Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Bixlozone (F9600)

Volume 3 – B.7 (AS)

Residue Data

Great Britain

July 2022

Version History

When	What
July 2022	Initial DAR
September 2022	Updated post July 2022 Expert Committee on Pesticides (ECP) meeting Independent Scientific Advice (ISA)

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B.7. RESIDUE DATA

This section B.7 document evaluates the residues information available to support the new active substance bixlozone (F9600). The list of guidance and guidelines applied are outlined in the appendices to Volume 1.

B.7.1. STORAGE STABILITY OF RESIDUES

Table 7-1 Outline of crops for which data have been submitted in this assessment to support primary crop and rotational crop (denoted in *italics*) field trial samples stored prior to analysis

Analytes	Crop	Commodity group (storage stability)
bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 2,2-dimethyl-3-hydroxy propionic acid, *bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone*	Wheat straw	No group (representative of dry commodities)
bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 2,2-dimethyl-3-hydroxy propionic acid	Potato tuber	High starch
	Oilseed rape seed	High oil
	Grape	High acid
bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone	Radish root	High starch
	Leaf lettuce	High water
	Wheat grain	High starch

B.7.1.1. Stability of bixlozone and its metabolites 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.1-01, Rawle, N., 2018
Title:	Storage stability study of F9600 and its metabolites (2,4-dichlorobenzoic acid, 5-hydroxy-F9600 and 2,2-dimethyl-3-hydroxy propionic acid) in crop samples stored under frozen conditions.
Report No.:	CEMR-7268
Guidelines:	EC Regulation 1107/2009, EC Guideline 1607/VI/97 rev.2, Appendix H 7032/VI/95 rev.5 storage stability in residue samples, OECD Guideline for the testing of chemicals Test No 506: Stability of pesticide residues in stored commodities (2007), EPA Residues chemistry test guidelines: OPPTS 860.1380 Storage stability data (1996)
GLP	yes

Materials and methods

The stability of bixlozone and its metabolites (2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid for 24 months in deep frozen storage (<-18°C) was investigated in oilseed rape seed (high oil content commodity), potato tuber (high starch content commodity), grapes (high acid content commodity) and wheat straw (it is noted that straw fits into no specific grouping in accordance with the OECD Guideline 506, however straw is considered most representative of dry commodities).

Untreated samples of wheat straw, oilseed rape seed, potato tuber and grape (5 g) were each fortified with a known amount of a mixed standard solution containing bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid at a rate of 0.1 mg/kg. Untreated specimens were prepared by homogenisation and dry ice. Six additional spare sets of fortified specimens of each matrix were prepared at the start of the study to allow

for any extra time points or repeat analysis. On day zero, three freshly fortified samples, one untreated control sample and two control samples freshly fortified at 0.1 mg/kg for procedural recoveries were analysed for each matrix. For each other storage period, one untreated control and three fortified samples that were stored deep frozen at ≤ -18 °C until analysis and two control samples freshly fortified at 0.1 mg/kg for procedural recoveries were analysed. After time intervals of 3 (4 for wheat straw), 6, 12, 18 and 24 months, samples were removed from storage and analysed for bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid. It is noted that residues of 5-hydroxy-bixlozone were not analysed at 24 months due to an error.

The same sample was fortified with a mixed standard of bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid. This approach is not recommended in accordance with OECD Guideline 506. However, as no clear instability or decline has been observed in this case, there are no concerns with the use of mixed standards in this study.

Bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid residues were analysed according to CEMAS analytical method CAM-0154/001 (Bragado de Jesús, A., 2016, Report No. CEMS-7267). Full details and validation data for this method can be found in DAR CA B5 Section B.5.1.2.5. The method has been considered fully validated in accordance with SANCO/3029/99 rev. 4. In brief, the specimen material is extracted with 1N hydrochloric acid. Subsequently a salt/ acetonitrile mixture is added to all matrix extracts. The samples are shaken, and the extract is centrifuged. An aliquot of the acetonitrile layer is added to a dispersive SPE mixture containing magnesium sulphate and end capped C18 sorbent. The extract is diluted and the final sample as prepared for analysis is determined for residues of bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid by HPLC-MS/MS. The LOQ for bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid in all investigated matrices is 0.01 mg/kg. Note: samples were spiked at 10 x LOQ (0.1 mg/kg).

Sample extracts were stored for up to 7 days between extraction and analysis. All unfortified specimens used for controls and procedural recoveries as well as the stored fortified specimens were stored in a freezer set to maintain a specimen temperature of <-18ºC. The specimens remained frozen throughout the storage unless removed for analysis. The stability of residues in extracts has not been investigated.

In both the residues trials (section B.7.3) and the storage stability trials here, the samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "*the stability of extracts shall be investigated if extracts are not analysed immediately*." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required.

It is noted that residues of 2,2-dimethyl-3-hydroxy propionic acid were detected above 30% LOQ in the control samples of wheat straw and therefore matrix matched standards were used which showed that the calculated residue values in control were below 30% LOQ. Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1.

The study report states that: 'The analysis of the untreated control specimens showed that no residues were present above 30% of the LOQ of each analyte. Although detectable background levels of 2,2-dimethyl-3-hydroxy propionic acid were found in wheat straw, the use of matrix-matched standards showed that calculated residue values in the control specimens were below 30% of LOQ.' The untreated control material was the same source of material (laboratory control system stocks) that was used for fortifications. The results for each untreated control sample are not detailed in the study report however the chromatograms of example untreated control samples provided support this statement.

Results and discussion

The residue levels of bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid from wheat straw, potato tuber, oilseed rape seed and grape after the various storage periods are summarised in Table 7-2 - Table 7-5. bixlozone and its metabolites, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid remained stable over the entire storage period of 24 months in wheat straw, potato tuber, oilseed rape seed and grape. The metabolite 5-hydroxy-bixlozone remained stable over the period of 18 months in wheat straw, potato tuber, oilseed rape seed and grape.

The majority of the mean procedural recoveries (freshly spiked control samples at 0.1 mg/kg) were in the range 70 – 110% demonstrating the effectiveness of the method at each time interval, with the exception of the 0 day mean procedural recoveries of bixlozone in straw, potato and grape (113, 120 and 116% respectively) and 0 day mean procedural recoveries of 5-hydroxy-bixlozone in straw, potato, oilseed rape and grape (117, 112, 111 and 111%

respectively). The mean percentage of the day zero residues (uncorrected) of 2,2-dimethyl-3-hydroxy propionic acid in the 6 month wheat straw sample is <70% (66%). However, the procedural recovery at this time point is slightly low in comparison to recoveries at other sampling points. Furthermore, as significant decline is not observed in the samples for any other metabolite, and the mean recoveries at the later time points are acceptable, it is unlikely that significant breakdown has occurred. This recovery can be considered acceptable.

Table 7-2 Storage stability of bixlozone in wheat straw, potato tuber, oilseed rape seed and grape

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Wheat straw	0.1	0	0.1061, 0.1045, 0.1079	0.1062	100	97, 128 (113)
		4	0.1038, 0.0866, 0.0859	0.0921	87	104, 111 (107)
		6	0.0946, 0.1123, 0.1163	0.1077	101	101, 120 (110)
		12	0.1020, 0.1128, 0.1178	0.1108	104	86, 89 (87)
		18	0.0953, 0.1115, 0.1051	0.1040	98	99, 103 (101)
		24	0.0799, 0.0759, 0.0769†	0.0776	73	70, 71 (71)
Potato tuber	0.1	0	0.1055, 0.0997, 0.0993	0.1015	100	102, 137 (120)
		3	0.0888, 0.1047, 0.0938	0.0958	94	97, 106 (101)
		6	0.0953, 0.0965, 0.0897	0.0938	92	100, 105 (102)
		12	0.1014, 0.0935, 0.0880	0.0943	93	83, 95 (89)
		18	0.1204, 0.1038, 0.0914	0.1052	104	96, 119 (107)
		24	0.1019, 0.1088, 0.1064	0.1057	104	101, 107 (104)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Oilseed rape seed	0.1	0	0.0975, 0.0813, 0.0878	0.0889	100	90, 111 (100)
		3	0.0864, 0.0835, 0.0857	0.0852	96	76, 78 (77)
		6	0.0898, 0.0886, 0.0807	0.0864	97	89, 93 (91)
		12	0.0727, 0.0806, 0.0815	0.0783	88	69, 73 (71)
		18	0.0828, 0.0820, 0.0798	0.0815	92	89, 92 (91)
		24	0.0718, 0.0744, 0.0675†	0.0713	80	76, 78 (77)
Grape	0.1	0	0.1158, 0.0992, 0.1034	0.1061	100	109, 123 (116)
		3	0.1013, 0.0964, 0.0969	0.0982	93	90, 97 (94)
		6	0.0857, 0.0943, 0.0916	0.0905	85	77, 92 (84)
		12	0.1181, 0.1073, 0.1123	0.1126	106	98, 106 (102)
		18	0.1277, 0.0897, 0.1075	0.1083	102	105, 108 (106)
		24	0.1092, 0.1107, 0.1026	0.1075	101	109, 110 (110)

†Although the level of residue seems to have declined, it is considered that all samples are sufficiently stable over 24 months as the procedural recoveries at the 24 month sampling time point are more towards the lower end than those in the earlier time points.

Table 7-3 Storage stability of 2,4-dichlorobenzoic acid in wheat straw, potato tuber, oilseed rape seed and grape

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Wheat straw	0.1	0	0.1015, 0.1029, 0.1017	0.1020	100	102, 107 (105)
		4	0.0853, 0.0850, 0.0874	0.0859	84	89, 99 (94)
		6	0.0925, 0.0937, 0.0927	0.0930	91	95, 98 (96)
		12	0.0980, 0.1012, 0.0998	0.0997	98	94, 101 (97)
		18	0.0978, 0.1030, 0.1046	0.1018	100	98, 99 (98)
		24	0.1006, 0.0993, 0.1014	0.1004	98	86, 88 (87)
Potato tuber	0.1	0	0.0959, 0.1068, 0.1051	0.1026	100	104, 105 (105)
		3	0.0885, 0.0995, 0.0893	0.0924	90	95, 103 (99)
		6	0.0912, 0.0904, 0.0906	0.0907	88	92, 93 (92)
		12	0.0960, 0.0951, 0.1004	0.0971	95	93, 94 (94)
		18	0.1028, 0.1100, 0.0864	0.0997	97	96, 110 (103)
		24	0.1015, 0.1006, 0.1014	0.1012	99	96, 106 (101)
Oilseed rape seed	0.1	0	0.1033, 0.1030, 0.1043	0.1035	100	99, 103 (101)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		3	0.0920, 0.0927, 0.0941	0.0929	90	86, 86 (86)
		6	0.0949, 0.0937, 0.0943	0.0943	91	93, 93 (93)
		12	0.0927, 0.0965, 0.0980	0.0957	92	92, 94 (93)
		18	0.1069, 0.0982, 0.0942	0.0998	96	89, 93 (91)
		24	0.1025, 0.1001, 0.1053	0.1027	99	102, 107 (105)
Grape	0.1	0	0.1072, 0.1061, 0.1035	0.1056	100	105, 106 (106)
		3	0.0983, 0.0944, 0.0974	0.0967	92	91, 93 (92)
		6	0.0891, 0.0877, 0.0901	0.0890	84	89, 90 (90)
		12	0.0969, 0.0939, 0.0952	0.0954	90	101, 101 (101)
		18	0.1065, 0.1061, 0.1060	0.1062	101	95, 100 (98)
		24	0.0998, 0.1052, 0.1028	0.1026	97	104, 106 (105)

Table 7-4 Storage stability of 5-hydroxy-bixlozone in wheat straw, potato tuber, oilseed rape seed and grape

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Wheat straw	0.1	0	0.1000, 0.1072, 0.1060	0.1044	100	98, 136 (117)
		4	0.1068, 0.0882, 0.0901	0.0950	91	98, 104 (101)
		6	0.0877, 0.1191, 0.1199	0.1089	104	93, 128 (110)
		12	0.1044, 0.1126, 0.1144	0.1105	106	100, 112 (106)
		18	0.1061, 0.1337, 0.1106	0.1168	112	92, 93 (93)
Potato tuber	0.1	0	0.0949, 0.0989, 0.1012	0.0984	100	108, 115 (112)
		3	0.0892, 0.1016, 0.0962	0.0956	97	98, 101 (99)
		6	0.0971, 0.0969, 0.0952	0.0964	98	99, 102 (100)
		12	0.1165, 0.115, 0.1089	0.1123	114	97, 101 (99)
		18	0.1050, 0.1186, 0.0787	0.1008	102	96, 120 (108)
Oilseed rape seed	0.1	0	0.1117, 0.1106, 0.1094	0.1106	100	107, 114 (111)
		3	0.1027, 0.1015, 0.1024	0.1022	92	91, 93 (92)
		6	0.1042, 0.1014, 0.1013	0.1023	93	102, 102 (102)
		12	0.1058, 0.1188, 0.1159	0.1135	103	104, 105 (104)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		18	0.1043, 0.1233, 0.0908	0.1061	96	86, 93 (90)
Grape	0.1	0	0.0949, 0.0989, 0.1012	0.0984	100	109, 113 (111)
		3	0.0892, 0.1016, 0.0962	0.0956	97	95, 95 (95)
		6	0.0971, 0.0969, 0.0952	0.0964	98	89, 95 (92)
		12	0.1165, 0.1115, 0.1089	0.1123	114	103, 105 (104)
		18	0.1050, 0.1186, 0.0787	0.1008	102	100, 101 (101)

Table 7-5 Storage stability of 2,2-dimethyl-3-hydroxy propionic acid in wheat straw, potato tuber, oilseed rape seed and grape

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Wheat straw	0.1	0	0.1132, 0.1069, 0.1158	0.1120	100	73, 86 (80)
		4	0.0935, 0.0988, 0.0886	0.0870	78	95, 104 (99)
		6	0.0809, 0.0756, 0.0653	0.0739	66	78, 84 (81)
		12	0.1362, 0.0798, 0.0929	0.1029	92	67, 92 (79)
		18	0.0925, 0.1100, 0.1643	0.1222	109	84, 95 (90)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		24	0.0862, 0.0919, 0.0917	0.0899	80	73, 73 (73)
Potato tuber	0.1	0	0.0869, 0.0969, 0.0983	0.0940	100	91, 92 (92)
		3	0.0842, 0.0827, 0.0872	0.0847	90	91, 93 (92)
		6	0.0812, 0.0802, 0.0841	0.0818	87	73, 77 (75)
		12	0.0979, 0.0918, 0.1079	0.0992	106	90, 97 (94)
		18	0.0931, 0.1025, 0.0783	0.0913	97	89, 107 (98)
		24	0.0946, 0.0977, 0.0998	0.0974	104	84, 95 (90)
Oilseed rape seed	0.1	0	0.0891, 0.0872, 0.0860	0.0874	100	85, 86 (86)
		3	0.0750, 0.0985, 0.0977	0.0904	103	89, 92 (90)
		6	0.0811, 0.0787, 0.0791	0.0797	91	73, 82 (78)
		12	0.0864, 0.0949, 0.0934	0.0916	105	82, 85 (83)
		18	0.0926, 0.0893, 0.0868	0.0896	102	87, 87 (87)
		24	0.0932, 0.0969, 0.0680	0.0860	98	87, 101 (94)
Grape	0.1	0	0.0983, 0.0970, 0.0978	0.0977	100	98, 100 (99)

Commodity	Fortification level (mg/kg)	Storage period (months)	Stored sample residues			Procedural recovery for freshly fortified control samples (%) (mean)
			Individual values uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		3	0.1102, 0.0860, 0.0939	0.0967	99	99, 106 (103)
		6	0.0708, 0.0792, 0.0737	0.0746	76	67, 77 (72)
		12	0.0948, 0.0764, 0.0957	0.0890	91	59, 88 (73)
		18	0.0863, 0.0791, 0.0977	0.0877	90	73, 86 (80)
		24	0.0990, 0.0981, 0.0851	0.0941	96	97, 98 (98)

Conclusions

Bixlozone and its metabolites, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid are stable in wheat straw, potato tuber, oilseed rape seed and grape for at least 24 months when stored under deep frozen conditions. The metabolite 5-hydroxy-bixlozone is stable in wheat straw, potato tuber, oilseed rape seed and grape for at least 18 months when stored under deep frozen conditions.

The samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "the stability of extracts shall be investigated if extracts are not analysed immediately." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required in this case.

B.7.1.2. Stability of the metabolite 5'-hydroxy-bixlozone

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.1-02, Kennedy, S., 2019
Title:	Storage stability study of 5'-hydroxy-F9600 in crop samples stored under frozen conditions.
Report No.:	CEMR-8008
Guidelines:	EC Regulation 1107/2009, EC Guideline 1607/VI/97 rev.2, Appendix H 7032/VI/95 rev.5 storage stability in residue samples, OECD Guideline for the testing of chemicals Test No 506: Stability of pesticide residues in stored commodities (2007), EPA Residues chemistry test guidelines: OPPTS 860.1380 Storage stability data (1996)
GLP	yes

Materials and methods

The stability of the metabolites 5'-hydroxy-bixlozone for 24 months in deep frozen storage (<-18 °C) was investigated in oilseed rape seed (high oil content commodity), potato tuber (high starch content commodity), grapes (high acid content commodity) and wheat straw (it is noted that straw fits into no specific grouping in accordance with the OECD Guideline 506, however straw is considered most representative of dry commodities).

Untreated samples of wheat straw, oilseed rape seed, potato tuber and grape (10 g) were each fortified with a known amount of a standard solution containing 5'-hydroxy-bixlozone at a concentration of 0.1 mg/kg. Each specimen was left to stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction or frozen storage. Eight additional spare sets of fortified specimens of each matrix were prepared at the start of the study to allow for any extra time points or repeat analysis. On day zero, three freshly fortified samples, one untreated control sample and two control samples freshly fortified at 0.1 mg/kg for procedural recoveries were analysed for each matrix. For each other storage period, one untreated control and three fortified samples that were stored deep frozen at ≤ -18 °C until analysis and two control samples freshly fortified at 0.1 mg/kg for procedural recoveries were analysed. After time intervals of 3, 6, 12, 18 and 24 months, samples were removed from storage and analysed for 5'-hydroxy-bixlozone.

5'-hydroxy-bixlozone residues were analysed according to CEMAS analytical method CAM-0180/002 (Langridge, G., 2017, Report No. CEMS-0180). Full details and validation data for this method can be found in DAR CA B5 Section B.5.1.2.5. The method is considered fully validated in accordance with SANCO/3029/99 rev. 4. In brief, the specimen material is extracted with 1N hydrochloric acid. A further QuEChERS liquid/liquid extraction is then carried out and the upper acetonitrile layer is cleaned up further using dispersive SPE. The supernatant is diluted in 0.05% acetic acid in water and analysed for 5'-hydroxy-bixlozone by HPLC-MS/MS. The LOQ for 5'-hydroxy-bixlozone in all investigated matrices is 0.01 mg/kg. Note: samples were spiked at 10 x LOQ (0.1 mg/kg).

Sample extracts were stored for up to 5 days between extraction and analysis. All unfortified specimens used for controls and procedural recoveries as well as the stored fortified specimens were stored in a freezer set to maintain a specimen temperature of <-18ºC. The specimens remained frozen throughout the storage unless removed for analysis. The stability of residues in extracts has not been investigated.

In both the residues trials (section B.7.3) and the storage stability study here, the samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "*the stability of extracts shall be investigated if extracts are not analysed immediately*." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100%) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required.

The study report states that: 'All untreated control samples analysed at each time-point demonstrated that no residues of 5'-hydroxy-bixlozone were present above 30% of the LOQ.' The untreated control material was the same source of material (laboratory control system stocks) that was used for fortifications. The results for each untreated control sample are not detailed in the study report however the chromatograms of example untreated control samples provided support this statement.

Results and discussion

The residue levels of 5'-hydroxy-bixlozone from wheat straw, potato tuber, oilseed rape seed and grape after the various storage periods are summarised in Table 7-6. The metabolite 5'-hydroxy-bixlozone remained stable under deep frozen storage over the period of 24 months in wheat straw, potato tuber, oilseed rape seed and grape.

The mean procedural recoveries (freshly spiked control samples at 0.1 mg/kg) were in the range 70 – 110% demonstrating the effectiveness of the method at each time interval.

The percentage of the day zero uncorrected residues found in potato tuber samples are high at all time points (3-24 months). This can be attributed to the low uncorrected residue level observed in the day zero sample (0.071 mg/kg reported for a sample spiked with 0.1 mg/kg). It is noted that the procedural recovery at day zero is also relatively low (74%).

Table 7-6 Storage stability of 5'-hydroxy-bixlozone in wheat straw, potato tuber, oilseed rape seed and grape

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Wheat straw	0.1	0	0.07758, 0.09260, 0.08345	0.0845	100	93, 95 (94)
		3	0.08793, 0.08492, 0.09364	0.0888	105	88, 88 (88)
		9	0.09684, 0.09677, 0.08938	0.0943	112	89, 90 (90)
		12	0.09309, 0.09977, 0.10490	0.0993	118	93, 100 (97)
		18	0.08136, 0.09241, 0.08570	0.0865	102	87, 89 (88)
		24	0.09506, 0.08477, 0.08260	0.0875	104	86, 94 (90)
Potato tuber	0.1	0	0.07453, 0.06783, 0.06981	0.0707	100	72, 76 (74)
		3	0.08588, 0.08039, 0.08936	0.0852	121	80, 85 (83)
		9	0.10483, 0.10383, 0.10673	0.1051	149	90, 90 (90)
		12	0.09646, 0.09954, 0.09968	0.0986	139	83, 92 (88)
		18	0.08981, 0.08683, 0.08762	0.0881	125	76, 85 (81)
		24	0.08916, 0.08341, 0.08476	0.0858	121	92, 103 (98)
Oilseed rape seed	0.1	0	0.10359, 0.10524, 0.10742	0.1054	100	106, 108 (107)
		3	0.09117, 0.09346, 0.09675	0.0938	89	90, 91 (91)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		9	0.11552, 0.11427, 0.11528	0.1150	109	105, 106 (106)
		12	0.10900, 0.11199, 0.10959	0.1102	105	104, 106 (105)
		18	0.10274, 0.09943, 0.10376	0.1020	97	99, 102 (101)
		24	0.08587, 0.08495, 0.08997	0.0869	82	80, 83 (82)
Grape	0.1	0	0.09493, 0.09319, 0.09696	0.0950	100	101, 103 (102)
		3	0.09663, 0.06387, 0.08994	0.0835	88	89, 92 (91)
		9	0.09049, 0.08910, 0.09346	0.0910	96	78, 78 (78)
		12	0.08199, 0.08917, 0.08900	0.0867	91	79, 83 (81)
		18	0.09105, 0.08689, 0.08829	0.0887	93	87, 87 (87)
		24	0.07804, 0.07465, 0.07728	0.0767	81	75, 81 (78)

Conclusions

The metabolite, 5'-hydroxy-bixlozone is stable in wheat straw, potato tuber, oilseed rape seed and grape for at least 24 months when stored under deep frozen conditions.

The samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "the stability of extracts shall be investigated if extracts are not analysed immediately." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required in this case.

B.7.1.3. Stability of bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	Fritzsch S., 2020
Title:	Storage stability of F9600-dimethyl-malonamide, F9600-OH-isobutyramide and 4-hydroxymethyl-F9600 in Radish, Leaf Lettuce, Wheat Grain and Straw under Deep Frozen Conditions
Report No.:	S18-04053 (FMC-1801L)
Guidelines:	EC Regulation 1107/2009, EC Guideline 1607/VI/97 rev.2, Appendix H 7032/VI/95 rev.5 storage stability in residue samples, OECD Guideline for the testing of chemicals Test No 506: Stability of pesticide residues in stored commodities (2007), EPA Residues chemistry test guidelines: OPPTS 860.1380 Storage stability data (1996)
GLP	Yes

Materials and methods

The stability of the metabolites bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone for 24 months in deep frozen storage (<-18 °C) was investigated in radish roots (high starch commodity), leaf lettuce (high water commodity), wheat grain (high starch commodity) and wheat straw (it is noted that straw fits into no specific grouping in accordance with the OECD Guideline 506, however straw is considered most representative of dry commodities).

