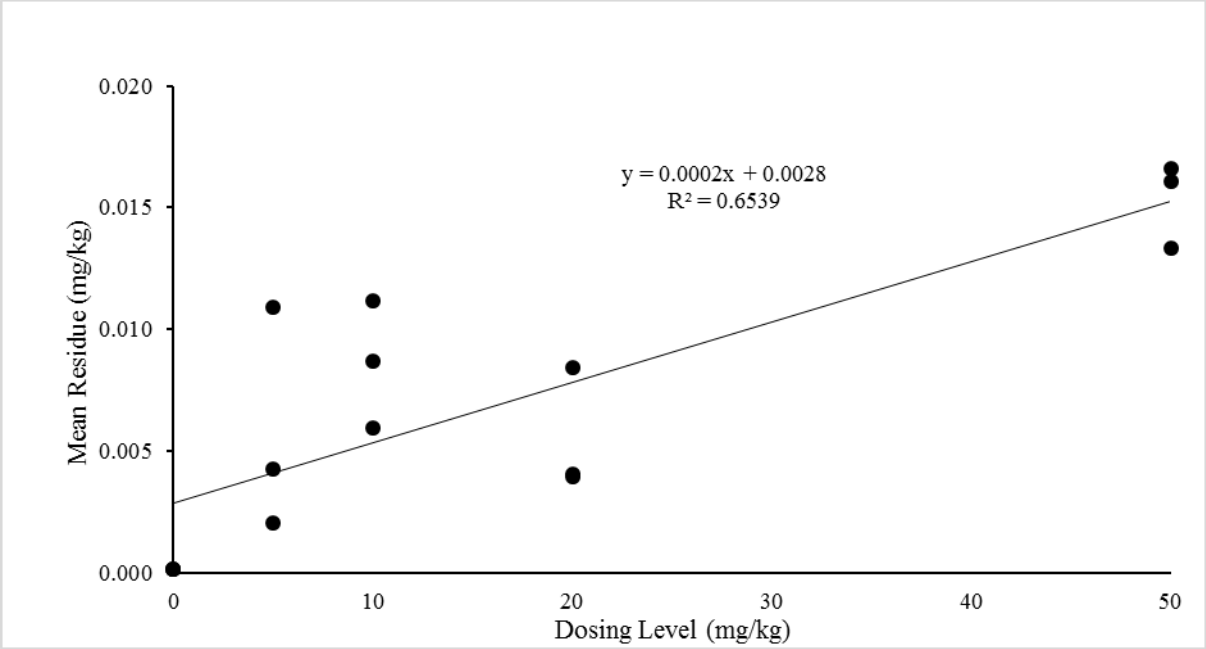


Figure B.7.4.1.3-6: Linear regression of clopyralid residue concentration (mg/kg) in muscle vs. feeding level (mg/kg)

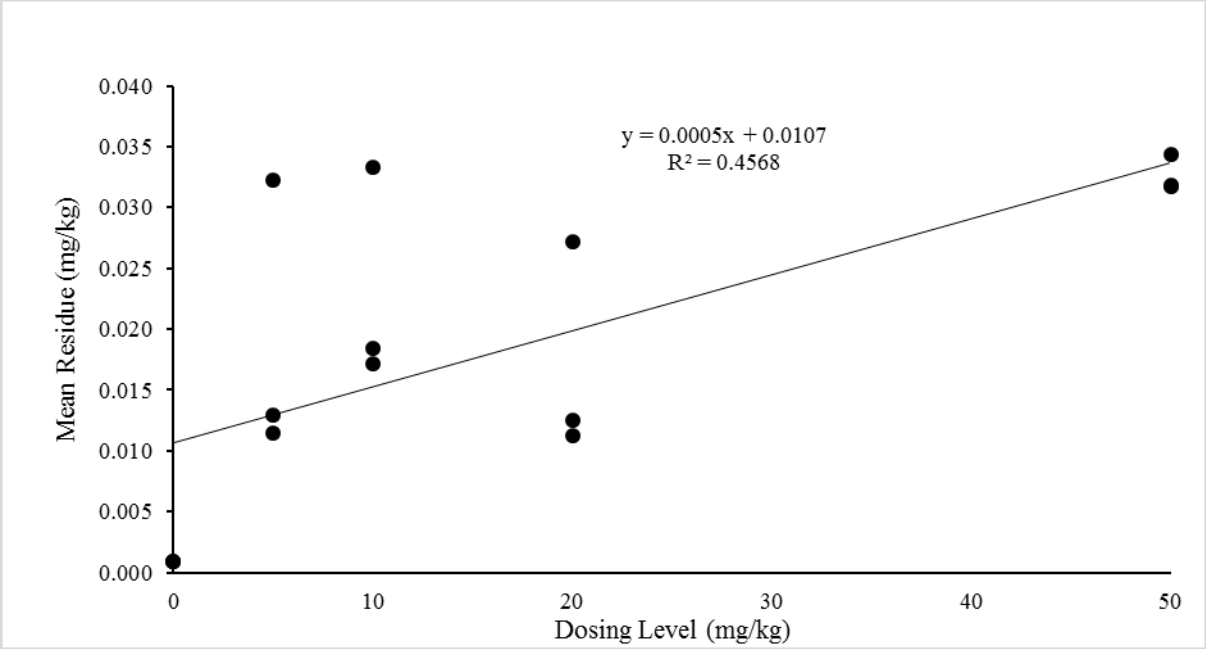


Figure B.7.4.1.3-7: Linear regression of clopyralid residue concentration (mg/kg) in liver vs. feeding level (mg/kg)

CONCLUSION

On Day 1 of the study (second day of dosing, which was the first sampling interval after dosing began) residues of clopyralid started to appear in the eggs from the 4x and 10x treatment groups. Residues appeared to plateau within 7 days of the initiation of dosing. Residues of clopyralid in eggs increased from the lowest to highest dose groups. In the depuration animals, average residues of clopyralid in the 10x treatment group fell to below the LOQ by Day 30 of the study (3 days after withdrawal of the dose from the hens' diet).

Residues of clopyralid above the LOQ were found in the muscle of one replicate in the 1x and 2x dosing groups and in all replicates in the 10x dosing group. Residues of clopyralid were found in the liver of treated hens in the 1x, 2x, 4x and 10x treatment groups. No residues of clopyralid above the LOQ were found in the fat from birds in any treatment group.

Residues of clopyralid in muscle and liver increased from the lowest to highest dose groups. In the 1x, 2x and 4x treatment groups, the residue in Replicate 1 was significantly higher than the residues found in the Replicates R2 and R3. The Replicate 1 was sampled earlier than the Replicates R2 and R3 and it is believed that the higher residue is caused by the relatively short interval between dosing and necropsy. In the 10x treatment group, the sampling interval for the Replicate 1 was longer and the effect is not seen. Additionally, although the interval between dosing and necropsy was less than 6 hours for all hens, except those used in the depuration phase of the study, the interval between dosing and necropsy for hens in the 10x dose group was longer than for the lower dose levels. This longer interval together with rapid removal of clopyralid from tissues may have resulted in somewhat lower levels of residue in the tissues in the 10x dose group than would have been observed had the interval between dosing and sampling been more similar to that for the 1x and 2x dose levels.

Residues of clopyralid in the tissues of the depuration animals fell to below the LOQ by 3 days after withdrawal of the test item from the hens' diet.

B.7.4.2 Ruminants

Ruminants

Results from cattle feeding studies have previously been evaluated during Annex I inclusion / Active Approval (Clopyralid Draft Assessment Report, Vol.3, B7.8, February, 2005). These data were considerd valid for decision making, but there were some unresolved questions on certain experimental details, including confirmation of whether or not dose levels in the diet were expressed on a dry matter basis. A new cattle feeding study was conducted since some experimental detail were not well documented in the earlier studies and since they were not GLP studies and did not follow current study guidelines because the studies were conducted prior to initation of GLP requirements and development of guidelines for these studies. Additionally, residues were generally found at equal ot greater levels in the new study than in the previous studies. Therefore, it is proposed that the new cattle feeding study be used to evaluate transfer of clopyralid residues from the diet to milk and tissues.

In the new cattle feeding study lactating dairy cows were dosed orally for 28 or 29 consecutive days via gelatine feeding capsules containing clopyralid. Gelatine capsules containing the test item were administered to each cow on two occasions each day (AM and PM feeding). Based on actual feed consumption during the period of dosing, the average dose levels of clopyralid based on concentration in the diet (DM feed basis) were 16.7 mg/kg (0.3x), 56.6 mg/kg (1x), 309.8 mg/kg (5x) and 1019.5 mg/kg (18x). If the daily dosage of clopyralid is expressed on the basis of bodyweight of the individual cows (mg/kg bw/day), the average dosage over the four weeks of dosing was 0.451 mg/kg bw/day, 1.670 mg/kg bw/day, 8.517 mg/kg bw/day and 30.538 mg/kg bw/day, for the 0.3x, 1x, 5x and 18x treatment groups, respectively.

No adverse treatment-related effects were observed on body weight, feed consumption or milk production. No treatment-related behavioural reactions or systemic signs of toxicity were noted and gross necropsies showed no effects that appeared to be treatment-related.

Residues of clopyralid were measured using an analytical method based on LC-MS/MS with a limit of detection (LOD) and limit of quantitation (LOQ) in all sample matrices of 0.003 mg/kg and 0.01 mg/kg, respectively. Overall average procedural recovery for clopyralid in all matrices ranged from 71% to 82%.

Residues of clopyralid above the LOQ of 0.01 mg/kg were found in muscle samples from cows in the 1x, 5x and 18x treatment groups and in the liver, kidney, subcutaneous fat, mesenteric fat and perirenal fat from cows in the 0.3x, 1x, 5x and 18x treatment groups. Residues of clopyralid in whole milk appeared to reach a plateau within the first 2 days of dosing. The average level of clopyralid in whole milk from samples collected from the second day of dosing (Study Day 2) until the end of the dosing period was ND, <0.01 mg/kg, 0.040 mg/kg and 0.153 mg/kg for the 0.3x, 1x, 5x and 18x treatment groups, respectively.

Regression analysis of clopyralid in milk, skimmed milk, cream and tissues (muscle, liver, kidney and fat) demonstrated a generally linear relationship between the dose level and the resulting residue concentration.

Depuration data generated using the 12 cows in the 18x dose level showed that residues of clopyralid declined rapidly following withdrawal of the test items from the cows' diet.

Results from livestock feeding studies have previously been evaluated during Annex I inclusion / Active Approval (Clopyralid Draft Assessment Report, Vol.3, B7.8, February, 2005). These data were considered valid for decision making, but there were some unresolved questions on certain experimental details, including confirmation of whether or not dose levels in the diet were expressed on a dry matter basis. As indicated below, a new cattle feeding study was conducted to provide additional, updated information since the previous studies were not conducted fully in compliance with current guidelines and were not GLP studies in addition to questions about certain experimental details, including whether or not the dose level in the diet was expressed on a dry feed basis. A summary concerning the justification for use of a different study rather than those evaluated for the Active Approval / original Annex I inclusion follows:

Data point/Study	Rationale
6.4.2/4	The cattle feeding studies (documented in three study reports) evaluated for the Active Approval were not a GLP studies and were not conducted according to current guidelines. A new GLP study was conducted according to current guidelines to provide additional information for determination of the transfer of clopyralid residues from the diet to ruminant milk and tissues. Residue levels observed in the new study were generally similar to or greater than those observed in the original studies. Considering the new study meets current guideline requirements, is GLP compliant and indicates residues at approximately equal or greater levels compared to the original studies, it is proposed to use the new cattle feeding study rather than the original studies for purposes of determining the transfer of residues from the diet to milk and ruminant tissues in the Annex I Renewal evaluation.

Although a summary of the new ruminant feeding study is presented in B.7.4.2/4, reference to the previous cattle feeding studies evaluated during the Active Approval along with a brief presentation of key results are presented below for comparison with the new study.

B.7.4.2.1 [redacted],1974 Milk Residue Study with Dairy Cows

Report	[redacted] 1974
Report title	Milk Residue Study with Dairy Cows Fed Lontrel Herbicide, Nellite Nematocide and 2,4-D Herbicide: Animal Care, Sampling and Production Records
DAS Study / Report number	Study report no. GH-A 579
Guidelines	N/A
GLP	Non-GLP. This study was conducted prior to the effective date of the final rule, 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

This report provides the live-phase information for generation of samples that are analyzed and reported in report no. GH-C 745, which is listed below in B.7.4.2.2.

B.7.4.2.2 [redacted] (1974) Residues in Milk and Cream from Cows Fed with clopyralid

Report	IIA 6.4/01 [redacted] 1974
Report title	Residues of Dowco 290 (3,6-dichloropicolini acid) in Milk and Cream from Cows Fed the Herbicide
DAS Study / Report number	Study report no. GH-C 745
Guidelines	N/A
GLP	Non-GLP. This study was conducted prior to the effective date of the final rule, 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

A summary of residue results in milk is provided in Table B.7.4.2.2-1.

Table B.7.4.2.2-1: Residues of clopyralid in milk from a dairy cow feeding study (Study report no. GH-C 745, 1974) *

Dose Level (mg/kg bw/d) [mg/kg feed DM]	**No**	**Results in milk**	
		Mean (mg/kg)	**Max. (mg/kg)**
0.36 (10)	**3**	**<0.01**	**<0.01**
1.09 (30)	**3**	**<0.01**	**<0.01**
3.63 (100)	**3**	**0.02**	**0.03**
10.90 (300)	**3**	**0.05**	**0.06**
36.36 (1000)	**3**	**0.14**	**0.17**

* Taken from: EFSA Journal 2011; 9(10): 2418. 'Reasoned Opinion. Modification of the existing MRLs for clopyralid in various commodities.'

B.7.4.2.3 [redacted] (1975) Residues in Bovine Tissues from Calves Fed the Clopyralid

Report	IIA 6.4/02 [redacted] 1975
Report title	Residues of Dowco 290 (3,6-dichloropicolinic acid) in Bovine Tissues from Calves Fed the Herbicide
DAS Study / Report number	Study report no. GH-C 811
Guidelines	N/A
GLP	Non-GLP. This study was conducted prior to the effective date of the final rule, 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

A summary of residue results in bovine tissues for clopyralid (3,6-dichloropicolinic acid) is provided in Table B.7.4.2/3-1.

Table B.7.4.2.3-1: Residues of clopyralid in tissues from the livestock feeding study *

Dose Level (mg/kg bw/d) [mg/kg feed DM]	Results in tissues							
	Meat		Fat		Liver		Kidney	
	Mean (mg/kg)	Max. (mg/kg)	Mean (mg/kg)	Max. (mg/kg)	Mean (mg/kg)	Max. (mg/kg)	Mean (mg/kg)	Max. (mg/kg)
0.08 (3)	n.a	n.a	n.a	n.a	n.a	n.a	<0.05	<0.05
0.17 (10)	<0.05	<0.05	n.a	n.a	<0.05	<0.05	0.07	0.07
0.81 (30)	<0.05	<0.05	n.a	n.a	<0.05	<0.05	0.3	0.3
2.95 (100)	0.11	0.11	<0.05	<0.05	0.07	0.07	0.48	0.48
8.84 (300)	0.07	0.07	0.05	0.05	0.32	0.32	4	4
27.9 (1000)	0.35	0.35	0.16	0.16	1.34	1.34	14.7	14.7

n.a Not analysed

* Taken from: EFSA Journal 2011; 9(10): 2418. 'Reasoned Opinion. Modification of the existing MRLs for clopyralid in various commodities.'

B.7.4.2.4 [redacted] (2015) Clopyralid Livestock Feeding Study: Magnitude of Residue in Milk, Muscle, Liver, Kidney and Fat of Lactating Dairy Cattle

REFERENCE	[redacted] 2015; Summary of Clopyralid Livestock Feeding Study: Magnitude of Residue in Milk, Muscle, Liver, Kidney and Fat of Lactating Dairy Cattle; [redacted], UK; Lab Study No. CEMS-6968; DAS Study No. 150030; 09 September 2015; Unpublished
Guideline(s):	EC Council Directive 91/414/EEC – Working Document 7031/VI/95 rev. 4, OECD Guidance Document: Overview for Residue Chemistry Studies (As Revised in 2009), OECD Guidelines for the Testing of Chemicals, No. 505: Residues in Livestock (2007), EPA Residue Chemistry Test Guideline OPPTS 860.1480 – Meat/Milk/Poultry/Eggs, APVMA Residue Guideline No. 1 – Animal Transfer Studies
US EPA Guideline(s):	EPA Residue Chemistry Test Guideline OPPTS 860.1480 – Meat/Milk/Poultry/Eggs
Deviations:	None
Dates of work:	29 March 2015 to 20 August 2015
GLP status:	Yes

Lactating Friesian/Holstein dairy cows were dosed orally for 28 or 29 consecutive days via gelatine feeding capsules containing the test item, clopyralid. Gelatine capsules containing the test item were administered to each cow on two occasions each day (AM and PM feeding). The animals were divided into 5 separate treatment groups. One treatment group of 3 cows was an untreated control group, which was dosed with capsules that did not contain test item. The remaining groups were treated with clopyralid, targeted at a nominal dose equivalent to a concentration in the animals' diet (on a dry matter (DM) basis) of:

15 mg/kg clopyralid (0.3x, 4 cows)

50 mg/kg clopyralid (1x, 4 cows)

250 mg/kg clopyralid (5x, 4 cows)

900 mg/kg clopyralid (18x, 16 cows)

The animals were dosed for 28 or 29 consecutive days. Twelve of the cows in the 18x treatment group were used to generate depuration data. At the end of the dosing period, they were transferred to the control diet to measure the decline in residues following withdrawal of the test item from the diet.

Based on actual feed consumption during the period of dosing, the average dose levels of clopyralid based on concentration in the diet (DM feed basis) were 16.7 mg/kg (0.3x), 56.6 mg/kg (1x), 309.8 mg/kg (5x) and 1019.5 mg/kg (18x). These dose levels represent 112%, 113%, 124% and 113% of the nominal/target dose levels for the 0.3x, 1x, 5x and 18x treatment groups, respectively. If the daily dosage of clopyralid is expressed on the basis of bodyweight of the individual cows (mg/kg bw/day), the average dosage over the four weeks of dosing was 0.451 mg/kg bw/day, 1.670 mg/kg bw/day, 8.517 mg/kg bw/day and 30.538 mg/kg bw/day, for the 0.3x, 1x, 5x and 18x treatment groups, respectively.

All animals were observed at least twice daily for general health. No adverse treatment-related effects were observed on body weight, feed consumption or milk production. Additionally, no treatment-related behavioural reactions or systemic signs of toxicity were noted. Gross necropsies showed no effects that appeared to be treatment-related.

Residues of clopyralid in milk and tissues were measured using an analytical method based on LC-MS/MS. The limit of detection (LOD) and limit of quantitation (LOQ) for clopyralid in milk, skimmed milk, cream, muscle, liver, kidney, subcutaneous fat, mesenteric fat and perirenal fat were 0.003 mg/kg and 0.01 mg/kg, respectively. Overall average procedural recovery for clopyralid in all matrices (whole milk, skimmed milk, cream, muscle, liver, kidney, subcutaneous fat, mesenteric fat and perirenal fat) ranged from 71% to 82%.

Residues of clopyralid above the LOQ of 0.01 mg/kg were found in muscle samples from cows in the 1x, 5x and 18x treatment groups and in the liver, kidney, subcutaneous fat, mesenteric fat and perirenal fat from cows in the 0.3x, 1x, 5x and 18x treatment groups. Although results are provided separately for subcutaneous fat, mesenteric fat and perirenal fat, OECD Test Guideline 505 indicates that a composite sample of fat consisting of approximately equal amounts of subcutaneous, mesenteric and perirenal fat may be collected. Therefore, average values for clopyralid residues in subcutaneous, mesenteric and perirenal fat were calculated and are also presented in summary tables as the value that would apply for the corresponding samples of composite fat. Residues of clopyralid in whole milk appeared to reach a plateau within the first 2 days of dosing. The average level of clopyralid in whole milk from samples collected from the second day of dosing (Study Day 2) until the end of the dosing period was ND, (0.008) mg/kg, 0.040 mg/kg and 0.153 mg/kg for the 0.3x, 1x, 5x and 18x treatment groups, respectively.

Regression analysis of clopyralid in milk, skimmed milk, cream and tissues (muscle, liver, kidney and fat) demonstrated a generally linear relationship between the dose level and the resulting residue concentration.

Depuration data generated using the 12 cows in the 18x dose level showed that residues of clopyralid declined rapidly following withdrawal of the test items from the cows' diet. Residues of clopyralid were below the LOQ of 0.01 mg/kg by Day 31 of the study (3 days after withdrawal) in whole milk, muscle and perirenal fat samples, by Day 42 of the study (14 days after withdrawal) in subcutaneous fat samples and by Day 49 of the study (21 days after withdrawal) in kidney samples. Clopyralid residues of up to 0.067 mg/kg were found in in liver samples and up to 0.026 mg/kg in mesenteric fat samples 21 days following withdrawal of the test item from the cows' diet.

BACKGROUND INFORMATION

The use of clopyralid may result in residues in feed commodities that are consumed by cattle. The purpose of this study is to determine the magnitude of residues of clopyralid in milk and tissues (muscle, fat, liver and kidney) from lactating dairy cattle dosed with clopyralid for a minimum of 28 days. Information generated by this study on the transfer of residues from dietary intake to milk, muscle, liver, kidney and fat is intended for use in supporting MRLs (tolerances) in these commodities, if needed.

Materials and Methods

Test Item(s)

ISO Common name:	Clopyralid
IUPAC chemical name	3,6-dichloropyridine-2-carboxylic acid
Test item (chemical/other name)	Clopyralid (technical grade)
Purity:	96.5% (w/w)

Methods

In-life Phase

Thirty one lactating Friesian/Holstein or Swedish red and white cross dairy cows were assigned for use in this study and were individually identified with unique statutory ear tags and freeze brands. The animals were divided into 5 separate treatment groups. The individual milk yields were measured daily and the cows were allocated to their treatment groups on the basis of milk yield.

The cows were arranged in homogeneous blocks. Three blocks of nine cows and one block of the four lowest yielding cows were formed based on their recent milk yields. For the purposes of allocation to treatment the different slaughter dates within Group E were treated as separate treatments. From within each block, cows were allocated at random. From blocks one to three, one cow was allocated to each treatment. From block four, one cow was allocated to each of treatments B, C, D and E.

One treatment group of 3 cows was an untreated control group, which was dosed with capsules that did nto contain the test item. The remaining groups were treated with clopyralid, targeted at a nominal dose equivalent to a concentration in the animals' diet (on a dry matter (DM) basis) of:

15 mg/kg clopyralid (0.3x, 4 cows)

50 mg/kg clopyralid (1x, 4 cows)

250 mg/kg clopyralid (5x, 4 cows)

900 mg/kg clopyralid (18x, 16 cows)

The animals were dosed for 28 or 29 consecutive days. Clopyralid was administered orally twice per day with the use of gelatine dosing capsules. Twelve of the cows in the 18x

treatment group were used to generate depuration data. At the end of the dosing period, they were transferred to the control diet to measure the decline in residues following withdrawal of the test item from the diet.

Table B.7.4.2.4-1: Description of livestock used in the feeding study

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Lactating dairy cows	Friesian/Holstein or Swedish red and white cross	2.5 to 7.5 years at the time dosing began	Average body weight by dose group at the start of dosing was 583.3–682.5.	All cows selected for use in the study were examined and identified as fit, healthy and suitable for use in the study prior to dosing.	Animals were housed under natural light with artificial lighting where necessary. Animals within each treatment group were housed adjacent to one another with nose-to-nose contact possible. Where cows from different treatment groups were housed next to each other, contact was prevented by a barrier. The buildings were ventilated naturally

The cows were offered grass silage fed to appetite throughout the study. The silage was chopped prior to feeding to reduce the amount dragged by the cows onto the feeding areas and provide a better assessment of feed intake. Silage remaining uneaten from the previous day was weighed and discarded and the weight of fresh silage offered was recorded. Samples of the silage offered were taken three times each week and stored at 5±3°C. Once each week, the three samples were mixed to form a composite sample, which was used for a weekly dry matter analysis.

In addition, all animals were offered 8 kg of a pelleted compound feed daily, split into three feeds. The feed was composed mainly of wheat, palm kernel expeller and rape seed meal. Other ingredients included wheat feed, sunflower seed meal, sugar cane molasses, soyabean meal, minerals and vitamins. Each week, a representative sample of the compound feed was taken for dry matter determination. Total dry matter consumption for each cow on a daily basis was determined.

Table B.7.4.2/4-2: Test animal dietary regimen

Composition of diet (fresh weight or as-fed basis)	**Feed consumption (kg/day) – Average consumption of feed (dry weight basis) during the dosing period**	**Water**	**Acclimation, dosing and withdrawal period**
Grass silage fed to appetite. In addition, all animals were offered 8 kg per day of a pelleted dairy compound feed, composed mainly of wheat, palm kernel expeller and rape seed meal Average dry matter content of the silage was 35.90% to 49.80%% during dosing period. Average dry matter content of the compound feed was 87.12% to 87.90% during dosing period.	0x (A) Control group: 18.82 0.3x (Dose Group B): 18.71 1x (Dose Group C): 19.34 5x (Dose Group D): 17.56 18x (Dose Group E): 17.82	Water offered *ad libitum*.	A 16-day acclimation period was used in this study. Dietary regime was the same through all phases of the study.

Dosage Rates

The maximum dietary residue intake for ruminant livestock used for selection of the 1x dose level (Group C) in this study took into consideration crop residue data previously evaluated for purposes of setting MRLs for clopyralid in the EU. For dairy and beef cattle, the maximum dietary intake of residues was based on use of clopyralid in pasture. At the time dose selection for this study took place, results from additional pasture grass residue trials that were on-going were not yet available. Based on existing pasture grass residue data and the use pattern to be supported, it was expected that the maximum residue level in grass was unlikely to exceed 10 mg/kg. According to Appendix G of the EU Guidance document on livestock feeding studies, grass may comprise up to 100% of the diet for beef and dairy cattle and is expected to have a dry matter content of 20%. Based on these inputs, dietary intake of clopyralid residues in ruminant livestock of up to 50 mg/kg in dry feed (DM basis) may be expected [i.e. (10 mg/kg in grass / 20% DM) x 100% of diet = 50 mg/kg in diet, DM basis]. Therefore, the 1x dose level for Group C was set at 50 mg/kg in the diet. A dose level of 0.3x at 15 mg/kg was also included in the study to provide information for lower levels of residue intake.

Two additional dose levels were included in the study targeted at 250 mg/kg (5x) and 900 mg/kg (18x) in the diet on a dry feed (DM) basis in order to cover other regions of the world where the use patterns in pasture and other crops may contribute higher levels of residue intake in cattle (e.g. higher application rates, reduced pre-grazing intervals).

To summarize, the dosing levels were targeted at 15 mg/kg (0.3x, Group B), 50 mg/kg (1x, Group C), 250 mg/kg (5x, Group D) and 900 mg/kg (18x, Group E). The study also contained an untreated control group (0x, Group A).