Untreated samples of radish roots, leaf lettuce, wheat grain and wheat straw (10 g) were each fortified with a known amount of a standard solution containing bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone at a concentration of 0.1 mg/kg for each analyte. The sample was mixed by brief swirling to ensure distribution of the test items in the respective matrices. The solvent was allowed to evaporate for approx. 10 minutes. On day zero, three freshly fortified samples and one untreated control sample were analysed for each matrix. For each other storage period, one untreated control and three fortified samples that were stored deep frozen at ≤ -18 °C until analysis and two control samples freshly fortified at 0.1 mg/kg for procedural recoveries were analysed. After time intervals of 3, 6, 9, 12, 18 and 24 months, samples were removed from storage and analysed for bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone.

The same sample was fortified with a mixed standard of bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone. This approach is not recommended in accordance with OECD Guideline 506. However, as no clear instability or decline has been observed in this case, there are no concerns with the use of mixed standards in this study.

Bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone residues were analysed according to CEMAS analytical method CAM-0180/001. Full details and validation data for this method can be found in DAR CA B5 Section B.5.2.1. The method is considered fully validated in accordance with SANCO/3029/99 rev. 4. In brief, the specimen material is extracted with 1N hydrochloric acid. A further QuEChERS liquid/liquid extraction is then carried out and the upper acetonitrile layer is cleaned up further using dispersive SPE. The supernatant is diluted in 0.05% acetic acid in water and analysed by LC-MS/MS. The LOQ for bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone in all investigated matrices is 0.01 mg/kg. Note: samples were spiked at 10 x LOQ (0.1 mg/kg).

All unfortified specimens used for controls and procedural recoveries as well as the stored fortified specimens were stored in a freezer set to maintain a specimen temperature of <-18°C. The specimens remained frozen throughout the storage unless removed for analysis. Sample extracts were stored for up to 2 days under refrigerated conditions (1-10°C) between extraction and analysis. The stability of residues in extracts has not been investigated. As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100%) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required.

In both the residues trials (section B.7.3) and the storage stability study here, the samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "*the stability of extracts shall be investigated if extracts are not analysed immediately*." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not

always close to 100%) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required.

The study report states that: 'The control samples showed no significant interference (above 30% of LOQ)'. The untreated control material was the same source of material (purchased at local markets or from GLP study S16-01153, residue field trial on wheat reported in Section 7.3) that was used for fortifications. The results for each untreated control sample are not detailed in the study report however the chromatograms of example untreated control samples provided support this statement.

Results and discussion

The residue levels of bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone from radish roots, leaf lettuce, wheat grain and wheat straw after the various storage periods are summarised in Table 7-7, Table 7-8 and Table 7-9. The metabolites bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone remained stable under deep frozen storage over the period of 24 months in radish roots, leaf lettuce, wheat grain and wheat straw. Taking account of the procedural recoveries, which were variable in some instances across the timepoints investigated, it is difficult to conclude that any clear decline is occurring. The data support a conclusion of overall stability of the analytes studied for the period of storage investigated (24 months).

The mean procedural recoveries (freshly spiked control samples at 0.1 mg/kg) were in the range 70 – 110% demonstrating the effectiveness of the method at each time interval. Separate fortified samples which could be considered procedural recoveries were not determined at the zero day time point.

Table 7-7 Storage stability of bixlozone-dimethyl-malonamide in radish roots, leaf lettuce, wheat grain and wheat straw

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Radish Roots	0.1	0	0.094 0.102 0.102	0.099	100	N/A[$]
		3	0.084 0.085 0.092	0.087	88	74, 90 (82)
		6	0.080 0.084 0.089	0.084	85	78, 80 (79)
		9	0.080 0.080 0.089	0.083	84	84, 88 (86)
		12	0.084 0.089 0.090	0.088	89	91, 93 (92)
		18	0.085 0.086 0.087	0.086	87	90, 98 (94)
		24	0.072 0.080 0.089	0.080	81	91, 96 (94)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Leaf Lettuce	0.1	0	0.091 0.109 0.114	0.105	100	N/A[$]
		3	0.090 0.098 0.099	0.096	91	99, 100 (100)
		6	0.090 0.095 0.096	0.094	90	96, 99 (98)
		12	0.082 0.084 0.089	0.085	81	98, 100 (99)
		18	0.095 0.099 0.102	0.099	94	103, 105 (104)
		24	0.070 0.072 0.088	0.077	73	89, 93 (91)
Wheat Grain	0.1	0	0.090 0.092 0.094	0.092	100	N/A[$]
		3	0.069 0.073 0.076	0.073	79	81, 84 (83)
		6	0.069 0.070 0.072	0.070	76	82, 86 (84)
		9	0.065 0.071 0.075	0.070	76	81, 84 (83)
		12	0.077 0.079 0.080	0.079	86	100, 104 (102)
		18	0.068 0.069 0.078	0.072	78	71, 74 (73)
		24	0.071 0.072 0.073	0.072	78	76, 81 (79)
Wheat Straw	0.1	0	0.083 0.086 0.087	0.085	100	N/A[$]

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		3	0.083 0.089 0.090	0.087	102	83, 87 (85)
		6	0.089 0.091 0.094	0.091	107	87, 95 (91)
		9	0.072 0.073 0.074	0.073	86	77, 80 (79)
		12	0.088 0.091 0.093	0.091	107	93, 96 (95)
		18	0.083 0.093 0.099	0.092	108	82, 82 (82)
		24	0.075 0.077 0.079	0.077	91	76, 79 (78)

Table 7-8 Storage stability of bixlozone-OH-isobutyramide in radish roots, leaf lettuce, wheat grain and wheat straw

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Radish Roots	0.1	0	0.085 0.085 0.085	0.085	100	N/A[$]
		3	0.081 0.086 0.092	0.086	101	73, 88 (81)
		6	0.075 0.077 0.080	0.077	91	72, 75 (74)
		9	0.073 0.075 0.082	0.077	91	82, 83 (83)
		12	0.080 0.084 0.088	0.084	99	84, 88 (86)
		18	0.076 0.077 0.078	0.077	91	79, 85 (82)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		24	0.067 0.070 0.076	0.071	84	79, 85 (82)
Leaf Lettuce	0.1	0	0.092 0.096 0.098	0.095	100	N/A[$]
		3	0.094 0.094 0.099	0.096	101	95, 96 (96)
		6	0.087 0.088 0.091	0.089	94	87, 87 (87)
		12	0.079 0.083 0.086	0.083	87	94, 97 (96)
		18	0.093 0.093 0.098	0.095	100	96, 96 (96)
		24	0.067 0.070 0.078	0.072	76	79, 82 (81)
Wheat Grain	0.1	0	0.087 0.087 0.092	0.089	100	N/A[$]
		3	0.075 0.079 0.084	0.079	89	83, 84 (84)
		6	0.071 0.072 0.078	0.074	83	75, 82 (79)
		9	0.074 0.076 0.083	0.078	88	81, 82 (82)
		12	0.084 0.086 0.091	0.087	98	97, 105 (101)
		18	0.072 0.073 0.079	0.075	84	69, 72 (71)
		24	0.075 0.080 0.082	0.079	89	72, 80 (76)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Wheat Straw	0.1	0	0.090 0.094 0.096	0.093	100	N/A[$]
		3	0.093 0.094 0.094	0.094	101	86, 88 (87)
		6	0.087 0.088 0.090	0.088	95	83, 84 (84)
		9	0.082 0.082 0.085	0.083	89	84, 86 (85)
		12	0.095 0.095 0.095	0.095	102	95, 98 (97)
		18	0.083 0.093 0.093	0.090	97	84, 87 (86)
		24	0.069 0.070 0.073	0.071	76	80, 82 (81)

Table 7-9 Storage stability of 4-hydroxymethyl-bixlozone in radish root, leaf lettuce, wheat grain and wheat straw

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
Radish Roots	0.1	0	0.097 0.103 0.108	0.103	100	N/A[$]
		3	0.084 0.092 0.096	0.091	88	78, 93 (86)
		6	0.076 0.089 0.098	0.088	85	72, 87 (80)
		9	0.083 0.084 0.090	0.086	83	90, 91 (91)
		12	0.080 0.082 0.089	0.084	82	96, 99 (98)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		18	0.084 0.084 0.088	0.085	83	89, 98 (94)
		24	0.072 0.075 0.083	0.077	75	89, 93 (91)
Leaf Lettuce	0.1	0	0.094 0.096 0.103	0.098	100	N/A[$]
		3	0.089 0.092 0.095	0.092	94	97, 100 (99)
		6	0.095 0.098 0.099	0.097	99	94, 108 (101)
		12	0.080 0.083 0.085	0.083	85	101, 107 (104)
		18	0.097 0.098 0.100	0.098	100	108, 110 (109)
		24	0.072 0.078 0.078	0.076	78	90, 90 (90)
Wheat Grain	0.1	0	0.094 0.097 0.102	0.098	100	N/A[$]
		3	0.077 0.079 0.080	0.079	81	89, 91 (90)
		6	0.069 0.072 0.073	0.071	72	80, 84 (82)
		9	0.072 0.073 0.080	0.075	77	88, 90 (89)
		12	0.081 0.083 0.085	0.083	85	100, 104 (102)
		18	0.071 0.072 0.081	0.075	77	75, 80 (78)

Commodity	**Fortification level** (mg/kg)	**Storage period** (months)	**Stored sample residues**			**Procedural recovery for freshly fortified control samples** (%) (mean)
			Individual uncorrected residue (mg/kg)	Mean uncorrected residue (mg/kg)	% of day 0 uncorrected residues (%)	
		24	0.067 0.071 0.071	0.070	71	82, 84 (83)
Wheat Straw	0.1	0	0.084 0.087 0.089	0.087	100	N/A[$]
		3	0.093 0.096 0.098	0.096	110	88, 92 (90)
		6	0.090 0.092 0.096	0.093	107	86, 88 (87)
		9	0.084 0.085 0.087	0.085	98	88, 91 (90)
		12	0.094 0.094 0.094	0.094	108	92, 99 (96)
		18	0.080 0.096 0.101	0.092	106	84, 85 (85)
		24	0.070 0.075 0.079	0.075	86	76, 80 (78)

[$] Procedural recoveries were not determined at day 0; three freshly fortified samples and a control sample were analysed at this time point. The freshly fortified sample results have been reported as the 'uncorrected residue' results and are also reported as recovery samples analysed as part of the supporting method validation. No additional procedural recovery data are available for this time point.

Conclusions

The metabolites, bixlozone-dimethyl-malonamide, bixlozone-OH-isobutyramide and 4-hydroxymethyl-bixlozone remained stable under deep frozen (<-18°C) storage over the period of 24 months in radish roots, leaf lettuce, wheat grain and wheat straw.

The samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "the stability of extracts shall be investigated if extracts are not analysed immediately." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required in this case.

B.7.1.4. Storage stability in animal matrices

No data has been generated to support the storage stability of residues of bixlozone in animal matrices.

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

The bixlozone (F9600) molecule is a two-ring structure, as shown in Figure 7-1. It consists of the dichloro-phenyl ring and the dimethyl-isoxazolidinone ring at the opposite side of the molecular backbone connected by the aliphatic CH_2 group in between.

The metabolism and distribution of bixlozone (F9600) in plants and animals (hens and goat) was investigated using the active substance radiolabelled in the dichloro-phenyl ring ([phenyl-U-^{14}C]-label) or on the carbonyl carbon of the dimethyl-isoxazolidin-3-one ring ([carbonyl-C5-^{14}C]-label). The molecular structures and the positions of the labels are shown in Figure 7-2. These labelling positions are considered appropriate for the study of metabolic pathways in primary crops, rotational crops and livestock animals. Throughout this evaluation P-label and C-label is used to denote phenyl label and carbonyl label respectively.

Test Material:	bixlozone
IUPAC Name:	2-(2,4-dichlorobenzyl)-4,4-dimethylisoxazolidin-3-one
CAS Name:	2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone
Molecular Weight:	274.14 g/mol
CAS No.:	81777-95-9
Development Code:	F9600

Figure 7-1 Structure of non-radiolabelled bixlozone

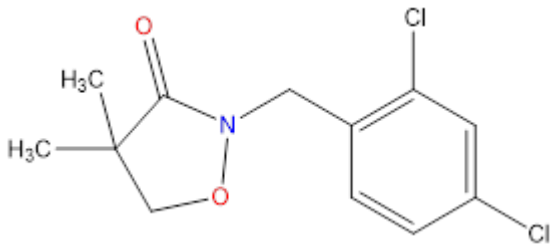
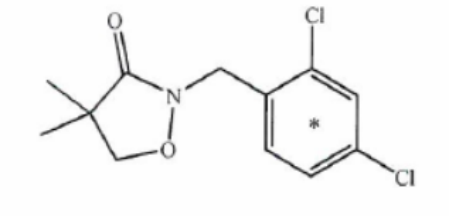
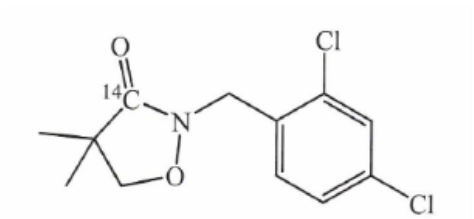


Figure 7-2 Structures of radiolabelled bixlozone

	
[Phenyl -U-^{14}C]bixlozone **(***denotes ^{14}C position) "Phenyl label"	[Carbonyl -C5-^{14}C]bixlozone "Carbonyl label"

In this section, all residue values given in mg/kg refer to parent compound equivalents if not indicated otherwise. All total radioactive (TRR) levels reported for each metabolite/extract were determined based on the TRR determined by combustion (prior to extraction). The determination and expression of residue levels across all metabolism studies

(plants and livestock animals) is the same. All mg/kg expression of residues in all the metabolism studies are as mg parent equivalents/kg (mg parent eq./kg).

Numerous metabolites were identified in the metabolism studies. The chemical structures and report names used in the summaries are given in the appendices to Volume 1.

Each metabolism study write-up indicates the reference standards used in the studies. As the metabolism evaluation write-ups refers to 'unknowns' the reader is referred to the list of reference standards in each study in order to give the context of metabolites sought (and not found, versus not sought).

The primary crop metabolism studies (wheat, canola/oilseed rape, sugar beet and rice) and rotational crop studies were performed outdoors in the US, and each of these studies involved application of bixlozone (F9600) prepared in an emulsifiable concentrate formulation 'blank'. All of the primary crop and rotational crop metabolism studies were conducted in California except for the canola study which was conducted in Illinois. The data on fate and behaviour in the environment do not indicate that photolysis is a rapid route of degradation of bixlozone in the environment. In section CAB.8.1.1.3 (photolysis in soil) bixlozone degraded slowly on soil surfaces following irradiation for up to 15 days alongside dark control samples (irraditation treatment approximately equivalent to 34 days of natural summer sunlight at latitude 30-50°N). The rate of slow degradation was described as geomean DT_{50} = 100 days, converted to natural summer light, with no degradates > 5% AR being observed in either irradiated or dark control samples. In a direct photolysis study (investigating photochemical degradation in water), see section CAB.8.2.1, bixlozone was slowly degraded to minor photoproducts after 13 days continuous irradiation. In the primary and rotational crop metabolism studies conducted in the sunnier climate of California (and also Illinois) US, and considering the long pre-harvest intervals in the primary crop studies, there could have been a faster rate of photolysis in these studies compared to under the conditions for UK GAP, however these outdoor metabolism studies are acceptable for reliance on the data in a semi-quantitative fashion. It is not typically expected that different metabolism studies should need to be available conducted in different outdoor climates.

The applicant was asked by HSE to comment on why they did not seek the metabolite dichlorobenzyl alcohol in the primary crop metabolism studies for bixlozone as it was noted that clomazone (for which applicant is also a data holder) is a very similar structure to bixlozone. 2,4-dichlorobenzoic acid has been included in the residues investigations for bixlozone and not the corresponding alcohol. In the assessment of clomazone, the 2-chlorobenzyl alcohol was the major metabolite found in crops (MRL Review for clomazone, EFSA, 2011). The applicant responded that 2,4-dichlorobenzyl alcohol was sought in primary crop samples. The applicant provided evidence (example chromatograms for the canola metabolism study provided in applicant correspondence dated 19th June 2020) that no radioactive peak corresponding to the retention of the reference 2,4-dichlorobenzyl alcohol standard was observed in any of the organic extracts (before hydrolysis) or in hydrolysates (after hydrolysis samples) in oilseed rape. The applicant explained that since results from the HPLC-radio-chromatographic analysis of the primary crop samples showed the absence of this potential metabolite, the chromatogram of this compound was not included in the primary crop metabolism reports, although it was recorded and retained in the study files. It is not clear whether the metabolite dichlorobenzyl alcohol was sought after in the cereal metabolism studies (the reference standard for this component was listed in the canola, sugar beet and rotational crop metabolism study but not the cereal metabolism studies). In terms of rationale (regarding the potential for dichlorobenzyl alcohol to be found) the applicant has stated:

> "Clomazone (CLZ) is the first molecule discovered in the class of isoxazolidinone herbicides. CLZ has a single chlorine substitution (on C-2) on the phenyl ring. In crops, upon cleavage of the parent molecule, orthochlorobenzyl alcohol (also referred to as OCB alcohol) is formed as one of the major residues.
>
> Bixlozone (F9600) which is the second molecule discovered in this class of chemistry, has two chlorine atoms (C-2 and C-4) on the phenyl ring. From the available metabolism studies in crops and animals, it is evident that 2,4-dichlorobenzoic acid (also referred to as 2,4-DCBA) is formed as the major residue upon cleavage of bixlozone. On the other hand, no detectable levels of 2,4-dichlorobenzyl alcohol (2,4-DCB alcohol) have been seen in crops or animal matrices. This is likely due to the presence of an additional chlorine in bixlozone, which results in an enhanced resonance effect with the aromatic ring."

Please see Vol 1, section 2.7.4 regarding the finding of 2,2-dimethyl-3-hydroxy propionic acid in trials as a possible natural source of finding this residue. It is noted that radiolabelled residues of this component were determined in the metabolism studies, so it seems that this metabolite does form as a direct result of the proposed pesticide treatment. Residues of 2,2-dimethyl-3-hydroxy propionic acid were not found in control material in the metabolism studies (in contrast to the crop field trials, where the residues were found in both treated plots as well as untreated control plant material). In these primary crop metabolism studies the control and the treated containers were separately located by a large distance (wheat/rice: at least 60 m distance; canola: at least 22 m distance and a plastic barrier at application between them; sugar beet: at least 60 m distance and a plastic barrier at application between them).

The efficiency of the extraction procedures for the major components of the residue in plants has been considered only for wheat straw (to compare extraction of the proposed enforcement method CAM-180) with the extractability observed in the metabolism study. See Volume 3 CA Section B.5.2.1. The conclusions made in section B.5.2.1 are in regard of 2,4-dichlorobenzoic acid or 5'-Hydroxy-bixlozone. Due to the lack of residues of bixlozone and the 2-2-dimethyl-3-hydroxy propionic acid in the samples tested in the efficiency extraction work (section B.5.2.1), no conclusions are made in regard of these components. Please see Volume 1, section 2.7.3 which discusses the proposed residue definition. Extraction efficiency of analytical methods for residues of bixlozone in animal products has been considered, see section B.5.2.6.

Sample storage periods

The final analysis of samples took place after an excessive period in each of the metabolism studies (1.5 years for livestock studies to 3.5 years for wheat metabolism studies). Samples, whether as raw commodity samples, homogenised powders and extracts, were stored frozen during the course of the studies. OECD Guidelines 501, 502 and 503 indicate that metabolism studies should be completed within an analysis period of six months, or otherwise be appropriately supported by storage stability investigations performed in the context of the metabolism studies. Each of the metabolism reports stated the period of time until 'initial analysis'; this was usually within 6 months (or not exceeding by a large amount). The metabolic profile storage stability (chromatographic comparison) investigations performed within each of the wheat, sugar beet and poultry metabolism studies have been written up in each of the metabolism study evaluations below but are considered limited. Further studies and reports considering the stability of incurred residues in the context of the primary crop metabolism studies (wheat, canola, sugar beet and rice) were submitted and evaluated. These contained some further metabolic profile storage stability work (chromatographic comparisons) and are reported at the end of B.7.2.1.

It is noted that none of the non-radiolabelled freezer storage stability data available for metabolites, as written up in section B.7.1 (see also summary in Vol 1, section 2.7.1) suggests that there is a concern with any instability of residues. The issue of sample storage periods in the context of metabolism work is discussed further in Vol 1 (section 2.7.2).

B.7.2.1. Plants

B.7.2.1.1. *Wheat*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.2.1-01, Desai, M., 2019
Title:	Nature of the residue/metabolism of [^{14}C]F9600 in/on wheat crop
Report No.:	14003-RPT03661 (Report amendment date: October 30, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 501 Metabolism in Crops, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996)
GLP	yes

Materials and methods

Materials

1. Test Material

Test Material:	[Phenyl -U-^{14}C] bixlozone
Lot/batch No.:	CFQ42017
Radiochemical Purity:	99.6% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 21.57 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.89 MBq/mg (certified)

2. Test Material

Test Material:	[Carbonyl -C5-^{14}C] bixlozone
Lot/batch No.:	CFQ42018

Radiochemical Purity:	99.9% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 20.49 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.75 MBq/mg (certified)

Methods

Test system

A metabolism study on spring wheat (variety Certified WB Patron) grown outdoors in Madera, California, USA was carried out in 2014. Spring wheat plants were cultivated in containers (wooden boxes, each lined with a heavy gauge plastic liner, each 1.0 m^2) with sandy loam soil (soil column depth ~50 cm, pH in 1:1 soil: water ratio = 7.0, Percentage sand = 69%). No crops had been grown, or pesticides applied to the test system for 3 years before the trial. No radiolabelled material had ever been applied to the planting area prior to the trial. Weather data were reported for the trials and no exceptional events were noted.

Applications

One foliar spray application of test substance in EC formulation 'blank' of either phenyl or carbonyl labelled bixlozone (F9600) was made. Two test containers (one C-label one P-label) were treated with a single application at a target rate of 300 g a.s./ha per application at early-post emergence (BBCH 09-11, timings in line with the proposed GAP for post-emergence use on wheat and barley). Actual application rates are summarised in Table 7-10. A summary of the study design is given in Table 7-11. Comparisons to the GAP 'N' rates for the achieved application rates are provided in the table below, and were around 1.5N with regard to the wheat and barley GAP and around 0.8N with regard to the maize GAP. A further plot was used as a control plot and not treated. The control container was located more than 60 m from the treated plots to limit the risk of contamination. Plastic sheeting approximately two feet high was erected all around the plot to block the wind during each application.

Two further test containers were treated with a lower dose application rate of 100 g a.s./ha, however the low dose applications were not analysed and are not considered further in the evaluation write up.

Table 7-10 Summary of treatment rates of [^{14}C]bixlozone

Label	Target treatment rate (g a.s./ha)	Target radioactivity treated (µCi)	Determined radioactivity treated (µCi)	% of target	Application rate achieved (g as/ha)	Application rate achieved N rate compared to wheat/barley GAP	Application rate achieved N rate compared to maize GAP
Phenyl	300	2.362	2.418	102.4	307	1.53	0.82
Carbonyl	300	2.243	2.156	96.1	288	1.44	0.77

Table 7-11 Study design: plant uptake part (wheat)

Applications of radiolabelled bixlozone		
intended use rate [g a.s./ha]	300	
application number	1	
application growth stage	BBCH 09-11	
sampled matrices	forage, hay, grain, straw	
sampling [DALA] *1)*	forage	28
	hay	48
	grain	60
	straw	60

1) days after last application

Sampling

Samples of immature wheat forage were taken 28 days after the application (BBCH 39). Samples of hay were taken 48 days after the application (BBCH 73). Samples of grain and straw were collected ca. 60 days after the last

application (both BBCH 89). Approximately 15 percent of the crop was taken for the sample at initial sampling point as wheat forage sample and another 15 percent of the crop was taken at hay stage and the remaining crop was harvested at maturity. Forage samples were cut above the ground with pruning clippers. Hay samples were collected similarly by cutting at two inches above soil line. Wheat grain and straw were collected from the plots by removing heads from the plants, as per typical harvest practices. Grain was separated from the seed heads by use of threshing blocks. Seed and resulting chaff fell into collecting trays. Grain was separated from the chaff by using moving air to blow the lighter chaff, allowing the grain to drop into a separate collection. Samples were then stored in a freezer at ≤-15°C.

Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 6 months from RAC harvest (ca. 8.5 months for grain samples, since grain samples were first stored frozen for 12 days, then homogenised (and stored frozen for a further ca. two months), then extracted and then stored as extracts for around 6 months before analysis). The latest analyses of samples were conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Combustion analysis for the determination of the TRR in plant samples was performed using finely homogenized/ground crops (forage, hay, straw, and grains). Triplicate aliquots (ca. 150 mg) of each representative plant sample were used for combustion. The TRR was determined using biological sample oxidizer and measured evolved ^{14}Carbon dioxide ($^{14}CO_2$) by LSC.

Extraction

The following extraction procedure was carried out for wheat forage, hay, straw and gain samples. Wheat samples were first extracted by blending with acetonitrile/water (80:20). The mixture was centrifuged and filtered under vacuum to separate solids. The remaining solids were then re-blended with a solution of acetonitrile/water (80:20) and the process repeated twice more. The extraction procedure was then repeated three more times with methanol: water (50:50). All extracts were combined, assigned Ext-1 fraction, and assayed by LSC. Ext-1 fraction was concentrated and analysed by HPLC. A fraction of Ext-1 was also concentrated to dryness and reconstituted in aqueous solution (AQ-1), which was subjected to acid hydrolysis (1N HCl) under reflux. The mixture was cooled, neutralised and concentrated under nitrogen evaporation then reconstituted in methanol: water (1:1) before assay by HPLC/LC-MS/MS analysis. AQ-1 fractions from a mature hay sample were subjected to dichloromethane (DCM) partitioning for the separation of aglycons. The DCM and aqueous (AQ-2) fractions were separately concentrated, reconstituted in methanol: water and analysed by HPLC.

The remaining post-extraction solid samples (PES-1) from the initial solvent extraction were air-dried and combusted, followed by LSC. Subsamples of PES-1 were subjected to further processing by enzyme hydrolysis. Samples were individually suspended in 100mM sodium acetate buffer (pH ~5.0). Cellulase solution was prepared in 100 mM sodium acetate buffer. PES-1 fractions were then mixed with the cellulase solution and incubated for ~24 hours. After incubation the mixture was centrifuged. The supernatant was transferred to a centrifuge tube and aliquots were taken for LSC. The procedure was repeated on PES-1 subsamples to demonstrate sequential enzyme hydrolysis for amylase, pectinase, and protease enzymes. The solid residues (PES-2) were transferred into a container for further hydrolysis.

PES-2 samples were subjected to mild acid and then base hydrolysis (sequentially). The entire PES-2 fraction from each sample was suspended in 1 N HCl. The mixture was incubated at 37 degrees C ca. 24 hours and then centrifuged. A single aliquot of the aqueous fraction was taken for LSC. The solid fraction after acid hydrolysis was suspended in 1 N NaOH and the mixture stirred at ambient temperature for ca. 24 hours. The sample was then centrifuged, neutralized and the volume measured, a single aliquot of the aqueous fraction was taken for LSC.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, dimethyl malonic acid, 2,2-dimethyl-3-hydroxypropionic acid, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, bixlozone dimethyl malonamide, bixlozone-hydroxy-isobutyramide, 3'-hydroxy-bixlozone, 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report (tabular 'Summary of bixlozone reference standards assayed by LC/MS for wheat') is as follows: bixlozone (F9600),

2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), 5-hydroxy-bixlozone (M289/1), 5'-hydroxy-bixlozone (M289/3), 3'-hydroxy-bixlozone (M289/6), 6'-hydroxy-bixlozone).

Selected extracts and isolated materials were analysed by LC/RAM/ESI-MS[1] and MS/MS. TLC-Radiochromatography was used as the secondary method for the characterization of some of the radioactive components in the sample extract with known reference standards. Components of the residue were analysed by LC/MS. The proposed structures were supported by CID-MS[2] or HCD-MS[3]. All metabolites were further confirmed by comparison of the LC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards.

A list of metabolites confirmed by LC-MS/MS stated in the metabolism study report as 'Summary of metabolites of F9600 identified by LC-MS/MS' is as follows: 2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), 5'-hydroxy-bixlozone (M289/3), 4-hydroxymethyl-5'-hydroxyl-bixlozone (M305/1), and 5-hydroxy-5'-hydroxy-bixlozone (M305/2). These were metabolites identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

Storage stability

As stated above, samples were not all analysed within a period of 6 months since sampling. Samples were stored frozen whether as collected field samples, homogenised samples (powder) or as extracts. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses':

> Wheat grain analysed at 8.5 months (12 days as raw samples, then as a fine homogenised powder for a further ca. two months, and a further 6 months storage as extract).
>
> All other matrices (forage, hay straw) analysed within 6 months.

Frozen storage up to the time in which analysis on the matrices were completed in this study:

> All matrices (grain forage, hay straw) up to 3.4 years

Storage stability investigations

A comparison was made between chromatograms from the initial and subsequent analyses of the representative hay (same extract) and straw (re-extract and acid hydrolysis), with the extract (hay) or sample (straw) having been stored in the freezer in the in-between period. The hay extract was stored at -20ºC for 17.5 months prior to re-analysis. The comparative work done on the straw sample was done by having analysed an acid hydrolysed extract of straw 'initial sample' and then storing the sample (not as an extract) for a period of 20 months at -20ºC. An aliquot of the stored straw sample was then extracted, subject to acid hydrolysis and analysed for comparison of this to the 'initial'. See information provided below under '*Storage stability investigations- results'.*

Results and discussion

Total radioactive residue

The TRR with the Phenyl-label were highest in hay at 1.896 mg/kg, lower in straw at 1.399 mg/kg and forage at 0.969 mg/kg, and lowest in grain with 0.135 mg/kg. A similar distribution was seen with the Carbonyl-label (with some lower residues across some of the matrices (compared to the levels in the Phenyl-label) and the TRR in straw was similar to that in hay).

No total radioactive residues were found in plants grown in the control test material (≤ 0.001 mg/kg).

A summary of the TRRs in the treated plants is presented in Table 7-12.

[1] Liquid Chromatography/Radioactivity monitor/Electrospray Ionization with mass spectroscopy detection and tandem mass spectroscopy detection
[2] Collision Induced Dissociation with Mass Spectroscopy detection
[3] Higher-energy collisional dissociation with Mass Spectroscopy detection

Table 7-12 Total radioactive residue after foliar spray application of bixlozone

Matrix [BBCH]	DALA	TRR measured 1) *[mg parent eq./kg]*
Phenyl-label		
forage [39]	28	0.969
hay [73]	48	1.896
straw [89]	60	1.399
grain [89]	60	0.135
Carbonyl-label		
forage [39]	28	0.938
hay [73]	48	1.013
straw [89]	60	1.088
grain [89]	60	0.085

1) TRR measured directly via combustion LSC.

Extractability

The extractabilities of ^{14}C residues from wheat forage, hay, straw and grain are summarized in Table 7-13.

The wheat samples were extracted with a combination of acetonitrile/water and methanol/water solvent mixtures from all commodities. High extractability of ^{14}C residue was seen in forage (>94% TRR for total extract for both labels), straw and hay (>85% TRR for total extract for both labels in both commodities). The extractability from grain samples was lower, ca 55 to 60% of TRR, for the phenyl-label and the carbonyl-label respectively.

One representative hay sample extract from each label was also subjected to dichloromethane (DCM) partitioning to determine the presence of non-polar metabolites, ca. 7 to 10% of TRR was extracted in DCM. It was proposed by the applicant that the majority of the residues in the original hay extracts therefore, might be metabolites that are polar in nature, and possibly conjugates.

Table 7-13 Extractability of radioactive residue from wheat commodities. (mg/kg = mg parent eq./kg)

Commodity	Forage		Hay		Straw		Grain	
Residue Levels	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Phenyl-label								
TRR		0.969		1.896		1.399		0.135
Extracted residue (acetonitrile/ methanol/ water)	95.33	0.924	85.50	1.622	85.69	1.199	60.29	0.081
Extracted residue (DCM phase)	N/A	N/A	10.3	0.196	N/A	N/A	N/A	N/A
Extracted residue (Aqueous phase)	N/A	N/A	75.7	1.435	N/A	N/A	N/A	N/A
Non-extracted PES-1	4.67	0.045	14.04	0.275	14.31	0.200	39.71	0.054
Enzyme hydrolysis of PES-1 (done sequentially in the below order on PES1)								
Cellulase	0.96	0.009	2.43	0.046	2.95	0.041	3.47	0.005
Alpha amylase	0.95	0.009	5.46	0.104	2.53	0.035	6.82	0.009
Pectinase	-	-	2.50	0.048	1.25	0.018	1.39	0.002
Protease	-	-	1.55	0.029	0.91	0.013	5.19	0.007
Sequential acid then base hydrolysis of PES-2 (which remained after sequential enzyme hydrolysis)								
Acid (1N)	-	-	0.50	0.009	0.62	0.009	4.22	0.006

Base (1N)	-	-	3.58	0.067	3.32	0.046	6.70	0.009
Non-extracted PES (final)	2.44	0.024	0.22	0.004	2.60	0.036	6.61	0.009
Carbonyl-label								
TRR		0.938		1.013		1.088		0.085
Extracted residue (acetonitrile/ methanol/ water)	94.93	0.890	85.32	0.864	85.21	0.927	54.80	0.047
Extracted residue (DCM phase)	N/A	N/A	6.9	0.070	N/A	N/A	N/A	N/A
Extracted residue (Aqueous phase)	N/A	N/A	80.8	0.818	N/A	N/A	N/A	N/A
Non-extracted PES-1	5.07	0.048	14.68	0.149	14.79	0.161	45.20	0.038
Enzyme hydrolysis of PES-1 (done sequentially in the below order on PES1)								
Cellulase	0.98	0.009	1.55	0.016	2.41	0.023	9.94	0.008
Alpha amylase	0.92	0.009	4.70	0.043	2.61	0.029	9.78	0.008
Pectinase	-	-	2.42	0.025	1.67	0.018	6.15	0.006
Protease	-	-	1.69	0.017	2.05	0.022	3.19	0.003
Sequential acid then base hydrolysis of PES-2 (which remained after sequential enzyme hydrolysis)								
Acid (1N)	-	-	0.85	0.009	0.77	0.008	3.65	0.003
Base (1N)	-	-	-	-	5.46	0.059	-	-
Non-extracted PES (final)	2.62	0.025	4.29	0.043	3.15	0.034	7.95	0.007

N/A = extraction technique not performed for commodity
- = no TRR detected

Following the solvent extraction approaches, approximately 5% of radioactivity remained as unextracted residues from forage samples, while the PES-1 in hay and straw samples were determined to be ca. 15%. In grain samples ca. >40% of radioactivity remained as PES-1. Though hay and straw samples showed that the unextracted residues represented less than ca.15% of TRR, the actual concentrations were significant in terms of ppm values (> 0.050 mg/kg). These (PES-1) unextracted residues accounted for 0.275 mg/kg for hay and 0.200 mg/kg for straw (14.5% and 14.3% of TRR respectively) for the phenyl-label and 0.149 mg/kg for hay and 0.161 mg/kg for straw (14.7% and 14.8% of TRR respectively) for the carbonyl-label. PES-1 was further worked on and sequential enzyme treatments each released appreciable amounts of radioactivity. PES-2 was further worked on (where residues were high enough to conduct the further work) and acid (then base) treatments, also released measured amounts of radioactivity. Following the enzyme and acid/base hydrolysis, the final PES remaining was up to only 8% TRR or 0.00.04 mg/kg) showing a good degree of characterisation of the overall residues.

Characterisation and Identification

For all matrices similar results were observed with both radiolabels. No unchanged parent was detected for any matrix using either label. An overview over the components of the extracted residue is given below in Table 7-14 - Table 7-17. Metabolites found at >10% TRR are discussed in detail for each matrix below. Structures of the metabolites are outlined in the appendices to Volume 1.

Forage

The most significant metabolite identified after organic extraction with ACN:H_2O and MeOH:H_2O was the M289/3 conjugate for both labels, characterised based on the hydrolysis behaviour, with 38.4% TRR (0.372 mg/kg) and 40.5% TRR (0.380 mg/kg) for the phenyl-label and the carbonyl-label respectively. 2,4-dichlorobenzoic acid (M190/1) was identified at 10.8% TRR (0.104 mg/kg) in the phenyl-label organic extract. The total of all unknowns within the organic extracts contained 46.2% TRR (0.447 mg/kg) and 54.4% TRR (0.511 mg/kg) for the phenyl and carbonyl labels respectively. In the phenyl label 6 unknowns were present, the largest of which contained 13.0% TRR (0.126 mg/kg). For the carbonyl label 7 unidentified regions were present, the largest of which contained 16.8% TRR (0.158 mg/kg). After acid hydrolysis the majority of these unknown regions were not detected.

For the phenyl-label, following acid hydrolysis of the organic extracts, the radioactivity was identified as containing 20.7% TRR (0.201 mg/kg) of the metabolite M190/1 and four other metabolites, 5'-Hydroxy-bixlozone (M289/3) at

49.4% TRR (0.478 mg/kg), M305/1 at 12.3% TRR (0.119 mg/kg), M305/2 at 10.6% TRR (0.103 mg/kg, and a low level region (2.3% TRR, 0.023 mg/kg) which was characterised as 6'-hydroxy-bixlozone by retention time matching only. The applicant did not assign the metabolite an M code so HSE has referred to this characterisation as Met.1 throughout. No unknown regions remained after acid hydrolysis of the phenyl-label.

For the carbonyl-label, four metabolites were identified after acid hydrolysis. M289/3 was present at 47.0% TRR (0.441 mg/kg) and M118/1 was present at 18.6% TRR (0.174 mg/kg). Two other metabolites (M305/1 and Met 1.) were found at a maximum level of 7.4% TRR (0.07 mg/kg). A further 20.3% TRR (0.190 mg/kg) of unknown metabolites were detected, which includes four unidentified regions, the largest of which contained 7.81% TRR (0.073 mg/kg). In accordance with OECD 501, for such a level, characterisation/identification should be decided on a case by case basis taking account of how much has been identified. Also, forage is not consumed directly by humans.

In forage samples the PES after organic extraction represented <5% TRR for both label positions. Cellulase hydrolysis and alpha amylase hydrolysis each extracted ca. 1% TRR in the phenyl and the carbonyl labelled samples. After exhaustive extraction a total of 2.44% TRR (0.024 mg/kg) unextracted residue remained in the phenyl-label, whereas 2.62% TRR (0.025 mg/kg) unextracted residue remained in the carbonyl-label.

Hay

Similarly, to the forage samples, the most significant metabolite after organic extraction with ACN:H_2O and MeOH:H_2O was characterised, based on the hydrolysis behaviour, as the M289/3 conjugate for both labels, with 37.5% TRR (0.711 mg/kg) and 34.0% TRR (0.344 mg/kg) for the phenyl-label and the carbonyl-label respectively.

The total of all unknowns within the organic extracts was 40.3% TRR (0.764 mg/kg) and 51.3% TRR (0.52 mg/kg) for the phenyl and carbonyl labels respectively. In the phenyl label 5 unidentified regions were present, the largest of which contained 11.3% TRR (0.214 mg/kg). For the carbonyl label 6 unidentified regions were present, the largest of which contained 12.6% TRR (0.128 mg/kg). It is noted that after acid hydrolysis the majority of these unknown regions were not detected.

For the phenyl-label, the acid hydrolysis of the organic extracts contained five identified metabolites, as in the forage samples, the most significant of these was 5'-Hydroxy-bixlozone (M289/3) at 43.6% TRR (0.827 mg/kg). M190/1 was also identified at 18.4% TRR (0.349 mg/kg). Three other metabolites were identified at a maximum level of 8.2% TRR (0.156 mg/kg). One unidentified region containing 5.73% TRR (0.109 mg/kg) was detected in the acid hydrolysed extract.

For the carbonyl-label, the acid hydrolysis of the organic extracts contained six identified metabolites. M289/3 was present at 31.5% TRR (0.319 mg/kg) [and its conjugate present at 3% TRR (0.03 mg/kg] and M118/1 was present at 17.2% TRR (0.174 mg/kg). The other metabolites were found at a maximum level of 6.0% TRR (0.06 mg/kg). A further 20.4% TRR (0.207 mg/kg) of unknown metabolites were detected, which includes four unidentified regions, the largest of which contained 7.09% TRR (0.072 mg/kg). In accordance with OECD 501, for such a level, characterisation/identification should be decided on a case by case basis taking account of how much has been identified. Also, hay is not consumed directly by humans.

In hay samples the PES after organic extraction represented ~14% TRR for both label positions. Sequential enzymatic (various), acid and base extraction was observed with similar results (fairy low amounts of radioactivity released at each step, aside from alpha amylase) across both label positions, with the exception of base (1N) hydrolysis which extracted no measurable residues in the carbonyl label. Alpha amylase hydrolysis extracted the most significant amount of PES for both label positions (5.46% TRR, 0.104 mg/kg, in the phenyl-label and 4.30% TRR, 0.043 mg/kg, in the carbonyl-label). After exhaustive extraction a total of 0.22% TRR (0.004 mg/kg) unextracted residue remained in the phenyl-label, whereas 4.29% TRR (0.043 mg/kg) unextracted residue remained in the carbonyl-label.

Straw

The most significant metabolite present in both labelled samples after organic extraction with ACN:H_2O and MeOH:H_2O was the M289/3 conjugate which was detected at 39.2% TRR (0.549 mg/kg) in the phenyl label and at 36.5% TRR (0.397 mg/kg) in the carbonyl label.

The total of all unknowns within the organic extracts amounted to 38.7% TRR (0.541 mg/kg) and 48.7% TRR (0.530 mg/kg) for the phenyl and carbonyl labels respectively. In the phenyl label 6 unknown regions were present, the largest of which contained 12.7% TRR (0.177 mg/kg). For the carbonyl label 6 unknown regions were present, the largest of which contained 15.4% TRR (0.167 mg/kg). In accordance with OECD 501, regions at this level should be identified by all possible means, which has not been achieved. However, it is noted that the HPLC chromatograms shows that the largest unidentified region appears to consist of at least two different metabolites. Furthermore, the

majority of the unknown regions were not detected after acid hydrolysis (more metabolites were identified in the acid hydrolysed extracts).

Four metabolites were detected after acid hydrolysis of the organic extracts for the phenyl label. The most significant metabolite was M289/3 which was detected at 47.9% TRR (0.670 mg/kg), followed by M190/1 at 14.5% TRR (0.203 mg/kg) and M305/2 at 12.1% TRR (0.169 mg/kg). One other metabolite was found at a maximum of 4.6% TRR (0.064 mg/kg). One unknown metabolite was detected at 6.6% TRR (0.093 mg/kg).

For the carbonyl label, five metabolites were detected after acid hydrolysis of the organic extracts. M118/1 was identified at 22.4% TRR (0.244 mg/kg) and M289/3 at 30.5% TRR (0.332 mg/kg). Three other metabolites were identified at a maximum level of 9% TRR (0.098 mg/kg). A total of four unknown regions were detected, totalling 18.6% TRR (0.202 mg/kg), the largest region contained 8.3% TRR (0.09 mg/kg). In accordance with OECD 501, for such a level, characterisation/identification should be decided on a case by case basis taking account of how much has been identified. Also, straw is not consumed directly by humans.

The PES after organic extraction represented ~14% TRR for both label positions. Sequential enzymatic (various), acid and base extraction was observed with similar results (fairy low amounts of radioactivity released at each step). Base (1N) hydrolysis extracted the highest %TRR of the PES for both label positions (3.32% TRR, 0.046 mg/kg, in the phenyl-label and 5.46% TRR, 0.059 mg/kg, in the carbonyl-label). After exhaustive extraction a total of 2.60% TRR (0.036 mg/kg) unextracted residue remained in the phenyl-label, and 3.15% TRR (0.034 mg/kg) unextracted residue remained in the carbonyl-label.

Grain

In grain samples low levels of radioactivity were detected in comparison with the other plant commodities (forage, hay and straw). The majority of the extracted residues after organic solvent extraction with ACN:H_2O and MeOH:H_2O consisted of unknowns. In the phenyl label one metabolite (M190/1, 2,4-dichlorobenzoic acid) was identified at a maximum level of 8.6% TRR (0.012 mg/kg) and in the carbonyl label a total of three metabolites were identified at a maximum level of 6.9% TRR (0.006 mg/kg). Of these three, 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was present in the highest amount (and the other two metabolites were M289/3 and Met 1.).

The total of all unknowns in the organic solvent extract amounted to 47.0% TRR (0.065 mg/kg) in the phenyl-label and 45.4% TRR (0.039 mg/kg) in the carbonyl-label. In the phenyl-label 19 unidentified regions were detected, the largest of which contained 11% TRR (0.015 mg/kg). In the carbonyl-label 14 unidentified regions were detected, the largest of which contained 19% TRR (0.016 mg/kg). In accordance with OECD 501, significant attempts to identify the metabolites should be made when present at this level. Attempts to characterise these residues by LC-MS and comparison to the known reference standards were made. Also, a further understanding of the residues was gained by the acid hydrolysis work as explained below.

In the phenyl-label acid hydrolysis 25.5% TRR (0.034 mg/kg) was accounted for by the metabolite M190/1 (at a much higher level therefore than in the organic extract) and one other metabolite at a maximum level of 0.51% TRR (0.001 mg/kg). This left 32.2% TRR (0.044 mg/kg) as unknown metabolites, including 12 unidentified regions, the largest of which contained 7.5% TRR (0.010 mg/kg).

In the carbonyl-label acid hydrolysis of the organic extracts, the metabolite M118/1 was found at 44.4% TRR (0.038 mg/kg), much higher than the level of this metabolite found in the organic extract (unhydrolysed by acid). The acid hydrolysed extract also contained 10.3% TRR (0.009 mg/kg) unknowns, made up of 4 regions, the largest of which contained 3.5% TRR (0.003 mg/kg).

In the phenyl-labelled grain samples the initial PES after the solvent extraction contained 39.7% TRR (0.054 mg/kg). Following sequential enzyme (various) and then acid followed by base hydrolysis, alpha amylase hydrolysis and base (1N) hydrolysis extracted the most significant levels of PEC at 6.82% TRR (0.009 mg/kg) and 6.70% (0.009 mg/kg) TRR respectively. In the carbonyl-label samples 45.2% TRR was attributed to PES after the initial solvent extraction. The hydrolysis pattern was different to the phenyl-label with the most significant extract detected following cellulase hydrolysis and the alpha amylase hydrolysis at 9.9% TRR (0.008 mg/kg) and 9.8% (0.008 mg/kg) respectively, whilst no metabolites were detected upon base (1N) hydrolysis for the carbonyl label. Although the percentages of released radioactivity from various PES fractionations were low (less than 10% of TRR), the fractions were analysed further by HPLC however no meaningful metabolite identification data could be generated from such analysis.

In grain samples, each enzyme/acid or base hydrolysis step released relatively small amounts of TRR (less than 0.01 mg/kg) from the PES fractions (remaining after the initial organic extraction). The applicant suggested that unextracted radioactivity (that was released in each sequential extraction step a bit more each time, different enzymes followed by acid/base) might have undergone some natural incorporation, which is a plausible proposal.

After exhaustive extraction a total of 6.61% TRR (0.009 mg/kg) unextracted residue remained in the phenyl-label, and 7.95% TRR (0.007 mg/kg) unextracted residue remained in the carbonyl-label.