Dose Capsule Preparation and Administration

Dosing capsules were prepared up to 4 days before use. In each case, the correct amount of test item was weighed into the capsule and white plain flour was used to fill up the capsule. Each prepared capsule was transferred to a labelled plastic bag and transferred to a refrigerator until use. On one occasion, six Group A capsules and twelve Group C capsules were prepared for stability testing, in order to demonstrate that the test item was stable in the capsule when stored at 5°C for up to 4 days, which covers the maximum period of storage for the capsules when containing the test item. Three Group A capsules and six Group C capsules were transferred immediately to a freezer at below -18°C; the remaining nine capsules were stored at approximately 5°C for 4 days and then transferred to the freezer.

Half of each dose was administered in the morning and half in the evening. The amount of clopyralid administered was adjusted as needed for each animal individually in accordance with the recent actual amount of dry matter consumption (i.e. dry matter intake over a five day period near the end of the acclimation period) in order to more closely match the target dose level in that animal's diet. The capsule was placed at the rear of the tongue so that the cow swallowed the capsule intact.

Table B.7.4.2.4-3: Test animal dosing regime

Treatment group	Treatment Type	Average clopyralid dose expressed as level in diet – dry feed basis (mg/kg) [a, b]	Average clopyralid dose expressed as mg/kg bw/day [a, c]	Vehicle [c]	Timing/ duration
Untreated control (Dose Group A)	**Oral – twice per day**	**Clopyralid: 0**	**Clopyralid: 0**	**Gelatine capsule**	**30 days flour only added**
Dose Group B (0.3x)	**Oral – twice per day**	**Clopyralid: 16.7**	**Clopyralid: 0.451**	**Gelatine capsule**	**29 days**
Dose Group C (1x)	**Oral – twice per day**	**Clopyralid: 56.8**	**Clopyralid: 1.670**	**Gelatine capsule**	**28 days**
Dose Group D (5x)	**Oral – twice per day**	**Clopyralid: 309.8**	**Clopyralid: 8.517**	**Gelatine capsule**	**29 days**
Dose Group E (18x)	**Oral – twice per day**	**Clopyralid: 1019.5**	**Clopyralid: 30.538**	**Gelatine capsule**	**28 days**

a *Average for treatment group over dosing period*

b *Calculated based on average daily dry matter consumption of 18.82, 18.71, 19.34, 17.56 and 17.82 kg for Dose Groups A, B, C, D and E, respectively.*

c *Test Average clopyralid dose expressed as mg/kg body weight / day based on amount of clopyralid administered to each animal and the associated body weights.*

On specified days, samples of milk, cream and skimmed milk were collected for analysis to quantify residues of clopyralid.

Samples of whole milk (approximately 500 mL) were taken from each cow at each milking (PM and AM) on Days -1, 2, 6, 8, 10, 14, 18, 22, 27, 28, 29, 30, 31, 33, 35, 38, 40, 42, 45, 48 and 49.

The samples were placed in a refrigerator set to 5 ± 3°C immediately after collection. For each cow, a combined proportional sample was constructed from the PM and AM samples, on the basis of milk yields recorded at the corresponding milkings. For example, the proportional sample for Day 10 consisted of the morning sample for Day 10 and the evening sample for Day 9. A combined sample, of at least 200 mL, was constructed within 24 hours of collection of all contributing samples. After thorough mixing, 4 replicate sub-samples (R1

to R4) of approximately 50 mL were transferred to HDPE screw-cap bottles and transferred to a freezer set to maintain a temperature of less than -18°C.

Processing of milk samples was carried out in order of increasing treatment / dose level, with control samples processed first.

On Days 22 and 27, an additional proportional sample of approximately 2 L was retained from the combined evening and morning milk yield for each cow and stored at approximately 5°C overnight. After overnight storage, a combined proportional sample was constructed for each cow from the AM and PM samples, on the basis of milk yields recorded at the corresponding milkings. They were then separated into cream and skimmed milk samples by centrifugation in glass bottles for approximately 20 minutes at 2000 rpm. Four replicate samples (R1 to R4) of approximately 50 mL skimmed milk, two replicate samples (R1 and R2) of approximately 20 g of cream and a further replicate sample (R3) of approximately 50 g cream were collected and transferred to a freezer set to maintain a sample temperature of less than -18°C.

All cows were sacrificed within 6 hours of administration of the final dose, with the exception of the cows used to collect depuration data. Tissue samples were collected from each cow immediately after slaughter. For all tissues, two replicate specimens (R1 and R2) of approximately 1 kg (or as available) were taken. Skeletal muscle specimens comprised approximately equal pieces of hind leg or flank, loin and diaphragm muscle. On eight occasions, the amount of subcutaneous fat was limited and on one occasion the amount of mesenteric and perirenal fat was limited. On each of these occasions, only an R1 replicate sample was collected. Liver samples were taken from at least 6 subsamples from different areas of the organ. Kidney samples were taken from at least three subsamples of each kidney. The weight of each tissue sample was recorded. All tissue samples were double-wrapped in polythene bags. Immediately upon collection, the samples were stored in a polystyrene box containing ice packs to reduce the temperature and then transferred to a freezer set to maintain a temperature of less than -18°C within three hours of collection.

Table B.7.4.2.4-4: Sample collection

Milk collected	**Amount of milk produced during normal production**	**Interval from last dose to sacrifice (days)**	**Tissues harvested and analysed**
<u>Milk:</u> Day before dosing (study day -1) and dosing (study) days 2, 6, 8, 10, 14, 18, 22, 27, 28, 29 and 30 <u>Depuration period:</u> Milk collected on study days 29, 30, 31, 33, 35, 38, 40, 42, 45, 48 and 49	Average yields during the dosing period in the study were 23.35 kg, 20.53 kg, 22.43 kg, 19.14 kg and 22.82 kg per day for the 0x, 0.3x 1x, 5x and 18x treatment groups respectively.	Less than 6 hours Depuration of residues in tissues also evaluated at 3, 7, 14 and 21 days after administration of final dose.	Muscle – approx. 1 kg Liver – approx. 1 kg Kidney – approx. 1 kg Subcutaneous fat – approx. 1 kg Mesenteric fat – approx. 1 kg Perirenal fat – approx. 1 kg

Sampling, Handling and Preparation

All tissue samples were double-wrapped in polythene bags. Immediately upon collection, the samples were stored in a polystyrene box containing ice packs to reduce the temperature and then transferred to a freezer set to maintain a temperature of less than -18°C within three hours of collection. All samples remained frozen during shipment and were stored in temperature-monitored freezers at ≤-18°C on arrival.

Complete samples of muscle, fat, liver and kidney were homogenised in a Robot Coupe processor, in the presence of dry ice in the case of fat samples. After appropriate mixing of each sample, samples were transferred to HDPE plastic containers. No preparation was required for milk, skimmed milk or cream samples.

Analytical Phase

Residues of clopyralid were determined according to the validated analytical method described in Dow AgroSciences Study Number 120483 ([redacted] 2012. [redacted] Study Number 68447). The method principle is listed below.

Residues of clopyralid were extracted from animal milk and tissue samples with 2.5 N NaOH with heating at approximately 105°C for a minimum of 2 hours. An aliquot of the extract was acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) clean-up and elution with dichloromethane. After removal of the dichloromethane using nitrogen blow down, the sample was reconstituted in acetonitrile: 0.1% formic acid (10:90, v/v). The final extract was filtered through a 0.2-µm PTFE syringe filter and then analysed by liquid chromatography coupled with negative-ion electrospray (ESI) tandem mass spectrometry (LC/MS/MS).

The limit of detection (LOD) and limit of quantitation (LOQ) of clopyralid in all matrices were 0.003 mg/kg and 0.01 mg/kg, respectively.

Results and Discussion

Animal Health

Treatment did not appear to affect body weights, feed intake or milk yields. There were no clinical observations made during the study that were attributed to treatment. In general, all health issues observed were relatively minor and typical for cows of this age and situation.

Storage Stability

The maximum interval of frozen storage for samples between collection and extraction for analysis along with supporting frozen storage stability study references is presented in Table B.7.4.2/4-5. The maximum interval between collection of samples and extraction for analysis of clopyralid for whole milk, cream, skim milk, muscle, fat (subcutaneous, mesenteric and perirenal), liver and kidney was 96, 74, 70, 92, 93, 97 and 81 days, respectively. In an earlier study, residues of clopyralid in milk, muscle, liver and kidney were found to remain stable in frozen storage at approximately -20°C for a period of at least 569 days, which was the maximum storage interval evaluated ([redacted] 2004. Dow AgroSciences Study ID 020120.01). Additionally, clopyralid was found to remain stable in bovine fat for a period of 24 months, which was the maximum interval evaluated, when stored frozen at approximately -20°C ([redacted] 2015. Dow AgroSciences Study ID 120602). Therefore, based on results

from these studies, residues of clopyralid are expected to remain stable in frozen storage for the maximum periods of storage encountered in this study.

Table B.7.4.2.4-5: Summary of sample frozen storage conditions

Matrix	Storage temperature (°C)	Maximum storage duration (days)	Interval of demonstrated frozen storage stability (days)
Whole milk	**≤-18**	**96**	**569** [a]
Skimmed milk	**≤-18**	**70**	**569** [a] **(Based on milk)**
Cream	**≤-18**	**74**	**569** [a] **(Based on milk)**
Muscle	**≤-18**	**92**	**569** [a]
Liver	**≤-18**	**97**	**569** [a]
Kidney	**≤-18**	**81**	**569** [a]
Subcutaneous fat	**≤-18**	**75**	**730** [b]
Mesenteric fat	**≤-18**	**93**	**730** [b]
Perirenal fat	**≤-18**	**87**	**730** [b]

[a] [redacted] 2004. 'Frozen Storage Stability of Clopyralid in Beef Muscle, Liver, Kidney, Milk, and Chicken Egg.' Dow AgroSciences Study ID 020120.01.

[b] [redacted] 2015. 'Frozen Storage Stability of Clopyralid in Bovine Fat.' Dow AgroSciences Study ID 120602.

Analytical Method Performance

The efficiency of the analytical method was determined at the time of analysis of each set of samples by fortifying aliquots of the appropriate control matrix with analyte and analysing according to the method. Each analytical batch contained at least three procedural recovery samples fortified at the LOQ of 0.01 mg/kg and at least two procedural recovery samples fortified at a higher level. An unfortified control matrix, reagent blank, and control matrix fortified at the limit of detection (LOD) were included in each set as well. The LOD recovery samples were analysed only to demonstrate observable peaks at the LOD level. Therefore, the percent recovery of analyte is not reported for these samples. Recoveries were corrected for any apparent residue in the corresponding control sample. A summary of procedural recovery results by matrix and by fortification level is presented in Table B.7.4.2/4-6. Overall average procedural recovery for clopyralid in all matrices (whole milk, skimmed milk, cream, muscle, liver, kidney, subcutaneous fat, mesenteric fat and perirenal fat) ranged from 71% to 82%.

Residue Results – Milk and Tissues

Residue results in milk, cream and tissues are discussed briefly below. Additionally, data from the cattle feeding study are presented in a number of Tables and Figures that follow.

Residues of clopyralid in milk during the period when the test material was being administered to the cattle are presented in Table B.7.4.2/4-7, while residues of clopyralid in milk during the depuration period when dosing with the test material has ended are presented in Table B.7.4.2/4-8. Residues of clopyralid in tissues are presented in Table B.7.4.2/4-9. A summary of residue results for clopyralid in milk and tissues is presented in Table B.7.4.2/4-10. Residues of clopyralid in milk during the dosing period are shown in Figure B.7.4.2/4-1. Residues of clopyralid in milk and tissues during the depuration period are shown in Figure B.7.4.2/4-2 to Figure B.7.4.2/4-8. Linear regression results for clopyralid residues in milk, cream and tissues by dose level of clopyralid are presented in Figure B.7.4.2/4-9 to Figure B.7.4.2/4-17.

Residues in Whole and Skimmed Milk

Residues of clopyralid whole milk and skimmed milk during the dosing period were below the LOQ (<0.010 mg/kg) in the 0.3x dosing group and above the LOQ in the 1x, 5x and 18x dosing groups. Residues appeared to reach a plateau in whole milk within the first 2 days of dosing. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Cream

Residues of clopyralid in cream were ND (<0.003 mg/kg) in the 0.3x dosing group, below the LOQ (<0.01 mg/kg) in the 1x dosing group and above the LOQ in the 5x and 18x dosing groups. Residues did not concentrate in cream compared to whole milk. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Muscle

Residues of clopyralid in muscle were below the LOQ (<0.010 mg/kg) in the 0.3x dosing group and above the LOQ in the 1x, 5x and 18x dosing groups. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Liver

Residues of clopyralid in liver were above the LOQ in the 0.3x, 1x, 5x and 18x dosing groups. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Kidney

Residues of clopyralid in kidney were above the LOQ in the 0.3x, 1x, 5x and 18x dosing groups. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Subcutaneous Fat

Residues of clopyralid in subcutaneous fat were below the LOQ (<0.010 mg/kg) in the 0.3x dosing group and above the LOQ in the 1x, 5x and 18x dosing groups. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Mesenteric Fat

Residues of clopyralid in mesenteric fat were below the LOQ (<0.010 mg/kg) in the 0.3x dosing group and above the LOQ in the 1x, 5x and 18x dosing groups. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Residues in Perirenal Fat

Residues of clopyralid in perirenal fat were above the LOQ in the 0.3x, 1x, 5x and 18x dosing groups. There was a linear relationship between the residue and the level of clopyralid test item in the cows' diet.

Table B.7.4.2.4-6: Summary of procedural recoveries for clopyralid

Matrix	Fortification level (mg/kg)	n	Recovery (%)	Mean recovery (%)	RSD (%)
Whole milk	0.01	51	72, 72, 75, 73, 72, 77, 74, 73, 71, 70, 73, 71, 71, 72, 79, 72, 70, 71, 70, 70, 73, 75, 70, 66, 81, 70, 77, 71, 81, 73, 78, 78, 79, 91, 84, 82, 86, 86, 86, 71, 69, 71, 77, 76, 84, 85, 72, 85, 73, 69, 77	75	7.6
	0.10	32	74, ,74, 76, 77, 70, 70, 71, 70, 74, 73, 72, 67, 76, 72, 71, 69, 73, 70, 78, 70, 91, 83, 88, 85, 76, 70, 86, 73, 80, 79, 72, 69	75	8.0
	1.0	2	81, 82	82	0.9
Skimmed milk	0.01	6	75, 80, 84, 80,79, 78	79	5.7
	0.10	4	83, 88, 79, 81	83	4.7
	0.5	2	93, 87	90	4.7
Cream	0.01	6	72, 68, 70, 70, 71, 73	71	2.5
	0.10	4	70, 69, 75, 69	71	4.1
Muscle	0.01	6	76, 86, 74, 71, 77, 67	75	8.6
	0.10	2	69, 73, 66, 71	70	4.3
	30.0	2	90, 75	88	4.0
Liver	0.01	6	69, 81, 67, 85, 84, 66	75	11.8
	0.50	2	68, 68	68	0.0
	30.0	2	87, 91	89	3.2
Kidney	0.01	6	70, 72, 78, 65, 74, 78	73	6.9
	0.10	2	73, 79	76	5.6
	50.0	2	104, 118	111	8.9

Matrix	Fortification level (mg/kg)	n	Recovery (%)	Mean recovery (%)	RSD (%)
Subcutaneous fat	0.01	6	81, 82, 82, 70, 70, 72	76	8.0
	0.10	4	80, 77, 72, 70	75	6.1
	2.0	2	96, 85	91	8.6
Mesenteric fat	0.01	6	80, 89, 90, 77, 74, 79	82	8.0
	0.10	2	81, 78	80	2.7
	5.0	2	85, 91	88	4.8
Perirenal fat	0.01	6	72, 73, 72, 71, 72, 69	72	1.9
	0.10	2	71, 66	69	5.2
	5.0	2	83, 89	86	4.9

Table B.7.4.2.4-7: Residue data for clopyralid in milk from ruminant feeding study with clopyralid during dosing

Dose	Cow No.	Study day											
		-1	2	6	8	10	14	18	22	27	28	29	30
A	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	ND
A	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	ND
A	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	ND
B	4	ND	ND	ND	ND	ND	ND	(0.005)	ND	(0.003)	-	(0.005)	-
B	5	ND	(0.003)	ND	(0.003)	ND	ND	(0.004)	(0.003)	(0.005)	-	(0.003)	-
B	6	ND	(0.003)	(0.003)	(0.004)	(0.003)	ND	(0.004)	ND	(0.005)	-	ND	-
B	7	ND	ND	ND	ND	ND	ND	(0.003)	(0.003)	ND	-	(0.003)	-
C	8	ND	0.012	(0.007)	(0.009)	(0.008)	(0.009)	0.014	0.017	0.012	(0.008)	-	-
C	9	ND	(0.005)	(0.005)	(0.004)	(0.004)	(0.005)	(0.009)	(0.005)	(0.007)	(0.005)	-	-

Dose	Cow No.	Study day											
		-1	2	6	8	10	14	18	22	27	28	29	30
C	10	ND	(0.007)	(0.009)	(0.007)	(0.007)	(0.008)	0.012	0.014	0.013	(0.010)	-	-
C	11	ND	(0.007)	(0.006)	(0.004)	(0.005)	(0.005)	(0.007)	(0.006)	0.010	(0.007)	-	-
D	12	ND	0.038	0.033	0.029	0.033	0.030	0.068	0.037	0.052	-	0.033	-
D	13	ND	0.050	0.041	0.043	0.041	0.048	0.070	0.044	0.058	-	0.046	-
D	14	ND	0.032	0.034	0.036	0.034	0.036	0.064	0.045	0.044	-	0.041	-
D	15	ND	0.031	0.023	0.026	0.030	0.033	0.027	0.033	0.036	-	0.034	-
E	16	ND	0.091	0.090	0.090	0.100	0.096	0.106	0.079	0.134	0.075	-	-
E	17	ND	0.175	0.189	0.205	0.176	0.186	0.170	0.191	0.189	0.138	-	-
E	18	ND	0.143	0.134	0.124	0.116	0.113	0.145	0.138	0.141	0.146	-	-

Dose	Cow No.	Study day											
		-1	2	6	8	10	14	18	22	27	28	29	30
E	19	ND	0.101	0.101	0.104	0.090	0.104	0.132	0.178	0.114	0.104	-	-
E	20	ND	-	-	-	-	0.146	-	0.083	-	0.202	0.097	-
E	21	ND	-	-	-	-	0.121	-	0.266	-	0.120	0.098	-
E	22	ND	-	-	-	-	0.105	-	0.110	-	0.084	0.068	-
E	23	ND	-	-	-	-	0.217	-	0.237	-	0.164	0.123	-
E	24	ND	-	-	-	-	0.189	-	0.530	-	0.133	0.134	-
E	25	ND	-	-	-	-	0.227	-	0.382	-	0.192	0.139	-
E	26	ND	-	-	-	-	0.118	-	0.154	-	0.090	0.050	-
E	27	ND	-	-	-	-	0.133	-	0.250	-	0.132	0.076	-

Dose	Cow No.	Study day											
		-1	2	6	8	10	14	18	22	27	28	29	30
E	28	ND	-	-	-	-	0.199	-	0.236	-	0.146	0.097	-
E	29	ND	-	-	-	-	0.143	-	0.246	-	0.141	0.081	-
E	30	ND	-	-	-	-	0.141	-	0.142	-	0.122	0.078	-
E	31	ND	-	-	-	-	0.121	-	0.159	-	0.127	0.066	-

[a] *Average clopyralid concentration in the diet (dry feed (DM) basis) for Dose Groups A, B, C, D and E was: 0 mg/kg, 16.7 mg/kg, 56.6 mg/kg, 309.8 mg/kg and 1019.5 mg/kg, respectively. If expressed on the basis of bodyweight, the average dosage for Dose Groups A, B, C, D and E was: 0.000 mg/kg bw/day, 0.451 mg/kg bw/day, 1.670 mg/kg bw/day, 8.571 mg/kg bw/day and 30.538 mg/kg bw/day, respectively.*

Table B.7.4.2.4-8: Residue data for clopyralid in milk from ruminant feeding study with clopyralid during depuration

Dose	Cow No.	Days after final dose (study day)										
		0 (28)	1 (29)	3 (31)	5 (33)	7 (35)	10 (38)	12 (40)	14 (42)	17 (45)	20 (48)	21 (49)
E	20	0.202	0.097	ND	-	-	-	-	-	-	-	-
E	21	0.120	0.098	ND	-	-	-	-	-	-	-	-
E	22	0.084	0.068	ND	-	-	-	-	-	-	-	-
E	23	0.164	0.123	(0.003	ND	ND	-	-	-	-	-	-
E	24	0.133	0.134	ND	ND	ND	-	-	-	-	-	-
E	25	0.192	0.139	ND	ND	ND	-	-	-	-	-	-
E	26	0.090	0.050	ND	ND	ND	ND	ND	ND	-	-	-
E	27	0.132	0.076	ND	ND	ND	ND	ND	ND	-	-	-

Dose	Cow No.	Days after final dose (study day)										
		0 (28)	1 (29)	3 (31)	5 (33)	7 (35)	10 (38)	12 (40)	14 (42)	17 (45)	20 (48)	21 (49)
E	**28**	**0.146**	**0.097**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**-**	**-**	**-**
E	**29**	**0.141**	**0.081**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
E	**30**	**0.122**	**0.078**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
E	**31**	**0.127**	**0.066**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**

[a] Average clopyralid concentration in the diet (dry feed (DM) basis) for Dose Group E was: 1019.5 mg/kg. If expressed on the basis of bodyweight, the average dosage for Dose Group E was: 30.538 mg/kg bw/day.

Table B.7.4.2.4-9: Residue data for clopyralid from ruminant feeding study with clopyralid upon completion of dosing and during depuration

Dose group [a]	Cow No.	Study day	Days after last dose	Residues(mg/kg)						
				Muscle	Liver	Kidney	Subcutaneous fat	Mesenteric fat	Perirenal fat	Composite / Average Fat
A	1	30	-	ND	ND	(0.003)	ND	ND	ND	ND
A	2	30	-	ND	ND	(0.004)	ND	ND	ND	ND
A	3	30	-	ND	(0.005)	ND	ND	ND	ND	ND
B	4	29	-	(0.007)	0.029	0.356	0.014	0.014	0.032	0.020
B	5	29	-	(0.007)	0.035	0.427	0.012	(0.007)	0.041	0.020
B	6	29	-	(0.007)	0.036	0.606	(0.004)	(0.004)	(0.007)	(0.005)
B	7	29	-	(0.005)	0.028	0.325	(0.006)	ND	0.012	(0.006)
C	8	28	-	0.029	0.145	1.396	0.066	0.116	0.264	0.149
C	9	28	-	0.020	0.088	1.528	0.032	0.021	0.124	0.059

Dose group [a]	Cow No.	Study day	Days after last dose	Residues(mg/kg)						
				Muscle	Liver	Kidney	Subcutaneous fat	Mesenteric fat	Perirenal fat	Composite / Average Fat
C	10	28	-	0.022	0.111	1.559	0.022	0.016	0.035	0.024
C	11	28	-	0.021	0.105	1.355	0.012	0.020	0.012	0.015
D	12	29	-	0.113	0.527	4.743	0.307	0.549	1.048	0.635
D	13	29	-	0.110	0.560	4.923	0.340	0.211	0.622	0.391
D	14	29	-	0.112	0.497	6.030	0.172	0.104	0.330	0.202
D	15	29	-	0.079	0.425	4.705	0.057	0.027	0.075	0.053
E	16	28	-	0.411	1.962	25.304	0.448	0.370	0.856	0.558
E	17	28	-	0.484	1.874	22.872	1.453	1.282	3.658	2.131
E	18	28	-	0.259	1.423	13.662	0.191	0.356	0.366	0.304
E	19	28	-	0.251	1.809	18.300	0.173	0.296	0.241	0.237

Dose group [a]	Cow No.	Study day	Days after last dose	Residues(mg/kg)						
				Muscle	Liver	Kidney	Subcutaneous fat	Mesenteric fat	Perirenal fat	Composite / Average Fat
E	20	31	3	(0.006)	0.238	0.039	0.030	0.013	(0.005)	0.016
E	21	31	3	ND	0.140	0.024	(0.005)	(0.005)	ND	(0.003)
E	22	31	3	(0.006)	0.194	0.025	ND	ND	ND	ND
E	23	35	7	ND	0.305	0.012	0.014	(0.004)	(0.004)	(0.007)
E	24	35	7	ND	0.246	0.026	0.014	0.016	(0.004)	0.011
E	25	35	7	ND	0.179	0.017	(0.007)	0.012	ND	(0.006)
E	26	42	14	ND	0.085	0.015	ND	0.026	ND	(0.009)
E	27	42	14	ND	0.147	0.014	(0.003)	0.012	ND	(0.005)
E	28	42	14	ND	0.131	0.021	ND	(0.004)	ND	ND
E	29	49	21	ND	0.067	(0.006)	(0.005)	0.020	ND	(0.008)

Dose group [a]	Cow No.	Study day	Days after last dose	Residues(mg/kg)						
				Muscle	Liver	Kidney	Subcutaneous fat	Mesenteric fat	Perirenal fat	Composite / Average Fat
E	30	49	21	ND	0.066	(0.007)	ND	0.026	ND	(0.009)
E	31	49	21	ND	0.054	(0.006)	ND	ND	ND	ND

[a] Average clopyralid concentration in the diet (dry feed (DM) basis) for Dose Groups A, B, C, D and E was: 0 mg/kg, 16.7 mg/kg, 56.6 mg/kg, 309.8 mg/kg and 1019.5 mg/kg, respectively. If expressed on the basis of bodyweight, the average dosage for Dose Groups A, B, C, D and E was: 0.000 mg/kg bw/day, 0.451 mg/kg bw/day, 1.670 mg/kg bw/day, 8.571 mg/kg bw/day and 30.538 mg/kg bw/day, respectively.