Table 7-14 Distribution of [^{14}C]bixlozone and its metabolites in wheat forage. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl label				Carbonyl label			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		Acid hydrolysis of organic extract		Organic extract *(ACN:H_2O and MeOH:H_2O)*		Acid hydrolysis of organic extract	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total TRR by combustion (mg/kg)	**0.969**				**0.938**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	-	-	18.578	0.174
2,4-Dichlorobenzoic Acid (M190/1)	10.768	0.104	20.709	0.201	-	-	-	-
4-OH-Me, 5'-OH-bixlozone (M305/1)	-	-	12.322	0.119	-	-	7.375	0.069
5-OH, 5'-OH-bixlozone (M305/2)	-	-	10.611	0.103	-	-	-	-
5'-Hydroxy-bixlozone (M289/3)	-	-	49.359	0.478	-	-	46.989	0.441
M289/3 conjugate	38.411	0.372	-	-	40.511	0.38	-	-
6'-Hydroxy-bixlozone (Met.1)	-	-	2.328	0.023	-	-	1.67	0.016
Total unknown	46.151[a]	0.447[a]	0	0	54.418[b]	0.511[b]	20.317[c]	0.190[c]
Total 'identified'	**49.179**	**0.476**	**95.33**	**0.924**	**40.511**	**0.38**	**74.612**	**0.700**
	Phenyl label				**Carbonyl label**			
	%TRR		**mg/kg**		**%TRR**		**mg/kg**	
Un-extracted residue after solvent extraction	4.67		0.045		5.07		0.048	
Sequential treatment as follow:								
Cellulase hydrolysis	0.96		0.009		0.98		0.009	
Alpha amylase hydrolysis	0.95		0.009		0.92		0.009	
Pectinase hydrolysis	-		-		-		-	
Protease hydrolysis	-		-		-		-	
Acid (1N) hydrolysis	-		-		-		-	
Base (1N) hydrolysis	-		-		-		-	
Un-extracted residue after exhaustive extraction	**2.44**		**0.024**		**2.62**		**0.025**	

' - ' considered but not detected (no associated radioactivity)

a - Includes 6 unidentified regions, the largest of which contained 13.0% TRR, 0.126 mg/kg

b - Includes 7 unidentified regions, the largest of which contained 16.8% TRR, 0.158 mg/kg
c – Includes 4 unidentified regions, the largest of which contained 7.81% TRR, 0.073 mg/kg

Table 7-15 Distribution of [^{14}C]bixlozone and its metabolites in wheat hay. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl label				Carbonyl label			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**1.896**				**1.013**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	-	-	17.174	0.174
2,4-Dichlorobenzoic Acid (M190/1)	7.865	0.149	18.421	0.349	-	-	-	-
M289/3 conjugate	37.301	0.707	-	-	33.985	0.344	2.996	0.03
4-OH-Me, 5'-OH-bixlozone (M305/1)	-	-	4.544	0.086	-	-	5.991	0.061
5-OH, 5'-OH-bixlozone (M305/2)	-	-	8.241	0.156	-	-	5.442	0.055
5'-Hydroxy-bixlozone (M289/3)	-	-	43.598	0.827	-	-	31.502	0.319
6'-Hydroxy-bixlozone (Met.1)	-	-	4.968	0.094	-	-	1.797	0.018
Unknown	40.335[a]	0.764[a]	5.726[b]	0.109[b]	51.334[c]	0.52[c]	20.418[d]	0.207[d]
Total 'identified'	**45.166**	**0.856**	**79.772**	**1.51**	**33.985**	**0.344**	**64.902**	**0.657**
	Phenyl label				**Carbonyl label**			
	%TRR		**mg/kg**		**%TRR**		**mg/kg**	
Un-extracted residue after solvent extraction	14.50		0.275		14.68		0.149	
Cellulase hydrolysis	2.43		0.046		1.55		0.016	
Alpha amylase hydrolysis	5.46		0.104		4.30		0.043	
Pectinase hydrolysis	2.50		0.048		2.42		0.025	
Protease hydrolysis	1.55		0.029		1.69		0.017	
Acid (1N) hydrolysis	0.50		0.009		0.85		0.009	
Base (1N) hydrolysis	3.58		0.067		-		-	
Un-extracted residue after exhaustive extraction	**0.22**		**0.004**		**4.29**		**0.043**	

' - ' considered but not detected (no associated radioactivity)

a – Includes 5 unidentified regions, the largest of which contained 11.3% TRR, 0.214 mg/kg

b – Includes 1 unidentified region containing 5.73% TRR, 0.109 mg/kg

c – Includes 6 unidentified regions, the largest of which contained 12.6% TRR, 0.128 mg/kg

d – Includes 4 unidentified regions, the largest of which contained 7.09% TRR, 0.072 mg/kg

Table 7-16 Distribution of [^{14}C]bixlozone and its metabolites in wheat straw. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl label				Carbonyl label			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**1.399**				**1.088**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	-	-	22.408	0.244
2,4-Dichlorobenzoic Acid (M190/1)	7.776	0.109	14.541	0.203	-	-	-	-
M289/3 conjugate	39.22	0.549	-	-	36.467	0.397	3.003	0.033
5-OH, 5'-OH-bixlozone (M305/2)	-	-	12.064	0.169	-	-	9.008	0.098
5'-Hydroxy-bixlozone (M289/3)	-	-	47.878	0.67	-	-	30.48	0.332
6'-Hydroxy-bixlozone (Met.1)	-	-	4.572	0.064	-	-	1.7	0.018
Unknown	38.694[a]	0.541[a]	6.636[b]	0.093[b]	48.742[c]	0.530[c]	18.611[d]	0.202[d]
Total 'identified'	**46.996**	**0.658**	**79.055**	**1.106**	**36.467**	**0.397**	**66.599**	**0.725**
	Phenyl label				**Carbonyl label**			
	%TRR		**mg/kg**		**%TRR**		**mg/kg**	
Un-extracted residue after solvent extraction	14.31		0.200		14.79		0.161	
Cellulase hydrolysis	2.95		0.041		2.10		0.023	
Alpha amylase hydrolysis	2.53		0.035		2.61		0.029	
Pectinase hydrolysis	1.25		0.018		1.67		0.018	
Protease hydrolysis	0.91		0.013		2.05		0.022	
Acid (1N) hydrolysis	0.62		0.009		0.77		0.008	
Base (1N) hydrolysis	3.32		0.046		5.46		0.059	
Un-extracted residue after exhaustive extraction	**2.60**		**0.036**		**3.15**		**0.034**	

' - ' considered but not detected (no associated radioactivity)

a – Includes 6 unidentified regions, the largest of which contained 12.7% TRR, 0.177 mg/kg

b - Includes 1 unidentified region containing 6.64% TRR, 0.093 mg/kg

c – Includes 6 unidentified regions, the largest of which contained 15.4% TRR, 0.167 mg/kg

d – Includes 4 unidentified regions, the largest of which contained 8.27% TRR, 0.090 mg/kg

Table 7-17 Distribution of [^{14}C]bixlozone and its metabolites in wheat grain . (mg/kg = mg parent eq./kg)

Metabolite	Phenyl label				Carbonyl label			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		Acid hydrolysis of organic extract		Organic extract *(ACN:H_2O and MeOH:H_2O)*		Acid hydrolysis of organic extract	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total TRR by combustion (mg/kg)	**0.135**				**0.085**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	6.902	0.006	44.438	0.038
2,4-Dichlorobenzoic Acid (M190/1)	8.621	0.012	25.503	0.034	-	-	-	-
5'-Hydroxy-bixlozone (M289/3)	-	-	0.51	0.001	1.803	0.002	-	-
6'-Hydroxy-bixlozone (Met.1)	-	-	-	-	0.684	0.001	-	-
Unknown	47.016[a]	0.065[a]	32.236[b]	0.044[b]	45.391[c]	0.039[c]	10.342[d]	0.009[d]
Total 'identified'	**8.621**	**0.012**	**26.013**	**0.035**	**9.389**	**0.009**	**44.438**	**0.038**
	Phenyl label				**Carbonyl label**			
	%TRR		**mg/kg**		**%TRR**		**mg/kg**	
Un-extracted residue after solvent extraction	39.71		0.054		45.20		0.038	
Cellulase hydrolysis	3.47		0.005		9.94		0.008	
Alpha amylase hydrolysis	6.82		0.009		9.78		0.008	
Pectinase hydrolysis	1.39		0.002		6.15		0.006	
Protease hydrolysis	5.19		0.007		3.19		0.003	
Acid (1N) hydrolysis	4.22		0.006		3.65		0.003	
Base (1N) hydrolysis	6.70		0.009		-		-	
Un-extracted residue after exhaustive extraction	**6.61**		**0.009**		**7.95**		**0.007**	

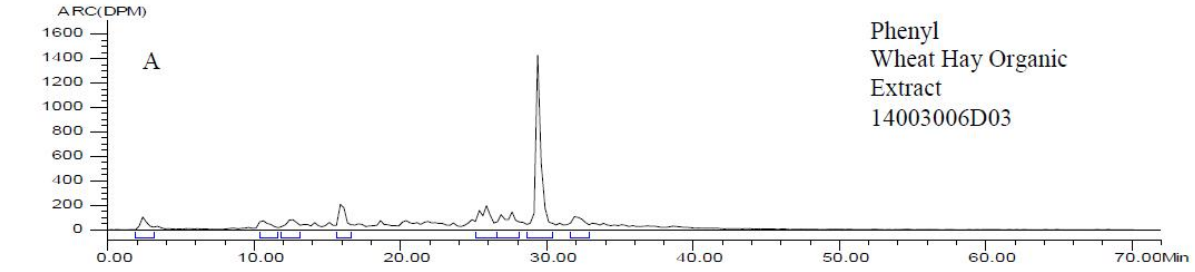
' - ' considered but not detected (no associated radioactivity)
a – Includes 19 unidentified regions, the largest of which contained 11.0% TRR, 0.015 mg/kg
b – Includes 12 unidentified regions, the largest of which contained 7.55% TRR, 0.010 mg/kg
c – Includes 14 unidentified regions, the largest of which contained 19.1% TRR, 0.016 mg/kg
d – Includes 4 unidentified regions, the largest of which contained 3.47% TRR, 0.003 mg/kg

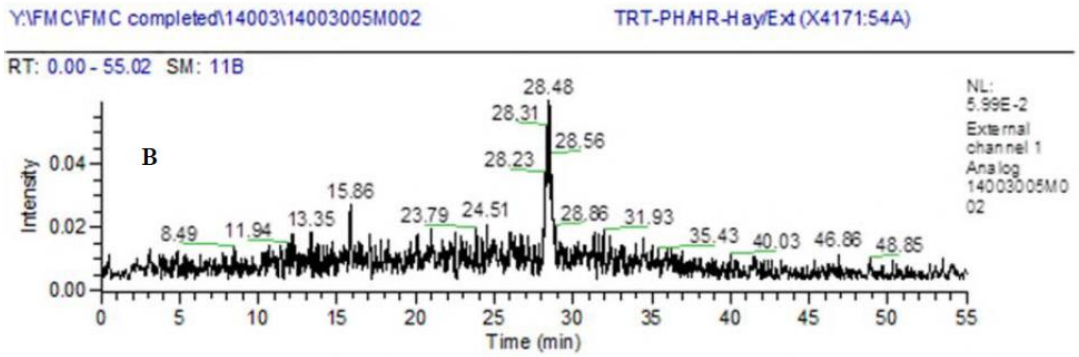
Storage stability investigations- results

The applicant considers that the data provided (some example chromatograms) lend support to the view that samples remained stable over frozen storage for a period of over a year. HSE considers that the information provided on whether samples/extracts are unchanged over a storage period in the context of the metabolism study is limited as explained below.

A comparison was made between chromatograms from the initial and subsequent analyses of the representative hay (same extract) and straw (re-extract and acid hydrolysis), with the extract (hay) or sample (straw) having been stored in the freezer in the in-between period. The hay extract was stored at -20ºC for 17.5 months prior to re-analysis. The comparative work done on the straw sample was done by having analysed an acid hydrolysed extract of straw 'initial sample' and then storing the sample (not as an extract) for a period of 20 months at -20ºC. An aliquot of the stored straw sample was then extracted, subject to acid hydrolysis and analysed for comparison of this to the 'initial'.

HSE finds it difficult to draw firm conclusions from the chromatography. The comparison for the hay extract is given below. The 'initial' chromatogram is at the top, and the 'after' chromatogram is at the bottom. The chromatogram is unlabelled (metabolite peaks are not labelled), and the y axis scale is different in the two chromatograms. It is assumed that as this extract is phenyl hay organic extract that the main peak is M289/3 Conjugate (see summary metabolism tables in Vol 1). Aside from saying that the main peak remained present in the 'after' compared to the 'initial' chromatogram, it is not possible to draw comparisons about possible changes to other peaks. The difference in the ratio of the main peak to the baseline variation could indicate that radioactive peak amounts have decreased. It is difficult to make any quantitative or qualitative conclusions (aside from the main peak remaining present).





The results for the straw are not presented here, although chromatograms were made available by the applicant (carbonyl straw extract after acid hydrolysis). Apart from the main peak (which might be M289/3, see metabolism summary tables in Vol 1), it was not possible to conclude that the chromatograms were similar.

In response to an HSE question regarding grain, the applicant referred to a comparison of phenyl labelled extracts in the wheat grain (primary crop samples-before and after acid hydrolysis) and compared these chromatograms to the rotational crop study wheat grain samples (phenyl label before and after acid hydrolysis) which seemed not to have been stored for so long (possibly around a month prior to analysis). However, although one main peak (labelled as M190/1, 2,4-dichlorobenzoic acid) was common to both sets of samples (primary crop wheat grain and rotational crop wheat grain), the chromatograms were again different quantitatively and qualitatively (in both the before and after acid hydrolysis sets, perhaps not unexpected as one set was from primary crop study and the other a rotational crop study, so the same extracts are not being compared 'before' and 'after' storage for a period).

It is difficult to draw firm conclusions from the chromatograms compared that suggest the stability of samples is sufficiently maintained over the long course of the study.

Translocation and proposed metabolic pathway

The unchanged parent bixlozone was not detected in any of the wheat commodities analysed showing that the compound is readily metabolised into various metabolite compounds. The total radioactive residue uptake and translocation into wheat grains amounted to only 0.135 mg/kg and 0.085 mg/kg for the phenyl and carbonyl labels respectively.

When the results from both labels are considered together the data demonstrate consistent metabolic pathways in wheat forage, hay, straw and grain (more limited information was available for grain based on the degree of identification being lower in grain). More information was gained about the metabolism following the analysis of the acid hydrolysed extracts (of the initial organic solvent extracts). The applicant's proposed metabolic pathway is outlined in Figure 7-4.

For grain, the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound, to form the metabolites 2,4-dichlorobenzoic acid (M190/1) and 2,2-dimethyl-3-hydroxy-propionic acid (M118/1). A significant level of the radioactivity remained initially unextracted (~40% in the phenyl label and ~45% in the carbonyl label) followed by iterative further release of small amounts of radioactivity following a stepwise sequential process of enzyme (cellulase, alpha amylase, pectinase, protease) and acid/base treatment. The applicant suggested that this might indicate possible natural incorporation into the grain matrix.

In forage, hay and straw the most significant metabolic conversion of the parent compound is by hydroxylation in the 5'-position of the dichloro-phenyl ring to form the metabolite 5'-OH-bixlozone (M289/3) and subsequent conjugation of this metabolite to form the 5'-OH-bixlozone conjugate (M289/3 conjugate). The metabolites formed upon oxidative ring opening of the isoxazolidin-3-one ring (M118/1 and M190/1) were also found, especially after acid hydrolysis of the organic extracts.

Conclusion

The metabolism of bixlozone was investigated in wheat by applying phenyl-labelled or carbonyl-labelled bixlozone. The overall residue levels (TRR) in the phenyl-labelled forage, hay, straw and grain were 0.97, 1.90, 1.40 and 0.14 mg/kg respectively. For carbonyl-labelled forage, hay, straw and grain the levels were 0.94, 1.01, 1.09 and 0.09 mg/kg.

For both labels, solvent extractability was high for forage, hay and straw (at least 85% TRR). Sequential enzyme hydrolysis of straw and hay released 7-12% of the TRR and acid hydrolysis of straw and hay released 0.8-6% of the TRR. In forage sequential enzyme hydrolysis released ~2% TRR.

For grain, solvent extraction retrieved 60% TRR and 55% TRR for the phenyl- and carbonyl-labels respectively. In the phenyl-label sequential enzyme hydrolysis steps released a further 17% TRR and then sequential acid and base hydrolysis released a further 11% TRR. In the carbonyl-label sequential enzyme hydrolysis released 29% TRR and then further sequential acid and base hydrolysis released a further 4% TRR. In grain the final unextracted residue was 6 to 8 %TRR (0.007 to 0.009 mg/kg).

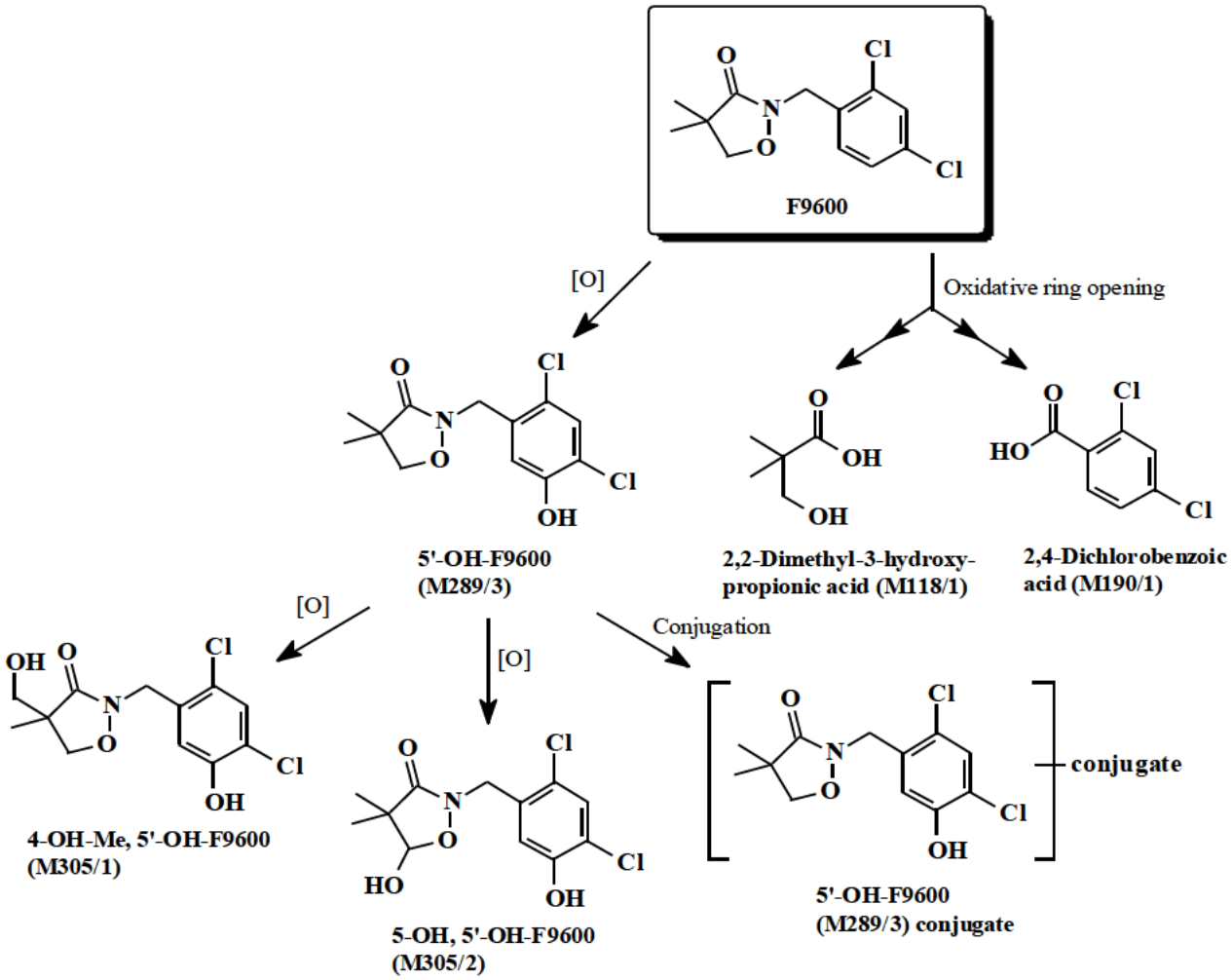
Metabolism of bixlozone includes primarily, the hydroxylation at the 5'-position and oxidative ring opening. For both labels, unchanged parent bixlozone was not detected in any of the commodities. In forage, hay and straw the metabolites M289/3 and its conjugate accounted for the highest proportion of the radioactive residue (accounting for ~31-49% TRR). In contrast, the metabolite M289/3 is only detected at very low levels in grain (~0.5-2% TRR), and the major metabolites detected were M190/1 and M118/1 (accounting for 25% and 44% TRR respectively after the acid hydrolysis of the organic solvent extract). Additional hydroxylation's at the 4 and 5 positions of the 5-membered ring, were also observed, with these metabolites (M305/1 and M305/2) individually being found at a maximum level of up to 12% TRR in straw, hay and forage. A high portion of the TRR was unidentified (<47% TRR). The majority of the unknown regions detected in the organic extracts were not detected after acid hydrolysis of the sample, perhaps indicating that these might be conjugates that were released via the acid treatment of the extract. The proportion remaining unidentified was far less in the acid hydrolysed extracts. The highest proportion of unidentified residues in the acid hydrolysed extracts was for wheat grain at 32.2%TRR, however this was a number of different unknown chromatographic regions (12).

Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in foliar applied wheat. Vol 1 also further considers the overview of the metabolism from the rice metabolism study, in consideration of cereals as a group.

Overall, this study has enabled metabolism in wheat to be reasonably well-elucidated (see the applicants proposed pathway in Figure 7-3). HSE has the following remarks/observations based on this wheat metabolism study.

- Especially for forage, hay and straw, some more metabolites were found/identified in the acid hydrolysed extract compared to the initial solvent extraction extract. The number of unknowns was markedly reduced in the acid hydrolysed fraction compared to the initial organic extract. It could be that the additional metabolites observed in the acid hydrolysate were conjugated in the organic extract and could only be 'seen' following the acid hydrolysis step. In grain metabolite M118/1 was shown to be present in a higher amount following the acid hydrolysis step. Due to the way in which (on working on post extraction solids) the sequential enzyme (various), acid/base treatments released further radioactivity (seen especially in the grain, but also hay and straw), the applicant suggested that the PES unextracted radioactivity might have undergone some natural incorporation, which is a plausible proposal.
- A number of successive extractions and treatments (including enzyme/acid/base) seemed to release radioactivity so individual fractions were well characterised. However, the success of identification of the large number of fractions was not very high (likely constrained by the level of radioactivity in each fraction). The highest number of reference standards was used for initial scoping e.g. where M132/1, dimethyl malonic acid, was included but it is not clear how comprehensively this reference standard was used to check residues in metabolite fractions, since this component (M132/1) was not designated as an identified residue, and this metabolite (and its identification) was not discussed in the wheat metabolism study report. Metabolite M132/1 was found in rice grain and straw (in broadly similar proportions to the finding of M118/1 in rice grain, please see the evaluation of the rice metabolism study at the end of this section (B.7.2.1)).
- Further data on the stability of incurred primary crop residues in the metabolism context is presented at the end of section B.7.2.1 and a discussion of storage stability of residues is presented in Vol 1, Section 2.7.2

Figure 7-3 Applicant's proposed metabolic pathway of bixlozone (F9600) in wheat



B.7.2.1.2. ***Canola/(Oilseed rape)***

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.2.1-02, Desai, M., 2019
Title:	Nature of the residue/metabolism of [^{14}C]F9600 in/on canola crop
Report No.:	14004-RPT04034 (Report amendment date: December 19, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 501 Metabolism in Crops, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996)
GLP	yes

Materials and methods

Materials

1. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42017
Radiochemical Purity:	99.6% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 21.57 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.89 MBq/mg (certified)

2. Test Material

Test Material:	[Carbonyl -C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42018
Radiochemical Purity:	99.9% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 20.49 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.75 MBq/mg (certified)

Methods

Test system

A metabolism study on canola (variety SCN900 canola seeds) grown outdoors in Seymour, Illinois, USA was carried out in 2014. Canola plants were cultivated simulating normal agricultural practices (as far as possible) in five containers (wooden boxes, each lined with a heavy gauge plastic liner, each 1.0 m^2) with clay loam soil (soil column depth ~50 cm, pH in 1:1 soil: water ratio = 6.3, Percentage sand = 20%). No pesticides had been applied to the test system for 3 years before the trial. No radiolabelled material had ever been applied to the planting area prior to the trial. Weather data were reported for the trials and no exceptional events were noted.

Applications

One foliar spray application of test substance in EC formulation 'blank' of either phenyl or carbonyl labelled bixlozone (F9600) was made. Two test containers (one C-label one P-label) were treated with a single application at a target rate of 300 g a.s./ha (1N for oilseed rape) per application at around 10% crop emergence from the soil (BBCH 09-11, timings in line with the proposed GAP). Actual application rates are summarised in Table 7-10. A summary of the study design is given in Table 7-10. Comparisons to the GAP 'N' rates for the achieved application rates are provided in the table below, and were around 0.95N with regard to the oilseed rape GAP. A further plot was used as a control plot and not treated. The treated plots were located more than 22 m downwind from the control plot to limit the risk of contamination. At application, each plot was also surrounded by a plastic barrier to prevent spray drift contamination.

Two further test containers were treated with a lower dose application rate of 100 g a.s./ha, however the low dose applications were not analysed and are not considered further in the evaluation write up.

Table 7-18 Summary of treatment rates of [^{14}C]bixlozone (canola)

Label	Target treatment rate (g a.s./ha)	Target radioactivity treated (µCi)	Determined radioactivity treated (µCi)	% of target	Application rate achieved (g as/ha)	Application rate achieved N rate compared to OSR GAP
Phenyl	300	2.362	2.258	95.6	287	0.96
Carbonyl	300	2.243	2.067	92.1	276	0.92

Table 7-19 Study design: plant uptake part (canola)

Applications of radiolabelled bixlozone		
Intended use rate [g a.s./ha]	300	
Application number	1	
Application growth stage	BBCH 09-11	
Sampled matrices	Immature forage, mature straw, seeds	
Sampling [DALA]	Immature forage	36
	Mature straw	70-71
	Seeds	70-71

Sampling

Samples of immature canola forage were taken 36 days after the application. Samples of straw were taken 70-71 days after the application. Samples of seeds were collected 70-71 days after the last application. To avoid seed loss in the field, plants were cut green and moved into a greenhouse to dry 58 days after application. Approximately 15 percent

of the crop was taken for the sample at initial sampling point as canola forage sample and the remaining crop was harvested at maturity. Forage samples were cut near the ground surface. After drying, pods were removed from the plants, crushed by hand and the seed was separated from the chaff using screen and vacuum separator. Straw samples were collected concurrently with the seed samples and were comprised of whole dried above ground portions of the plants with seed pods removed. Pods were returned to this sample after removal of the seed. Samples were then stored in a freezer at -23 to -20 °C.

Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 6 months from RAC harvest (ca. 8.5 months for straw extracts prior to initial analysis). The latest analyses of samples was conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Combustion analysis for the determination of the TRR in plant samples was performed on forage, straw and seed samples. Samples analysed to produce extraction data were either counted in single, duplicate (direct counting) or triplicate (combustion analysis). Samples analysed for metabolite profiling and identification were usually limited to single or duplicate analysis, depending on sample availability. The TRR was determined using biological sample oxidizer and measured evolved $^{14}CO_2$ by LSC.

Extraction

The following extraction procedure was carried out for canola forage, straw and seed samples (except for seed where no data were presented for acid hydrolysis of the organic solvent extract). Canola samples were first extracted by blending with acetonitrile/water (3x, 80:20). The mixture was centrifuged and filtered under vacuum to separate solids. The remaining solids were then re-blended with a solution of acetonitrile/water (80:20) and the process repeated four more times. The extraction procedure was then repeated three more times with methanol: water (3x, 50:50). All extracts were combined, assigned Ext-1 fraction, and assayed by LSC. Ext-1 fraction was concentrated and analysed by HPLC. A fraction of Ext-1 was also concentrated to dryness and reconstituted in aqueous solution (AQ-1), which was subjected to acid hydrolysis (1N HCl) under reflux. The mixture was cooled, neutralised and concentrated under nitrogen evaporation then reconstituted in methanol: water (1:1) before assay by HPLC/LC-MS/MS analysis. PES samples were not subjected to further processing and analysis.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, dimethyl malonic acid, 2,2-dimethyl-3-hydroxypropionic acid, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, bixlozone dimethyl malonamide, bixlozone-hydroxy-isobutyramide, 3'-hydroxy-bixlozone, 'bixlozone ring open acid', 2-4-dichlorobenzyl alcohol, 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report ('Summary of F9600 reference standards assayed by LC/MS') is as follows: bixlozone (F9600), 2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), F9600-hydroxy-isobutyramide (M261/1), and bixlozone dimethyl malonamide (M289/2).

The proposed structures were supported by SID[4]-MS analysis. All metabolites were further confirmed by comparison of LC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards. In addition to LC/MS analysis, metabolites in straw and seed of canola crop treated with phenyl labelled bixlozone were characterised by TLC in comparison to reference standards.

A list of metabolites confirmed by LC-MS/MS stated in the metabolism study report ('Summary of metabolites of F9600 identified by LC-MS/MS') is as follows: 2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), F9600-hydroxy-isobutyramide (M261/1), bixlozone dimethyl malonamide (M289/2), and bixlozone dimethyl malonamide methyl ester (M303/1). These were metabolites identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those

[4] SID- surface induced dissociation

characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

Storage stability

As stated above, samples were not all analysed within a period of 6 months since sampling. Samples were stored frozen whether as collected field samples, homogenised samples (powder) or as extracts. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses':

Canola straw was analysed after frozen storage for 8.5 months. The plant material was stored in different forms over this period, for straw - stored frozen as raw agricultural commodity for 16 days, as homogenised powder for a further three days, and the remainder of time stored as extract.

All other matrices (forage, seed) analysed within 6 months.

Frozen storage up to the time in which analysis on the matrices were completed in this study:

All matrices (straw, forage and seed) up to 2.7 years

Storage stability investigations

No storage stability comparisons were made for canola within this metabolism study (e.g. chromatogram comparisons for extracts analysed initially and later on in the study). In a letter response to HSE, the applicant stated the following "*the straw samples were stored frozen for 257 days, however, the forage samples were assayed within the confines of the OECD guidelines for sample storage (with ca. 3 months or 96 days), and the metabolites radioactive-profile of both the straw (stored frozen for 257 days) and the forage samples (with ca. 3 months or 96 days), were similar in nature, indicating the stability of F9600 residues in canola straw and forage.*" It is noted that the metabolism summary table (in Vol 1) does show some similarities/some differences in metabolic findings and levels of metabolites for canola straw and forage.

Results and discussion

Total radioactive residue

The TRR with the phenyl-label were highest in straw at 0.058 mg/kg. The TRR in forage and seeds were both relatively low with 0.017 mg/kg and 0.015 mg/kg respectively. A similar distribution was seen with the carbonyl-label. The TRR in seed for the carbonyl label was lower than observed in the Phenyl-label with a TRR of 0.009 mg/kg.

No total radioactive residues were found in plants grown in the control test material (≤ 0.001 mg/kg).

A summary of the TRRs in the treated plants is presented in Table 7-20.

Table 7-20 Total radioactive residue after foliar spray application of bixlozone in canola

Matrix	**DALA**	**TRR measured** [1)] *[mg parent eq./kg]*
Phenyl-label		
forage	36	0.017
straw	70-71	0.058
seeds	70-71	0.015
Carbonyl-label		
forage	36	0.026
straw	70-71	0.074
seeds	70-71	0.009

1) TRR measured directly via combustion LSC.

Extractability

The extractabilities of ^{14}C residues from canola forage, straw and seeds are summarized in Table 7-21.

The canola samples were extracted with a combination of acetonitrile/water and methanol/water solvent mixtures from all commodities. High extractability of ^{14}C residue was seen in forage and straw (both commodities >90% TRR for total extract for both labels). The extractability from grain samples was lower, ca 65% and 42% of TRR, for the phenyl-label and the carbonyl-label respectively.

Table 7-21 Extractability of radioactive residue from canola commodities. (mg/kg = mg parent eq./kg)

Commodity	TRR (mg/kg)	Organic/ aqueous extract *(MeOH:H_2O)*		PES	
		% TRR	mg/kg	% TRR	mg/kg
Phenyl-label					
Forage	0.017	90.9	0.015	9.1	0.002
Straw	0.058	92.3	0.054	7.7	0.004
Seeds	0.015	64.9	0.010	35.1	0.005
Carbonyl-label					
Forage	0.026	90.1	0.023	9.9	0.003
Straw	0.074	90.9	0.067	9.1	0.007
Seeds	0.009	41.9	0.004	58.2	0.005

Less than 10% of radioactivity remained as unextracted residues from forage and straw samples. In seed samples ca. 35% and ca. 59% of radioactivity remained as PES in the phenyl- and carbonyl-labels respectively. In all commodities the unextracted residues in terms of actual concentrations were low (<0.007 mg/kg) and subsequently the PES was not subjected to further processing or analysis.

Characterisation and Identification

For all matrices similar results were observed with both radiolabels. No unchanged parent was detected for any matrix using either label. An overview over the components of the extracted residue is given below in Table 7-22 - Table 7-24. Metabolites found at >10% TRR are discussed in detail for each matrix below. Structures of the metabolites are outlined in the appendices to Volume 1.

Forage

No parent bixlozone was found in forage in any of the extracts.

The majority of the TRR after organic extraction with ACN:H_2O and MeOH:H_2O consisted of unknown metabolites for both labels, totalling 81.4% TRR (0.014 mg/kg) and 84.6% TRR (0.021 mg/kg) for the phenyl-label and the carbonyl-label respectively. In the phenyl label 12 unknown regions were present, the largest of which contained 13.4% TRR (0.002 mg/kg). For the carbonyl label 14 unidentified regions were present, the largest of which contained 13.1% TRR (0.003 mg/kg). It is noted that the majority of the unknown regions that were detected in the organic extracts were not detected, or were reduced, after the acid hydrolysis. The metabolite, bixlozone-hydroxy-isobutyramide (M261/1) was the only identified metabolite in the organic extract (prior to acid hydrolysis) and was present in both labels at a maximum level of 7.4% TRR (0.001 mg/kg).

For the phenyl-label, the acid hydrolysis of the organic extracts contained five identified metabolites (M289/4 and M303/1 are regarded as tentatively identified). M261/1 was found in the highest amount at 14.8% TRR (0.002 mg/kg) and a metabolite region at 10.9% TRR (0.002 mg/kg) was postulated (TLC analysis) as M289/4. Three other metabolites were identified at a maximum level of 5.7% TRR (0.001 mg/kg). A large amount of radioactivity (46.0% TRR (0.007 mg/kg)) represented unknown regions, containing 17 unidentified regions, the largest of which contained 5.1% TRR (0.001 mg/kg).

For the carbonyl-label, 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was identified at 14.3% TRR (0.004 mg/kg) after acid hydrolysis. A further three metabolites were found at a maximum level of 6.8% TRR (0.002 mg/kg). As per the phenyl label, a large amount of radioactivity (55.1% TRR (0.015 mg/kg)) represented unknown regions, containing 16 unidentified regions, the largest of which contained 11.3% TRR (0.003 mg/kg).

In forage samples the PES following organic extraction represented <10% TRR (<0.003 mg/kg) for both label positions therefore further extraction and characterisation was not carried out.

Straw

No parent bixlozone was found in straw in any of the extracts.

As seen in the forage samples, the majority of the TRR in both labelled samples after organic extraction with ACN:H_2O and MeOH:H_2O was made up of unknowns. The total of all unknowns within the organic extracts contained 70.6% TRR (0.041 mg/kg) and 78.5% TRR (0.0059 mg/kg) for the phenyl and carbonyl labels respectively. In the phenyl label 10 unknown regions were present, the largest of which contained 11.8% TRR (0.007 mg/kg). For the carbonyl label 14 unidentified regions were present, the largest of which contained 9.2% TRR (0.007 mg/kg). It is noted that the majority of the unknown regions detected in the organic extracts were not detected, or were reduced, after the acid hydrolysis.

Some metabolites were present in the organic extract prior to acid hydrolysis but as more were found in the acid hydrolysate, the below paras focus on the metabolites present following acid hydrolysis.

Five metabolites were detected after acid hydrolysis of the organic extracts for the phenyl label. The most significant metabolite was M261/1 which was detected at 15.0% TRR (0.009 mg/kg), followed by a metabolite region at 14.7% TRR (0.009 mg/kg) which was postulated (following TLC analysis) to be M289/4. M303/1 (tentatively identified by LC-MS) at 11.4% TRR (0.007mg/kg) and M190/1 which was identified at 10.7% TRR (0.006 mg/kg). One other metabolite was found at a maximum of 7.2% TRR (0.004 mg/kg). A large amount of radioactivity (33.2% TRR (0.02 mg/kg)) represented unknown regions, containing 9 unidentified regions, the largest of which contained 6.9% TRR (0.004 mg/kg).

For the carbonyl label, four metabolites were detected after acid hydrolysis of the organic extracts. M118/1 was identified at 30.6% TRR (0.023 mg/kg). Three other metabolites were identified at a maximum level of 6.8% TRR (0.005 mg/kg). A large amount of radioactivity (41.8% TRR (0.031 mg/kg) represented unknown regions, containing 6 unidentified regions, the largest of which contained 23.5% TRR (0.017 mg/kg). In accordance with OECD 501, significant attempts to identify metabolites at this level should have been made ("Significant attempts to identify should be made especially if needed to establish a pathway, ultimately characterisation might be accepted." Canola straw is not feed commodity in the UK (OECD Guidance Document on Residues in Livestock, No. 73), and canola straw is not directly consumed by humans.

The PES represented up to 9% TRR (<0.004 mg/kg) for both label positions therefore further extraction and characterisation was not carried out.

Seeds

No parent bixlozone was found in grain in the extracts (following organic solvent extraction).

In seed samples low levels of radioactivity were detected in comparison with the other plant commodities (forage and straw). For the carbonyl label the total TRR was <0.01 mg/kg and therefore no differentiation of the radioactivity is required in accordance with OECD 501. Further characterisation by HPLC was not carried out for this sample. In the phenyl label, the residues were further analysed (the TRR was 0.015 mg/kg). The most significant metabolite identified after organic extraction with ACN:H_2O and MeOH:H_2O was 2,4-dichlorobenzoic acid (M190/1) at 34.7% TRR (0.005 mg/kg). M289/2 was identified at low levels (4.0% TRR, 0.001 mg/kg).

The total of all unknowns was represented by 26.4% TRR (0.065 mg/kg) in the phenyl-label and contained 10 unidentified regions, the largest of which contained 6.1% TRR (0.001 mg/kg).

For seed, no data were presented for acid hydrolysis of the organic solvent extract.

The remaining PES (after organic solvent extraction) amounted to 35.1% TRR for the phenyl label and 58.2%TRR for the carbonyl label (both representing 0.005 mg/kg).

Based on the very low levels of the unidentified regions in seed suitable characterisation has been achieved in seed in accordance with OECD 501.

Table 7-22 Distribution of [^{14}C]bixlozone and its metabolites in canola forage. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.017**				**0.026**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	-	-	14.3	0.004
2,4-Dichlorobenzoic Acid (M190/1)	-	-	5.4	0.001	-	-	-	-
Dimethyl-malonamide-bixlozone (M289/2)	-	-	4.1	0.001	-	-	6.7	0.002
bixlozone-hydroxy-Isobutyramide (M261/1)	7.4	0.001	14.8	0.002	5.40	0.001	6.8	0.002
4-Hydroxy-Methyl-bixlozone (M289/4)	-	-	10.9	0.002	-	-	6.2	0.002
bixlozone-dimethyl-malonamide methyl ester (M303/1)	-	-	5.7	0.001	-	-	-	-
Unknown	81.4[a]	0.013[a]	46.0[b]	0.007[b]	84.6[c]	0.021[c]	55.1[d]	0.015[d]
Unextracted residue after solvent extraction								
PES	9.1	0.002	9.1	0.002	9.9	0.003	9.9	0.003
Total 'identified'	**7.4**	**0.001**	**40.9**	**0.007**	**5.40**	**0.001**	**34.0**	**0.01**

' - ' considered but not detected (no associated radioactivity)
a - Includes 12 unidentified regions, the largest of which contained 13.4% TRR, 0.002 mg/kg
b – Includes 17 unidentified regions, the largest of which contained 5.1% TRR, 0.001 mg/kg
c – Includes 14 unidentified regions, the largest of which contained 13.1% TRR, 0.003 mg/kg
d – Includes 16 unidentified regions, the largest of which contained 11.3% TRR, 0.003 mg/kg

Table 7-23 Distribution of [^{14}C]bixlozone and its metabolites in canola straw. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.058**				**0.074**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	-	-	30.6	0.023
2,4-Dichlorobenzoic Acid (M190/1)	-	-	10.7	0.006	-	-	-	-
Dimethyl-malonamide-bixlozone (M289/2)	7.4	0.004	7.2	0.004	7.5	0.006	6.2	0.005
bixlozone-hydroxy-Isobutyramide (M261/1)	6.8	0.004	15.0	0.009	5.0	0.004	6.8	0.005
4-Hydroxy-Methyl-bixlozone (M289/4)	4.9	0.003	14.7	0.009	-	-	3.8	0.003
bixlozone-dimethyl-malonamide methyl ester (M303/1)	-	-	11.4	0.007	-	-	-	-
Unknown	70.6[a]	0.041[a]	30.4[b]	0.02[b]	78.5[c]	0.059[c]	41.8[d]	0.031[d]
Unextracted residue after solvent extraction								
PES	7.7	0.004	7.7	0.004	9.1	0.003	9.1	0.003
Total 'identified'	**19.1**	**0.011**	**59.0**	**0.035**	**12.5**	**0.010**	**47.4**	**0.036**

' - ' considered but not detected (no associated radioactivity)
a - Includes 10 unidentified regions, the largest of which contained 11.8% TRR, 0.007 mg/kg
b – Includes 10 unidentified regions, the largest of which contained 6.9% TRR, 0.004 mg/kg
c – Includes 14 unidentified regions, the largest of which contained 9.2% TRR, 0.007 mg/kg
d – Includes 6 unidentified regions, the largest of which contained 23.5% TRR, 0.017 mg/kg

Table 7-24 Distribution of [^{14}C]bixlozone and its metabolites in canola seeds. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl	
	Organic Extract	
	%TRR	mg/kg
Total TRR by combustion (mg/kg)	**0.015**	
bixlozone	-	-
2,4-Dichlorobenzoic Acid (M190/1)	34.7	0.005
Dimethyl-malonamide-bixlozone (M289/2)	4.0	0.001
Unknown	26.4[a]	0.003[a]
Unextracted residue after solvent extraction		
PES	35.1	0.005
Total 'identified'	**38.7**	**0.006**

' - ' considered but not detected (no associated radioactivity)
a - Includes 10 unidentified regions, the largest of which contained 6.1% TRR, 0.001 mg/kg

Storage stability investigations- results

No storage stability comparisons were made for canola in this metabolism study (chromatogram comparisons for extracts analysed initially and later on in the study). The applicant stated the following "*the straw samples were stored frozen for 257 days, however, the forage samples were assayed within the confines of the OECD guidelines for sample storage (with ca. 3 months or 96 days), and the metabolites radioactive-profile of both the straw (stored frozen for 257 days) and the forage samples (with ca. 3 months or 96 days), were similar in nature, indicating the stability of F9600 residues in canola straw and forage.*" It is noted that the metabolism summary table (in Vol 1) does show some similarities/some differences in metabolic findings and levels of metabolites for canola straw and forage.

Translocation and proposed metabolic pathway

The unchanged parent bixlozone was not detected in any of the canola matrices analysed showing that the compound is readily metabolised. The total radioactive residue uptake and translocation into canola seeds amounted to only 0.015 mg/kg and 0.009 mg/kg for the phenyl and carbonyl labels respectively. The TRR level of residues in the forage and the straw were also fairly low (0.074 mg/kg was the highest TRR – straw).

Most information on identified metabolites was obtained from analysis of acid hydrolysed material (following the initial solvent extraction), as this tended to enable more metabolites to be identified. The results for the seed (on acid hydrolysis of the organic solvent extract) were not available. The work was affected by the low levels present, and many unknown metabolites were not identified.

For seeds, the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound, to form the metabolite 2,4-dichlorobenzoic acid (M190/1). A significant level of the radioactivity remained unextracted (~40% in the phenyl label and ~45% in the carbonyl label) suggesting a possibly high level of natural incorporation into seed matrix, however as the residues were low further efforts to release and further characterise radioactivity (to support such a proposition) were not made.

In forage and straw, M118/1 was found in significant amounts (also indicative of oxidative ring opening of the isoxazolidin-3-one ring), and metabolites M261/1, M289/2, M289/4 and M303/1 were found. In forage and straw, a route of metabolic conversion of the parent compound is by reduction of the dimethyl-oxazolidone, followed by an oxidation, decarboxylation and hydroxylation step to form the metabolite bixlozone-hydroxy-isobutyramide (M261/1). The acid hydrolysis identified a number of metabolites, suggesting possible conjugation of metabolites within forage and straw. Further metabolic pathways following acid hydrolysis included oxidative ring opening, identified in the carbonyl-label, and oxidation of the dimethyl-oxazolidone ring.

Based on the information on identified metabolites (both labels) a metabolic pathway was proposed by the applicant is outlined in Figure 7-4.

Conclusion

The metabolism of bixlozone was investigated in canola by applying phenyl-labelled or carbonyl-labelled bixlozone. The TRR in the phenyl-labelled forage, straw and seeds were 0.017, 0.058 and 0.015 mg/kg respectively. For carbonyl-labelled forage, straw and seeds the levels were 0.026, 0.074 and 0.009 mg/kg. Hence, TRR for forage, straw and seeds were broadly similar cross both labels.

For both labels, solvent extractability was high for forage and straw (at least 90% TRR) For seeds, solvent extraction retrieved 65% TRR and 42% TRR for the phenyl- and carbonyl-labels respectively. In grain the final unextracted residue was 35.1 %TRR (0.005 mg/kg) for the phenyl-label and 58.2% TRR (0.005 mg/kg) for the carbonyl-label. The actual concentrations of the PES were low and therefore no further extraction techniques were investigated.

Due to the overall only low (seed and forage) to moderate (straw) levels of radioactivity as mg/kg amounts, and relatively large number of unknown fractions (albeit with individual components being present at low levels), the metabolic pathway is based on only a relatively small proportion of overall identified metabolites. Most information on the known metabolites comes from the elucidation of metabolites in the acid hydrolysed extracts of forage and straw. It was suggested that some metabolites (in the organic extract) prior to acid hydrolysis might be present as conjugates.

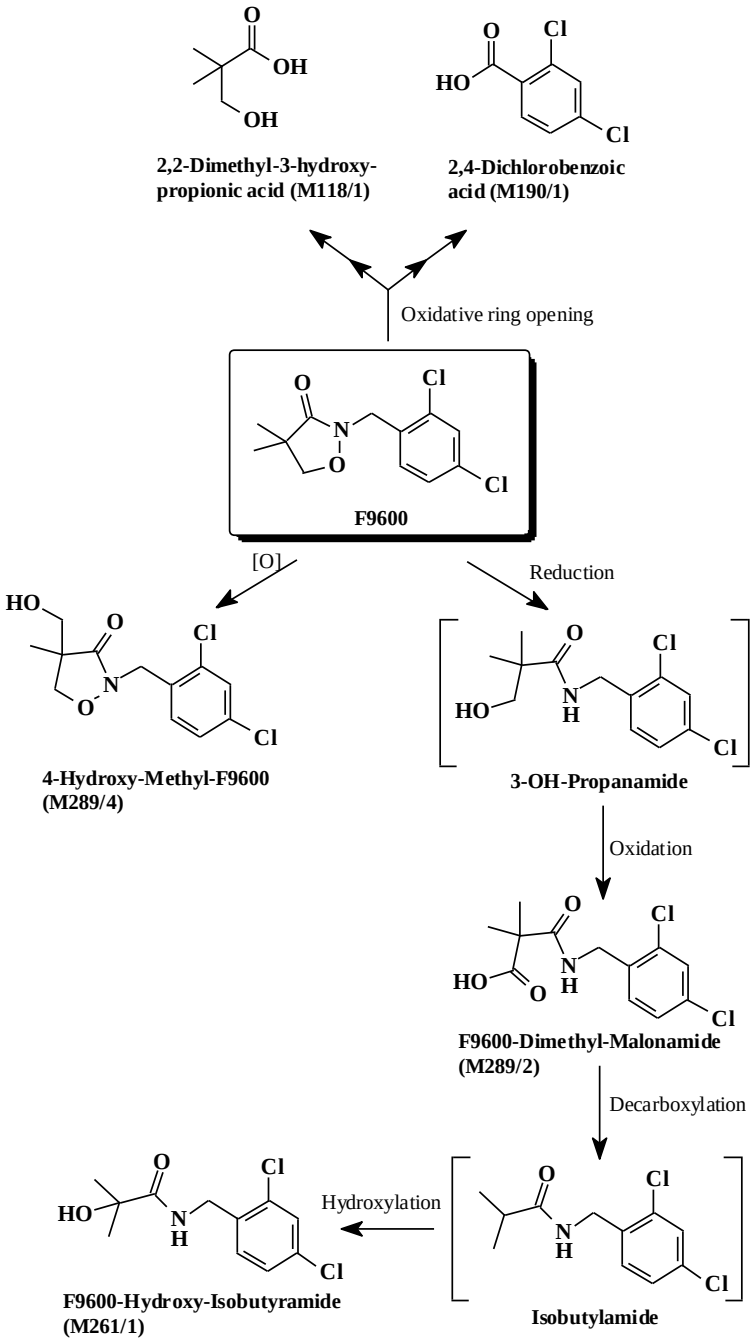
Metabolism of bixlozone in canola includes primarily the reduction and subsequent oxidation, decarboxylation and hydroxylation of the dimethyl-oxazolidone ring and oxidative ring opening. For both labels, unchanged parent bixlozone was not detected in any of the commodities. In forage and straw, the metabolites M261/1 and M118/1 accounted for the highest proportion of the radioactive residue in the phenyl-label and carbonyl-label respectively (M118 up to 31%TRR and 0.023 mg/kg in straw and M261 up to 15%TRR and 0.009 mg/kg in straw).