Table B.7.4.2.4-10: Summary of residue data for clopyralid from ruminant feeding study with clopyralid

Matrix	Feeding level – dry feed (mg/kg) [a]	Residue levels (mg/kg)					
		n	Min.	Max.	Median (STMdR)	Mean (STMR)	Std. Dev.
Whole milk	**16.7**	**36**	ND	(0.005)	ND	ND	0.002
Days 2 – 28/29	**56.6**	**36**	(0.004)	0.017	(0.007)	(0.008)	0.003
	309.8	**36**	0.023	0.070	0.036	0.040	0.011
	1019.5	**72**	0.075	0.53	0.138	0.153	0.070
Skimmed milk	**16.7**	**4**	**ND**	**(0.004)**	**(0.004)**	**ND**	**0.002**
Study Day 27	**56.6**	**4**	**(0.006)**	**0.012**	**(0.008)**	**(0.008)**	**0.003**
	309.8	**4**	**0.026**	**0.056**	**0.044**	**0.043**	**0.012**
	1019.5	**4**	**0.096**	**0.217**	**0.120**	**0.136**	**0.058**
Cream	**16.7**	**4**	**ND**	**ND**	**ND**	**ND**	**0.000**
Study Day 27	**56.6**	**4**	**ND**	**(0.004)**	**ND**	**ND**	**0.002**
	309.8	**4**	**(0.01)**	**0.016**	**0.016**	**0.015**	**0.003**
	1019.5	**4**	**0.026**	**0.075**	**0.041**	**0.046**	**0.021**
Muscle	**16.7**	**4**	**(0.005)**	**(0.007)**	**(0.007)**	**(0.007)**	**0.001**
	56.6	**4**	**0.020**	**0.029**	**0.022**	**0.023**	**0.004**
	309.8	**4**	**0.079**	**0.113**	**0.111**	**0.104**	**0.016**
	1019.5	**4**	**0.251**	**0.484**	**0.335**	**0.351**	**0.115**

Matrix	Feeding level – dry feed (mg/kg) [a]	Residue levels (mg/kg)					
		n	Min.	Max.	Median (STMdR)	Mean (STMR)	Std. Dev.
Liver	16.7	4	0.028	0.036	0.032	0.032	0.004
	56.6	4	0.088	0.145	0.108	0.112	0.024
	309.8	4	0.425	0.560	0.512	0.502	0.058
	1019.5	4	1.423	1.962	1.842	1.767	0.238
Kidney	16.7	4	0.325	0.606	0.392	0.429	0.126
	56.6	4	1.355	1.559	1.462	1.460	0.099
	309.8	4	4.705	6.030	4.833	5.100	0.627
	1019.5	4	13.662	25.304	20.586	20.035	5.146
Subcutaneous fat	16.7	4	(0.004)	0.014	(0.009)	(0.009)	0.005
	56.6	4	0.012	0.066	0.027	0.033	0.023
	309.8	4	0.057	0.340	0.240	0.219	0.130
	1019.5	4	0.173	1.453	0.320	0.566	0.604
Mesenteric fat	16.7	4	ND	0.014	(0.006)	(0.006)	0.006
	56.6	4	0.016	0.116	0.021	0.043	0.049
	309.8	4	0.027	0.549	0.158	0.223	0.230
	1019.5	4	0.296	1.282	0.363	0.576	0.472
Perirenal fat	16.7	4	(0.007)	0.041	0.022	0.023	0.016
	56.6	4	0.012	0.264	0.080	0.109	0.114

Matrix	Feeding level – dry feed (mg/kg) [a]	Residue levels (mg/kg)					
		n	Min.	Max.	Median (STMdR)	Mean (STMR)	Std. Dev.
	309.8	4	0.075	1.048	0.476	0.519	0.418
	1019.5	4	0.241	3.658	0.611	1.280	1.607
Composite fat	16.7	4	(0.005)	0.020	0.013	0.013	0.008
	56.6	4	0.015	0.149	0.042	0.062	0.061
	309.8	4	0.053	0.635	0.297	0.320	0.251
	1019.5	4	0.237	2.131	0.431	0.808	0.893

[a] Average clopyralid concentration in the diet expressed on a dry feed (DM) basis.

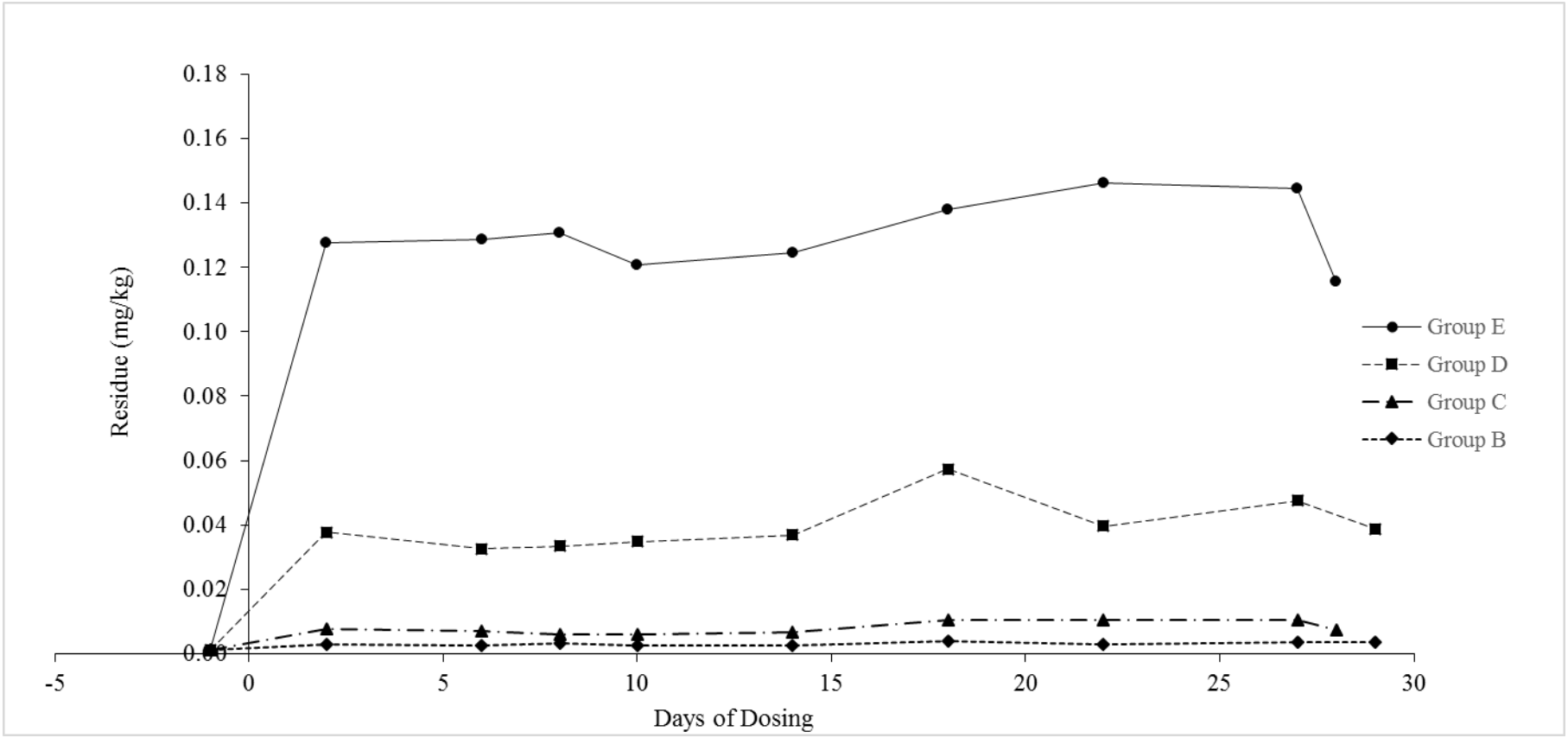


Figure B.7.4.2.4-1: Average residues of clopyralid in whole milk during 28- or 29-day dosing period

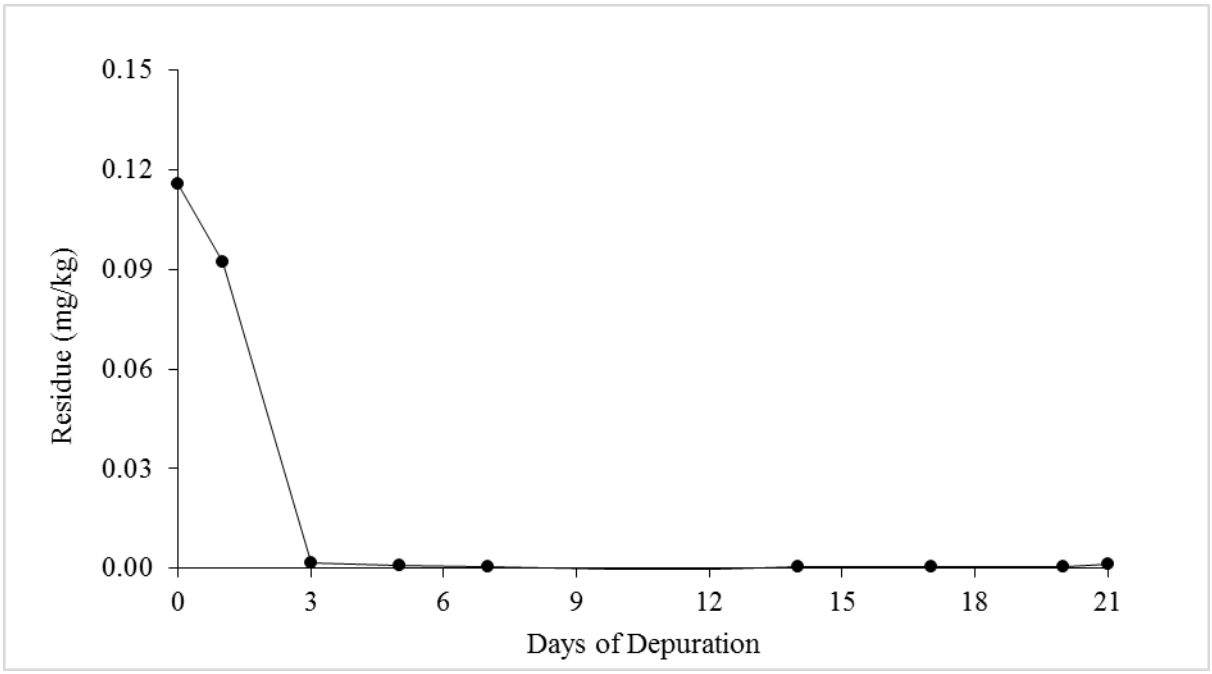


Figure B.7.4.2.4-2: Average residues of clopyralid in whole milk during depuration period

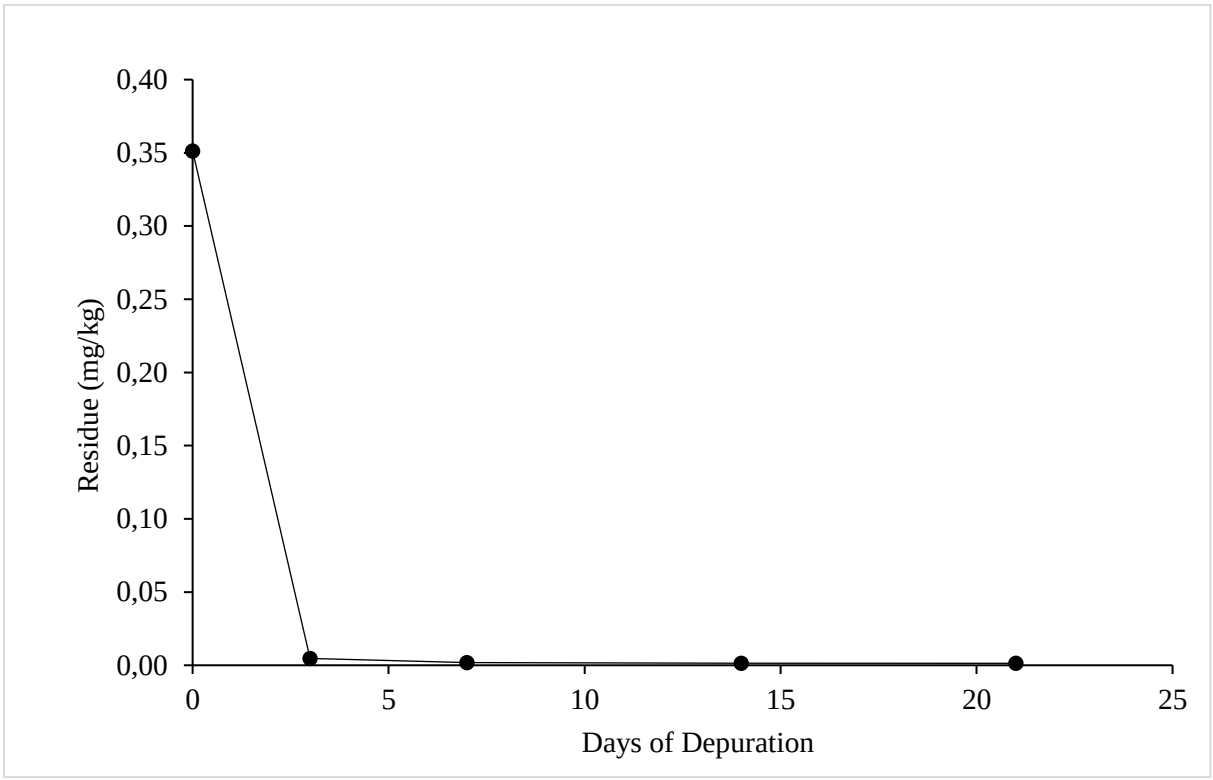


Figure B.7.4.2.4-3: Average residues of clopyralid in muscle during depuration period

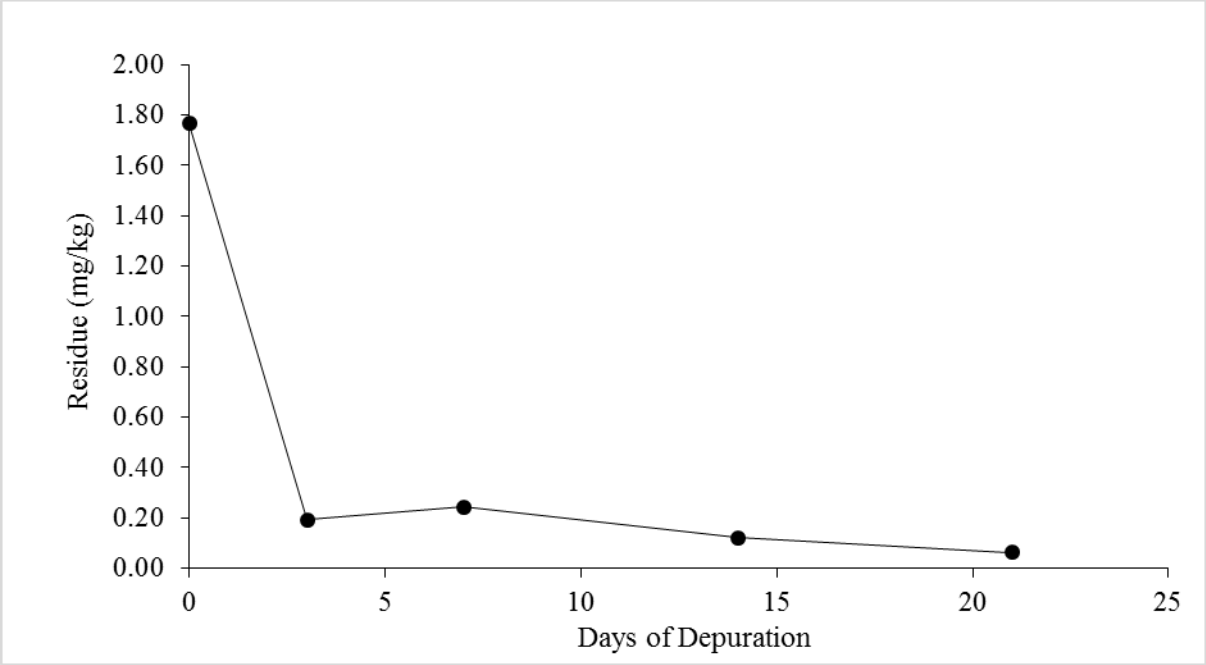


Figure B.7.4.2.4-4: Average residues of clopyralid in liver during depuration period

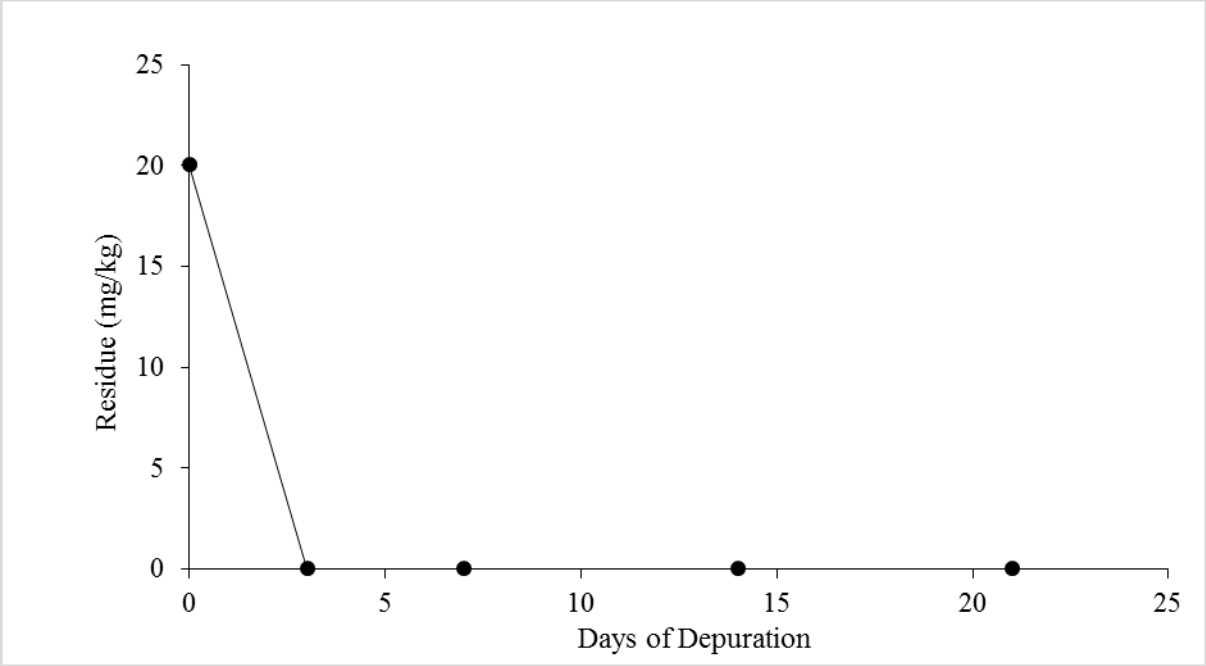


Figure B.7.4.2.4-5: Average residues of clopyralid in kidney during depuration period

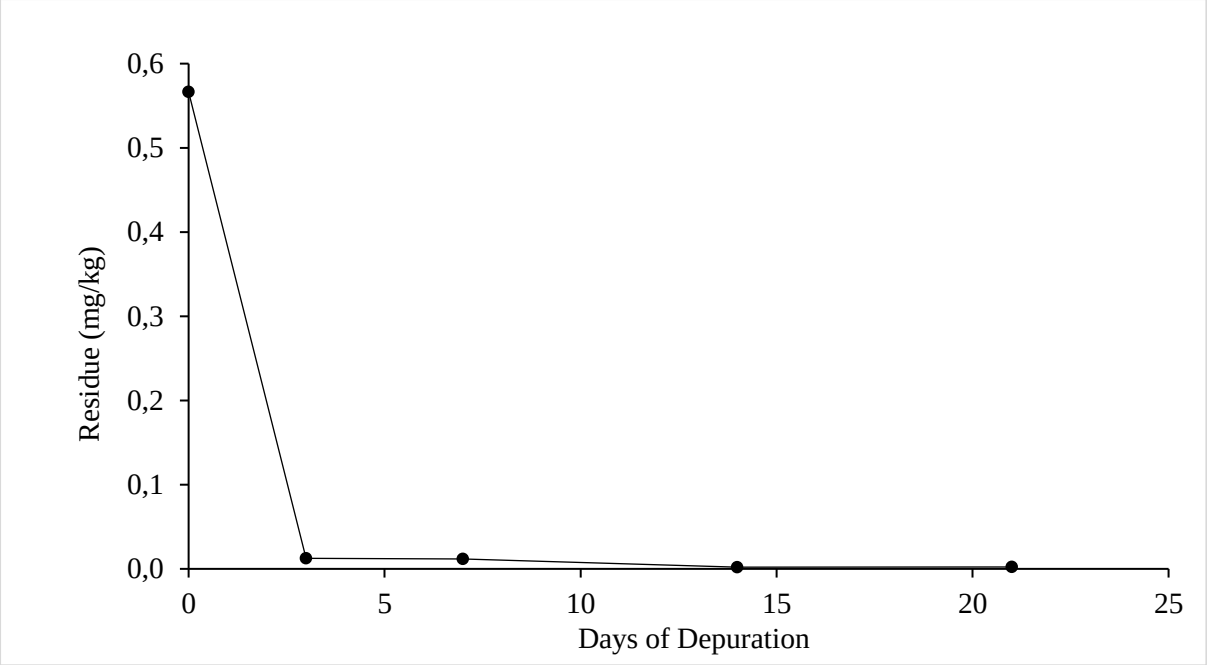


Figure B.7.4.2.4-6: Average residues of clopyralid in subcutaneous fat during depuration period

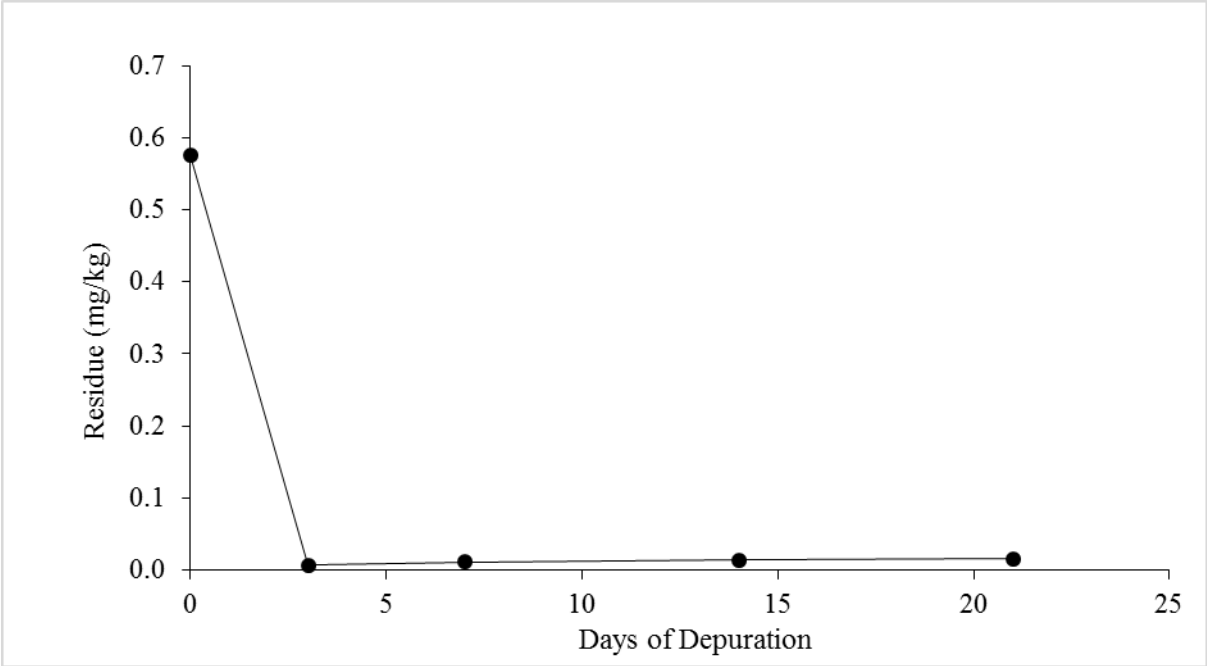


Figure B.7.4.2.4-7: Average residues of clopyralid in mesenteric fat during depuration period

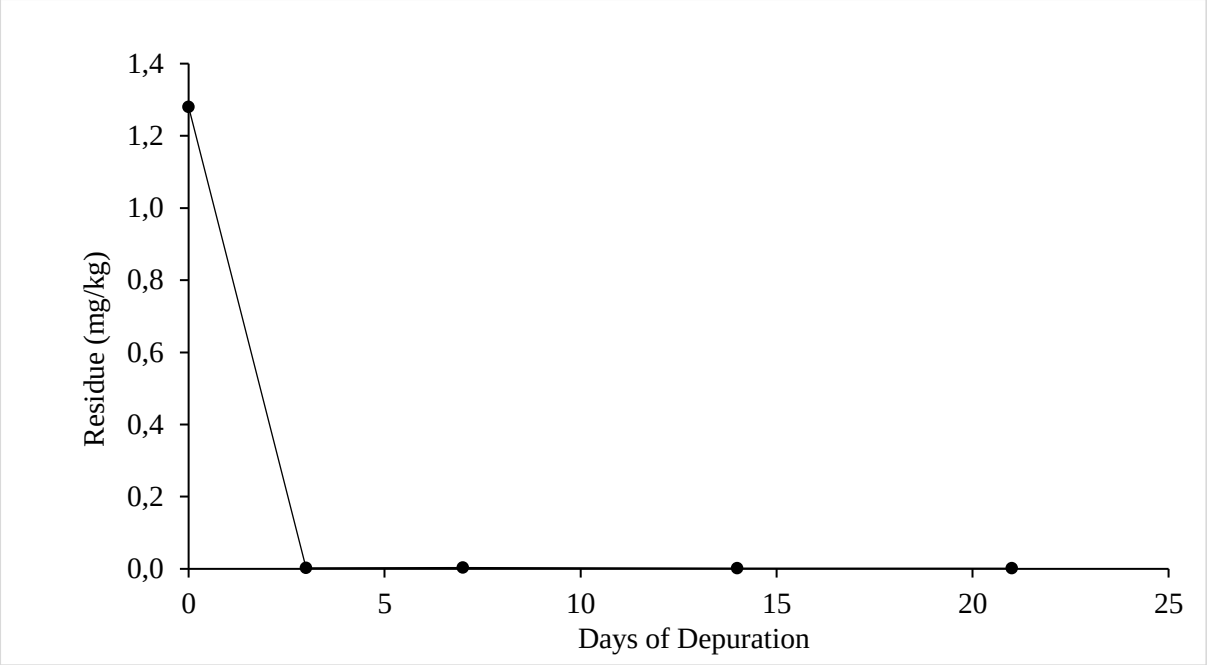


Figure B.7.4.2.4-8: Average residues of clopyralid in perirenal fat during depuration period

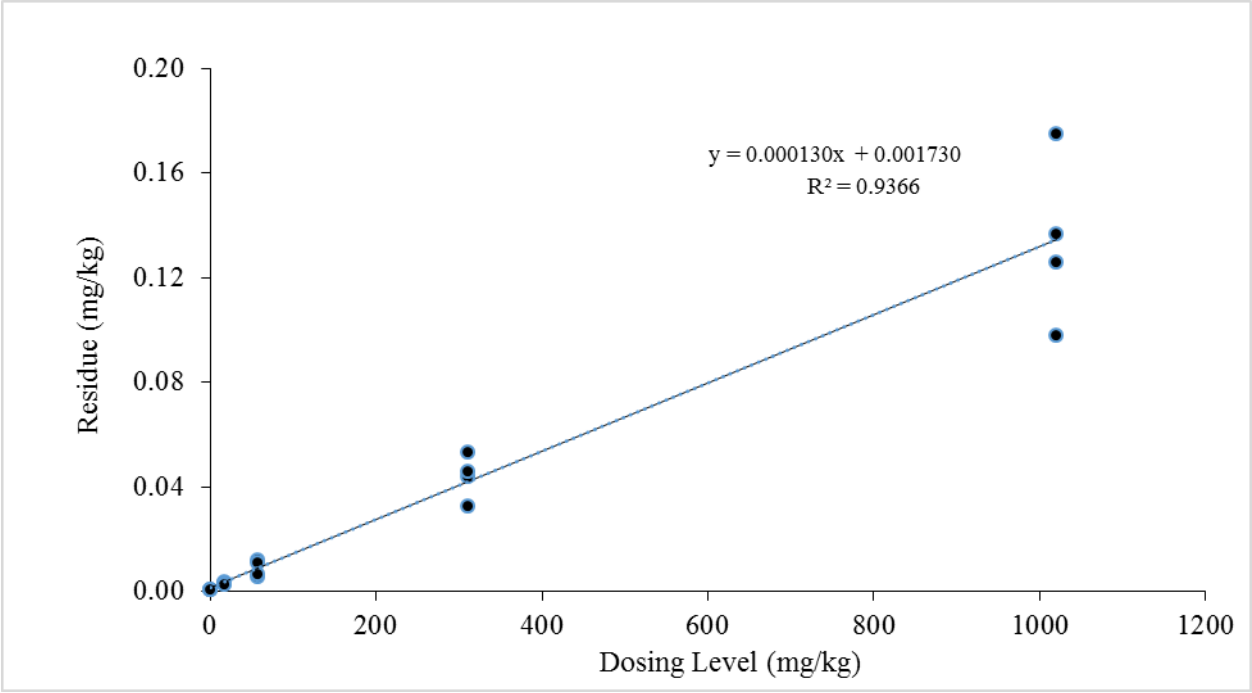


Figure B.7.4.2.4-9: Linear regression of clopyralid residue concentration (mg/kg) in whole milk vs. feeding level (mg/kg)

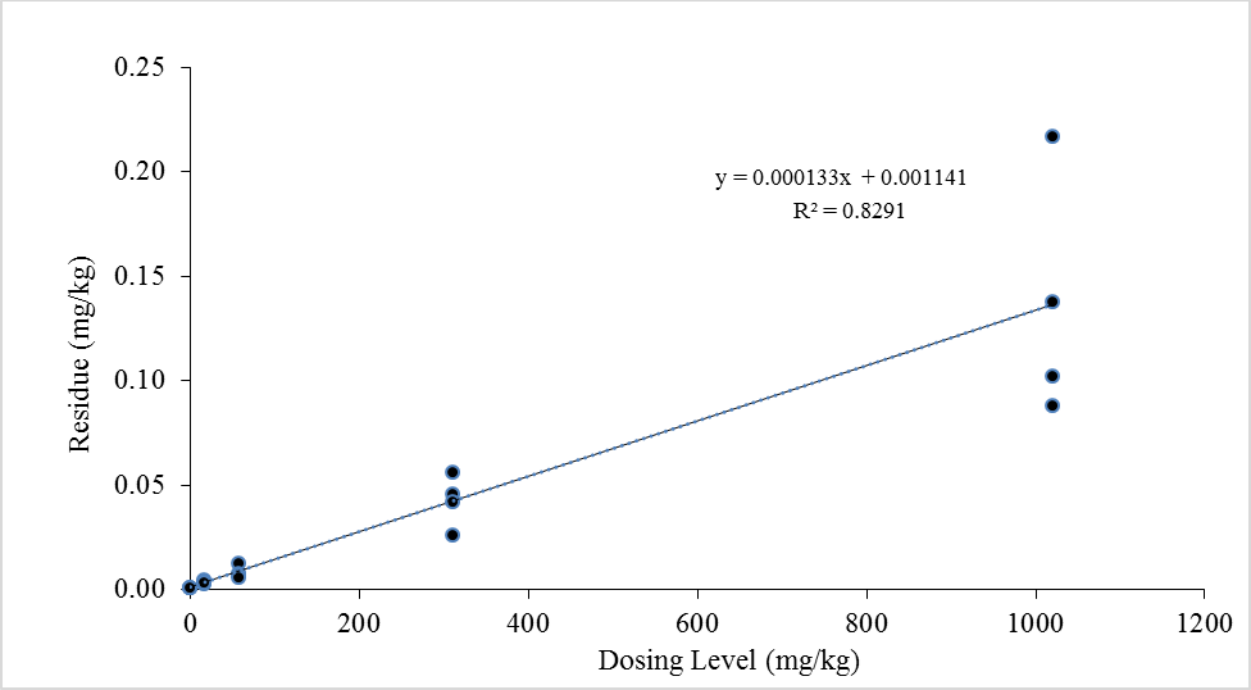


Figure B.7.4.2.4-10: Linear regression of clopyralid residue concentration (mg/kg) in skimmed milk vs. feeding level (mg/kg)

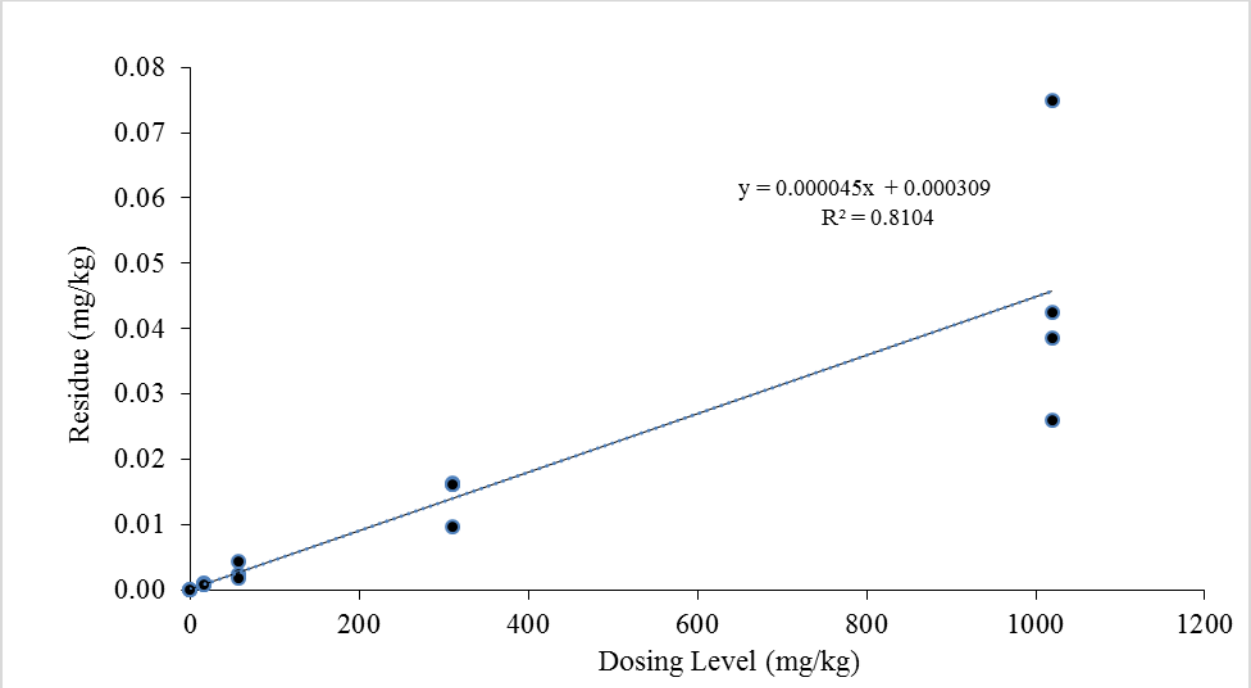


Figure B.7.4.2.4-11: Linear regression of clopyralid residue concentration (mg/kg) in cream vs. feeding level (mg/kg)

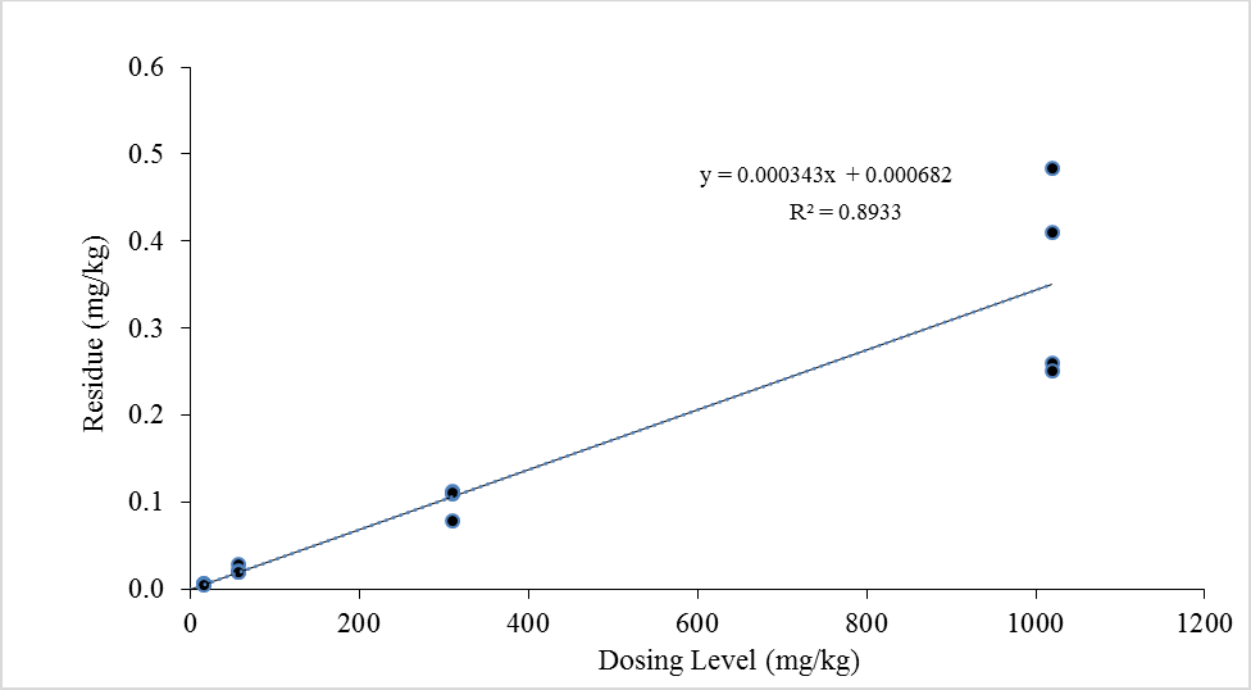


Figure B.7.4.2.4-12: Linear regression of clopyralid residue concentration (mg/kg) in muscle vs. feeding level (mg/kg)

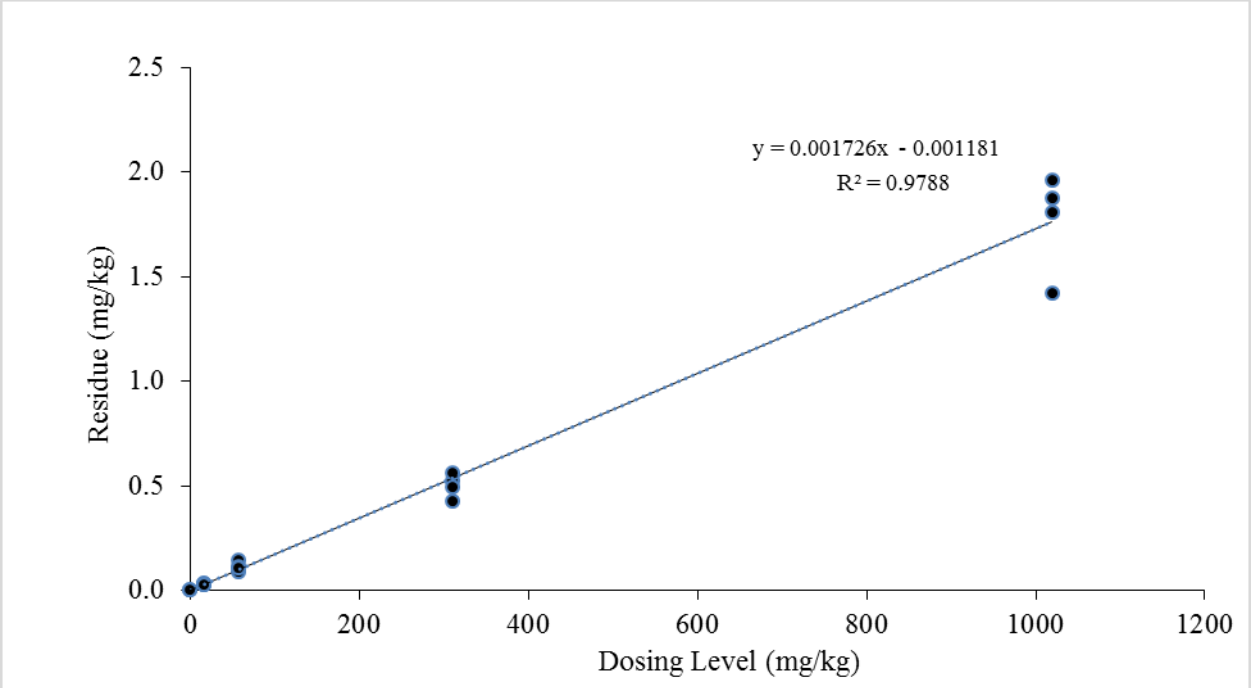


Figure B.7.4.2.4-13: Linear regression of clopyralid residue concentration (mg/kg) in liver vs. feeding level (mg/kg)

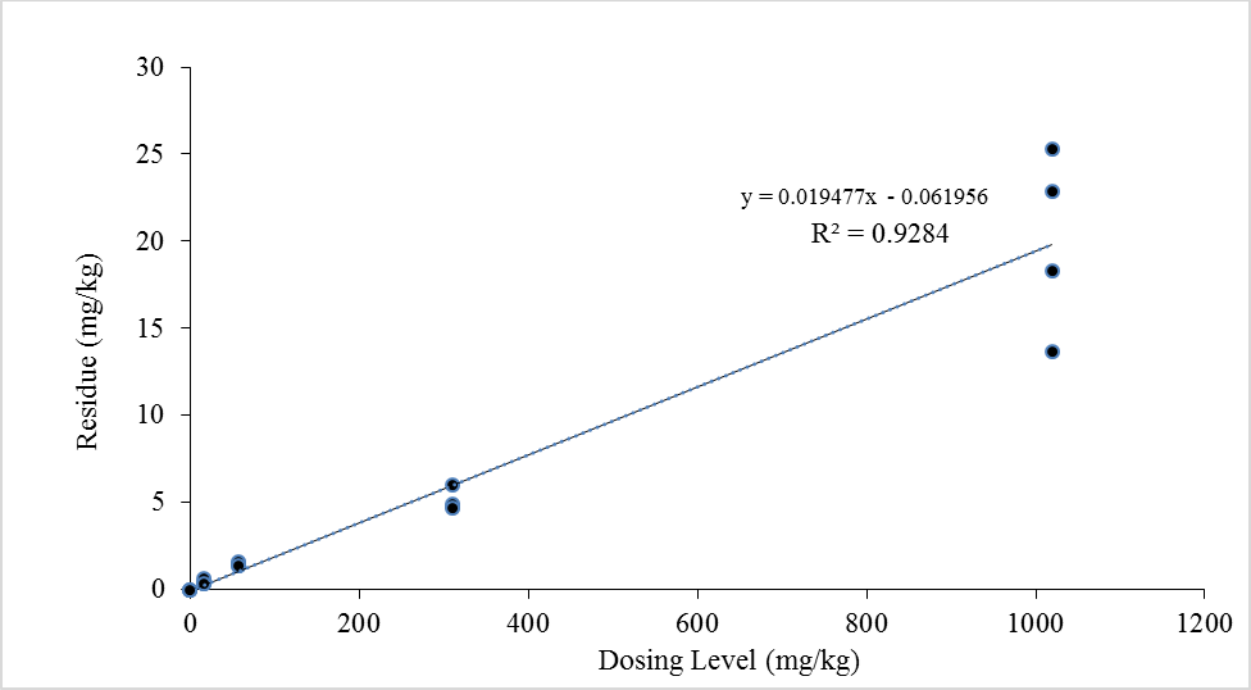


Figure B.7.4.2.4-14: Linear regression of clopyralid residue concentration (mg/kg) in kidney vs. feeding level (mg/kg)

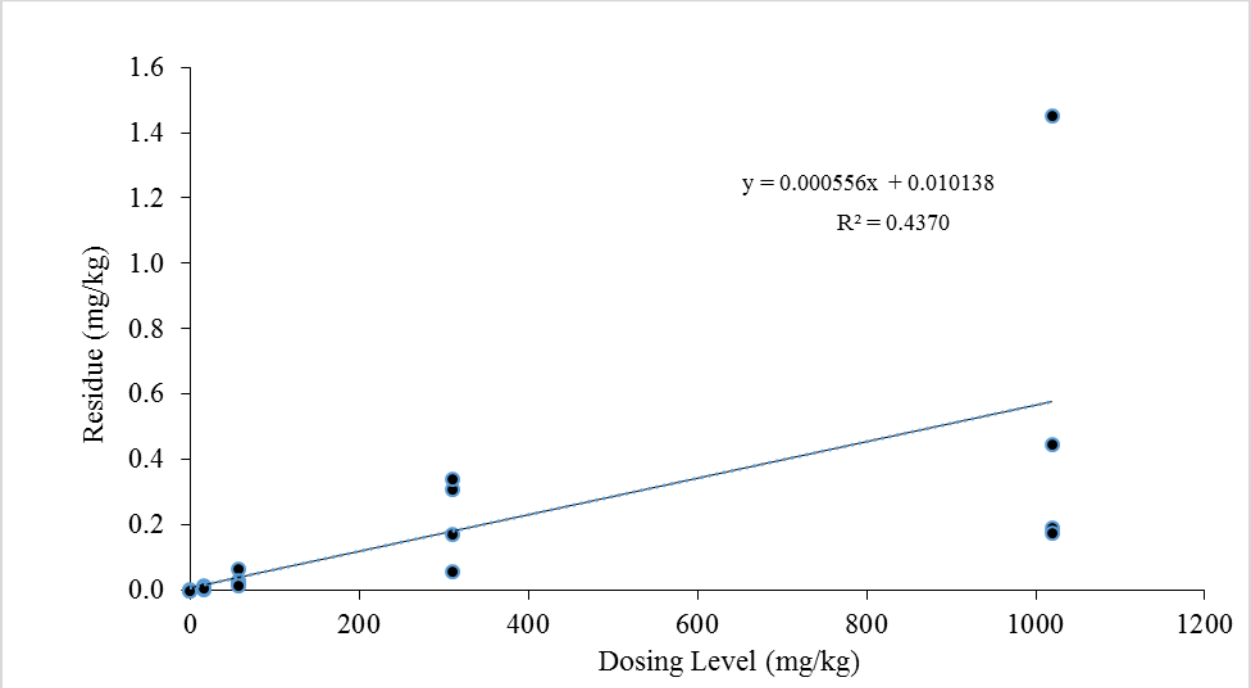


Figure B.7.4.2.4-15: Linear regression of clopyralid residue concentration (mg/kg) in subcutaneous fat vs. feeding level (mg/kg)

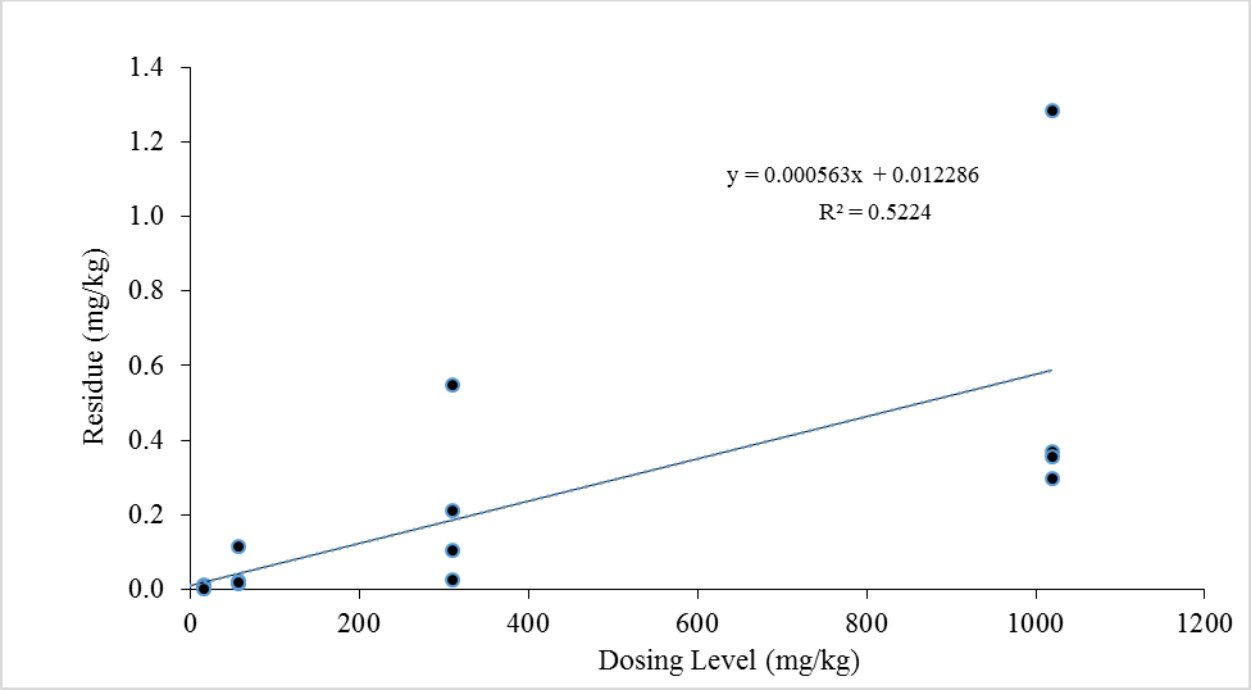


Figure B.7.4.2.4-16: Linear regression of clopyralid residue concentration (mg/kg) in mesenteric fat vs. feeding level (mg/kg)

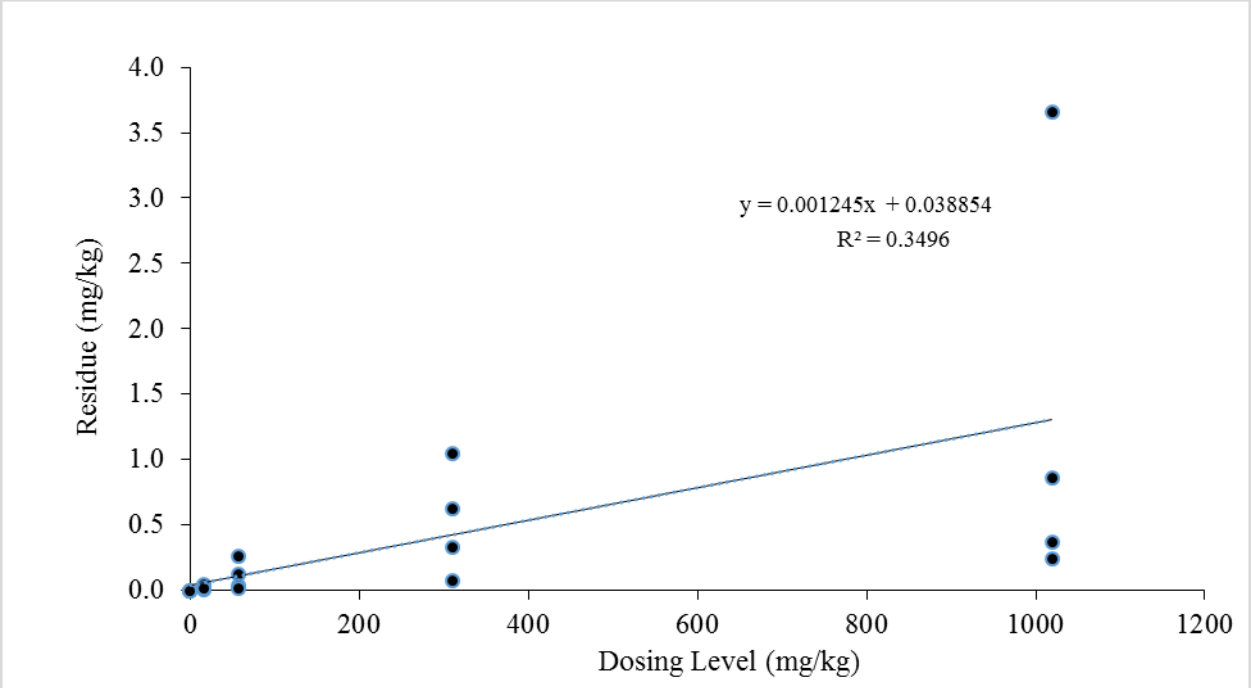


Figure B.7.4.2.4-17: Linear regression of clopyralid residue concentration (mg/kg) in perirenal fat vs. feeding level (mg/kg)

CONCLUSION

Residues of clopyralid transfer into whole milk at the 1x, 5x and 18x dosing levels and appeared to reach a plateau within the first 2 days of dosing. Clopyralid residues above the LOQ were found in whole milk and skimmed milk from cows in the 1x, 5x and 18x treatment groups and in cream from cows in the 5x and 18x treatment groups. Clopyralid did not concentrate in the cream fraction. Residues of clopyralid above the LOQ of 0.01 mg/kg were found in muscle samples from cows in the 1x, 5x and 18x treatment groups and in the liver, kidney, subcutaneous fat, mesenteric fat and perirenal fat from cows in the 0.3x, 1x, 5x and 18x treatment groups.

Regression analysis of clopyralid in milk, skimmed milk, cream and tissues (muscle, liver, kidney and fat) demonstrated a generally linear relationship between the dose level and the resulting residue concentration.

Depuration data generated using the 12 cows in the 18x dose level showed that residues of clopyralid declined rapidly following withdrawal of the test items from the cows' diet. Following withdrawal of the test item from the cows' diet, residues of clopyralid decreased rapidly and were below the LOQ of 0.01 mg/kg by Day 31 of the study (3 days after withdrawal) in whole milk, muscle and perirenal fat samples, by Day 42 of the study (14 days after withdrawal) in subcutaneous fat samples and by Day 49 of the study (21 days after withdrawal) in kidney samples. Clopyralid residues of up to 0.067 mg/kg were found in in liver samples and up to 0.026 mg/kg in mesenteric fat samples 21 days following withdrawal of the test item from the cows' diet.

B.7.4.3 Pigs

The metabolism of clopyralid in ruminants (goats) and non-ruminants (rats, poultry) is similar, and therefore metabolism and feeding studies in pigs are not required. It is possible to extrapolate results from the cattle feeding study to pigs.

No supplementary study or data are required or submitted.

Results from a swine feeding study conducted with clopyralid have previously been evaluated during Annex I inclusion / Active Approval (Clopyralid Draft Assessment Report, Vol.3, B7.8, February, 2005). The study was not required and was considered supplemental since metabolism of clopyralid in ruminants (goats) was not considered to be different than metabolism in non-ruminants (poultry and rodents (rats)). Since metabolism in ruminants and non-ruminants is considered to be similar, the cattle feeding study may be used for determination of the transfer of residue from the diet to swine tissues. Therefore, as indicated below, it is proposed that the new cattle feeding study which is compliant with GLP and current guidelines be used to determine potential levels of residue in swine tissues. A summary concerning the justification for use of the new cattle feeding study rather than the swine feeding study follows:

Data point/Study	Rationale
6.4.2/4	The swine feeding study ([redacted] 1975) evaluated for the Active Approval was not a GLP study and was not conducted according to current guidelines. A new GLP feeding study with clopyralid in dairy cattle was conducted according to current guidelines and is presented in 6.4.2/4. Since metabolism of clopyralid in ruminants (goats) does not differ significantly from metabolism in non-ruminants (poultry and rodents/rats), the cattle feeding study may be extrapolated for use in estimating the level of residue transferred to swine tissues from their diet. Residue levels observed in the new cattle feeding study were generally similar to or greater than those observed at comparable dose levels in the previously submitted swine feeding study. Considering the new cattle feeding study meets current guideline requirements, is GLP compliant and indicates residues at approximately equal or greater levels compared to the previously submitted swine study, it is proposed to use the new cattle feeding study rather than the swine feed study to estimate the transfer of residues from the diet to swine tissues in the Annex I Renewal evaluation.