In contrast, these metabolites were not detected in seeds, and the major metabolite in seeds detected was M190/1 (accounting for 34.7% TRR, 0.005 mg/kg). In seeds, a high portion of the TRR was unextracted (35%TRR) and 26% TRR was extracted but unidentified (with very low levels for any individual unidentified regions).

Overall, metabolism of bixlozone in canola, has been adequately studied, however the degree of identification of residues is not as high in this study (due to low residue levels) compared to other crop metabolism studies (such as wheat metabolism). The elucidated metabolism for canola (oilseeds) is outlined in the applicant's metabolic pathway (see the applicants proposed pathway in Figure 7-4). Similar pathways/routes of metabolism have been observed in the other plant metabolism studies. HSE has the following remarks/observations based on this canola metabolism study:

- The overall levels of radioactivity were lower in this study compared to the wheat metabolism (section B.7.2.1.1). Both the wheat metabolism study and the canola (oilseed rape) metabolism study contained a relatively large number of unknown metabolic fractions. It seems that the relatively low TRR in the canola (oilseed rape) metabolism study (up to 0.07 mg/kg in straw) constrained the extent to which the metabolic pathway in canola could be more fully elucidated. The differences in the proposed metabolic pathways therefore (wheat and canola) might be reflective of the different degrees of identification rather than there being significant differences in the metabolic profile.
- For forage and straw, more metabolites were found/identified in the acid hydrolysed extract compared to the initial solvent extraction extract. The %TRR radioactivity of unknowns was reduced in the acid hydrolysed fraction compared to the initial organic extract, although the number of unknown regions (in the acid hydrolysed material) was still relatively large (but these individually tended to represent low (<0.01 mg/kg) levels of radioactivity). It could be that the additional metabolites observed in the acid hydrolysate were conjugated in the organic extract and could only be 'seen' following the acid hydrolysis step. Seed results for acid hydrolysis step were not presented.
- The highest number of reference standards was used for initial scoping e.g. where M132/1, dimethyl malonic acid was included. This component (M132/1) was only noted in the metabolism study report text that dimethyl malonic acid had been identified in [14C-Carbonyl]bixlozone label samples (it was not reported in tables of results). M132/1 was found in rice grain in the rice metabolism study (it was also found in sugar beet metabolism) alongside M118/1 and M190/1. The amounts of any individual component metabolite residues in canola seed (and in canola forage and straw) are low.
- Further data on the stability of incurred primary crop residues in the metabolism context is presented at the end of section B.7.2.1 and a discussion of storage stability of residues is presented in Vol 1, Section 2.7.2.

Figure 7-4 Applicant's proposed metabolic pathway for bixlozone (F9600) in canola



B.7.2.1.3. *Sugar beet*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.2.1-03, Desai, M., 2019
Title:	Nature of the residue: metabolism of [^{14}C]F9600 in/on sugar beet crop
Report No.:	14005-RPT03978 (Report amendment date: December 19, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 501 Metabolism in Crops, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996)
GLP	yes

Materials and methods

Materials

1. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42017
Radiochemical Purity:	99.6% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 21.57 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.89 MBq/mg (certified)

2. Test Material

Test Material:	[Carbonyl -C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42018
Radiochemical Purity:	99.9% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 20.49 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.75 MBq/mg (certified)

Methods

Test system

A metabolism study on sugar beet (variety Eden Brothers sugar beet) grown outdoors in Madera, California, USA was carried out in 2014. Sugar beet plants were cultivated simulating normal agricultural practices (as far as possible) in five containers (wooden boxes, each lined with a heavy gauge plastic liner, each 1.0 m^2) with sandy loam soil (soil column depth ~50 cm, pH in 1:1 soil: water ratio = 7.0, Percentage sand = 69%). No crops had been grown or pesticides applied to the test system for 3 years before the trial. No radiolabelled material had ever been applied to the planting area prior to the trial. Weather data were reported for the trials and no exceptional events were noted.

Applications

One foliar spray application of test substance in EC formulation 'blank' of either phenyl or carbonyl labelled bixlozone (F9600) was made. Two test containers (one C-label one P-label) were treated with a single application at a target rate of 300 g a.s./ha per application at around 10% crop emergence from the soil (BBCH 09-11). A further plot was used as a control plot and not treated. The control plot was located more than 60 m from the treated plots to limit the risk of contamination. At application, the treated plots were also surrounded by a plastic barrier to prevent spray drift contamination.

Two further test containers were treated with a lower dose application rate of 100 g a.s./ha, however the low dose applications were not analysed and are not considered further in the evaluation write up.

Actual application rates were 298 g a.s./ha (phenyl label) and 293 g a.s./ha (carbonyl label). A summary of the study design is given in Table 7-25.

Table 7-25 Study design: plant uptake part (sugar beet)

Applications of radiolabelled bixlozone		
Intended use rate [g a.s./ha]	300 (actual rates achieved were 298 and 293 g as/ha)	
Application number	1	
Application growth stage	BBCH 09-11	
Sampled matrices	Immature tops, mature tops, roots	
Sampling [DALA]	Immature tops	28
	Mature tops	173
	Roots	173

Sampling

Samples of immature sugar beet tops were taken 28 days after the application (BBCH 39). Samples of mature tops and roots were taken 173 days after the application (BBCH 49). Approximately 15 percent of the crop was taken for the sample at initial sampling point as the immature sugar beet tops sample and the remaining crop was harvested at maturity. Immature and mature top samples were cut (thinned) with a grape knife. Dead leaves and soil were not collected. Sugar beet roots were pulled from the plot and rinsed with water over the plot to remove soil. Sugar beet top samples were then stored in a freezer at -20 °C and roots were stored at 4 °C until shipment. Samples were shipped frozen on dry ice. In the laboratory, raw agricultural commodities, processed (cryogenically ground) and extracted samples were all stored at *ca* -20 °C.

Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 6 months from RAC harvest. The latest analyses of samples was conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Combustion analysis for the determination of the TRR in plant samples was performed using triplicate aliquots from immature sugar beet tops, mature sugar beet tops and sugar beet root samples. The TRR was determined using biological sample oxidizer and measured evolved ^{14}Carbon dioxide ($^{14}CO_2$) by LSC.

Extraction

The following extraction procedure was carried out for immature and mature tops and mature root samples. Sugar beet samples were first extracted by blending with acetonitrile/water (80:20). The mixture was centrifuged and filtered under vacuum to separate solids. The remaining solids were then re-blended with a solution of acetonitrile/water (80:20) and the process repeated three more times. The extraction procedure was then repeated three more times with methanol: water (50:50). All extracts were combined, assigned Ext-1 fraction, and assayed by LSC. Ext-1 fraction was concentrated and analysed by HPLC. A fraction of Ext-1 was also concentrated to dryness and reconstituted in aqueous solution (AQ-1), which was subjected to acid hydrolysis (1N HCl) under reflux. The mixture was cooled, neutralised and concentrated under nitrogen evaporation then reconstituted in methanol: water (1:1) before assay by HPLC/LC-MS/MS analysis. The initial profiling data showed that only partial hydrolysis had occurred, therefore the samples were then individually subjected to 2N acid hydrolysis under reflux (at 100°C for *ca*. 1 hour), cooled, neutralized, concentrated and reconstituted in methanol: water (1:1) and assayed by HPLC/LC-MS/MS analysis. The results provided in this evaluation for acid hydrolysed residues (following acid hydrolysis of the organic solvent extract) are following the 2N (reflux) treatment.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, dimethyl malonic acid, 2,2-dimethyl-3-hydroxypropionic acid, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, bixlozone dimethyl malonamide, bixlozone-hydroxy-isobutyramide, 3'-hydroxy-bixlozone, 'bixlozone open ring acid', 2,4-dichlorobenzyl alcohol, 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report (tabular 'Summary of F9600 reference standards assayed by LC/MS') is as follows: bixlozone (F9600), 2,2-dimethyl-3-hydroxypropionic acid (M118/1), dimethyl malonic acid (M132/1), 2,4-dichlorobenzoic acid (M190/1), 5-hydroxy-bixlozone (M289/1), bixlozone-dimethyl malonamide (M289/2)).

The proposed structures were supported by CID[5] or HCD-MS[6] analysis. All metabolites were further confirmed by comparison of LC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards. In addition to LC/MS analysis, the identification of metabolite 5-OH-bixlozone (M289/1) in sugar beet samples was also supported by TLC in comparison to a reference standard.

A list of metabolites confirmed by LC-MS/MS stated in the metabolism study report ('Summary of metabolites of bixlozone identified by LC-MS/MS') is as follows: 2,2-dimethyl-3-hydroxypropionic acid (M118/1), dimethyl malonic acid (M132/1), 2,4-dichlorobenzoic acid (M190/1), 5-OH-bixlozone (M289/1), bixlozone (bixlozone)-Dimethyl-malonamide (M289/2), bixlozone (F9600)-[O, glucoside conjugates] (M451/1 and 451/2), and Dihydroxy -bixlozone (F9600) glucoside conjugate (M467/1). These were metabolites identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

Storage stability

Samples were stored frozen whether as collected field samples, homogenised samples (powder) or as extracts. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses':

All matrices (immature tops, mature tops, mature roots) analysed within 6 months.

Frozen storage up to the time in which analysis on the matrices were completed in this study:

All matrices (immature tops, mature tops, mature roots) up to 3.3 years

Storage stability investigations

A comparison was made between chromatograms from the initial and subsequent analyses of the immature tops extract (phenyl label) with the extract having been stored in the freezer in the in-between period. The immature tops extract was stored at -20ºC for almost 8 months prior to re-analysis. See information provided below under '*Storage stability investigations- results'.*

Results and discussion

Total radioactive residue

The TRR for both radiolabels was highest in immature tops at 0.159 mg/kg and 0.176 mg/kg in the phenyl- and carbonyl-labels respectively. The TRR in mature tops and roots were both relatively low with 0.007 mg/kg and 0.011 mg/kg for the phenyl and carbonyl-labels respectively for mature tops and with 0.031 mg/kg and 0.014 mg/kg for the phenyl- and carbonyl-labels respectively for mature roots. A similar distribution was across both the phenyl- and carbonyl-labels. However in mature root samples the TRR was slightly higher in the phenyl-label compared to the carbonyl-label.

No radioactive residues were found in plants grown in the control test material (≤ 0.001 mg/kg).

A summary of the TRRs in the treated plants is presented in Table 7-26.

Table 7-26 Total radioactive residue after foliar spray application of bixlozone in sugar beet

Matrix	**DALA**	**TRR measured** [1)] *[mg parent eq./kg]*
Phenyl-label		
Immature tops	28	0.159
Mature tops	173	0.007
Mature roots	173	0.031

[5] Collision Induced Dissociation with Mass Spectroscopy detection

[6] Higher-energy collisional dissociation with Mass Spectroscopy detection

Carbonyl-label		
Immature tops	28	0.176
Mature tops	173	0.011
Mature roots	173	0.014

1) TRR measured directly via combustion LSC.

Extractability

The extractabilities of ^{14}C residues from sugar beet immature and mature tops and mature roots are summarized in Table 7-27.

The sugar beet samples were extracted with a combination of acetonitrile/water and methanol/water solvent mixtures from all commodities. High extractability of ^{14}C residue was seen all sugar beet commodities (>90% TRR for both labels).

Table 7-27 Extractability of radioactive residue from sugar beet commodities. (mg/kg = mg parent eq./kg)

Commodity	**TRR (mg/kg)**	**Organic/ aqueous extract** *(MeOH:H_2O)*		**PES**	
		% TRR	**mg/kg**	**% TRR**	**mg/kg**
Phenyl-label					
Immature tops	0.159	91.7	0.146	8.3	0.013
Mature tops	0.007	97.0	0.007	3.0	<0.0001
Roots	0.031	96.7	0.030	3.4	0.001
Carbonyl-label					
Immature tops	0.176	91.5	0.161	8.5	0.015
Mature tops	0.011	92.0	0.010	8.0	0.001
Roots	0.014	95.2	0.013	4.8	0.001

Less than 10% of radioactivity remained as unextracted residues in immature and mature tops, and in mature roots. In all commodities as the unextracted residues were <10% and the actual concentrations were low (up to 0.015 mg/kg in immature tops and up to 0.001 mg/kg in mature tops and roots mg/kg) and subsequently the PES was subjected to further processing or analysis.

Characterisation and Identification

For all matrices similar results were observed with both radiolabels. No unchanged parent was detected for any matrix using either label. An overview over the components of the extracted residue is given below in Table 7-14 - Table 7-17. Structures of the metabolites are outlined in the appendices to Volume 1.

In the following sections for the various matrices of sugar beet, the results provided for acid hydrolysed residues (following acid hydrolysis of the organic solvent extract) are following the 2N (reflux) treatment. This was because the initial profiling data showed that only partial hydrolysis had occurred (following 1N acid treatment under reflux), therefore the samples were then individually subjected to 2N acid hydrolysis under reflux (at 100°C for *ca*. 1 hour), cooled, neutralized, concentrated and reconstituted in methanol: water (1:1) and assayed.

Immature tops

No parent bixlozone was found in immature tops in any of the extracts.

The most significant metabolite after organic extraction with ACN:H_2O and MeOH:H_2O was postulated as the M289/1 conjugate for both labels, with 22.9% TRR (0.036 mg/kg) and 24.7% TRR (0.043 mg/kg) for the phenyl label and the carbonyl label respectively. {This metabolite was characterized based on the released aglycone 5-OH-bixlozone (in the acid hydrolysate of the organic solvent extract in the phenyl label work). Four other metabolites were identified in the organic solvent extract at a maximum level of 11.2% TRR (0.02 mg/kg) [M467/1].

The total of all unknowns consisted of 47.1 % TRR (0.075 mg/kg) and 39.2% TRR (0.068 mg/kg) for the phenyl and carbonyl labels respectively. In the phenyl label 11 unknown regions were present, the largest of which contained 14.0% TRR (0.022 mg/kg). For the carbonyl label 7 unidentified regions were present, the largest of which contained

12.8% TRR (0.022 mg/kg). It is noted that the majority of the unknown regions detected in the organic extracts were not detected, or were reduced, after the acid hydrolysis.

For the phenyl-label, five metabolites were observed following acid hydrolysis of the organic extracts. M190/1 was found at 35.3% TRR (0.056 mg/kg), M289/2 was found at 29.7% TRR (0.047 mg/kg) and M289/1 was found at 16.4% TRR (0.026 mg/kg). Two other metabolites were found at a maximum level of 4.0% TRR (0.006 mg/kg). One unknown metabolite was detected at 3.1% TRR (0.005 mg/kg).

For the carbonyl label, three metabolites were observed following acid hydrolysis of the organic extracts. 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was found at 64.7% TRR (0.114 mg/kg) after acid hydrolysis. This had been present at only 4.4% in the initial organic solvent extract. The metabolite M289/2 was also found in the acid hydrolysate at 10.5% TRR (0.018 mg/kg) and M289/1 was identified at 5.3% TRR (0.009 mg/kg). A further 11.1% TRR (0.020 mg/kg) of unknown metabolites were detected, which includes 3 unidentified regions, the largest of which contained 5.0% TRR (0.009 mg/kg).

In immature tops samples the PES represented <9% TRR (<0.015 mg/kg) for both label positions therefore further extraction and characterisation of the PES was not carried out.

Mature tops

Overall TRRs were fairly low in these samples (up to 0.011 mg/kg).

No parent bixlozone was found in mature tops in any of the extracts.

In the phenyl-label, the most significant metabolite present after organic extraction with ACN:H_2O and MeOH:H_2O was postulated as the M289/1 conjugate which was detected at 30.3% TRR (0.002 mg/kg). {This metabolite was characterized based on the released aglycone 5-OH-bixlozone (in the acid hydrolysate of the organic solvent extract). Two other metabolites were tentatively characterised at >10% TRR: (M289/4) 4-hydroxy-methyl-bixlozone (18.8% TRR, 0.001 mg/kg) and (M451/1) a hydroxy glucoside conjugate of bixlozone (F9600) (17.5% TRR, 0.001 mg/kg). Two other metabolites were found at a maximum level of 4.5% TRR (<0.001 mg/kg). A total of 21.5% TRR (0.002 mg/kg) unknown regions were detected within the organic extracts of the phenyl-label sample, containing 2 regions, the largest of which contained 12.1% TRR (0.001 mg/kg).

In the carbonyl-label, the most significant peak present after organic extraction with ACN:H_2O and MeOH:H_2O was tentatively characterised as the metabolite M451/1 which was detected at 23.5% TRR (0.003 mg/kg). M118/1 was also detected at 15.8% TRR (0.002 mg/kg), along with 5 other metabolites that were identified at a maximum level of 9.7% TRR (0.001 mg/kg). The total of all unknowns within the organic extracts for the carbonyl-label contained 26.8% TRR (0.003 mg/kg) and included 3 unidentified regions, the largest of which contained 14.9% TRR (0.002 mg/kg). It is noted that the all of the unknown regions detected in the organic extracts (carbonyl label) were not detected after the acid hydrolysis.

Five metabolites were detected after acid hydrolysis of the organic extracts for the phenyl label. The most significant metabolite was M289/2 which was detected at 34.0% TRR (0.002 mg/kg), followed by M190/1 at 19.0% TRR (0.001 mg/kg), M467/1 was tentatively characterised at 17.3% TRR (0.001 mg/kg) and the M289/1 conjugate was postulated as a region of 11.1% TRR (0.001 mg/kg). M289/1 was found at a maximum of 6.2% TRR (<0.001 mg/kg). A further 9.5% TRR (<0.001 mg/kg) of unknowns were identified, including 2 unidentified regions, the largest of which contained 5.5% TRR (<0.001 mg/kg).

For the carbonyl label, four metabolites were detected after acid hydrolysis of the organic extracts. As seen in the phenyl label, the most significant metabolite identified was M289/2, at 54.6% TRR (0.006 mg/kg). The M289/1 conjugate was postulated at 13.2% TRR (0.001 mg/kg). M467/1 was tentatively characterised at 12.8% TRR (0.001 mg/kg) and M289/1 was also identified at 11.4% TRR (0.001 mg/kg) respectively. No unknown metabolites were detected.

In mature tops samples the PES represented <8% TRR (<0.001 mg/kg) for both label positions therefore further extraction and characterisation of the PES was not carried out.

Mature Roots

No parent bixlozone was found in mature roots in any of the extracts.

In the phenyl-label, the most significant metabolite present after organic extraction with ACN:H_2O and MeOH:H_2O was M289/2 which was detected at 41.1% TRR (0.013 mg/kg). M467/1 was tentatively characterised at 10.5% TRR (0.003 mg/kg). Two other metabolites were identified at a maximum level of 5.4% TRR (0.002 mg/kg). A total of 32.3% TRR (0.010 mg/kg) of unknown metabolites were detected, including 2 regions, the largest of which contains

26.7% TRR, (0.008 mg/kg). It is noted that the majority of the unknown regions detected in the organic extracts were not detected, or were reduced, after the acid hydrolysis.

In the carbonyl-label, M132/1 was the most significant metabolite present after organic extraction with ACN:H_2O and MeOH:H_2O and was detected at 40.8% TRR (0.006 mg/kg). Two other metabolites were found at a maximum level of 6.6% TRR (0.001 mg/kg) [M118/1]. The total of all unknowns within the organic extracts for the carbonyl-label contained 56.7% TRR (0.008 mg/kg) and included 4 unidentified regions, the largest of which contained 21.2% TRR (0.003 mg/kg).

Acid hydrolysis of the organic extracts for the phenyl label revealed five identified metabolites in the acid hydrolysate. The most significant metabolite was M190/1 which was detected at 34.8% TRR (0.011 mg/kg), followed by M289/2 at 29.8% TRR (0.009 mg/kg). Three other metabolites were identified at a maximum level of 9.0% TRR (0.003 mg/kg). 17.7% TRR (0.005 mg/kg) were unknown residues, which includes three unidentified regions, the largest of which contains 12.5% TRR (0.004 mg/kg).

For the carbonyl label, three identified metabolites were found after acid hydrolysis of the organic extracts. The most significant metabolite identified was M118/1 at 43.4% TRR (0.006 mg/kg). M132/1 was also identified at 34.0% TRR (0.005 mg/kg). One other metabolite was detected at a maximum level of 3.5% TRR (<0.001 mg/kg). One unknown region was detected at 14.3% TRR (0.002 mg/kg).

In mature root samples the PES totalled <5% TRR (<0.001 mg/kg) for both label positions therefore further extraction and characterisation of the PES was not carried out.