Although it is proposed that the cattle feeding study completed in 2015, which is summarized in 6.4.2/4 be used to estimate transfer of residues from the diet to swine tissues, the Reference for the swine feeding study previously submitted during the Active Approval is listed below for reference and completeness.

B.7.4.3.1 Residues of Dowco 290 (3,6-dichloropicolinic acid) in Tissues of Swine Fed the Clopyralide

Report	IIA 6.4/04 [redacted] 1975
Report title	Residues of Dowco 290 (3,6-dichloropicolinic acid) in Tissues of Swine Fed the Herbicide
DAS Study / Report number	Study report no. GH-C 874
Guidelines	N/A
GLP	Non-GLP. This study was conducted prior to the effective date of the final rule, 40 CFR Part 160, EPA FIFRA Good Laboratory Practice Standard.

The study is rather old and do not meet GLP requirements. Consequently it is not dealt further.

B.7.4.4 Fish

At the time of compilation of the data a fish feeding study was not considered by the Notifier as there were currently no final, approved guidance documents or test guidelines for determining dietary burden / potential residue intake in the diet or methodology for conducting a fish feeding study. Vegetable proteins are used in fish meals and often wheat is used as a binder in the pelleted formulations.

As far as OECD guidelines are considered, no approved guidance does not exist so far. For the EU, "Working document on the nature of pesticide residues in fish", SANCO/11187/2013 (31 January 2013 rev. 3) is feasible for the purpose. This guidance document is applicable from 1 January 2014 on for all active substances for which an application for approval and all plant protection products for which an application for authorisation is made.

Table B.7.4.4-1The first step is to calculate dietary burden for the carp and the trout.

Crop	Commodity	IFN Code	Class	Residue Input value	CP** (%) of DM	NfE (%) of DM	CL (%) of DM	DM (%)	Carp* (max. % of diet)	Trout* (max. % of diet)	STMR mg/kg
Wheat	Grain (extruded)	4-05-211	CC	STMR	13.8	79.4	2.9	89	35	20	3.61
Barley	Bran fractions	4-00-515	CC	STMR-P	16.4	68.0	6.6	88	35	15	5.61
Brewer's grain	Dried	5-00-516	CC	STMR-P	25.9	44.5	7.0	92	35	15	
Wheat	Gluten		PC	STMR-P	80.1	17.2	1.5	91.4	15	15	
Wheat	Extruded grain	4-12-208	CC	STMR-P	13.5	80.2	1.9	87.7	15	15	
Wheat	Bran	4-05-190	CC	STMR-P	15.6	61.8	4.7	88.7	35	15	5.61
Wheat	Flour	4-05-199	CC	STMR-P	14.3	81.9	1.7	88.0	15	15	
Wheat	Germ	5-05.218	CC	STMR-P	28.5	54.3	8.8	88.7	5	5	
Wheat	Middlings	4-06-749	CC	STMR-P	16.9	66.2	4.4	89.4	40	25	
Potato	Protein		PC	STMR-P	81.8	12.2	2.8	89.4	3	-	
Oilseed rape	Meal (toxic)	5-26-093	PC	STMR-P	37.3	33.2	1.9	91	5	5	0.5
Canola	Meal	5-08-136	PC	STMR-P	37.3	33.2	1.9	91	35	20	

The next step is to optimize the diet to provide both maximum residue levels and adequate nutritient intake.

PC (%CP) = X
TARGET (%CP) = Z
Amount of CC% = (X-Z)*100/((Y-Z)+(X-Z)
CC (%CP) = Y
Amount of PC% = (Y-Z)*100/((Y-Z)+(X-Z)

Table B.7.4.4-2 Selection of protein supplement and basal ingredient (Example)

CP(%)	CL(%)	STMR-P	(mg	/kg	DM)	
1.Peanut_meal	(PC)	46.5	1.0	0.09		
2.Soybean_meal	(PC)	49.8	0.8	0.05		
1.Corn meal	(CC)	10.2	4.8	0.3		
2.Rice_(broken_grains)	(CC)	8.1	0.6	0.02		
1.Vegetable_oil	(F)	0	100	0.01		
Wheat bran milled products						
Wheat grain						

Table B.7.4.4-3Selection of protein supplement and basal ingredient (Example)

		CP(%)	CL(%)	STMR-P (mg/kg DM)
1.Peanut meal	(PC)	46.5	1.0	0.09
2.Soybean_meal	(PC)	49.8	0.8	0.05
1.Corn meal	(CC)	10.2	4.8	0.3
2.Rice (broken grains)	(CC)	8.1	0.6	0.02
1.Vegetable_oil	(F)	0	100	0.01

Effects of Processing

Table B.7.5-1: Summary of processing factors

Crop	Processed Commodity	Number of trials	Mean Transfer Factor
Wheat	White flour	4	0.3
	Wholemeal flour	2	1.0
	Wheat germ	2	3.3
	Bran	4	6.1
	Wholemeal bread	2	0.6
	White bread	2	0.1
Barley	Malt sprouts	2	0.2
	Brewing malt	2	0.6
	Spent grains and flocs	2	0.1
	Brewer's yeast	2	0.1
	Beer	2	0.1

[a] Based on information in the "Evaluation Report for Clopyralid", prepared by RMS Finland, 15-12-2008. For white flour and bran, mean Transfer Factors were calculated based on results from 4 trials by selecting the highest transfer factor from two treatments within the same trial for the two trials having two treated plots each rather than handling the treatments within a trial as separate trials.

B.7.5 Effects of Processing

B.7.5.1 Adusumilli 2014 Processing Study to Determine the Nature of Residues of ^{14}C -Clopyralid Following the Industrial or Household Preparation

REFERENCE	Adusumilli, H.; 2014; Processing Study to Determine the Nature of Residues of ^{14}C -Clopyralid Following the Industrial or Household Preparation; Dow AgroSciences LLC, Indianapolis, Indiana, USA; Lab Study No. 140574; DAS Study No. 140574; 01 October 2014; Unpublished
Guideline(s):	OECD 507
Deviations:	N/A
Site	outside EU
GLP status:	Yes

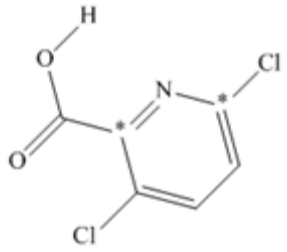
Materials and Methods

Test Item(s)

Non-radiolabelled test item #1

ISO Common name:	Clopyralid
Purity:	99.9%

Radiolabelled test item #1

Name:	Clopyralid-2,6-^{14}C
Test item (chemical/other name):	^{14}C-clopyralid 3,6-dichloropicolinic acid-2,6-^{14}C
Structural formula: Position of labelling (*)	
Radiochemical purity:	98.0%
Specific radioactivity:	32.8 mCi/mmol (379,250 dpm/µg

Methods

A total of fifteen samples were prepared with five replicates per set of hydrolysis conditions. Per replicate, 100 µL of the 70 µg/mL application solution was directly pipetted into a weighed and labeled vial containing 10 mL of buffer, for a target concentration of 0.700 µg/mL (equivalent to mg/L). Three replicates from each set were analyzed for radioactive content, one surrogate from each sample set was used to measure the pH before heating (this sample was not heated) and another surrogate was prepared to measure the pH before and after heating. The sample vials were capped with a Teflon-lined septum cap. The septum was pierced with a syringe needle, which remained in position during processing to prevent pressure build-up in the samples during heating. Each sample was weighed before heating.

Buffer Solutions

The study was performed with buffer solutions at three different pH values selected to simulate typical processing practice:

Table B.7.5.1-1 Test system – citrate buffer

pH 4 citrate buffer	500 mL 40 mM citric acid plus approximately 400 mL HPLC-grade water, pH adjusted to 4.01 with 2 N sodium hydroxide and diluted up to 1000 mL with HPLC-grade water.
pH 5 citrate buffer	500 mL 40 mM citric acid plus approximately 400 mL HPLC-grade water, pH adjusted to 5.00 with 2 N sodium hydroxide and diluted up to 1000 mL with HPLC-grade water.
pH 6 citrate buffer	500 mL 40 mM citric acid plus approximately 400 mL HPLC-grade water, pH adjusted to 6.00 with 2 N sodium hydroxide and diluted up to 1000 mL with HPLC-grade water.

The final concentration of the buffer solutions were 20 mM.

Test Vessels

Each sample was contained in a 20 mL sterile glass vial (volume of 10 mL). Five replicate of samples per buffer were prepared for each temperature conditions.

Sample Handling

A hydrolysis study was conducted using three sets of conditions representative of processing of raw agricultural commodities. To measure radioactive content triplicate buffered water samples, approximately 0.700 mg/L, were heated for 20 minutes at 90 °C (pH 4), boiled at 100 °C for 60 minutes (pH 5), or steamed at 120 °C for 20 minutes (pH 6). Average mass balance for the nine replicates for each test substance was measured.

Table B.7.5.1- 2: Industrial and household preparation of clopyralid experimental design

Parameter		Description
Test concentrations	**Nominal**	**0.700 mg/L**
	Measured	**0.718-0.724 mg/L**
Number of replicates		**5 per condition (3)**
Preparation of test medium	**Volume per buffer**	**10 mL**
Test item application	**Co-solvent (acetonitrile)**	**0.1 mL of methanol (1.0%)**
	Volume of application **solution used/treatment**	**10 mL dosed buffer**
	Application Method	**Use positive displacement pipette to spike 0.1 mL of application solution to 10 mL of buffer**
Test apparatus		**20-mL glass vials with Teflon-lined septum caps**
Test item sorption to walls of apparatus?		**No**
Other details		**0.02 mL of formic acid was added after heating** **pH measurements were on separate surrogates, one before heating and another after heating**

Five replicates per set of hydrolysis conditions were prepared, for a total of 3 per condition and 9 samples overall. The buffer solution (10 mL) was pipetted into each labelled vial and dosed separately. The 20-mL samples vials were capped with a Teflon-lined septum cap. The septum was pierced with a syringe needle, which remained in position during processing, to prevent pressure build-up in the samples during heating. Each sample was weighed before and after heating. The samples were heated in a water bath (90 or 100 ºC) or autoclave (120 ºC). Heating time did not include warm-up or cool-down periods.

Analytical Methodology

Total ^{14}C measurement

The liquid scintillation counters automatically converted the radioactivity counting rate in counts per minute (cpm) to disintegrations per minute (dpm) using an external standard to correct for sample quenching. The instrument was calibrated at least every six months with a set of ten quenched standards. Each day of use, the instrument was normalized and its performance was checked with respect to background cpm value, unquenched standard cpm value, and quenched standard dpm value for a range of quenched standards. The dpm value for a sample was determined by LSC after diluting an appropriate aliquot of the sample with scintillation cocktail and counting for at least five minutes.

High performance liquid chromatography (HPLC) for quantitation

The HPLC system used for this study consisted of an Agilent 1200 Series autoinjector, degasser, and binary pump, a 1200 Series variable wavelength detector, connected to a stop-flow controller and Radio LC-System Detector from IN/US systems (IN/US Systems, Inc., Tampa, FL). The flow-through radioactivity monitor (RAM) was used to quantify the amount of radioactivity present in each peak. The RAM contained a liquid cell. Laura™ software (IN/US Systems, Inc.) was used to control the HPLC, collect UV and RAM data, and process the data. A direct spike of each sample analysed by HPLC was assayed by LSC and compared to the sum of the radioactivity eluted from the column to determine chromatographic recovery. The HPLC column used was a Synergi 4 µm Hydro-RP, 150 x 4.6 mm (Phenomenex).

An UV detector at 254 nm wavelength was used to determine the retention times of non-radiolabeled standards.

Identification of potential transformation products was based on mass detector (LC/MS) and cochromatoraphy.

Results and Discussion

The final concentration of the dosing solutions were 0.718-0.724 µg/mL containing 0.1% co-solvent (methanol). Temperature was recorded during the pH 4 and pH 5 studies. A steam indicator strip was used to confirm the heating conditions for pH 6 studies. During the pH 4 and pH 5 sample heating, the target temperature was 90 °C for 20 minutes and 100 °C for 60 minutes, respectively. The average temperature was 89.4 ± 0.6 °C and 99.0 ± 0.2 °C during the actual heating intervals. For pH 6 samples, the steam chemical indicator strips proved that steam temperature (121 °C) was reached for at least 18 minutes.

Overall material balance for all nine replicates was > 99%. Material balance of ^{14}C-clopyralid averaged 102.1 ± 0.4%, 102.7 ± 0.3%, and 101.6 ± 0.4% at pH 4, 5, and 6, respectively. The material balance values demonstrate that the radioactivity did not dissipate from the test systems during the processing period.

No hydrolysis of ^{14}C-clopyralid occurred after heating using the three sets of conditions.

Table B.7.5.1- 3: High temperature hydrolysis of clopyralid

Temperature (°C)	Time (min)	pH	Processes represented	Parent/metabolite	% of initial dose
90	**20**	**4**	**Pasteurisation**	**Clopyralid**	**99.3**
100	**60**	**5**	**Baking, brewing, boiling**	**Clopyralid**	**96.9**
120	**20**	**6**	**Sterilisation**	**clopyralid**	**97.1**

Metabolic Pathway

No hydrolysis of ^{14C}-clopyralid occurred in pH 4 aqueous buffer heated to 90°C for 20 minutes, pH 5 aqueous buffer heated to 100°C for 60 minutes, or pH 6 aqueous buffer heated to 120°C for 20 minutes.

Any test regarding the behaviour of clopyralid conjugates has not been conducted.

B.7.5.2 Distribution of the residue in inedible peel and pulp

Pasture grass and cereals do not have inedible peel or pulp. Therefore, this point is not relevant for the crops considered in this submission.

In fact cereals have a fruit coat or pericarp. Pericarp is one of the consituents of bran, while for wheat species used for bread making pericarp is separated from the grain. It is not clear whether the consumption values in PRIMo e.g. for wheat reflect wholemeal flowers or white flowers. So far this contemplation does not have an effect on present clopyralid evaluation, but it is noted that more guidenance may be needed in this respect.

Whear grains have appr. 4.6 higher levels than flour.

B.7.5.3 Magnitude of residues in processed commodities

Data to address this point for cereals (wheat and barely) have been submitted to the RMS (Finland) following Active Approval in response to these studies being identified as needed during the peer review. The RMS has since prepared a summary for each of the studies that were submitted and this was presented in the “Evaluation Report for Clopyralid”, RMS Finland, 15-12-2008. For consistency in review, the summaries previously prepared by the RMS are repeated / presented for the two studies submitted for wheat (6.5.3/1 and 6.5.3/2) and the study submitted for barley (6.5.3/1).

B.7.5.3.1.1 Garbay 2005

Report	Garbay, M., 2005, Residue study with fluroxypyr and clopyralid and 2,4 MCPA (Bofix - EF-1498) in wheat in France (North and South) year 2003. DAS Study / Report number: Study report no. S03AHBOFIX
Guidelines	FAO Guidelines on Producing Pesticide Residue Data from Supervised Trials, Rome 1990
Storage	214 days at -20C
Analytical method	R-T-M76-0
GLP	Yes

Materials and Methods:

In 2003, six residue trials in wheat (five winter wheat and one hard wheat) were conducted in France. Each trial was composed of two treated plots plot 1 and 2, which received one application of clopyralid. In three of the trials, one plot remained untreated representing a control plot. The application rates of clopyralid were 80 g as/ha (plot 1) and 160 g as/ha (plot 2). Foliar application was made at growth stage BBCH 32. Results presented in this summary are limited to only clopyralid.

Grain samples were collected at harvest (BBCH 89), stored and shipped for analytical laboratory in deep freezer conditions. Samples were analysed for clopyralid by using analytical method R-T-M76-0. The method involves extraction by methanol, purification by dividing liquid/liquid, esterification and finally, analysis by GC-MS. The LOQ for clopyralid was 0.150 mg/kg in wheat grain/straw and 0.09 mg/kg in processed wheat fractions.

Based on levels of clopyralid in the grain, specimens of grain from two trials were subjected to processing. One of the studies was conducted in Southern France (S03DAH.BOFGL13) and another study was conducted in Northern France (S03DAH.BOFVO28). Grains were milled to produce flour and bran + middlings by the test facility Grands Moulins de Paris. The maximum interval between harvest and analysis for clopyralid was 12 months. Samples were stored frozen at ≤ -18°C.

Results:

Residues of clopyralid in treated samples of wheat grain were below the existing EU-MRL for cereals (2 mg/kg). Residues of clopyralid in untreated wheat samples were not detected or below the LOQ. The residue results are summarised in Table B.7.5.3/1-1

Table B.7.5.3-2: Residues of clopyralid in wheat grain at harvest

Location, year	Crop	Application rate (kg as/ha)	PHI (days)	Residue levels of clopyralid (mg/kg)	Report No/ Trial ID
Northern France, 2003	Winter wheat	0.080 0.160	82 82	n.d. n.d	S03DAHBOFIX/ S03DAH.BOFVO27
Northern France, 2003	Winter wheat	0.080 0.160	89 89	0.542 0.196	S03DAHBOFIX/ S03DAH.BOFVO28
Northern France, 2003	Winter wheat	Control 0.080 0.160	96 96 96	n.d. < LOQ 0.194	S03DAHBOFIX/ S03DAH.BOFJL17
Southern France, 2003	Winter wheat	0.080 0.160	69 69	0.218 0.420	S03DAHBOFIX/ S03DAH.BOFGL13
Southern France, 2003	Winter wheat	Control 0.080 0.160	73 73 73	n.d. 0.369 0.382	S03DAHBOFIX/ S03DAH.BOFPR07
Southern France, 2003	Hard wheat	Control 0.080 0.160	89 89 89	n.d. 0.467 0.865	S03DAHBOFIX/ S03DAH.BOFPR08

n.d = no detectable residues (residues below the limit of detection)

Limit of Determination (LOD): 0.050 mg/kg

Limit of Quantification (LOQ): 0.150 mg/kg

Residue results of clopyralid in wheat grain and processed fractions are summarised in Table B.7.5.3/1-2 below. The results indicate that clopyralid does not concentrate in flour, but concentrates in bran and middlings.

For purposes of calculating average transfer factors, the maximum transfer factor for flour and bran + middlings from the two treatments within each of the two trials were selected. This results in selection of 0.6 and 0.3 as transfer factors for flour and 10.4 and 4.3 as transfer factors for bran + middlings (values shown in bold in Table B.7.5.3/1-2).

Table B.7.5.3-3: Residues of clopyralid in samples of wheat grain and processed fractions at harvest

Crop	Application rate (kg as/ha)	Fraction	Residue levels of clopyralid (mg/kg)	Transfer factor for clopyralid	Report No/ Trial ID
Winter wheat	0.080	Grain Flour Bran +	0.542 0.049 0.788	- 0.1 1.5	S03DAHBOFIX/ S03DAH.BOFVO28

		middlings			
	0.160	Grain	0.196	-	
		Flour	0.123	**0.6**	
		Bran + middlings	2.042	**10.4**	
Winter wheat	0.080	Grain	0.218	-	S03DAHBOFIX/ S03DAH.BOFGL13
		Flour	0.056	**0.3**	
		Bran + middlings	0.751	3.4	
	0.160	Grain	0.420	-	
		Flour	0.073	0.2	
		Bran + middlings	1.809	**4.3**	

B.7.5.3.1 Residues of clopyralid in wheat and process fractions at harvest following a single application of EF-1498, Northern France - 2005

Report	Devine, H.C., 2006, Residues of clopyralid in wheat and process fractions at harvest following a single application of EF-1498, Northern France – 2005. DAS Study / Report number: Study report no. GHE-P-11274
Guidelines	Commission Directive 96/68/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market FAO Guidelines on Producing Pesticide Residue Data from Supervised Trials, Rome 1990
GLP	Yes

As mentioned previously, the summary that follows was taken from an earlier summary prepared by the RMS Finland and presented in the "Evaluation Report for Clopyralid", RMS Finland, 15-12-2008.

Materials and Methods:

A study was conducted in Northern France during 2005 in order to determine the residues of clopyralid in wheat grain, straw and processed fractions (white flour, whole meal flour, wheat germ, bran, white bread and wholemeal bread) at harvest. A single application of clopyralid was made at a nominal application rate of 60 g as/ha. Foliar application was done at growth stage BBCH 30, 31 or 32. Three plots were treated and one remained untreated representing a control plot.

For all plots, samples of grain and straw were collected at harvest (75-89 days after application). For the plots where the application occurred at BBCH 30 or 32, additional samples of grain were taken for processing. Samples were shipped to the analytical laboratory in deep freezer conditions. Samples were kept deep-frozen not more than 191 days before extraction and analysis. The processing was done according to the technical procedures in a laboratory scale comparable to the processes used for commercial or household production of the goods.

Before milling, grain samples were determined for seed moisture content; cleaned and divided into 2 sub-samples. One sub-sample was used to get the white flour, wheat germ and bran, the other sub-sample was used to get the whole meal flour. For milling, 2 rolling mill type machines were used. One was used for the whole meal flour and the other to get the white flour with all the fractions.

Baking procedure involved the following steps: 1) preparation of dough; 2) resting seasons and 3) baking process. Whole meat bread was heated in the oven at about 210 ºC and white bread at about 230 ºC.

Samples of grain and processed fractions were analysed for clopyralid by using analytical method GRM 01.16. LOQ was 0.01 mg/kg.

Results:

Mean recoveries of wheat samples were between 72 and 109 %. Residues of clopyralid in treated samples of wheat grain were below the existing EU-MRL for cereals (2 mg/kg). Residues of clopyralid in untreated wheat samples were not detected or below 0.01 mg/kg. Residues in treated wheat samples are summarised in Table B.7.5.3/2-1

The results indicate that clopyralid does not concentrate in flour, whole meal and white bread. Instead residues of clopyralid concentrate in germ and bran.

Table B.7.5.3.2-1: Residues of clopyralid in samples of wheat grain and processed fractions

Location, year	Crop	Application rate (kg as/ha)	Fraction	Residue levels of clopyralid (mg/kg)	Transfer factor for clopyralid	Report No/ Trial ID
Northern France, 2005	Winter wheat	0.060	Grain	0.41	-	GHE-P-11274/ CEMS-2711A
			Straw	0.80	-	
			White flour	0.05	0.1	
			Wholemeal flour	0.34	0.8	
			Wheat germ	0.93	2.3	
			Bran	1.44	3.5	
			Wholemeal bread	0.22	0.5	
			White bread	0.33	0.1	
Northern France, 2005	Winter wheat	0.060	Grain	0.19	-	GHE-P-11274/ CEMS-2711A
			Straw	0.55	-	
			White flour	0.04	0.2	
			Wholemeal flour	0.23	1.2	
			Wheat germ	0.82	4.3	
			Bran	1.16	6.1	
			Wholemeal bread	0.12	0.6	
			White bread	0.02	0.1	
Northern France, 2005	Winter wheat	0.060	Grain	0.22	-	GHE-P-11274/ CEMS-2711A
			Straw	0.87	-	

B.7.5.3.2 Residues of clopyralid in spring barley and process fractions at harvest and at intervals following a single application of Lontrel 100 (EF-1136), Southern France, EF-1498, Northern France - 2005

Report	Devine, H.C., 2006. Residues of clopyralid in spring barley and process fractions at harvest and at intervals following a single application of Lontrel 100 (EF-1136), Southern France, EF-1498, Northern France – 2005. DAS Study / Report number: Study report no. GHE-P-11684
Guidelines	Commission Directive 96/68/EC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market FAO Guidelines on Producing Pesticide Residue Data from Supervised Trials, Rome 1990
GLP	Yes

As mentioned previously, the summary that follows was taken from an earlier summary prepared by the RMS Finland and presented in the "Evaluation Report for Clopyralid", RMS Finland, 15-12-2008.

Materials and Methods:

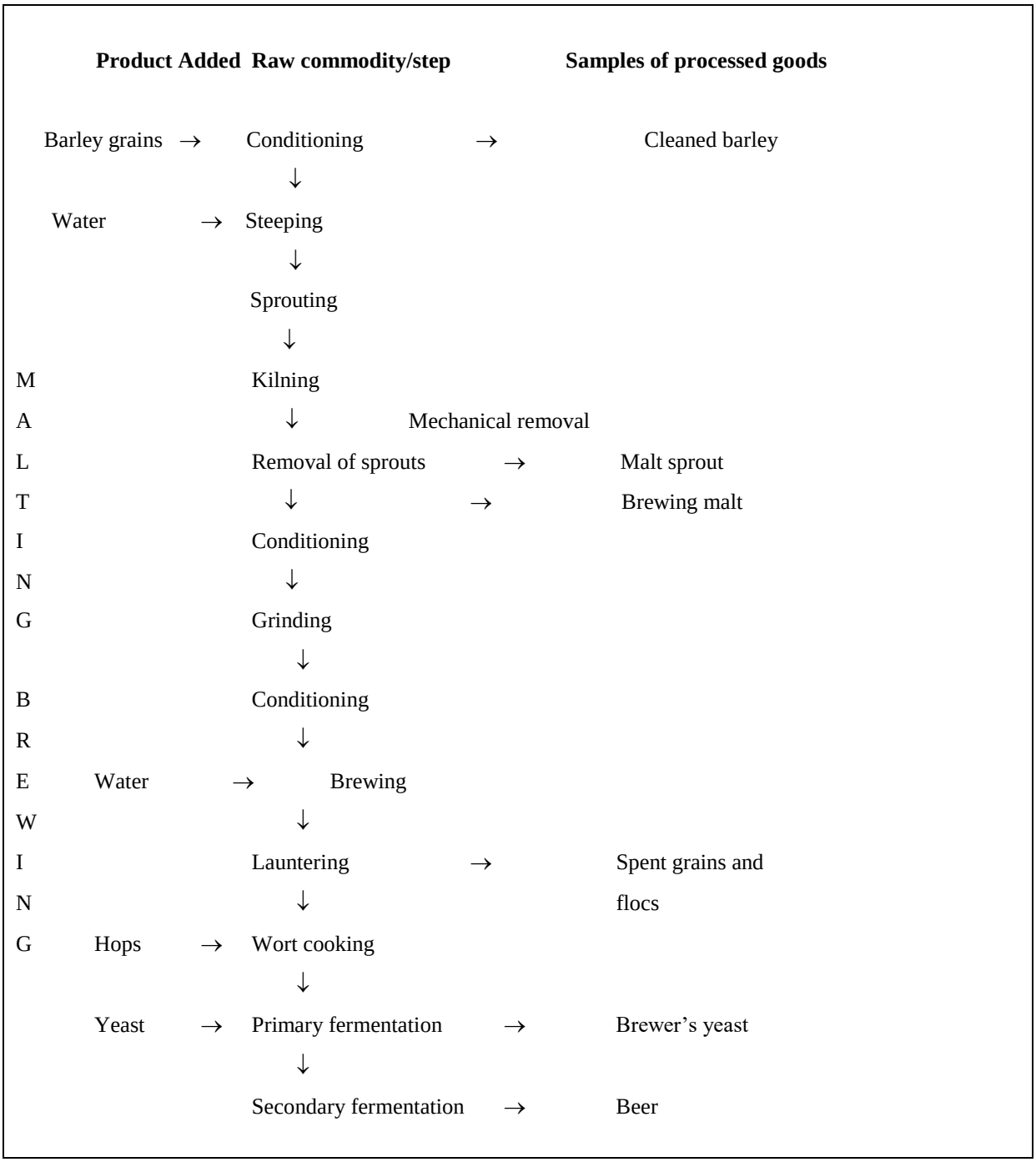
Four trials were conducted to determine the residues of clopyralid in spring barley at intervals or at harvest and in process fractions following a single application of clopyralid at a nominal application rate of 125 g as/ha at growth stage BBCH 39 or 41. Clopyralid was applied using conventional small plot application equipment at the proposed normal use rates and timings. The trials were conducted in regions of Greece, Spain and Southern France typical of Southern European barley growing areas.

For trials CEMS-2953A and CEMS-2953B samples of whole plant were taken before and after application and at intervals. Samples of grain and straw were taken at harvest (39-71 days after application). For trials CEMS-2953C and CEMS-2953C trials samples of grain and straw were taken at harvest (48-49 days after application). Additional samples were taken at harvest for processing.

The processing was carried out to obtain malt sprouts, brewing malt, spent grains and flocs, brewer's yeast and beer. The malting and brewing processing was carried out at the University of Honenheim, in Germany. Malting was done according to the spray steeping procedure in a micro matlhouse. The brewing procedure was done on a laborarory scale, but fully comparable to industrial brewing process. The scheme of malting and brewing is described in a Figure B.7.5.3/3-1.

Samples were analysed for clopyralid by using Dow AgroSciences Analytical Method GRM 01.16. The LOQ for clopyralid was 0.01 mg/kg.

Figure B.7.5.3.3-1 The scheme of malting and brewing (According to the report GHE-P-11684)



Results:

Mean recoveries of barley samples were between 68 and 109 %. Residues of clopyralid in treated samples of barley grain were below the existing EU-MRL for cereals (2 mg/kg). Residues of clopyralid in untreated wheat samples were not detected or below 0.01 mg/kg. Residues in treated barley samples are summarised in Table B.7.9 -4.

The results indicate that clopyralid does not concentrate during malting and brewing. Residue levels of clopyralid decreased in all processed fractions.

Table B.7.5.3.3-1. Residues of clopyralid in samples of barley grain and processed fractions

Location, year	Crop	Application rate (kg as/ha)	PHI (days)	Portion analysed	Residue levels of clopyralid (mg/kg)	Transfer factor for clopyralid	Report No/ Trial ID
Greece, 2006	Spring barley	0.120	0+	Whole plant	3.90	-	GHE-P-11684/ CEMS-2954A
			9	Whole plant	2.27	-	
			20	Whole plant	1.97	-	
			30	Whole plant	1.78	-	
			71	Grain	0.32	-	
			71	Straw	2.04	-	
Spain, 2006	Spring barley	0.128	0+	Whole plant	3.59	-	GHE-P-11684/ CEMS-2954B
			11	Whole plant	0.55	-	
			21	Whole plant	0.41	-	
			29	Whole plant	0.44	-	
			39	Grain	0.34	-	
			39	Straw	1.79	-	
Spain, 2006	Spring barley	0.123	49	Grain	1.56	-	GHE-P-11684/ CEMS-2954C
			49	Straw	2.30	-	
			49	Cleaned barley	0.99	0.6	
			49	Malt sprouts	0.35	0.2	
			49	Brewing malt	1.02	0.7	
			49	Spent grains and flocs	0.25	0.2	
			49	Brewer's yeast	0.16	0.1	
			49	Beer	0.14	0.1	
Southern France, 2006	Spring barley	0.130	47	Grain	1.63	-	GHE-P-11684/ CEMS-2954D
			47	Straw	5.62	-	
			47	Cleaned barley	0.86	0.5	
			47	Malt sprouts	0.40	0.2	
			47	Brewing malt	0.96	0.6	
			47	Spent grains and flocs	0.19	0.1	
			47	Brewer's yeast	0.14	0.1	
			47	Beer	0.13	0.1	

Conclusions – for processing of wheat and barley:

Residue trials have been conducted with clopyralid to determine residues in wheat and barley grain and processing fractions. Clopyralid was applied at normal application rates and timings. At harvest residues of clopyralid in wheat and barley grain were under the existing EU-MRL for cereals. The highest residue of clopyralid was 0.86 mg/kg in wheat grain and 1.63 mg/kg in barley grain.

Wheat and barley samples were processed according to the technical procedures on a laboratory scale comparable to the processes used for commercial or household production of the goods. Clopyralid concentrated in wheat germ and bran. Processing did not have effects on the residue levels in whole meal flour. Residue levels of clopyralid reduced in all other processed fractions of wheat. Residue levels were reduced also during malting and brewing of barley. An overview of the mean transfer factors for clopyralid in processed wheat and barley fractions is given in Table B.7.5.3-1.

Table B.7.5.3.3-2. Mean transfer factors for clopyralid in processed wheat and barley fractions

Crop	Commodity	Transfer factors	Mean Transfer factor [a]
Wheat	White flour	0.1, 0.2, 0.3, 0.6	0.3±0.2
	Wholemeal flour	0.8, 1.2	1.0
	Wheat germ	2.3, 4.3	3.3
	Bran	3.5, 4.3, 6.1, 10.4	6.1±3.1
	Wholemeal bread	0.5, 0.6	0.6
	White bread	0.1, 0.1	0.1
Barley	Cleaned barley	0.5, 0.6	0.6
	Malt sprouts	0.2, 0.2	0.2
	Brewing malt	0.6, 0.7	0.6
	Spent grains and flocs	0.1, 0.2	0.1
	Brewer's yeast	0.1, 0.1	0.1
	Beer	0.1, 0.1	0.1

[a] Average transfer factor for wheat across results presented in studies in 6.5.3/1 and 6.5.3/2 and average transfer factor for barley across two trials in 6.5.3/3. Only the maximum transfer factor was selected from the two treatments within each of the two trials in the study reported in 6.5.3/1.

B.7.6 Residues in Rotational Crops

A 30-day confined rotational crop study was conducted, since it was a data gap according to the current guideline and data requirements.

B.7.6.1 Metabolism in rotational crops

Data to address this point were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level.

In addition a short interval study (30-day) was conducted to meet the current guideline requirement.

According to present Volume 3 section B.8 (PPP) clopyralid is moderate to medium persistent (DT50 20ºC = 57 d – 215 d). Under anaerobic conditions there is practically no degradation and half-life is estimated to be greater than one year.

In the field studies clopyralid dissipated slightly faster being low to moderate persistent (DT50 field = 2 – 24 d). In laboratory conditions DT_{90lab} –values of 224, 331, 657 days were reported in the previous assessment.

In laboratory studies with an application rate below the maximum intended rate, the $DT_{90\ lab}$ exceeded 100 days (up to 217 days, mean 113 days). Degradation seems even to be slower when higher application rates are used.

At an application rate of 0.3 mg / kg (corresponding to 225 g a.s. / ha) and 40 % MWHC clopyralid is moderate to medium persistent (DT_{50} 20ºC = 13 d – 65 d). Degradation seems to be slower when higher application rates are used (1.0 mg / kg: DT_{50} 20ºC = 57 d – 215 d).

Under anaerobic conditions there is practically no degradation and half life is estimated to be greater than one year.

Furthermore, metabolism studies indicated that clopyralid is systemically taken into plants and readily translocated in plants. Soil –plant transition factors to estimate the residue situation in rotational crops have been calculated by RMS FI and presented in the evaluation meeting. The values indicate that there might be good uptake from soil or even accumulation in the plants, and soil residues above 0.001 mg/kg might be present at the time of harvesting rotational crops.

To clarify these issues notifer has submitted more data, which is evaluated in B:7.6.2.

B.7.6.1.1 Previously assessed studies on rotational crops:

A 10-1/2 Month Rotational Crops Study With ^{14}C-Labeled Clopyralid - MET90080

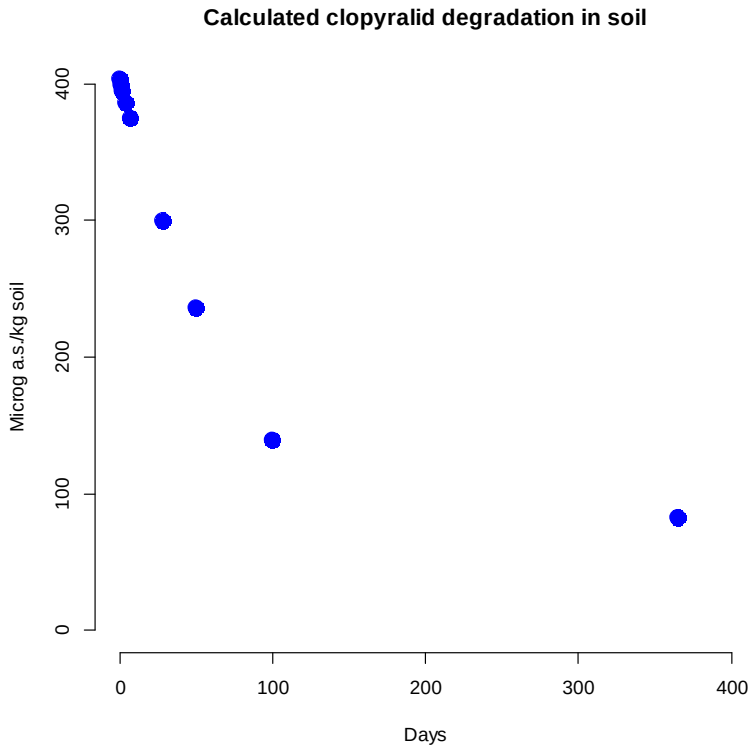
Report	MII 6.6, Yackovich, P. R. ; Lardie, T. S. ; Brink, D. L. , 1993, A 10-1/2 Month Rotational Crops Study With ^{14}C-Labeled Clopyralid - MET90080. DAS Study number: GH-C 2992
Guidelines	US EPA OPP 165-1
GLP	Yes

A 125-day Rotational Crops Study with ^{14}C Labelled Clopyralid

Report	MII 6.6, Yackovich, P.R.; Lardie T.S.; Miller J.H., 1989, A 125-Day Rotational Crops Study with ^{14}C Labelled Clopyralid. DAS Study number: GH-C 2277
Guidelines	US EPA OPP 165-1
GLP	Yes

The study has not been evaluated in the Draft Assessment Report (2003) for clopyralid.

Figure B.7.6.1: Worst-case time weighted average soil concentrations; data from Addendum 1 to Vol 3 Annex B, 2004, RMS FI (Table 8.3.3). Application rate 300 g clopyralid/ha.



B.7.6.1.2 Studies on rotational crops submitted for the present assessment:

REFERENCE	Hall, L. R.; 2015; ^{14}C-Clopyralid: Metabolism in Confined Rotational Crops with a 30-Day Plant-back Interval; ABC Laboratories, Inc., Columbia, Missouri 65202, USA; Lab Study No. 69725; DAS Study No. 130733; 12 January 2015; Unpublished
Guideline(s):	OECD 502
US EPA Guideline(s):	OCSPP 860.1850, OPPTS 860.
Deviations:	None
Formulation	The final specific activity of the test substance was 96,459 dpm/µg (8.34 mCi/mmol)
Application rate	Intended 300 g a.i./ha, actual rates 294.9 and 292.6 g a.i./ha.
Site	outside EU
GLP status:	Yes

BACKGROUND INFORMATION

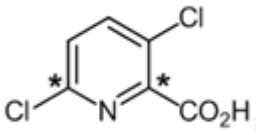
The previous confined rotational study was conducted at 125-day and 365-day plant-back interval.

A data gap was identified for the short plant-back interval in the EFSA conclusion. A new study with 30-day plant-back interval has therefore been conducted to meet the current guideline requirements.

Materials and Methods

Test Item(s)

Radiolabelled test item #1

Name:	^{14}C-Clopyralid
Test item (chemical/other name):	3,6-Dichloro-2-pyridine-2,6-$^{14}C_2$-carboxylic acid or 3,6-Dichloropicolinic acid-2,6-^{14}C
Structural formula: Position of labelling (*)	
Radiochemical purity:	99.9%
Specific radioactivity:	51.2 mCi/mmol

Methods

Wheat, cabbages, and radishes were grown in test plots containing a Missouri sandy loam soil. The bare soil was treated once with ^{14}C-clopyralid 30 days prior to planting. The specific activity of the ^{14}C-clopyralid in the treatment solution was 8.34 mCi/mmol. Untreated control plots were sown at the same time as the treated plots.

Treated wheat forage samples were harvested 62 days after treatment (DAT) at BBCH 43, which corresponded to mid-boot stage. Wheat hay samples were collected at BBCH 71, which corresponded to watery grain stage (78 DAT), and were allowed to air-dry in a greenhouse for 7 days prior to freezing and processing. Wheat straw and grain were collected at maturity (107 DAT, BBCH 97). Control wheat samples were collected on the same days as the treated samples.

Radish tops and roots were harvested at maturity (78 DAT, BBCH 53). Immature cabbage was harvested at 78 DAT (6-9 leafs/head, BBCH 53), and mature cabbage was harvested at 128 days (9+ leafs/head; heads failed to fully close due to heat, BBCH 53). Control cabbage and radish samples were collected on the same days as the treated samples.

All plant samples were homogenized, and total radioactive residues (TRR) levels were determined by combustion analysis. The TRR were 0.408, 0.803, 0.892, and 0.619 ppm in the wheat forage, hay, straw, and grain, respectively. The TRR in the immature and mature cabbage were 0.247 and 0.097 ppm, respectively. The TRR in the radish tops and roots were extremely low at 0.013 and 0.008 ppm, respectively. Radish roots were not analysed further.

Generally, at least 90% TRR was extracted with a acetonitrile/water (ACN/water, 80:20, v:v) followed by 0.125 N NaOH in MeOH/water (1:1) at room temperature. Non-extractable residues were <5% TRR in all tissues except straw. In straw >10% TRR was bound after extractions with ACN/water and 0.125 N NaOH in MeOH/water extraction. Extraction with 1

N NaOH at 100 °C solubilized the majority of these bound residues (*ca.* 9.2% TRR, 0.082 ppm) leaving <2% TRR unextracted in the residual solids.

The majority of the radioactivity extracted with ACN/water typically eluted much later than clopyralid and was comprised of several peaks. A minor component, which eluted earlier in the HPLC chromatogram and which was labelled as "polar radioactivity", was <4% TRR in all tissues except straw (7.1% TRR, 0.063 ppm) and radish tops (16.0% TRR, 0.002 ppm).

Basic extracts typically contained only clopyralid. The non-polar conjugates extracted by ACN/water converted to clopyralid upon base hydrolysis, which typically converted >93% of the radioactivity in these extracts to clopyralid. Base hydrolysis clearly showed that the later eluting material was comprised of clopyralid conjugates. In wheat straw, the conversion was slightly lower at *ca.* 80% TRR due to the formation of several minor additional products that were all <4.2% TRR (<0.037 ppm). The conversion to clopyralid after hydrolysis was confirmed by LC/MS-MS using multiple reaction monitoring (MRM).

Overall, >85% TRR was characterized as free or conjugated clopyralid in all matrices except wheat straw. The lowest identification was in straw, where free and conjugated clopyralid comprised 70.1% TRR. Further, the hydrolysis of the straw ACN/water extract produced several additional minor compounds (all <4.2% TRR and <0.037 ppm) other than clopyralid, which suggested that there were several other minor metabolites present in straw.

Aged residues in wheat forage, wheat straw, wheat grain, and immature cabbage were used to validate the extraction procedure (0.1 N NaOH in methanol/water, 1:1) used in the analytical method for clopyralid in plants. Clopyralid residues, as determined by the analytical method, were typically 77 to 87% of the value determined by the current confined rotational study in these raw agricultural commodities (RACs). These results also confirmed the overall results of the metabolism study since they demonstrated that the majority of the residue(s) in the plant tissue were either clopyralid or conjugates that readily hydrolysed to clopyralid.

Test Site Information

Testing environment:	outdoor test plots	
Container description:	2 m2 (wheat plot) and 1 m2 (cabbage and radish plot), 2 plots	
Soil type:	sandy loam	
Soil characteristics:	% sand	Not available
	% silt	Not available
	% clay	Not available
	% OM	1.07
	pH	5.9
	CEC	7.8
Any adverse weather conditions:	None	
Any adverse insect or disease problems:	None, except minor insect damage of control wheat	

Study Use Pattern

Application method:	soil-applied
Formulation type:	soluble liquid (SL)
Application rate:	300 g ai/ha (or g a.e/ha)
Number of plant-back intervals:	1
Plant-back intervals:	30-d
Plot maintenance during fallow periods:	watered

Test System

Table B.7.6.1.2- 1: Crop information

Crop/ crop group	Var.	Plant-back intervals (days)	Growth stage at harvest	Harvested RAC	Harvesting procedure
Cabbage/ leafy vegetable	Butter-crunch	30 day plant-back*	BBCH 51-53	Immature	cut by hand
			BBCH 53	Mature	cut by hand
Radish/ root crop	Cherry Belle	30 day plant-back*	BBCH 53	mature tops and roots	pulled up, tops and roots separated by cutting
Wheat/ cereal	Summit	30 day plant-back*	BBCH 43	Forage	cut by hand
			BBCH 71	Hay	cut by hand, dried
			BBCH 97	straw and grain	cut off heads, separated grain, cut straw

Sample Handling and Preparation

All plant samples, with the exception of wheat hay, were frozen at -20°C on the same day as harvest. Wheat hay was allowed to air-dry in a climate controlled greenhouse for 7 days prior to freezing (daily temperature in a similar on-site greenhouse averaged <28°C during drying). The frozen plant samples, with the exception of wheat grain, were ground and homogenized with dry ice using either a Straub, Hobart or a Robot Coupe processor. After processing and sublimation of the dry ice, the samples were stored frozen at approximately -20°C until extraction and analysis.

For wheat grain, the sample was transferred into a metal container containing liquid nitrogen. Samples were homogenized using a polytron PT3100 homogenizer. Once homogenized, the grain samples were transferred back to the HDPE containers and left overnight on dry ice. After processing and evaporation of the liquid nitrogen, the samples were stored frozen at approximately -20°C until extraction and analysis.

After processing, aliquots *ca.* 0.3 to 0.4 g (minimum of five) of each homogenized plant sample were analyzed by combustion analysis to determine the total ^{14}C residues. Evolved $^{14}CO_2$ was collected in a mixture of CarboSorb E® and Permafluor E+® (Perkin Elmer, Boston, MA) scintillation cocktail, and the radioactivity determined by LSC analysis. Total ^{14}C-residues in the samples were calculated and expressed as mg equivalents of clopyralid/kg plant matrix (ppm) on a fresh-weight basis.

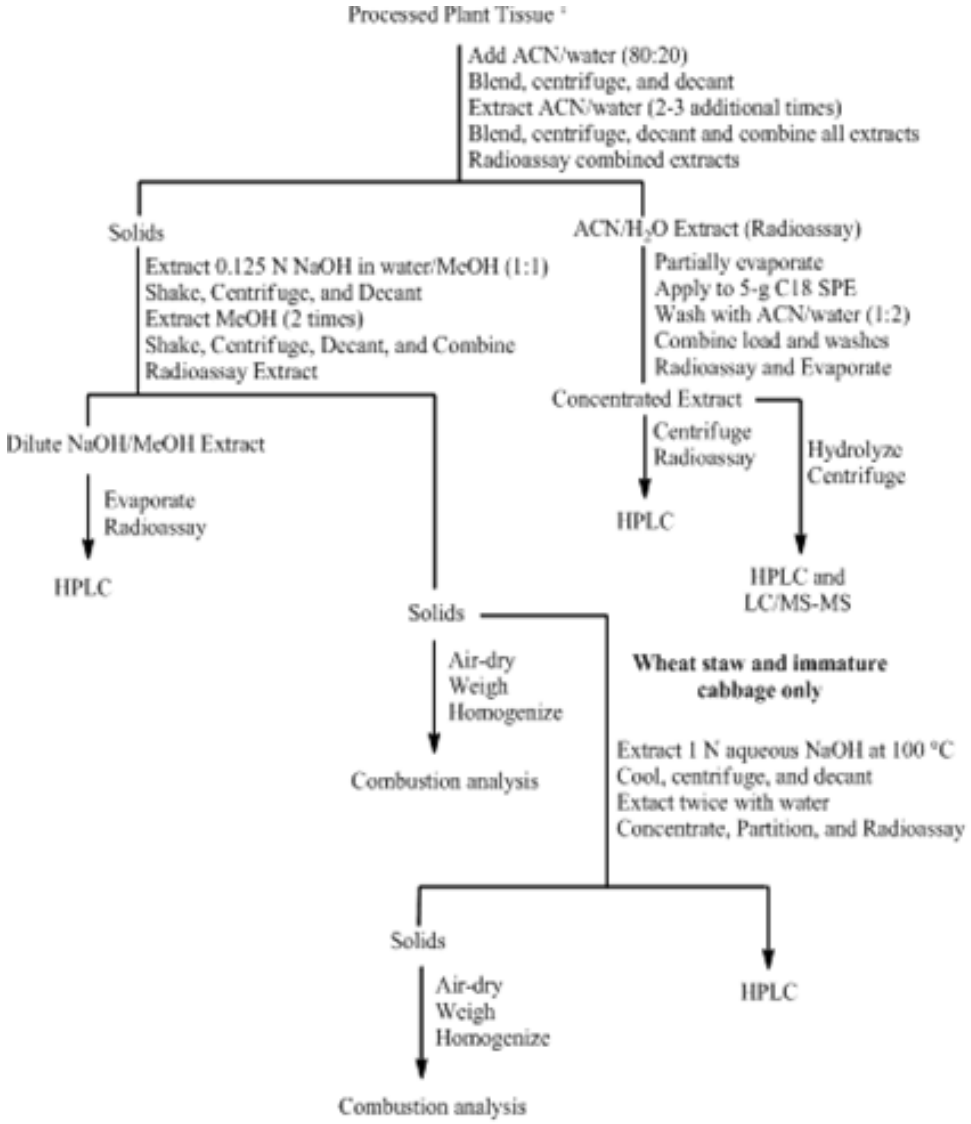
Extraction of Sample Residues

Processed plant material was weighed into 250-mL centrifuge bottle(s) and ACN/water (4:1) was added to the sample. The resultant slurries were blended for several minutes, and the probe was rinsed into the bottles with a small amount of the extraction solvent. The bottles were centrifuged at ≥4,000 rpm for 10 minutes, and the ACN/water was decanted into a graduated cylinder. The extraction was repeated 2-3 times using the same solvent. All extracts were combined and radioassayed. After partial evaporation, the combined ACN/water extracts were purified by passing the extracts through a 5-g C18 solid-phase extraction (SPE) cartridge. After further concentration, the ACN/water extracts were analysed by HPLC. The ACN/water extracts were also subjected to a combination of acid and base hydrolysis (see details below) to hydrolyse clopyralid conjugates to clopyralid for confirmation by HPLC and LC/MS-MS.

Sodium hydroxide (0.125 M NaOH) in methanol (MeOH)/water (1:1) was added to the extracted solids from the ACN/water extraction, and the mixture was shaken for *ca.* 1 hour. The solids were centrifuged at ≥4,000 rpm for 10 minutes. The dilute base extract was decanted into a graduated cylinder, and MeOH was added. The sample was shaken for approximately 10 minutes and centrifuged at ≥4,000 rpm for 10 minutes. The MeOH extract was decanted into the same graduated cylinder as the first NaOH extract. The extraction with MeOH was repeated, and all the extracts were combined. After concentration, the extracts were analysed by HPLC.

For all samples except wheat straw (immature cabbage was processed through this extraction, but it was not necessary based on combustion analysis), the ACN/water extraction followed by dilute NaOH in MeOH/ water was sufficient to extract the majority of the radioactive residues. For wheat straw, a third extraction with 1 N aqueous NaOH at 100 °C overnight was performed. After extensive purification described below, this was analysed by HPLC.

Figure B.7.6.1.2 - 1: Flow diagram for the extraction of [RAC 1]



[1] Duplicate plant samples were extracted and analyzed for wheat forage, wheat straw and radish tops. Only one sample was extracted and analyzed for all other matrices.

Analyses of Non-Extractable Residues

The post-extraction solids (PES) were air-dried and weighed. Five aliquots of each dried and homogenized sample were (*ca.* 0.3 to 0.4 g) oxidized with cellulose using a Packard Sample Model 307 Oxidizer to determine the non-extracted residue (NER) after extraction.

Hydrolysis of ACN/Water Extracts

An aliquot from each of the ACN/water extracts (with the exception of radish tops) was placed in a small vial or a microcentrifuge tube, and if necessary, the sample was evaporated to *ca.* 400 µL using a stream of nitrogen. Hydrochloric acid (10 N, 45 µL) was added to obtain a solution that was approximately 1 N in HCl. The vials were sealed and heated at *ca.* 90 °C for 2 hours in a heating block. After cooling to room temperature, 10 N KOH (100 µL) was added to obtain a solution that was approximately 1 N KOH. The vials were sealed and heated to *ca.* 80 °C for 4 hours. After cooling, 10 N HCl (55 µL) was added to

neutralize the samples. If the samples were hydrolysed in a vial, the contents were transferred to a microcentrifuge tube. All samples were centrifuged for at least 6 min at >14,000 rpm. The pellet was vortex mixed with water. After centrifugation as before, the two supernatants were combined and analysed by HPLC and LC/MS-MS.

Extraction and Clean-up Procedures for Metabolite Identification

Conjugates of clopyralid were not identified directly. Clopyralid conjugates in the ACN/water extracts from each tissue were hydrolysed in 1 N HCl to clopyralid and identified as described in the section above (Hydrolysis of ACN/water extracts). HPLC analysis confirmed the hydrolysis of the conjugates to clopyralid.

For confirmation of clopyralid, the hydrolysates were also analysed by LC/MS-MS using multiple reaction monitoring (MRM). LC/MS-MS was used to monitor two transitions (m/z 190 → m/z 146 and m/z 192 → m/z 148) for clopyralid. These two ions are used in the clopyralid analytical method to quantify and confirm the identity of clopyralid.

Analytical Methods

Samples (5.0 ± 0.02 g) of wheat forage, wheat straw, wheat grain, and immature cabbage that had been grown in soil treated with ^{14}C-clopyralid were weighed into separate Nalgene centrifuge bottles. Sodium hydroxide (0.1 N NaOH in methanol/water, 1:1; 100 mL) was added to each sample. The resultant slurry was blended with a polytron for at least one minute, and the samples were transferred to a reciprocating shaker. After shaking for an hour, the samples were allowed to stand at room temperature overnight. The extracts were radioassayed (3 × 0.5 mL).

Processing and Analysis of Wheat Forage, Wheat Straw, and Immature Cabbage

Samples of forage, straw, and cabbage extracts were processed through the analytical method using a slight modification. The fraction processed was increased five-fold to allow sufficient radioactivity for HPLC with online radiodetection to be utilized. Therefore, the amounts used at each step were increased 5x, and the SPE cartridge was increased from a 200-mg to a 1-g HLB Oasis cartridge (Waters).

A 25-mL aliquot of the extract was added to a clean glass tube, and the volume was marked on the tube. Sodium hydroxide (1 N; 5 mL) was added to the sample, and the methanol was removed using a stream of nitrogen (final volume should be approximately 5 mL). The sample was diluted back to the original volume with 1 N NaOH followed by vortex mixing and sonication.