Table 7-28 Distribution of [^{14}C]bixlozone and its metabolites in sugar beet immature tops. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.159**				**0.176**			
bixlozone	-	-	-	-	-	-	-	-
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	4.4	0.008	64.7	0.114
2,4-Dichlorobenzoic Acid (M190/1)	4.0	0.006	35.3	0.056	-	-	-	-
Di-OH-bixlozone conjugate (M467/1)	9.2	0.015	3.3	0.005	11.2	0.02	-	-
M289/1 conjugate	22.9	0.036	4.0	0.006	24.7	0.043	-	-
5-Hydroxy-bixlozone (M289/1)	-	-	16.4	0.026	7.2	0.013	5.3	0.009
bixlozone-Dimethyl-Malonamide (M289/2)	5.9	0.009	29.7	0.047	5.0	0.009	10.5	0.018
Unknown	47.1[a]	0.075[a]	3.1[b]	0.005[b]	39.2[c]	0.068[c]	11.1[d]	0.02[d]
Unextracted residue after solvent extraction								
PES	8.3	0.013	8.3	0.013	8.5	0.015	8.5	0.015
Total 'identified'	**42.0**	**0.066**	**88.7**	**0.14**	**52.5**	**0.084**	**80.5**	**0.141**

' - ' considered but not detected (no associated radioactivity)
a - Includes 11 unidentified regions, the largest of which contains 14.0% TRR, 0.022 mg/kg
b – Includes 1 unidentified region, containing 3.1% TRR, 0.005 mg/kg
c – Includes 7 unidentified regions, the largest of which contains 12.8% TRR, 0.022 mg/kg
d – Includes 3 unidentified regions, the largest of which contains 5.0% TRR, 0.009 mg/kg

Table 7-29 Distribution of [^{14}C]bixlozone and its metabolites in sugar beet mature tops. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.007**				**0.011**			
bixlozone	-	-	-	-	-	-	-	-
Dimethyl Malonic acid (M132/1)	-	-	-	-	9.7	0.001	-	-
2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	15.8	0.002	-	-
2,4-Dichlorobenzoic Acid (M190/1)	-	-	19.0	0.001	-	-	-	-
Di-OH-bixlozone conjugate (M467/1)	4.5	<0.001	17.3	0.001	-	-	12.8	0.001
5-Hydroxy-bixlozone (M289/1)	-	-	6.2	<0.001	-	-	11.4	0.001
bixlozone-Dimethyl-Malonamide (M289/2)	4.5	<0.001	34.0	0.002	2.5	<0.001	54.6	0.006
M289/1 conjugate	30.3	0.002	11.1	0.001	4.7	0.001	13.2	0.001
bixlozone-[O, glucoside] (M451/1)	17.5	0.001	-	-	23.5	0.003	-	-
4-hydroxy-methyl-bixlozone (M289/4)*	18.8	0.001	-	-	9.1	0.001	-	-
4-OH-Me, 5'-OH-bixlozone (M305/1)	-	-	-	-	2.5	<0.001	-	-
Unknown	21.5[a]	0.002[a]	9.5[b]	<0.001[b]	26.8[c]	0.003[c]	-	-
Unextracted residue after solvent extraction								
PES	3.0	<0.0001	3.0	<0.0001	8.0	0.001	8.0	0.001
Total 'identified'	**75.6**	**0.005**	**87.6**	**0.005**	**67.8**	**0.008**	**92.0**	**0.009**

' - ' considered but not detected (no associated radioactivity)
* 4-hydroxy-methyl-bixlozone characterised based on retention time of reference standard only.
a – Includes 2 unidentified regions, the largest of which contained 12.1% TRR, 0.001 mg/kg
b – Includes 2 unidentified regions, the largest of which contained 5.5% TRR, <0.001 mg/kg
c – Includes 3 unidentified regions, the largest of which contained 14.9% TRR, 0.002 mg/kg

Table 7-30 Distribution of [^{14}C]bixlozone and its metabolites in sugar beet root. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Organic extract *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**		**Organic extract** *(ACN:H_2O and MeOH:H_2O)*		**Acid Hydrolysis of organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.031**				**0.014**			
bixlozone	-	-	-	-	-	-	-	-
Dimethyl Malonic acid (M132/1)	-	-	-	-	40.8	0.006	34.0	0.005
2,2-Dimethyl-3-Hydroxypropionic Acid (M118/1)	-	-	-	-	6.6	0.001	43.4	0.006
2,4-Dichlorobenzoic Acid (M190/1)	-	-	34.8	0.011	-	-	-	-
Di-OH-bixlozone conjugate (M467/1)	10.5	0.003	-	-	-	-	-	-
5-Hydroxy-bixlozone (M289/1)	-	-	2.8	0.001	-	-	-	-
M289/1conjugate	5.4	0.002	9.0	0.003	-	-	-	-
bixlozone-Dimethyl-Malonamide (M289/2)	41.1	0.013	29.8	0.009	-	-	3.5	<0.001
bixlozone-[O, glucoside] (M451/1)	3.2	0.001	-	-	-	-	-	-
3'-hydroxy-bixlozone (M289/6)*	-	-	-	-	3.7	<0.001	-	-
4-hydroxy-methyl-bixlozone (M289/4)*	-	-	2.7	0.001	-	-	-	-
Unknown	32.3[a]	0.01[a]	17.7[b]	0.006[b]	56.7[c]	0.008[c]	14.3[d]	0.002[d]
Unextracted residue after solvent extraction								
PES	3.4	0.001	3.4	0.001	4.8	0.001	4.8	0.001
Total 'identified'	**60.2**	**0.019**	**79.1**	**0.025**	**51.1**	**0.007**	**80.9**	**0.011**

' - ' considered but not detected (no associated radioactivity)

* 4-hydroxy-methyl-bixlozone (M289/4) and 3'-hydroxy- bixlozone (M289/6) were characterised based on retention time of reference standard.

a - Includes 2 unidentified regions, the largest of which contains 26.7% TRR, 0.008 mg/kg.

b – Includes 3 unidentified regions, the largest of which contains 12.5% TRR, 0.004 mg/kg.

c – Includes 4 unidentified regions, the largest of which contains 21.2% TRR, 0.003 mg/kg

d – Includes 1 unidentified regions, containing 14.3% TRR, 0.002 mg/kg

Storage stability investigations- results

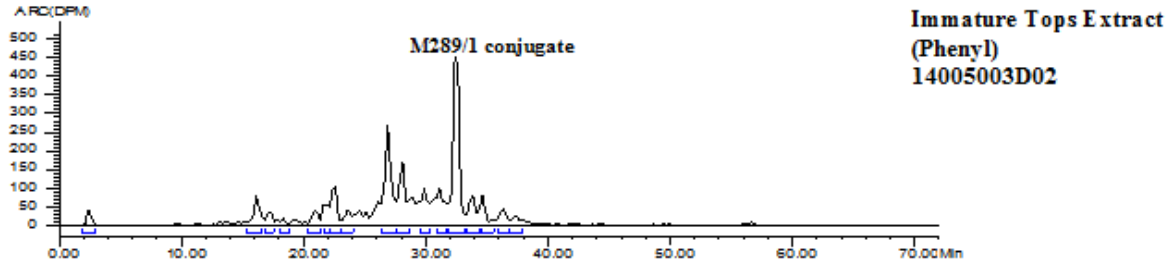
The applicant considers that the data provided (some example chromatograms) lend some support to the samples being stable over frozen storage for a period of over a year). HSE considers that the information provided on whether samples/extracts are unchanged over a storage period in the context of the metabolism study is limited as explained below.

A comparison was made between chromatograms from the initial and subsequent analyses of an immature tops extract-phenyl label (same extract), with the extract having been stored in the freezer in the in-between period. The immature tops extract was stored at -20ºC for almost 8 months prior to re-analysis.

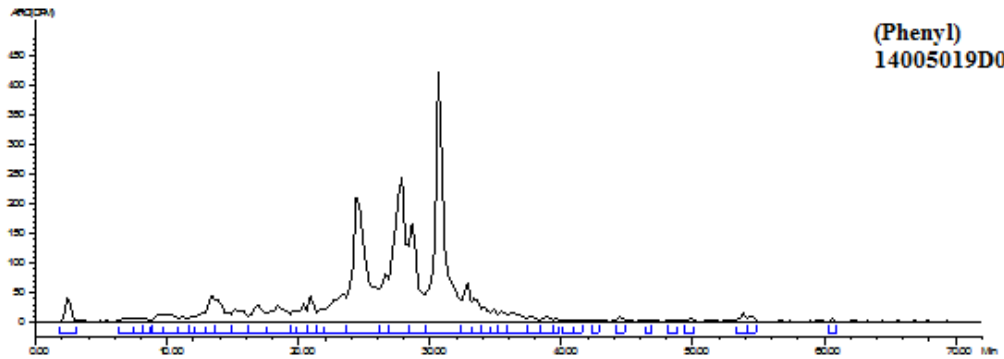
HSE finds it difficult to draw firm conclusions from the chromatography. The comparison for the immature tops extract is given below. The 'initial' chromatogram is at the top, and the 'after' chromatogram is at the bottom. Aside from the main peak (M289/1 conjugate) the chromatogram is unlabelled (further metabolite peaks are not labelled). Aside from saying that the main peak seems to remain present in the 'after' compared to the 'initial' chromatogram, it is not possible to draw comparisons about possible changes to other peaks. It is difficult to make any quantitative or qualitative conclusions (aside from the main peak remaining present).

Figure 2: HPLC radio-chromatograms of Treated Sugar beet Tops (Immature) Extracts

(A)



(B)



Note: First assay (A): 04 September 2014
Last assay (B): 24 April 2015

It is difficult to draw conclusions from the chromatograms compared (immature tops extract was stored frozen and reanalysed after 8 months) that suggest the stability of samples is sufficiently maintained over the long course of the study (up to 3.3 years storage after sampling).

Translocation and proposed metabolic pathway

The unchanged parent bixlozone was not detected in any of the sugar beet commodities analysed showing that the compound is readily metabolised into various metabolite compounds. The total radioactive residue uptake and translocation into the sugar beet roots amounted to 0.031 mg/kg and 0.014 mg/kg for the phenyl and carbonyl labels respectively. In mature samples, more radioactivity was detected in the roots compared to the tops. The analysis of the sugar beet immature tops harvested at an early growth stage provided the opportunity for higher amounts of radioactivity to be worked on as TRRs in immature tops were up to 0.176 mg/kg.

When the results from both labels are considered together the data demonstrate consistent information on metabolic pathways in sugar beet. A high level of extractability was achieved (using the organic solvent extraction) for all matrices (>91%TRR). More information was gained about the metabolism following the analysis of the acid hydrolysed extracts (of the initial organic solvent extracts). The work on mature roots was using material with low overall TRRs. The work was affected by the low levels present, and many unknown metabolites were not identified. This was also the case in immature and mature tops where a number of unknown regions were found.

For roots, the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound, to form the major metabolites 2,4-dichlorobenzoic acid (M190/1), 2,2-dimethyl-3-hydroxy-propionic acid (M118/1) and dimethyl malonic acid (M132/1). A major metabolite (observed in the phenyl label) was bixlozone (F9600)-Dimethyl-malonamide (M289/2) following hydroxylation, reduction and oxidation of the parent molecule. Another pathway involved hydroxylation of the 5 position to form the metabolite 5-hydroxy-bixlozone (M289/1) in the phenyl label.

The metabolic pattern in immature and mature tops was similar. The acid hydrolysis identified a number of free metabolites (the profiles were different in the acid hydrolysed extracts compared to the initial solvent extracts, suggesting some possible conjugation within the commodities. The presence of M289/1 and M289/1 conjugate was observed). In both labels the major metabolite M289/2 (bixlozone (F9600)-Dimethyl-malonamide) was detected in much greater amounts after acid hydrolysis. In the carbonyl label, oxidative ring opening forms the metabolite M118/1 (2,2-dimethyl-3-hydroxy-propionic acid), whereas M190/1 (2,4-dichlorobenzoic acid) was detected in all phenyl-labelled samples. Other more minor metabolites observed in sugar beet (glucoside conjugates of bixlozone {M451/1 and M467/1} and M289/4 [4-hydroxy-methyl-bixlozone (F9600)]) are also indicative of the way in which hydroxylation and conjugation can occur).

Based on the information on identified metabolites (both labels) a metabolic pathway was proposed by the applicant is outlined in Figure 7-5.

Conclusion

The metabolism of bixlozone was investigated in sugar beet by applying phenyl-labelled or carbonyl-labelled bixlozone. The overall residue levels (TRR) in the phenyl-labelled immature tops, mature tops and roots were 0.159, 0.007 and 0.031 mg/kg respectively. For carbonyl-labelled immature tops, mature tops and roots the levels were 0.176, 0.011 and 0.014 mg/kg respectively.

For both labels and all matrices, solvent extractability was high (at least 91% TRR). In all commodities the PES contained less than 9% TRR, furthermore the actual concentrations of the PES were low (up to 0.015 mg/kg) and therefore no further extraction techniques/investigation of the PES was taken forward.

Metabolism of bixlozone in sugar beet includes, primarily, the hydroxylation of the 5 position in the dimethyl-oxazolidone ring and oxidative ring opening. For both labels, unchanged parent bixlozone was not detected in any of the commodities. Bixlozone (F9600) is also metabolised in sugar beet via other hydroxylation reactions, reduction and oxidation (to form the main metabolite M289/2). This was present as in higher amounts in sugar beet tops after acid hydrolysis of the initial solvent extract, so it could be present as a conjugate.

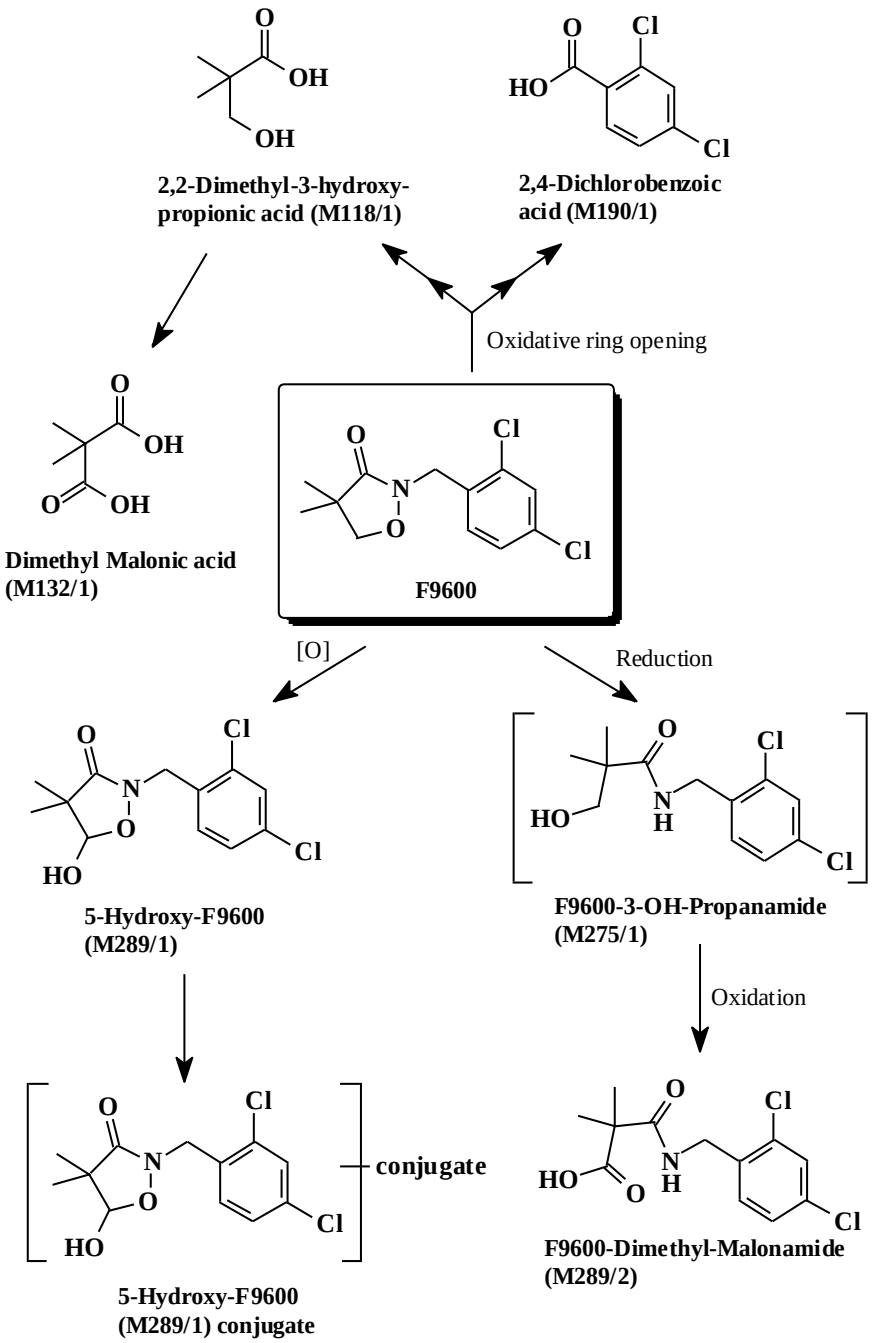
Across all commodities, the metabolites M289/1, M289/2, M190/1, M118/1 and M132/1 accounted for the majority proportion of the radioactive residues, with glucoside conjugates of bixlozone (M451/1 and M467/1) and M289/4 representing smaller amounts of radioactivity. A high portion of the TRR before acid hydrolysis was unidentified (<46% TRR in immature tops and up to 57% TRR in roots). However based on both low %TRR levels and the way in which the unknowns are representing a number of different regions seem to indicate that the unknowns are mostly very low (<0.01 mg/kg or <0.005 mg/kg in many cases). An exception to this was in sugar beet tops (immature), where the highest concentration of an unknown residue fraction was 0.022 mg/kg (in the solvent organic fraction),

however the majority of the unknown regions detected in the organic extracts were not detected after acid hydrolysis (and the highest level of any individual level of an unknown in the acid hydrolysed extract was 0.009 mg/kg) indicating that there is a potential for conjugation within the sugar beet.

Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in foliar applied sugar beet. Overall, metabolism of bixlozone in sugar beet, and by extrapolation, in the root and tuber crop group is considered reasonably well-elucidated (see the applicants proposed pathway in Figure 7-5). HSE has the following remarks/observations based on this sugar beet metabolism study.

- The overall levels of radioactivity were low to moderate in this sugar beet metabolism study compared to the wheat metabolism (section B.7.2.1.1). Despite this, and especially low TRR levels in sugar beet roots the metabolism work has enabled a fairly good elucidation of the metabolism in sugar beet, following high solvent extractability of the radioactive residues. As per the other metabolism studies, there were a number of unknown regions, that individually were present at low or very low levels in this sugar beet metabolism study. The differences in the metabolites observed for sugar beet therefore (compared to wheat, canola and rice) might be, in part, reflective of the different degrees of identification and the way that sugar beet is a food root crop with the below plant part analysed for residues, rather than there being an overall significant difference in the metabolic profile. Broad similarities in the metabolic pathways across the various primary crops are anyhow observed (the hydroxylation of the 5 position in the dimethyl-oxazolidone ring and oxidative ring opening).
- As per other metabolism studies, different metabolites were found/identified in the acid hydrolysed extract compared to the initial solvent extraction extract. The %TRR radioactivity of unknowns was reduced in the acid hydrolysed fraction compared to the initial organic extract, and those remaining tended to individually represent low (<0.01 mg/kg or in many cases <0.005 mg/kg) levels of radioactivity). It could be that the additional metabolites observed in the acid hydrolysate were conjugated in the organic extract and could only be ‘seen’ following the acid hydrolysis step.
- Further data on the stability of incurred primary crop residues in the metabolism context is presented at the end of section B.7.2.1 and a discussion of storage stability of residues is presented in Vol 1, Section 2.7.2.

Figure 7-5 Applicant's proposed metabolic pathway for bixlozone (F9600) in sugar beet



B.7.2.1.4. *Rice*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.2.1-04, Desai, M., 2019
Title:	Nature of the residue: metabolism of [^{14}C]F9600 in/on rice crop
Report No.:	15031-RPT03653 (Report amendment date: December 19, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 501 Metabolism in Crops, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996)
GLP	yes

Materials and methods

Materials

1. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42017
Radiochemical Purity:	99.6% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 21.50 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.88 MBq/mg (certified)

2. Test Material

Test Material:	[Carbonyl -C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42018
Radiochemical Purity:	99.9% (nominal) 99.5% (actual)
Specific Activity:	20 mCi/mmol (nominal) 20.74 mCi/mmol (certified) 2.68 MBq/mg (nominal) 2.78 MBq/mg (certified)

Methods

Test system

A metabolism study on rice (variety M-205) grown outdoors in Fresno, California, USA was carried out in 2015. Rice seeds for both paddy and dry land rice were cultivated simulating normal agricultural practices (as far as possible) in ten containers (each 1.0 m^2) with sandy loam soil (soil column depth ~50 cm, pH in 1:1 soil: water ratio = 6.9, Percentage sand = 72%). The containers were wooden boxes lined with heavy gauge plastic. No crops had been grown or pesticides applied to the test system for 3 years before the trial. No radiolabelled material had ever been applied to the planting area prior to the trial. Weather data were reported for the trials and no exceptional events were noted.

Applications

One foliar spray application of test substance in EC formulation 'blank' of either phenyl or carbonyl labelled bixlozone (F9600) was made. For paddy rice, there were two different treatment plots/containers, (one C-label one P-label), and likewise for dryland rice. These were treated with a single application high-dose, at a target rate of 375 g a.s./ha per application early-post emergence (BBCH 13-14). Actual application rates were 348 g as/ha (phenyl and carbonyl) for dry land rice and 350 g as/ha for carbonyl label paddy rice and 344 g as/ha for phenyl label paddy rice. Therefore although the target application rate was 375 g as/ha, the rate achieved was around 350 g as/ha (1.75 N wrt wheat/barley GAP and 0.93N wrt maize GAP). A further plot for each of paddy rice and dryland rice was used as a control plot and not treated. The control plots were located around 60 m from the treated plots to limit the risk of contamination. Plastic sheeting approximately two feet high was erected all around the plot to block the wind during each application. A summary of the study design is given in Table 7-31.

Two further test containers were treated with a lower dose application rate of 100 g a.s./ha, however the low dose applications were not analysed and are not considered further in the evaluation write up.

Table 7-31 Study design: plant uptake part (rice)

Applications of radiolabelled bixlozone		
Intended use rate [g a.s./ha]	375- see in the text above, the rates achieved were around 350 g as/ha , representing 1.75 N wrt wheat/barley GAP and 0.93N wrt maize GAP	
Application number	1	
Application growth stage	BBCH 13-14	
Sampled matrices	Straw and grain	
Sampling [DALA]	Straw	151-153
	Grain	151-153

Sampling

Samples of straw and grain were taken at maturity, 151-153 days after the application (BBCH 39). Heads were cut from standing plans using pruning shears. The standing stems were cut to approximately 5 cm above the soil line and then cut into approximately 10 cm lengths for the straw sample. The grain was separated from the panicles by stripping by hand. The empty panicles were added to the straw sample. Rice samples were then stored in a freezer at -15 °C until shipment to the laboratory.

Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 1 months from RAC harvest. The latest analyses of samples was conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Combustion analysis for the determination of the TRR in plant samples was performed using triplicate aliquots from rice straw and grain samples. The TRR was determined using biological sample oxidizer and measured evolved $^{14}CO_2$ by LSC.

Extraction

The following extraction procedure was carried out for straw and grain samples. Rice samples were first extracted by blending with acetonitrile/water (80:20). The mixture was centrifuged and filtered under vacuum to separate solids. The remaining solids were then re-blended with a solution of acetonitrile/water (80:20) and the process repeated two more times. The extraction procedure was then repeated three more times with methanol: water (50:50). All extracts were combined, assigned Ext-1 fraction, and assayed by LSC. Ext-1 fraction was concentrated and analysed by HPLC. A fraction of Ext-1 was also concentrated to dryness and reconstituted in aqueous solution (AQ-1), which was subjected to acid hydrolysis (1N HCl) under reflux for about an hour. The mixture was cooled, neutralised and concentrated under nitrogen evaporation then reconstituted in acetonitrile: water (1:1) before assay by HPLC/LC-MS/MS analysis. The post extraction solids (PES) were determined for straw and grain samples.

The PES samples (PES-1) were subjected to further hydrolysis using sequential enzyme treatments of cellulase, amylase, pectinase and protease. The remaining unextracted solids (PES-2) were collected for further analysis.

The PES-2 samples were subjected to further hydrolysis using sequential acid treatment (1N HCl), base treatment (1N NaOH). The results table also reported further 6N acid and base treatments which did not yield further release of radioactivity. The final remaining solid (final PES) was analysed by combustion and LSC.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, bixlozone dimethyl malonamide, bixlozone-hydroxy-isobutyramide, 3'-hydroxy-bixlozone, 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report (tabular 'Summary of F9600 reference standards assayed by LC/MS') is as follows: bixlozone (F9600), 2,2-dimethyl-3-hydroxypropionic acid (M118/1), dimethyl malonic acid (M132/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-3-OH-propanamide (M275/1), bixlozone-dimethyl malonamide (M289/2), bixlozone-dimethyloxoazoldinone.

The proposed structures were supported by HCD-MS. All metabolites were further confirmed by comparison of the LC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards. Minor components were also detected and were characterised based on HPLC and TLC.

A list of metabolites confirmed by LC-MS/MS stated in the metabolism study report ('Summary of metabolites of F9600 identified by LC-MS/MS') is as follows: 2,2-dimethyl-3-hydroxypropionic acid (M118/1), dimethyl malonic acid (M132/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-3-OH-propanamide (M275/1), bixlozone-dimethyl malonamide (M289/2), and bixlozone (F9600)-3-OH-propanamide-Glucoside (M437/1- conjugate of M275/1). These were metabolites identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

Storage stability

As stated above, samples were not all analysed within a period of 6 months since sampling. Samples were stored frozen whether as collected field samples, homogenised samples (powder) or as extracts. Samples were stored at <-20ºC in the laboratory. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses':

All matrices (Dryland straw, Dryland grain, Paddy straw, Paddy grain) analysed within 1 month.

Frozen storage up to the time in which analysis on the matrices were completed in this study:

All matrices (Dryland straw, Dryland grain, Paddy straw, Paddy grain) up to 2.1 years

Storage stability investigations

No storage stability comparisons were made for rice within this metabolism study (e.g. chromatogram comparisons for extracts analysed initially and later on in the study).

Results and discussion

Total radioactive residue

The TRR for both radiolabels in dry land rice commodities were higher than their respective paddy rice equivalents. The TRR in straw was higher than grain for both paddy and dry land rice for both the phenyl- and carbonyl-labels. For both dryland and paddy rice the TRR in the phenyl-labelled samples was higher than the carbonyl-labelled samples. In phenyl-labelled dry land rice the levels in grain and straw were 0.112 mg/kg and 0.908 mg/kg respectively whereas in the carbonyl-labelled samples of grain and straw the levels were 0.078 mg/kg and 0.525 mg/kg respectively. For paddy rice the levels in phenyl-labelled grain and straw were 0.077 mg/kg and 0.712 mg/kg respectively whereas in the carbonyl-labelled samples of grain and straw the levels were 0.038 mg/kg and 0.243 mg/kg respectively.

No total radioactive residues were found in plants grown in the control test material (≤ 0.001 mg/kg).

A summary of the TRRs in the treated plants is presented in Table 7-32.

Table 7-32 Total radioactive residue after foliar spray application of bixlozone in rice

Rice type	Matrix	DALA	TRR measured [1)] *[mg parent eq./kg]*
Phenyl-label			
Dry land rice	Straw	152	0.908
	Grain	152	0.112
Paddy rice	Straw	151	0.712
	Grain	151	0.077

Rice type	Matrix	DALA	TRR measured [1)] *[mg parent eq./kg]*
Carbonyl-label			
Dry land rice	Straw	153	0.525
	Grain	153	0.078
Paddy rice	Straw	152	0.243
	Grain	152	0.038

1) TRR measured directly via combustion LSC.