Dichloromethane (DCM; 25 mL) was added to each of the samples, and the samples were vortex mixed and sonicated. The samples were centrifuged for 2 min at *ca.* 3,000 rpm. A 20-mL aliquot of the water layer from each plant extract was transferred to a clean glass vial, and 1 N HCl (25 mL) was added. The acidified extract was then applied to a conditioned 1-g, HLB SPE cartridge (Waters). Each sample vial was rinsed with 1 N HCl (5 mL), and the rinse was also applied to the SPE. Each SPE was washed with ACN:1N formic acid (1:9, 20 mL), and vacuum dried for at least 30 min. The two washes were combined. Each SPE was then eluted with DCM (70 mL), and the washes and eluent were radioassayed (3 × 0.5 mL).

The DCM eluent was transferred to a pear shaped flask and rotary evaporated to dryness. The dry residues were dissolved in methanol/0.1% formic acid (1:9; 1 mL). Extensive vortex mixing and sonication was used since this was identified as a critical step in the analytical method. The samples were radioassayed (3 × 5.00 µL) and analysed by HPLC.

Processing and Analysis of Wheat Grain

Wheat grain could not be scaled up five-fold due to the nature of the matrix. Therefore, the radiovalidation for the grain was performed using the analytical method's extraction procedure and an alternate procedure for sample analysis as described below.

A 4-mL aliquot of the wheat grain extract was rotary evaporated to dryness and 1 N HCl (50 µL) was added to the residue. The sample was dissolved in methanol/0.1% formic acid (1:9; 3.95 mL) with extensive vortex mixing and sonication. The sample was transferred to microcentrifuge tubes and centrifuged at >13,000 rpm for 10 min. The supernatant was then applied to a 1.0-g C18 SPE, and the load fraction was collected. The SPE was eluted with ACN/water (3:7, 4 mL). The load and eluate were combined and transferred to a pear shaped flask. The sample was rotary evaporated to dryness. The dry residues were dissolved in methanol/0.1% formic acid (1:9; 4.00 mL) with extensive vortex mixing and sonication. The sample was radioassayed (3 × 125 µL) and analysed by HPLC.

HPLC was performed using the same conditions as was used for all other analyses. However, a total of 5, 250-µL injections were collected in 0.25-min fractions. These fractions were counted by LSC and plotted to obtain a histogram.

Metabolite Identification

Neutral organic solvent extracts for all matrices were purified using solid-phase extraction C18 SPE. These extracts were then analysed by HPLC and found to be comprised of primarily clopyralid and conjugates of clopyralid. Residues in this fraction were hydrolysed to clopyralid and confirmed by mass spectrometry.

Residues in the dilute base extractions for most matrices were found to be primarily clopyralid.

Results and Discussion

Results of In-Life Phase

^{14}C-Clopyralid (84.3 mg) was formulated as the olamine salt and applied to bare soil in the two treated plots. The rates for the wheat and radish/cabbage plots were 294.9 and 292.6 g a.i./ha, respectively, equivalent to the maximum field use rate of 300 g a.i./ha. The radiochemical purity for the ^{14}C-clopyralid used for preparation of the application solution and for the formulated ^{14}C-clopyralid in the application solutions both before and after application to the plots was 100.0% by HPLC.

After planting, wheat in both the treated and control plots grew normally with no significant differences in growth stages. Normal agricultural practices were followed to maintain optimum growth of the wheat. Fourteen days after planting (DAP), the control wheat had to

be treated with a light dusting of Sevin to control a severe grasshopper infestation. The treated wheat plot was treated at 17 and 19 DAP with Sevin, and both plots were again treated at 24 DAP with Sevin. At 53 DAP, the treated wheat plot was treated with insecticidal soap to control a slight aphid infestation.

After planting, cabbages grew rapidly and followed normal growth patterns in both treated and control plots. Normal agricultural practices were followed to maintain optimum growth of the wheat. However, as the plants started to reach maturity, growth switched from head formation and started moving towards flowering instead. The cabbages failed to form tight heads in both the treated and control plots. Light dustings of Sevin, insecticidal soap, and BT were used to control a variety of pests, but these infestations were minor in nature.

Radishes matured rapidly and followed normal growth patterns in both treated and control plots until near maturity. Normal agricultural practices were followed to maintain optimum growth of the wheat. Radishes, like cabbages, prefer cooler weather and are usually planted in early spring. As the radishes matured, growth again shifted towards flowering which led to reduced radish root (bulb) formation. However, sufficient radish roots (bulbs) were obtained for the study. Pest infestations were minor, and the plots were treated with Sevin, insecticidal soap, and BT.

Total Radioactive Residue (TRR) Levels

TRR levels in all samples, expressed as mg/kg of parent equivalents below.

Table B.7.6.1.2- 2: Total radioactive residue levels in the raw agricultural commodities after treatment with ^{14}C-clopyralid and 30 d plant-back interval and 294.9 and 292.6 g a.i./ha application rates.

Crop	RAC	mg eq/kg (ppm)
Wheat		
	Forage	**0.408**
	Straw	**0.892**
	Hay	**0.803**
	Grain	**0.619**
Cabbage		
	Immature	**0.247**
	Mature	**0.097**
Radish		
	Tops	**0.013**
	Roots	**0.008**

Characterisation and Identification of Residues

Wheat forage

The total radioactive residue (TRR) in wheat forage was 0.408 ppm.

ACN/water extracted on average 85.2% TRR (0.348 ppm).

The ACN/water extraction was followed with extraction in 0.125 N NaOH/MeOH which solubilized an additional 7.6% TRR (0.031 ppm). Therefore, an average of 92.8% TRR (0.379 ppm) was extracted from the forage using these two extractions.

Total accountability for radioactivity averaged 96.9% TRR (0.396 ppm).

HPLC of the two ACN/water forage extracts after purification on C18 SPE and evaporation showed at least three radioactive regions which were integrated and named as polar (comprised of near void volume radioactive residues), clopyralid (which eluted typically from 8-12 min depending on matrix and solvent loading), and conjugates of clopyralid (which was comprised of several components eluting after clopyralid, typically 18 to 25 min). Polar metabolites comprised on average 1.8% TRR (0.008 ppm). The clopyralid region comprised on average 31.8% TRR (0.130 ppm), and conjugated clopyralid comprised on average 51.6% TRR (0.211 ppm).

In order to obtain more consistent HPLC retention times for clopyralid, the ACN/water extracts were partitioned into MTBE. After evaporation and dissolution in water, the MTBE extracts were analysed by HPLC, and the clopyralid peak more closely matched the retention time for a clopyralid standard.

The radioactive residues in the ACN/water extracts were subjected to acid and base hydrolysis to remove all conjugation. Analysis of the hydrolysed ACN/water extracts showed that >96% of the radioactivity in the ACN/water extract was converted to clopyralid (average of 82.3% TRR or 0.336 ppm). The identification of clopyralid in this hydrolysate was confirmed by LC/MS, which monitored two transitions for clopyralid.

HPLC of the two 0.125 N NaOH in MeOH/Water (1:1) extracts after acidification, purification on C18 SPE, and evaporation showed a single component that was identified as clopyralid (average of 7.6% TRR or 0.031 ppm).

Overall, unconjugated clopyralid comprised on average 39.3% TRR (0.160 ppm) while conjugated clopyralid comprised at least 51.6% TRR (0.211 ppm). After hydrolysis, an average total of 89.9% TRR (0.367 ppm) was identified as free or conjugated clopyralid.

Confirmation that the majority of the radioactive residues in the wheat forage could be converted to clopyralid was obtained during the radiovalidation of the analytical method. The extraction and hydrolysis procedure for the method was considerably milder, but the results confirmed that at least 72.7% TRR (0.297 ppm) was clopyralid.

Wheat hay

The wheat hay TRR was 0.803 ppm.

ACN/water extracted 76.4% TRR (0.613 ppm) from the hay, and a second extraction with 0.125 N NaOH/MeOH solubilized an additional 19.3% TRR (0.155 ppm). A total of 95.7% TRR (0.768 ppm) was extracted from the hay using these two extractions.

Bound residues in the extracted solids comprised 2.1% TRR (0.017 ppm) and were not characterized further. The total accountability for radioactivity was 97.8% TRR (0.785 ppm).

HPLC of the ACN/water hay extract after purification on C18 SPE and evaporation showed at least three radioactive regions which were integrated and named as polar (comprised of near void volume radioactive residues), clopyralid (which eluted early in this sample due to solvent and matrix loading), and conjugates of clopyralid (which was comprised of several components eluting after clopyralid, typically 18 to 25 min). The major residue in the ACN/water extract was conjugated clopyralid which comprised 58.6% TRR (0.471 ppm).

The radioactive residue in the ACN/water hay extract was subjected to acid and base hydrolysis to remove all conjugation. Analysis of the hydrolysed ACN/water extract showed that approximately 94% of the radioactivity in the ACN/water extract was converted to clopyralid (72.1% TRR, 0.579 ppm). The identification of clopyralid in this hydrolysate was confirmed by LC/MS-MS which monitored two transitions for clopyralid.

HPLC of the 0.125 N NaOH/MeOH hay extract after acidification, purification on C18 SPE, and evaporation showed two components, a polar fraction (0.5% TRR, 0.004 ppm) and clopyralid (18.8% TRR and 0.151 ppm).

Overall, unconjugated clopyralid comprised 33.8% TRR (0.272 ppm) while conjugated clopyralid comprised at least 58.6% TRR (0.471 ppm). After hydrolysis, a total of 90.9% TRR (0.729 ppm) was identified as free or conjugated clopyralid. All of the residues in the various extracts were thoroughly characterized on the basis of extractability and hydrolysis, and no single component comprised >1.4% TRR (0.012 ppm).

Wheat straw

The wheat straw TRR was 0.892 ppm. ACN/water extracted on average 56.7% TRR (0.506 ppm).

The ACN/water extraction was followed with extraction in 0.125 N NaOH/MeOH which solubilized on average an additional 29.6% TRR (0.264 ppm). The NaOH/MeOH extracts were partitioned with MTBE resulting in an aqueous fraction (acidic water) which contained on average 5.2% TRR (0.046 ppm) and the MTBE extract which contained on average 24.4% TRR (0.218 ppm) as clopyralid.

A third extraction using 1 N NaOH was required to solubilize >90% TRR in wheat straw. This extract solubilized on average of 9.2% TRR (0.082 ppm). The amount of matrix in the extracts prevented direct analysis of this extract. Therefore, an attempt was made to purify the radioactivity in the extract by partitioning into MTBE. While basic, ≤0.1% TRR (<0.001 ppm) partitioned into MTBE. After acidification, only a relatively small fraction 2.5% TRR (0.022 ppm) partitioned into MTBE, which was surprising since clopyralid extracts readily partition into MTBE at *ca.* pH 1. Solids formed in the acidified water, and these were precipitated by centrifugation. Counting the acidic aqueous layer indicated that on average 4.7% TRR (0.042 ppm) remained in the water. The precipitated material was dissolved in base and counted, and the results suggested that some radioactive material was bound to biological matrix that was base soluble (2.0% TRR, 0.017 ppm). Clearly, the NaOH extract was comprised of several different compounds having distinctly different characteristics. However, since none of these minor components comprised >5% TRR or >0.05 ppm, no additional work was done with these fractions, which were thoroughly characterized as insoluble in ACN/water and dilute base in MeOH/water, soluble in 1 N NaOH after heating, and by partitioning among MTBE and water at basic and acidic pH.

Therefore, an average of 95.4% TRR (0.851 ppm) was extracted from the straw using these three extractions.

The total accountability for radioactivity averaged 96.8% TRR (0.864 ppm).

HPLC of the two ACN/water straw extracts after purification on C18 SPE and evaporation showed at least three radioactive regions that were integrated and named as polar (comprised of near void volume radioactive residues), clopyralid (which eluted typically from 8-12 min depending on matrix and solvent loading), and conjugates of clopyralid (comprised of several components eluting after clopyralid, typically 18 to 25 min). The major residue in the ACN/water straw extract was clopyralid and comprised on average 31.0% TRR (0.277 ppm).

The radioactive residues in the ACN/water straw extracts were subjected to acid and base hydrolysis to remove all conjugation. Analysis of the hydrolysed ACN/water extracts showed that approximately 80% of the radioactivity in the ACN/water extract was converted to

clopyralid (average of 45.7% TRR and 0.407 ppm). The identification of clopyralid in this hydrolysate was confirmed by LC/MS-MS, which monitored two transitions for clopyralid.

HPLC of the two 0.125 N NaOH/MeOH extracts of straw after acidification, purification on C18 SPE, partitioning into MTBE, and evaporation showed a single component that was identified as clopyralid (average of 24.4% TRR and 0.218 ppm).

Overall, unconjugated clopyralid comprised on average at least 55.5% TRR (0.495 ppm), while conjugated clopyralid comprised on average at least 18.5% TRR (0.166 ppm). After hydrolysis, an average of 70.1% TRR (0.625 ppm) was identified as free and conjugated clopyralid. All of the residues in the various extracts were thoroughly characterized on the basis of extractability, hydrolysis, and partitioning into MTBE. No single unidentified component comprised >5.2% TRR (>0.046 ppm).

Confirmation that the majority of the radioactive residues in the wheat straw could be converted to clopyralid was obtained during the radiovalidation of the analytical method. The extraction and hydrolysis procedure for the method was considerably milder, but the results confirmed that at least 61.0% TRR (0.545 ppm) was clopyralid.

Wheat grain

The wheat grain TRR was 0.619 ppm.

ACN/water extracted 78.2% TRR (0.484 ppm) from the grain, and a second extraction with 0.125 N NaOH/MeOH solubilized an additional 20.7% TRR (0.128 ppm). A total of 98.9% TRR (0.612 ppm) was extracted from the grain using these two extractions.

The total accountability for radioactivity was 102.7% TRR (0.636 ppm).

HPLC of the ACN/water grain extract after purification on C18 SPE and evaporation showed at least three radioactive regions. However, the distribution of the residues in the grain appeared slightly different than the other matrices. No “polar peak” was observed, and the clopyralid peak was split. Injection of a larger amount showed no real change other than a slight shift in retention times and the appearance of a small amount of the “polar peak” which suggests this may be an artefact caused by matrix in the sample. However, the second peak after clopyralid was still present. Therefore, the three regions were integrated as clopyralid, which eluted around 10 min; an unknown degradate (metabolite 1), which could be a conjugate and which eluted at around 11 min; and the typical conjugates of clopyralid, which was comprised of several components eluting around 22.6 min. The major residue in the ACN/water extract was clopyralid which comprised 38.2% TRR (0.236 ppm).

The radioactive residue in the ACN/water grain extract was subjected to acid and base hydrolysis to remove all conjugation. Analysis of the hydrolysed ACN/water extract showed that approximately 93% of the radioactivity in the ACN/water extract was converted to clopyralid (72.6% TRR, 0.450 ppm). The identification of clopyralid in this hydrolysate was confirmed by LC/MS-MS, which monitored two transitions for clopyralid.

HPLC of the 0.125 N NaOH/MeOH grain extract after acidification, purification on C18 SPE, and evaporation showed primarily one component. However, injection of a larger quantity showed that there was some conjugated material in the extract (1.1% TRR,

0.007 ppm) and clopyralid (16.0% TRR and 0.099 ppm). Further, there were some significant losses while processing this fraction (3.6% TRR, 0.023 ppm).

Overall, unconjugated clopyralid comprised approximately 54.1% TRR (0.335 ppm), while conjugates comprised the majority of the remaining residues. After hydrolysis, a total of 88.6% TRR (0.549 ppm) was identified as free and conjugated clopyralid. All of the residues in the various extracts were thoroughly characterized on the basis of extractability and hydrolysis. No single unidentified component comprised >3.8% TRR (0.023 ppm).

Confirmation that the majority of the radioactive residues in the wheat grain could be converted to clopyralid was obtained during the radiovalidation of the analytical method. The extraction and hydrolysis procedure for the method was considerably milder, but the results confirmed that at least 68.6% TRR (0.425 ppm) was clopyralid.

Immature cabbage

The immature cabbage TRR was 0.247 ppm.

ACN/water extracted 81.8% TRR (0.202 ppm) from the cabbage. The ACN/water extraction was followed with a 0.125 N NaOH/MeOH extraction, which solubilized an additional 5.6% TRR (0.014 ppm). Since these combined extracts had not solubilized >90% TRR based on initial combustion data, a third extraction with 1 N NaOH was used to solubilize an additional 2.0% TRR (0.005 ppm).

Combustion of the solids indicated that bound residues comprised 0.5% TRR (0.001 ppm). Therefore, the accountability of radioactivity was 90.0% TRR (0.222 ppm) based on initial combustion. This result suggested that the initial TRR for cabbage was *ca.* 10% too high. Further, the recovery data indicated that >99% TRR was extracted from the cabbage.

HPLC of the ACN/water cabbage extract after purification on C18 SPE and evaporation showed at least two radioactive regions, which were integrated as clopyralid (14.4% TRR, 0.036 ppm) and conjugates of clopyralid (comprised of several components eluting after clopyralid around 20.5 min). The major residue in the ACN/water extract was conjugated clopyralid and comprised 67.5% TRR (0.167 ppm).

The radioactive residues in the ACN/water cabbage extract were subjected to acid and base hydrolysis to remove all conjugation. Analysis of the hydrolysed ACN/water extract showed that approximately 98% of the radioactivity in the ACN/water extract was converted to clopyralid (80.2% TRR, 0.198 ppm. The identification of clopyralid in this hydrolysate was confirmed by LC/MS-MS, which monitored two transitions for clopyralid.

HPLC of the 0.125 N NaOH/MeOH cabbage extract after acidification, purification on C18 SPE, and evaporation showed one component, clopyralid, which comprised 5.5% TRR (0.014 ppm.

Overall, unconjugated clopyralid comprised 19.9% TRR (0.049 ppm), while conjugates comprised the at least 67.5% TRR (0.167 ppm). After hydrolysis, a total of 85.7% TRR (0.212 ppm) was identified as free and conjugated clopyralid. All of the residues in the various extracts were thoroughly characterized on the basis of extractability and hydrolysis. No single unidentified component comprised >0.5% TRR (0.001 ppm).

Bound residues comprised <0.5% (0.001 ppm) and were not further characterized.

Confirmation that the majority of the radioactive residues in the immature cabbage could be converted to clopyralid was obtained during the radiovalidation of the analytical method. The extraction and hydrolysis procedure for the method was considerably milder, but the results confirmed that at least 70.2% TRR (0.173 ppm) was clopyralid.

Mature cabbage

The mature cabbage TRR was 0.097 ppm.

ACN/water extracted 91.6% TRR (0.089 ppm) from the cabbage, and a second extraction with 0.125 N NaOH/MeOH solubilized an additional 3.1% TRR (0.003 ppm). A total of 94.7% TRR (0.092 ppm) was extracted from cabbage using these two extractions.

Bound residues in the extracted solids were 1.4% TRR (0.001 ppm) and were not further characterized. The total accountability for radioactivity was 96.0% TRR (0.094 ppm).

HPLC of the ACN/water cabbage extract after purification on C18 SPE and evaporation showed at least two radioactive regions that were integrated as clopyralid (10.7% TRR, 0.010 ppm) and conjugates of clopyralid that was comprised of several components eluting after clopyralid around 20.5 min (80.9% TRR, 0.079 ppm).

The radioactive residues in the ACN/water cabbage extract were subjected to acid and base hydrolysis to remove all conjugation. Analysis of the hydrolysed ACN/water extract showed that approximately 95% of the radioactivity in the ACN/water extract was converted to clopyralid (86.8% TRR, 0.085 ppm). The identification of clopyralid in this hydrolysate was confirmed by LC/MS-MS, which monitored two transitions for clopyralid.

Overall, unconjugated clopyralid comprised 10.7% TRR (0.010 ppm), while conjugates comprised at least 80.9% TRR (0.079 ppm). After hydrolysis, a total of 86.8% TRR (0.0.085 ppm) was identified as free and conjugated clopyralid. All of the residues in the various extracts were thoroughly characterized on the basis of extractability and hydrolysis. No single unidentified component comprised be >3.6% TRR (0.004 ppm).

Radish tops

The radish top TRR was 0.013 ppm.

ACN/water extracted on average 70.0% TRR (0.009 ppm). A second extraction with 0.125 N NaOH/MeOH solubilized on average an additional 6.9% TRR (0.001 ppm). An average total of 76.9% TRR (0.010 ppm) was extracted from radish tops using these two extractions.

The average total accountability for radioactivity was 86.5% TRR (0.011 ppm). While the unaccounted for radioactivity appears to be relatively large at 15% TRR, this is not an issue since the residue comprises approximately 0.002 ppm.

Since the residues were so low in the two ACN/water extracts, the samples were injected multiple times on HPLC with fractions collected and counted by LSC. The overall profile was similar to that observed for other ACN/water extracts in that three major regions were observed, and these were integrated as shown. The major residue in the radish tops was

conjugated clopyralid and comprised on average 51.1% TRR (0.007 ppm). Unconjugated clopyralid comprised on average 4.4% TRR (<0.001 ppm).

Due to the extremely low residue levels, no additional work was done on the radish top extract since all components comprised <0.010 ppm.

Radish roots

TRR in radish roots was 0.008 ppm. Due to the low residue levels in the radish root samples, no additional analyses were conducted.

Bound residues

Greater than 90% of the TRR was extracted from all matrices, except wheat straw and radish tops, using a combination of ACN/water followed by dilute NaOH in MeOH/water. Bound residues in these matrices comprised <0.05 ppm. All unidentified residues in the hydrolysed ACN/water extracts and in the dilute NaOH in MeOH/water extracts comprised <5% TRR and <0.05 ppm.

In wheat forage bound residues comprised on average 4.1% TRR (0.017 ppm) and in wheat grain comprised 3.8% TRR (0.023 ppm), respectively. These residues were not characterized further.

In wheat straw, the bound residues were solubilized using 1 N NaOH at 100 °C, and this extraction solubilized an additional 9.2% TRR (0.082 ppm) and left bound residues of 1.4% TRR and 0.012 ppm in the residual solids. The solubilized residues from wheat straw were thoroughly characterized by their extraction into strong base only after heating, and their partitioning behaviour between basic and acidic water using MTBE. After partitioning, no single residue comprised >5% TRR or >0.05 ppm. Therefore, no additional characterizations were performed with these fractions.

For radish tops, only 76.9% of the TRR (0.010 ppm) was extracted into ACN/water. However, no additional extractions or characterizations were performed since the bound residues in the extracted solids comprised <0.010 ppm (9.6% TRR) and were not further characterized.

Storage Stability

Since all extractions and analyses for the confined rotational crop study were performed within 5 months, no storage stability information was required. However, aged residues were used to radiovalidate the residue analytical method for clopyralid in crops. The validation extractions occurred on May 18, 2014, which corresponded to 278, 233, 233, and 262 days after collection of the wheat forage, wheat straw, wheat grain, and immature cabbage, respectively, for an interval of 7.8 to 9.3 months between harvest and extraction/analysis. The recovery of clopyralid in the radiovalidation ranged from *ca.* 77 to 87% of the clopyralid found in the confined rotational crop study using only a single mild extraction/hydrolysis in dilute base. Using multiple extractions, would probably have increased this value. Therefore, the majority of the residue appears to be stable for at least 8 months as a combination of conjugated and free clopyralid in wheat and cabbage matrices.

Table B.7.6.1.2- 3: Summary of characterisation and identification of radioactive residues in rotational crop matrices following application of radiolabeled clopyralid at 300 g ai/ha. Duplicate samples were extracted and the average for these is reported.

	Wheat Forage		Wheat Straw		Wheat Hay		Wheat Grain		Immature cabbage		Mature cabbage		Radish tops	
	Averaged Extracts		Averaged Extracts										Averaged Extracts	
	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]
TRR	100.0	0.408	100.0	0.892	100.0	0.803	100.0	0.619	100.0	0.247	100.0	0.097	100.0	0.013
Total extractable [b]	92.8	0.379	95.4	0.851	95.7	0.768	98.9	0.612	89.5	0.221	94.7	0.092	76.9	0.010
Parent clopyralid	39.3	0.160	55.5	0.495	33.8	0.272	54.1	0.335	19.9	0.049	10.7	0.010	4.4	<0.001
Polar radioactivity	1.8	0.008	7.1	0.063	3.2	0.026	--	--	0.1	<0.001	--	--	16.0	0.002
Conjugated clopyralid	51.6	0.211	18.5	0.166	58.6	0.471	20.8	0.129	67.5	0.167	80.9	0.079	51.1	0.007
Metabolite 1	--	--	--	--	--	--	20.3	0.126	--	--	--	--	--	--
Total clopyralid [c]	89.9	0.367	70.1	0.625	90.9	0.729	88.6	0.549	85.7	0.212	86.8	0.085	--	--
Total characterized [d]	2.9	0.012	25.3	0.226	4.8	0.039	10.3	0.064	1.8	0.004	7.9	0.008	--	--
Total unextractable	4.1	0.017	1.4	0.012	2.1	0.017	3.8	0.023	0.5	0.001	1.4	0.001	9.6	0.001
Accountability [e]	96.9	0.396	96.8	0.864	97.8	0.785	102.7	0.636	90.0	0.222	96.0	0.094	86.5	0.011

-- Not observed in most instances. For radish tops, these values were not obtained since the residues were so low (<0.010 ppm).

[a] mg/kg clopyralid equivalents (ppm)

[b] Total extractable includes all extracts (ACN/water, 0.125 N NaOH/MeOH, and 1 N NaOH at 100 °C)

[c] Total identified includes radioactive residues that hydrolyzed to clopyralid (i.e., clopyralid conjugates).

[d] Radioactivity that was characterized by extraction into ACN/water followed by hydrolysis or extraction into base followed by partitioning with methyl *t*-butyl ether. No single fraction comprised >5.2% TRR or 0.046 ppm (wheat straw). In most extracts this was much lower.

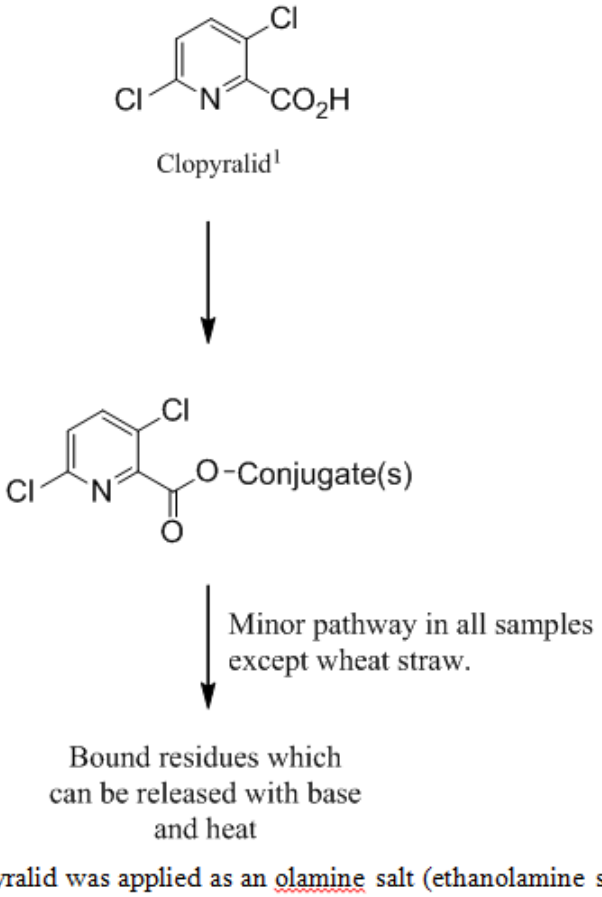
[e] Accountability = Total extractable + Total unextractable. For percent accountability, the accountability in mg/kg is divided by initial TRR and mutiplied by 100.

Metabolic Pathway

In plants, clopyralid is converted to at least one major conjugate that was readily hydrolysed in base to clopyralid. This result suggested that the conjugate was probably an ester formed by reaction with the carboxylic acid group in clopyralid. The nature of this residue, other than its hydrolysis to clopyralid, was not further investigated.

Another, usually minor pathway noted primarily in straw was binding to plant matrix. The majority of the bound residues in straw were solubilized by heating in 1 N NaOH, and this yielded several minor products that were characterized on the basis of distinctly different solubilities in MTBE, acid, and base.

Figure B.7.6.1/3- 2: Proposed metabolic pathway for clopyralid



[1]Clopyralid was applied as an olamine salt (ethanolamine salt)

B.7.6.2 Magnitude of residues in rotational crops

A magnitude of residue study in rotational crops was not carried out for use during the active approval. A magnitude of residue in rotational crops study is currently on-going to provide further information, but no data from the study is available at the time of writing.

Consideration of the available data from the confined rotational crop residue study is discussed below.

Data from the study addressed in B.7.6.1 indicates that following application of clopyralid at 280 g as/ha to bare soil, significant residues in rotational crops planted approximately 125 days later are not expected.

With regard to the GAPs proposed for clopyralid AIR, these results provide an overly conservative assessment of the potential for residues above the MRL in rotational crops since the maximum application rate considered for cereals in this submission is 80 g ae/ha, which is less than 30% of the rate used in the confined rotational crop residue study. Additionally, since the only use of clopyralid considered for cereals is a postemergence foliar application, the soil loading with clopyralid residues would be further reduced due to crop interception compared to the bare soil condition used in the confined rotational crop residue study. Use in established pasture would not normally be considered as relevant for rotational crop residues as crops are not normally grown in rotation with established grass pasture. However, even if rotation is considered, the maximum rate considered in pasture in this submission is 120 g ae/ha, which is less than 50% of the rate evaluated in confined rotational crop residue study. Additionally, in application to pasture, there would be a very high level of interception of clopyralid by the grass foliage with little of the spray reaching the soil. Another consideration is that existing EU MRLs in essentially all crops have been set at 0.5 mg/kg or higher. Consequently, an interval appreciably shorter than 125 days would be expected to be adequate to avoid significant residues in rotational crops or residues that would exceed existing MRLs. As mentioned previously, crops are not normally grown in rotation with established pasture as it is grown as a perennial crop. For clopyralid use in cereals, it is proposed that no further waiting period is required for sowing or planting succeeding crops after normal crop maturity and harvest. The latest growth stage for application is BBCH 39. Based on the GAPs proposed in this submission, the interval between application at BBCH 39 and normal crop maturity and harvest is expected to be adequate to ensure that residues are not found in rotational crops in significant levels.

B.7.7 Other Studies

B.7.7.1 Effects on the residue level in pollen and bee products

This is a new data requirement under Regulation 1107/2009 therefore no data have previously been considered.

Additionally, the representative uses evaluated in this submission involve application to grass pasture and cereals and these are not considerd to be crops that are attractive to bees.

There also are a wide range of supported uses of clopyralid containing products in production of various melliferous plants.

Bearing this issue and future MRL settings in mind, storage stability of honey should be set as a data gap, since such studies take considerable amount of time.

As far as rotational crops are concerned, EFSA guideline states that some crops (e.g. cauliflower, carrots, chicory) which usually do not flower during normal production, are indicated as attractive to bees because, in some cases, they can be cultivated for seed production.

Same likely holds true for raddish, a crop which has been evaluated in the present document as a rotational or succeeding crop.

Cabbages and other brassicas (*) such as Chinese, mustard cabbage, pak-choi (Brassica chinensis); white, red, Savoy cabbage, Brussels sprouts, collards, kale and kohlrabi (Brassica oleracea all varieties except botrytis) belong to crops visited by honey bees for pollen and/or nectar collection. Cabbage was evaluated in the present document as one of the rotational crops using a 30 day plant-back interval at BBCH 51 – 52. This is prior to BBCH 60 – BBCH 69, i.e. the flowering growth stage. In the study described and evaluated at B.7.6, mature cabbage was harvested at 128 days (9+ leafs/head).

At maturity the levels of the residues were approximately 0.1 mg/kg. At BBCH 53 (i.e. 78 DAT, 6-9 leafs/head), the levels in the whole cabbage plant was 0.247 mg/kg. This can be considered as a clear indication on that bees can be exposed to a level of clopyralid residues, which is likely reflected in honey and pollen products to a level (>0.05 mg/kg), which requires MRL setting.

Taking the decision making scheme for MRL setting in honey on board, as described proposed by AFSSA, the following notions can be made:

- Clopyralid has no described uses as a veterinary medicinal product.
- Crops, such as cabbage, are attractive to honeybees.
- Clopyralid is a selective systemic herbicide belonging to the chemical class of pyridines.
- Application before or during attractive period (flower – honeydew)
- The MRLs of clopyralid in the honey have been set at the limit of quantification 0.05 mg/kg as any data have not been available.

This is as close as one can get with currently available data. Consequently there are data gaps left :

- considering data on residue in aerial parts of the crop, flowers and pollen in particular including guttation

- considering data from studies on transfer from syrup
- considering data from field residue trials
- considering data on residue stability in honey

B.7.7.2 Literature data

Kucharski et al. (2005) studied Degradation dynamics and residues of clopyralid in surface and groundwater on fields of Lower Silesia. The objective of the studys is environmental.

Kucharski et al. (2006) studied residues of clopyralid in mustard seeds, following treatment according to a less critical use than considered in the DAR for oilseed rape, ranged from <0.001 (non-detected) to 0.005 mg/kg. The levels are significantly below the existing EU MRL of 0.5 mg/kg for mustard seeds and therefore no further consideration is required from a residues, MRLs and consumer exposure perspective.

Kucharski et al. (2009) studied effect of soil contamination with herbicides on the nitrification process. The study does not follow an appropriate guideline but methodology is comprehensively described. The study is relevant, but does not alter the existing risk assessment because the acute and chronic endpoints reported are greater than those currently used in the risk assessment.

In the study by Sadowski et al. (2010) residues of clopyralid in oilseed rape (seeds and straw) were found to be <0.01 mg/kg and 0.024 mg/kg, respectively. Rates and timings of application were not provided. The levels are significantly below the existing EU MRL of 0.5 mg/kg for rapeseeds and therefore no further consideration is required from a residues, MRLs and consumer exposure standpoint of view.

Crops	Definition	Honey bees	Bumble bees	Solitary bees	Extrafloral nectaries
Pollen				Nectar	
Rye grass for forage and silage	Italian ryegrass (*Lolium multiflorum*); English, perennial ryegrass (*L. perenne*). Quick-growing grasses	+ (1)	N/R	+ (3)	
Wheat	*Triticum* spp.: common (*T. aestivum*) durum (*T. durum*) spelt (*T. spelta*). Common and durum wheat are the main types. Among common wheat, the main varieties are spring and winter, hard and soft, and red and white.	+ (1)	N/R	+ (3)	
Cabbages and other brassicas (*)	Chinese, mustard cabbage, pak-choi (*Brassica chinensis*); white, red, Savoy cabbage, Brussels sprouts, collards, kale and kohlrabi (*Brassica oleracea* all varieties except *botrytis*)	+	+	+	+

Symbols in the table: **+** crop visited by pollen and/or nectar collection;

N/R= no relevant

1.- crop not attractive for bees,

3.- nectaries and/or anthers are not accessible

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
8.	Kucharski, M., Sadowski, M.	2005	Degradation dynamics and residues of clopyralid in surface and groundwater on fields of Lower Silesia	Progress in Plant Protection (2005) Vol. 45(1), pp. 242-247	N	N/A	Full article	There is no EU data requirement for degradation rates in groundwater. Methods were not validated
7.	Alder, L., Steinborn, A., Bergelt, S.	2011	Suitability of an orbitrap mass spectrometer for the screening of pesticide residues in extracts of fruits and vegetables	J AOAC Int (2011) Vol. 94(6), pp. 1661-73	N	N/A	N/A	Analytical detection of residues in foodstuffs

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
17.	Basa Cesnik, H., Bolta, S.V., Gregorcic, A.	2010	Pesticide residues in cauliflower, eggplant, endive, lettuce, pepper, potato and wheat of Slovene origin found in 2009	Acta Chimica Slovenica (2010) Vol. 57, Issue 4, p972-9	N	N/A	N/A	Analytical detection of residues in foodstuffs
18.	Basa Cesnik, H., Velikonja Bolta, S., Gregorcic, A.	2012	Pesticide residues in samples of apples, lettuce and potatoes from integrated pest management in Slovenia from 2005-2009	Acta Agriculturae Slovenica (2012) Vol. 99(1), pp. 49-56	N	N/A	N/A	Analytical detection of residues in foodstuffs

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
29.	Bol'shakov, D.S., Amelin, V.G., Tret'yakov, A.V.	2014	Determination of herbicides and their metabolites in natural waters by capillary zone electrophoresis combined with dispersive liquid-liquid microextraction and on-line preconcentration	Journal of Analytical Chemistry (2014) Vol. 69(1), pp. 72-82	N	N/A	N/A	Analytical detection of residues in water

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
95.	EPA	2012	Receipt of several pesticide petitions filed for residues of pesticide chemicals in or on various commodities	Federal Register (2012) Vol. 77(143), pp. 43562-43566	N	N/A	N/A	EPA pesticide petitions
103.	Felix, J., Doohan, D.J., Ditmarsen, S.C., Schultz, M.E., Wright, T.R., Flood, B.R.	2005	Effect of flumetsulam plus clopyralid soil residues on potatoes (Solanum tuberosum L.), lima beans (Phaseolus limensis, L.) and snap beans	Crop Protection (2005) Vol. 24(9), pp. 790-797	N	N/A	N/A	Effects of flumetsulam and clopyralid on following crops

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
			(Phaseolus vulgaris L.) grown in rotation					
111.	[redacted]	2012	Quality of pulp and molasses interms of their suitability as animal feed	Gazeta Cukrownicza (2012) Vol. 120(3), pp. 116-119	N	N/A	N/A	Detection of residues in foodstuffs

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
287.	[redacted]	2011	Chronic dietary risk characterization for pesticide residues: A ranking and scoring method integrating agricultural uses and food contamination data	Food Chem Toxicol (2011) Vol. 49(7), pp. 1484-510	N	N/A	N/A	Rank and scoring methodology for chronic dietary risk characterisation

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
307.	Polgár, L., García-Reyes, J.F., Fodor, P., Gyepes, A., Dernovics, M., Abrankó, L., Gilbert-López, B., Molina-Díaz, A.	2012	Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions	J Chromatogr A (2012) Vol. 1249, pp. 83-91	N	N/A	N/A	Analytical detection of residues in foodstuffs

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
334.	Rodriguez, N.	2005b	Carryover residues of herbicides and mixtures.: Cultivos de cosecha gruesa. Actualización 2005	Publicacion Tecnia - INTA 61 (2005) , pp. 179-206	N	N/A	N/A	Efficacy study
383.	Smyth, S.J., Gusta, M., Belcher, K., Phillips, P.W.B., Castle, D.	2011a	Changes in herbicide use after adoption of HR canola in Western Canada	Weed Technology (2011) Vol. 25(3), pp. 492-500	N	N/A	N/A	Herbicide use in canola production in Canada

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Vertebrate study (Y/N)	Data protection claimed (Y/N)	Rationale for data protection claim (A-L)	Owner	
435.	Wang, X., Hou, Z., Lu, Z.	2006	Residual dynamics of clopyralid in oil seed and soil	Journal of Jilin Agricultural University (2006) Vol. 28, pp. 430-432	N	N/A	N/A	No abstract available

B.7.8 References relied upon

For the new studies for which data protection (G) is claimed: *Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).*

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.1.1/1	Allen, L.	2013	Frozen Storage Stability of Residues of Clopyralid in Crop Matrices DAS Study No. 120939 CEM Analytical Services (CEMAS), North Ascot, Berkshire, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.1.1/2	Foster, D.R., Blakeslee, B.A., Rutherford, B.S.	1996	Frozen Storage Stability of Clopyralid, 2,4-D in Corn Grain, Straw and Fodder DAS Study No. RES93050.01 DowElanco, Indianapolis, Indiana, US GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.1.1/3	Clements, B, Bolton, A	1996	Determination of the Stability of Clopyralid Residues in Pasture under Frozen Storage Conditions DAS Study No. GHE-P-5350 CEM Analytical Services (CEMAS), North Ascot, Berkshire, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.1.2/1	[redacted]	2015	Frozen Storage Stability of Clopyralid in Bovine Fat DAS Study No. 120602 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.1.2/2	[redacted]	2004	Frozen Storage Stability of Clopyralid in Beef Muscle, Liver, Kidney, Milk and Chicken Egg DAS Study No. 020120.01 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.2.1/1	Chapleo, S. ; Caley, C. Y.	2002	The Metabolism of [14C]-Clopyralid in Sugar Beet DAS Study No. GHE-P-9939 Inveresk Research International, Tranent, East Lothian, United Kingdom GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.2.1/2	Guo, C.	1996	Metabolism of 14C -Clopyralid in Cabbage DAS Study No. RES95095 DAS Report No. GH-C-4289 ABC Laboratories Inc, Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.2.1/3	Chapleo, S., Caley, C. Y., White, D. E.	2002	The Metabolism of (14C)-Clopyralid in Oilseed Rape DAS Study No. GHE-P 9938 Inveresk Research International, Tranent, East Lothian, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.2.1/4	Gourlay, V.	2015	Plant uptake of 14C -labelled clopyralid in wheat and oilseed rape under greenhouse conditions DAS Study No. 150297 RLP AgroScience GmbH, 67435 Neustadt a.d. Weinstraße, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.2.2/1	[redacted]	2014	A Nature of the Residue Study in the Laying Hen with [14C]-Clopyralid DAS Study No. 130906 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No Dow AgroSciences LLC, Indianapolis, Indiana, USA	Yes	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.2.3/1	[redacted]	2015	A Nature of the Residue Study in the Ruminant with [14C]Clopyralid DAS Study No. 130202 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.1/1	Delmotte, R.	2015	Magnitude of the residues of clopyralid in grassland pasture (RAC fresh grass, hay and silage), following one application of GF-1966, Northern and Southern Europe – 2014 DAS Study No. 140653 Lab study No. CES-14-18931 STAPHYT, 23, Route de Moeuvres, 62860 Inchy en Artois, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.1/2	Freeman, J.M.H., Almond, R.H., MCDonald, I.A., Dawson, J., Ellis, S.E., Green, S.L.	1982	Determination of residues of 3,6-dichloropicolinic acid (DOWCO* 290) in grass & vegetable crops treated with FORMAT ** U.K. 1981 DAS Report No GHE-P-912 Huntingdon Research Centre (HRC) U.K. GLP/GEP (Y/N): No Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC Addendum 1 Rapporteur Member State Finland 30.12.2004
CA 6.3.1/3	Osborne, K.A (S Flatt – Student)	1988	Clopyralid residues in grass following application of SHIELD – UK 1987 DAS Study No. RT 51/87 DAS Report No. GHE-P-1881 Agricultural Products Research Development Centre, Letcombe Laboratory, UK GLP/GEP (Y/N): No Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.1/4	Wood, S.	1995	Residues of clopyralid in grass at intervals following a single application of DOW SHIELD (EF-584), UK – 1994 DAS Study No. R94-09[a] DAS Report No. GHE-P-3918 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.1/5	Wood, S.	1995	Residues of clopyralid in established grassland at intervals following application of DOW SHIELD (EF 584), UK – 1993 DAS Study No. R93-63 DAS Report No. GHE-P-3919 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.1/6	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in pasture at intervals under open field conditions following a single application of LONTREL 100 (EF 1136), Southern France and Spain – 2000 Study No. CEMS-1298 DAS Report No. GHE-P-9369 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.1/7	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in pasture at intervals following a single application of LONTREL 100 (EF 1136), EU Southern Zone – 2001 Study No. CEMS-1545 Report No. GHE-P-9386 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.2/1	Boissinot, J.C.	2015	Magnitude of the residues of clopyralid in spring barley (RAC whole plant, grain and straw), following one application of GF-1966, Northern and Southern Europe – 2014 DAS Study No. 140655 STAPHYT, 23, Route de Moeuvres, 62860 Inchy en Artois, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.2/2	Clements, B.	1997	Residues of fluroxypyr-BPE, clopyralid and MCPA in cereals at harvest following a single application of BOFIX (NEW) EF-1403, France (North and South) – 1996 DAS Study No. R96-138 Huntingdon Research Centre (HRC) U.K. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.2/3	Butler, R.E.	1998	Residues of clopyralid, fluroxypyr and MCPA in Winter Barley at harvest following a single application of BOFIX* BP (EF-1403), UK, 1997 DAS Study No. R97-104 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.2/4	Garbay, M.	2005	Determination of residue in Barley for malt and brewery in spring barley in France DAS Study No. S04DAR.BREGG39,JL34 Solevi, Crest, France; IFBM, Vandoeuvre, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.2/5	Garbay, M.	2005	Determination of residue in barley for malt and brewery in winter barley in France DAS Study No. S04DAR.BREGG40,JL35 Solevi, Crest, France; IFBM, Vandoeuvre, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.2/6	Freeman, JMH et al	1982	Effect of Length of Period Between Application of CYRONAL* and Harvest on Residues of 3,6-dichloropicolinic Acid (DOWCO 290**) in Winter Wheat, Winter Barley and Maize – Belgium 1981 DAS Report No. GHE-P-943 Huntingdon Research Centre (HRC) U.K. GLP/GEP (Y/N): No Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC Addendum 1 Rapporteur Member State Finland 30.12.2004
CA 6.3.2/7	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in barley at intervals and at harvest following a single application of LONTREL 100 (EF-1136), EU Northern Zone – 2011 Study No. CEMS-1542 Report No. GHE-P-9383 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.2/8	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in barley at intervals under open field conditions following a single application of LONTREL (EF-1136), UK – 2000 Study No. CEMS-1289 DAS Report No. GHE-P-9360 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.2/9	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in barley at harvest under open field conditions following a single application of LONTREL (EF-1136), UK – 2000 Study No. CEMS-1288 DAS Report No. GHE-P-9359 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.2/10	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in barley at harvest following a single application of LONTREL 100 (EF-1136), EU Southern Zone – 2001 Study No. CEMS-1543 DAS Report No. GHE-P-9384 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.3.2/11	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in barley at intervals under open field conditions following a single application of LONTREL 100 (EF-1136), Southern France and Italy – 2000 Study No. CEMS-1292 DAS Report No. GHE-P-9363 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.3/1	Pronier, I.	2013	Residues of fluroxypyr-meptyl, clopyralid MCPA-2-ethylhexyl in wheat at intervals and at harvest following a single application of GF-1681. Northern and Southern Zone – 2012 DAS Study No. 14SRFR12R03 SynTech Research France, 613 Route du Bois de Loyse, 71570 La Chapelle de Guinchay, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.3/2	Devine, H.C.	2006	Residues of clopyralid in wheat and process fractions at harvest following a single application of EF-1498, Northern France – 2005 Study No. CEMS-2711 DAS Report No. GHE-P-11274 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.3/3 Refer to CA 6.3.2/2	Clements, B.	1997	Residues of fluroxypyr-BPE, clopyralid and MCPA in cereals at harvest following a single application of BOFIX (NEW) EF-1403, France (North and South) – 1996 DAS Study No. R96-138 Huntingdon Research Centre (HRC) U.K. GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.3/4	Butler, R.E.	1998	Residues of clopyralid, fluroxypyr and MCPA in winter wheat at harvest following a single application of BOFIX* BP (EF-1403), Belgium, 1997 DAS Study No. R97-103 Redebel, Belgium GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.3/5	Garbay, M.	2005	Residue Study with Fluroxypyr and Clopyralid and 2,4-MCPA (Bofix = EF-1498) in or on Wheat in France (North And South); Analyse de Residus de Florasulam, Fluroxypyr, 2,4-MCPA et Clopyralid dans l'Orge, le Ble, la Farine et les Produits Transformes [Analysis of Residues of Florasulam, Fluroxypyr, 2,4-MCPA and Clopyralid in Barley, Corn, Flour and the Processed Products] DAS Study No. S03DAHBOFIX Solevi, Crest, France; IFBM, Vandoeuvre, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.3/6	Butler, R.E.	1998	Residues of clopyralid, fluroxypyr and MCPA in winter wheat and durum wheat at harvest following a single application of BOFIX* BP (EF-1403), Southern France, 1997 DAS Study No. R97-105 DowElanco Europe, Letcombe, UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.3.3/7 Refer to CA 6.3.2/6	Freeman, JMH et al	1982	Effect of Length of Period Between Application of CYRONAL* and Harvest on Residues of 3,6-dichloropicolinic Acid (DOWCO 290**) in Winter Wheat, Winter Barley and Maize – Belgium 1981 DAS Report No. GHE-P-943 Huntingdon Research Centre (HRC) U.K. GLP/GEP (Y/N): No Published (Y/N): No	No	No	N/A	DAS	Addendum 1 Rapporteur Member State Finland 30.12.2004

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.3/8	Freeman, JMH et al	1984	Clopyralid residues in wheat grain and straw treated with either LONPAR* or LONTREL* 100 from French trials, 1983 DAS Report No. GHE-P-1258 Huntingdon Research Centre (HRC) France GLP/GEP (Y/N): No Published (Y/N): No	No	No	N/A	DAS	Addendum 1 Rapporteur Member State Finland 30.12.2004
CA 6.3.3/9	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in wheat at intervals under open field conditions following a single application of LONTREL (EF-1136), UK and Germany – 2000 DAS Study No. CEMS-1287 DAS Report No. GHE-P-9358 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Addendum 1 Rapporteur Member State Finland 30.12.2004
CA 6.3.3/10	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in wheat at intervals following a single application of LONTREL 100 (EF-1136), EU Northern Zone – 2001 DAS Study No. CEMS-1544 DAS Report No. GHE-P-9385 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Addendum 1 Rapporteur Member State Finland 30.12.2004
CA 6.3.3/11	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in wheat at intervals under open field conditions following a single application of LONTREL 100 (EF-1136), Southern France and Italy – 2000 DAS Study No. CEMS-1242 DAS Report No. 9351 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Addendum 1 Rapporteur Member State Finland 30.12.2004

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.3.3/12	Rawle, N.W., Khoshab, A.	2002	Residues of clopyralid in wheat at harvest under open field conditions following a single application of LONTREL 100 (EF-1136), Greece – 2000 DAS Study No. CEMS-1290 DAS Report No. GHE-P-9361 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Addendum 1 Rapporteur Member State Finland 30.12.2004
CA 6.4.1/1	[redacted]	1974	Dowco 290 and 2,4-D Chicken Feeding Study DAS Study No. TA-517 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	Submitted for the purpose of renewal
CA 6.4.1/2	[redacted]	1975	Residues of Dowco 290 (3,6-dichloropicolinic acid) in Tissues of Chickens Fed the Herbicide DAS Study No. GH-C 819 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.4.1/3	[redacted]	2015	Summary of Clopyralid Livestock Feeding Study: Magnitude of Residue in Eggs, Muscle, Liver and Fat of Laying Hens DAS Study No. 150031 Lab Study No. CEMS-6921 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.4.2/1	[redacted]	1974	Milk Residue Study with Dairy Cows Fed Lontrel Herbicide, Nellite Nematocide and 2,4-D Herbicide: Animal Care, Sampling and Production Records DAS Study No. GH-A 579 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.4.2/2	[redacted]	1974	Residues of Dowco 290 (3,6-dichloropicolini acid) in Milk and Cream from Cows Fed the Herbicide DAS Study No. GH-C 745 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	Yes, evaluated for Annex I inclusion to Directive 91/414/EEC
CA 6.4.2/3	[redacted]	1975	Residues of Dowco 290 (3,6-dichloropicolinic acid) in Bovine Tissues from Calves Fed the Herbicide DAS Study No. GH-C 811 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.4.2/4	[redacted]	2015	Summary of Clopyralid Livestock Feeding Study: Magnitude of Residue in Milk, Muscle, Liver, Kidney and Fat of Lactating Dairy Cattle DAS Study No. 150030 Lab study No. CEMS-6968 [redacted] GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.4.3/1	[redacted]	1975	Residues of Dowco 290 (3,6-dichloropicolinic acid) in Tissues of Swine Fed the Herbicide DAS Study No. GH-C 874 [redacted] GLP/GEP (Y/N): No Published (Y/N): No	Yes	No	N/A	DAS	Yes, evaluated for inclusion to Annex I under Directive 91/414/EEC
CA 6.5.1/1	Adusumilli, H.	2014	Processing Study to Determine the Nature of Residues of 14C -Clopyralid Following the Industrial or Household Preparation DAS Study No. 140574 Dow AgroSciences LLC, Indianapolis, Indiana, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.5.3/1 Refer to 6.3.3/5	Garbay, M.	2005	Residue Study with Fluroxypyr and Clopyralid and 2,4-MCPA (Bofix = EF-1498) in or on Wheat in France (North And South); Analyse de Residus de Florasulam, Fluroxypyr, 2,4-MCPA et Clopyralid dans l'Orge, le Ble, la Farine et les Produits Transformes [Analysis of Residues of Florasulam, Fluroxypyr, 2,4-MCPA and Clopyralid in Barley, Corn, Flour and the Processed Products] DAS Study No. S03DAHBOFIX Solevi, Crest, France; IFBM, Vandoeuvre, France GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.5.3/2	Devine, H.C.	2006	Residues of clopyralid in wheat and process fractions at harvest following a single application of EF-1498, Northern France - 2005 DAS Study No. GHE-P-11274 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous assessments
CA 6.5.3/3	Devine, H.C.	2006	Residues of clopyralid in spring barley and process fractions at harvest and at intervals following a single application of Lontrel 100 (EF-1136), Southern Europe 2006 DAS Study No. GHE-P-11684 CEM Analytical Services - UK GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal
CA 6.6.1/1	Yackovich, P. R. ; Lardie, T. S. ; Brink, D. L.	1993	A 10-1/2 Month Rotational Crops Study With 14C -Labeled Clopyralid - MET90080 DAS Study No. GH-C 2992 Dow AgroSciences LLC, Indianapolis, Indiana, United States GLP/GEP (Y/N): Yes Published (Y/N): No	No	No	N/A	DAS	Submitted for the purpose of renewal
CA 6.6.1/2	Yackovich, P.R.; Lardie T.S.; Miller J.H.	1989	A 125-Day Rotational Crops Study with 14C Labelled Clopyralid DAS Study No. GH-C 2277 DowElanco, Midland, Michigan, USA Published (Y/N): No	No	No	N/A	DAS	Submitted for the purpose of renewal
CA 6.6.1/3	Hall, L. R.	2015	14C -Clopyralid: Metabolism in Confined Rotational Crops with a 30-Day Plant-back Interval DAS Study No. 130733 ABC Laboratories, Inc., Columbia, Missouri 65202, USA GLP/GEP (Y/N): Yes Published (Y/N): No	No	Yes	G	DAS	Submitted for the purpose of renewal