Extractability

The extractabilities of ^{14}C residues from rice commodities are summarized in Table 7-33.

The rice samples were extracted with a combination of acetonitrile/water and methanol/water solvent mixtures from all commodities. High extractability of ^{14}C residue was seen in straw samples for both paddy and dry land rice (>88% TRR for both labels). Extractability of grain samples was lower than for straw for both paddy and dry land rice (55-75% TRR for both labels).

Table 7-33 Extractability of radioactive residue from rice commodities. (mg/kg = mg parent eq./kg)

Commodity	Dry land Straw		Dry land Grain		Paddy Straw		Paddy Grain	
Residue Levels	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Phenyl-label								
TRR	100	0.908	100	0.112	100	0.712	100	0.077
Extracted residue (acetonitrile/ methanol/ water)	92.5	0.840	71.9	0.081	90.1	0.642	55.0	0.042
Non-extracted PES (PES-1)	7.5	0.068	28.1	0.031	9.9	0.070	45.0	0.035
Sequential enzyme hydrolysis of PES-1								
Cellulase	1.7	0.016	1.8	0.002	2.3	0.016	3.7	0.003
Alpha amylase	1.0	0.009	2.6	0.003	1.6	0.011	4.7	0.004
Pectinase	1.0	0.009	1.3	0.001	1.0	0.007	2.8	0.002
Protease	0.3	0.003	0.9	0.001	0.4	0.003	1.0	0.001
Sequential acid and base hydrolysis of PES-2								
Acid (1N)	-	-	7.8	0.009	-	-	11.2	0.009
Base (1N)	-	-	5.9	0.006	-	-	11.1	0.008
Acid (6N)	-	-	-	-	-	-	-	-
Base (6N)	-	-	-	-	-	-	-	-
Non-extracted PES following acid and base hydrolysis **(final PES)**	3.7	0.033	6.6	0.007	4.8	0.035	8.7	0.007
Carbonyl-label								
TRR	100	0.525	100	0.078	100	0.243	100	0.038
Extracted residue (acetonitrile/ methanol/ water)	90.8	0.477	74.2	0.058	88.0	0.214	72.9	0.028
Non-extracted PES (PES-1)	9.2	0.048	25.8	0.020	12.0	0.029	27.1	0.010
Sequential enzyme hydrolysis of PES-1								
Cellulase	-	-	4.4	0.003	3.4	0.008	8.7	0.003
Alpha amylase	-	-	3.6	0.003	-	-	7.1	0.003

Commodity	Dry land Straw		Dry land Grain		Paddy Straw		Paddy Grain	
Residue Levels	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Pectinase	-	-	2.1	0.002	-	-	5.6	0.002
Protease	-	-	1.0	0.001	-	-	2.9	0.001
Sequential acid and base hydrolysis of PES-2								
Acid (1N)	-	-	7.9	0.006	-	-	2.2	0.001
Base (1N)	-	-	3.4	0.003	-	-	8.0	0.003
Acid (6N)	-	-	-	-	-	-	-	-
Base (6N)	-	-	-	-	-	-	-	-
Non-extracted PES following acid and base hydrolysis **(final PES)**	-	-	7.7	0.006	9.0	0.022	15.8	0.006

N/A = extraction technique not performed for commodity
- = no TRR detected

In straw samples <12 % of radioactivity remained as unextracted residues following organic extraction. In grain samples the PES contained between 25 and 45% TRR following organic extraction. The highest level of PES was observed in phenyl-labelled paddy grain. The unextracted residues for grain accounted for 0.031 mg/kg and 0.035 mg/kg (28.1% and 45.0% of TRR) and 0.020 mg/kg and 0.010 mg/kg (25.8% to 27.1% of TRR) for phenyl and carbonyl labels respectively. The PES of both grain and straw were subjected to further hydrolysis using sequential enzymes (cellulase, alpha amylase, pectinase and protease), mild acid and base treatment to evaluate released residues and characterise their nature. Further to this, strong acid and base treatments were also used but released no further radioactivity. In all cases each individual enzyme extraction of the PES released up to 7% TRR. In grain where acid and base hydrolysis each extracted up to 11% TRR (up to 0.009 mg/kg in the phenyl labelled samples).

Characterisation and Identification

For all matrices similar results were observed with both radiolabels. No unchanged parent was detected for any matrix using either label. An overview over the components of the extracted residue is given below in Table 7-34 and Table 7-35. Metabolites found at >10% TRR are discussed in detail for each matrix below. Structures of the metabolites are outlined in the appendices to Volume 1.

In all samples, 2,2-dimethyl-3-hydroxypropionic acid (M118/1) (also referred to as 2,2-dimethyl-3-hydroxypivalic acid in the study reports) was explained in the study report as two co-eluting peaks at ca. 4-5 mins retention time. The applicant proposed that both peaks constituted a single metabolite. The reasoning given in the study amendment report states: '*The predicted pKa of the acid moiety in 2,2-dimethyl-3-hydroxypropionic acid is 4.55 which is close to the pH of the mobile phase (pH 5.6). Therefore 2,2-dimethyl-3-hydroxypropionic acid peak may split into ionised and un-ionised forms which can result in two separate peaks in the chromatogram.*' Furthermore, the conjugates of dimethyl malonic acid and 2,2-dimethyl-3-hydroxypropionic acid (M132/1 and M118/1 respectively) have been postulated as overlapping peaks at a retention time of ca. 9 minutes. The applicant's justification for this characterisation states: '*LC/MS data did not provide any definitive ion for ca. 9 mins. The characterisation was not achieved on the intact conjugate due to poor ionisation in MS. However, based on the results from hydrolysis of the conjugate and the released aglycon, it was deemed to be M132/1 and the M118/1 conjugate.*' Since metabolites M132/1 and M118/1 were found in other metabolism studies, the presence of these metabolites is plausible.

Dry land rice

Straw

For the phenyl-label, two metabolites were identified in the acid hydrolysis fraction of the organic extracts: M190/1 was identified at 67.5% TRR (0.613 mg/kg) and M289/2 was identified at 12.7% TRR (0.115 mg/kg). 12.3% TRR (0.112 mg/kg) of the radioactivity was assigned as unknown regions, containing 2 unidentified regions, the largest of which contained 7.3% TRR (0.066 mg/kg).

After the acid hydrolysis of the organic extracts for the carbonyl-label, two co-eluting peaks were postulated to be 2,2-dimethyl-3-hydroxypropionic acid (M118/1) – together these contained a total of 27.4% TRR (0.144 mg/kg) and the conjugates of M132/1 and M118/1 indicated to be overlapping regions containing 26.9% TRR (0.141 mg/kg) in

total. A different peak (14.8% TRR (0.078 mg/kg)) was identified as dimethyl malonic acid (M132/1). 21.6% TRR (0.114 mg/kg) of the radioactivity was assigned as unknown metabolites were detected, which includes 2 unidentified regions, the largest of which contained 7.3% TRR (0.066 mg/kg). In accordance with OECD 501, for such a level, characterisation/identification should be decided on a case by case basis taking account of how much has been identified. Also, straw is not consumed directly by humans.

In dry land rice straw samples, the PES after organic extraction and acid hydrolysis was 7.5% TRR (0.068 mg/kg) for the phenyl label and further enzymatic extraction was carried out. Cellulase hydrolysis extracted 1.7% TRR (0.016 mg/kg) and alpha amylase and pectinase each extracted 1% TRR (0.009 mg/kg). Protease also extracted 0.3% TRR (0.003 mg/kg). After exhaustive extraction a total of 3.7% TRR (0.03.3 mg/kg) unextracted residue remained in the phenyl-label. In the carbonyl label the PES after organic extraction and acid hydrolysis was 9.2% TRR (0.048 mg/kg) therefore further extraction and characterisation was not carried out.

Grain

Two metabolites were detected after acid hydrolysis of the organic extracts for the phenyl label. The most significant metabolite was M190/1 which was detected at 38.3% TRR (0.043 mg/kg). M289/2 was also detected at 7.9% TRR (0.009 mg/kg). 25.8% TRR (0.029 mg/kg) was assigned as unknown material, including 6 unidentified regions, the largest of which contained 7.0% TRR (0.008 mg/kg).

For the carbonyl label, the most significant region found after acid hydrolysis of the organic extracts was M118/1. Two co-eluting peaks were postulated to be 2,2-dimethyl-3-hydroxypropionic acid (M118/1) – together these contained a total of 21.9% TRR (0.017 mg/kg). Free M132/1 was found at 13.9% TRR (0.011 mg/kg) and the coeluting peak/region considered to be M118/1 and M132/1 conjugates were determined as 12.8% TRR (0.010 mg/kg) – total for both the conjugates. Two other metabolites were identified at a maximum level of 1.7% TRR (0.001 mg/kg). Radioactivity at 21.6% TRR (0.018 mg/kg) assigned as unknown material, including 9 regions, the largest of which contained 6.7% TRR (0.005 mg/kg).

In the phenyl labelled sample, the PES after organic extraction and acid hydrolysis contained 28.1% TRR (0.031 mg/kg) and in the carbonyl label the PES after organic extraction and acid hydrolysis was 25.8% TRR (0.020 mg/kg) and therefore further extraction and characterisation was carried out in both cases. In both labels 1N acid hydrolysis extracted the highest amount of the PES (7.8% TRR, 0.009 mg/kg and 7.9% TRR, 0.006 mg/kg for the phenyl and carbonyl labels respectively). In both labels, sequential cellulase, alpha amylase, pectinase, protease and 1N base hydrolysis also released radioactivity from the PES. After exhaustive extraction a total of 6.6% TRR (0.007 mg/kg) and 7.7% TRR (0.006 mg/kg) remained in the phenyl and carbonyl labels respectively.

Paddy rice

Straw

After acid hydrolysis of the organic extracts for the phenyl label, the most significant metabolite was M190/1 which was detected at 24.8% TRR (0.177 mg/kg), followed by M275/1 at 20.4% TRR (0.145 mg/kg) and M289/2 at 11.0% TRR (0.078 mg/kg). 30.2% TRR (0.215 mg/kg) was assigned as 'unknown', which included 6 unidentified regions, the largest containing 9.8% TRR (0.069 mg/kg).

For the carbonyl label, the most significant region detected after acid hydrolysis of the organic extracts was a co-eluting peak that was postulated to be conjugate of M118/1 and conjugate of M132/1 at a total level of 31.1% TRR (0.076 mg/kg). 14.5% TRR (0.035 mg/kg) was also tentatively identified as M118/1. Two other metabolites were detected at a maximum level of 8.9% TRR (0.022 mg/kg). The total of all the unknown radioactivity was 25.9% TRR (0.065 mg/kg) which included 8 unidentified regions, the largest of which contained 7.2% TRR (0.018 mg/kg).

In the phenyl labelled sample, the PES totalled 9.9% TRR (0.070 mg/kg) and in the carbonyl label the PES contained 12.0% TRR (0.029 mg/kg). Both samples were subjected to further extractions. In the carbonyl label, only cellulase hydrolysis extracted further radioactivity (3.4% TRR, 0.008 mg/kg). In the phenyl label, cellulase hydrolysis extracted the highest amount of radioactivity from the PES at 2.3% TRR (0.016 mg/kg) and alpha amylase, pectinase and protease hydrolysis extracted radioactivity, each at a maximum level of 1.6% TRR (0.011 mg/kg). After exhaustive extraction a total of 4.8% TRR (0.035 mg/kg) remained in the phenyl label and a total of 9.0% TRR (0.022 mg/kg) remained in the carbonyl label.

Grain

In paddy rice the acid hydrolysis of the organic extracts for the phenyl label identified 3 metabolites. The most significant metabolite was M190/1 which was detected at 21.4% TRR (0.016 mg/kg), followed by M275/1 at 13.5%

TRR (0.010 mg/kg). M289/2 was also identified at a maximum level of 3.8% TRR (0.003 mg/kg). 16.4% TRR (0.013 mg/kg) of unknowns were identified, which includes 8 unidentified regions, the largest containing 3.9% TRR (0.003 mg/kg).

After acid hydrolysis of the organic extracts of the carbonyl label, the most significant metabolite identified was dimethyl malonic acid (M132/1) at 19.6% TRR (0.008 mg/kg). A peak containing a total of 17.2% TRR (0.007 mg/kg) was postulated to be metabolite M118/1 and M275/1 was identified at 12.3% TRR (0.005 mg/kg). A further region (coeluting peaks) was considered to be a conjugate of M118/1 and M132/1 at a total level of 8.0% TRR (0.003 mg/kg). M289/2 was identified at 2.0% TRR (0.001 mg/kg). The total of all unknowns amounted to 14.0% TRR (0.005 mg/kg) which included 5 unidentified regions, the largest containing 6.1% TRR (0.002 mg/kg).

In the phenyl labelled sample, the PES totalled 45.0% TRR (0.035 mg/kg) and further extractions with 1N acid and 1N base hydrolysis extracted the most radioactivity at 11.2% TRR (0.009 mg/kg) and 11.1% TRR (0.008 mg/kg) respectively. Sequential cellulase, alpha amylase, pectinase and protease hydrolysis also extracted radioactivity from the PES at a maximum level of 4.7% TRR (0.004 mg/kg) [released by alpha amylase]. In the carbonyl labelled sample, the PES accounted for 27.1% TRR (0.010 mg/kg). Cellulase hydrolysis extracted 8.7% TRR (0.003 mg/kg) and 1N base hydrolysis extracted 8.0% TRR (0.003 mg/kg). Sequential alpha amylase, pectinase, protease and 1N acid hydrolysis extracted radioactivity at a maximum level of 7.1% TRR (0.003 mg/kg).

Table 7-34 Distribution of [^{14}C]bixlozone and its metabolites in paddy grown rice after initial organic/aqueous extraction and further 1N acid hydrolysis of the extracted residue. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Grain		Straw		Grain		Straw	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total TRR by combustion (mg/kg)	0.077		0.712		0.038		0.243	
bixlozone	-	-	-	-	-	-	-	-
Dimethyl malonic acid (M132/1)	-	-	-	-	19.6	0.008	8.9	0.022
2-2 dimethyl-3-OH –Propionic acid (M118/1)*	-	-	-	-	17.2	0.007	14.5	0.035
M132/1 & M118/1 conjugate	-	-	-	-	8.0	0.003	31.1	0.076
2,4-Dichlorobenzoic Acid (M190/1)	21.4	0.016	24.8	0.177	-	-	-	-
bixlozone-Dimethyl-Malonamide (M289/2)	3.8	0.003	11.0	0.078	2.0	0.001	2.5	0.006
bixlozone-3-OH-Propanamide (M275/1)	13.5	0.010	20.4	0.145	12.3	0.005	-	-
Unknown	16.4[a]	0.013[a]	30.2[b]	0.215[b]	14.0[c]	0.005[c]	25.9[d]	0.065[d]
Total 'identified'	**38.7**	**0.029**	**56.2**	**0.400**	**59.1**	**0.024**	**57.0**	**0.139**
	Post-extraction solids (PES-1)							
Un-extracted residue after initial extraction:	**45.0**	**0.035**	**9.9**	**0.070**	**27.1**	**0.010**	**12.0**	**0.029**
Cellulase	3.7	0.003	2.3	0.016	8.7	0.003	3.4	0.008
Alpha Amylase	4.7	0.004	1.6	0.011	7.1	0.003	-	-
Pectinase	2.8	0.002	1.0	0.007	5.6	0.002	-	-
Protease	1.0	0.001	0.4	0.003	2.9	0.001	-	-
Acid (1N HCl)	11.2	0.009	-	-	2.2	0.001	-	-
Base (1N NaOH)	11.1	0.008	-	-	8.0	0.003	-	-
Acid (6N HCl)	-	-	-	-	-	-	-	-
Base (6N NaOH)	-	-	-	-	-	-	-	-
Un-extracted residue after exhaustive extraction (final PES):	8.7	0.007	4.8	0.035	15.8	0.006	9.0	0.022

' - ' considered but not detected (no associated radioactivity)

* co-eluting peaks with retention time varying from 4.13-5.38

a – Includes 8 unidentified regions, the largest of which contains 3.9% TRR, 0.003 mg/kg
b – Includes 6 unidentified regions, the largest of which contains 9.8% TRR, 0.069 mg/kg
c – Includes 5 unidentified regions, the largest of which contains 6.1% TRR, 0.002 mg/kg
d – Includes 8 unidentified regions, the largest of which contains 7.2% TRR, 0.018 mg/kg

Table 7-35 Distribution of [^{14}C]bixlozone and its metabolites in dry land grown rice after initial organic/aqueous extraction and further 1N acid hydrolysis of the extracted residue. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Grain		Straw		Grain		Straw	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total TRR by combustion (mg/kg)	0.112		0.908		0.078		0.525	
bixlozone	-	-	-	-	-	-	-	-
Dimethyl malonic acid (M132/1)	-	-	-	-	13.9	0.011	14.8	0.078
2-2 dimethyl-3-OH –Propionic acid (M118/1)*	-	-	-	-	21.9	0.017	27.4	0.144
M132/1 & M118/1 conjugate	-	-	-	-	12.8	0.010	26.9	0.141
2,4-Dichlorobenzoic Acid (M190/1)	38.3	0.043	67.5	0.613	-	-	-	-
bixlozone-Dimethyl-Malonamide (M289/2)	7.9	0.009	12.7	0.115	1.7	0.001	-	-
bixlozone-3-OH-Propanamide (M275/1)	-	-	-	-	0.4	<0.001	-	-
Unknown	25.8[a]	0.029[a]	12.3[b]	0.112[b]	23.6[c]	0.020[c]	21.6[d]	0.114[d]
Total 'identified'	**46.2**	**0.052**	**80.2**	**0.728**	**50.3**	**0.040**	**69.1**	**0.363**
	Post-extraction solids (PES-1)							
Un-extracted residue after initial extraction:	**28.1**	**0.031**	**7.5**	**0.068**	**25.8**	**0.020**	**9.2**	**0.048**
Cellulase	1.8	0.002	1.7	0.016	4.4	0.003	-	-
Alpha Amylase	2.6	0.003	1.0	0.009	3.6	0.003	-	-
Pectinase	1.3	0.001	1.0	0.009	2.1	0.002	-	-
Protease	0.9	0.001	0.3	0.003	1.0	0.001	-	-
Acid (1N HCl)	7.8	0.009	-	-	7.9	0.006	-	-
Base (1N NaOH)	5.9	0.006	-	-	3.4	0.003	-	-
Acid (6N HCl)	-	-	-	-	-	-	-	-
Base (6N NaOH)	-	-	-	-	-	-	-	-
Un-extracted residue after exhaustive extraction: (final PES)	6.6	0.007	3.7	0.033	7.7	0.006	-	-

' - ' considered but not detected (no associated radioactivity)

* co-eluting peaks with retention time varying from 4.13-5.38 (for the purposes of this table, the two peaks identified in the study report have been summed)

a – Includes 6 unidentified regions, the largest of which contained 7.0% TRR, 0.008 mg/kg

b – Includes 2 unidentified regions, the largest of which contained 7.3% TRR, 0.066 mg/kg
c – Includes 9 unidentified regions, the largest of which contained 6.7% TRR, 0.005 mg/kg
d – Includes 6 unidentified regions, the largest of which contained 4.8% TRR, 0.025 mg/kg

Storage stability investigations-results

No storage stability comparisons were made for rice within this metabolism study (e.g. chromatogram comparisons for extracts analysed initially and later on in the study).

Translocation and proposed metabolic pathway

The unchanged parent bixlozone was not detected in any of the rice commodities analysed showing that the compound is readily metabolised into various metabolite compounds. The total radioactive residue uptake and translocation into the dry land or paddy rice grain amounted to up to 0.112 mg/kg. Significantly higher levels of radioactivity was observed in the straw than in the corresponding grain samples for both label and both rice types.

When the results from both labels are considered together the data demonstrate consistent metabolic pathways in rice straw and grain. The proposed metabolic pathway is outlined in Figure 7-6.

In dry land rice the main route of degradation in grain was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound, to form the metabolites 2,4-dichlorobenzoic acid (M190/1) in the phenyl labels and 2,2-dimethyl-3-hydroxy-propionic acid (M118/1) and dimethyl malonic acid (M132/1) in the carbonyl-label. The conjugates of M118/1 and M132/1 were also observed in significant portions in the carbonyl labelled samples.

Metabolites M275/1 and M289/2 were found in both the phenyl and carbonyl labelled work, in both grain and straw. In paddy rice grain samples, the metabolic pathway was similar to dry land rice with the oxidative ring opened metabolites forming the majority of the TRR. The reduction product M275/1 (bixlozone-3-hydroxy propenamide), which was not detected above <0.001 in dry land rice samples was observed in the paddy rice samples for both labels at *ca* 12-13% TRR.

Conclusion

The metabolism of bixlozone was investigated in dry land and paddy rice by applying phenyl-labelled or carbonyl-labelled bixlozone. The overall total radioactive residue levels (TRR) in dry land rice in the phenyl-labelled grain and straw were 0.112 and 0.908 mg/kg respectively and in the carbonyl-label grain and straw the levels were 0.078 and 0.525 mg/kg respectively. In paddy rice the overall residue levels (TRR) in the phenyl label were 0.077 and 0.712 mg/kg in grain and straw respectively and in the carbonyl label the levels were 0.038 and 0.243 mg/kg in grain and straw respectively. The TRR for straw and grain were similar with both labels however a difference was observed between dry land and paddy rice. Higher levels of TRR are observed in the dry land rice samples.

For dry land rice, in both labels solvent extractability was high for straw (>90% TRR) and in grain the extractability was lower at *ca*. 73% TRR. In all commodities the non-extractible PES contained less than 10% TRR after sequential enzyme, acid and base hydrolysis steps, indicating efforts to release radioactivity.

For paddy rice, in both labels solvent extractability was high for straw (>88% TRR) and in grain the extractability was slightly lower at around 55 and 73% TRR for the phenyl and carbonyl labels respectively. In all commodities the non-extractible PES contained less than 16% TRR (0.006 mg/kg grain) and less than 0.035 mg/kg in straw after sequential enzyme, acid and base hydrolysis steps, indicating efforts to release radioactivity.

Metabolism of bixlozone in rice includes primarily oxidative ring opening. For both labels, unchanged parent bixlozone was not detected in any of the commodities. In all commodities, the metabolites 2,4-dichlorobenzoic acid (M190/1), dimethyl malonic acid (M132/1) and 2,2-dimethyl-3-hydroxy-propionic acid (M118/1) accounted for the majority proportion of the identified radioactive residues.

Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in foliar applied rice. Similar pattern was observed in both paddy and dry land rice. Overall, metabolism of bixlozone in rice, and by extrapolation, in the cereal group is considered well-elucidated. Some differences in metabolism in rice and wheat metabolism studies were observed; this might in part be reflective of the use of some different reference standards used in the rice versus wheat metabolism study. The metabolic profile information for cereals should be taken from both the wheat and rice metabolism studies. HSE has the following remarks/observations based on this rice metabolism study:

- The rice metabolism study has enabled a further elucidation of the metabolic pathway in cereals (additionally to the wheat metabolism). There were analytical challenges with the analysis of 2,2-dimethyl-3-hydroxy-propionic acid (M118/1) and dimethyl malonic acid (M132/1) (both determined as conjugates as a co-eluting peak), and free 2,2-dimethyl-3-hydroxy-propionic acid (M118/1) was determined as two peaks. This was explained by the applicant as follows: '*The predicted pKa of the acid moiety in 2,2-dimethyl-3-hydroxypropionic acid is 4.55 which is close to the pH of the mobile phase*

(pH 5.6). Therefore 2,2-dimethyl-3-hydroxypropionic acid peak may split into ionised and un-ionised forms which can result in two separate peaks in the chromatogram.' Despite these challenges, this study did involve a more detailed investigation of the presence of dimethyl malonic acid (M132/1), dimethyl malonic acid, compared to the investigations done in other plant metabolism studies, such as the wheat study. Dimethyl malonic acid (M132/1) was identified as a major metabolite in rice (cereals) grain and straw.

- Whilst metabolite dimethyl malonic acid (M132/1) was sought and found in rice, one of the major wheat metabolites 5'-hydroxy bixlozone (M289/3) identified in the wheat metabolism study was not sought after or mentioned in the rice metabolism study. [As stated above, the metabolic profile information for cereals should be taken from both the wheat and rice metabolism studies, as different reference standards were included in the different studies].

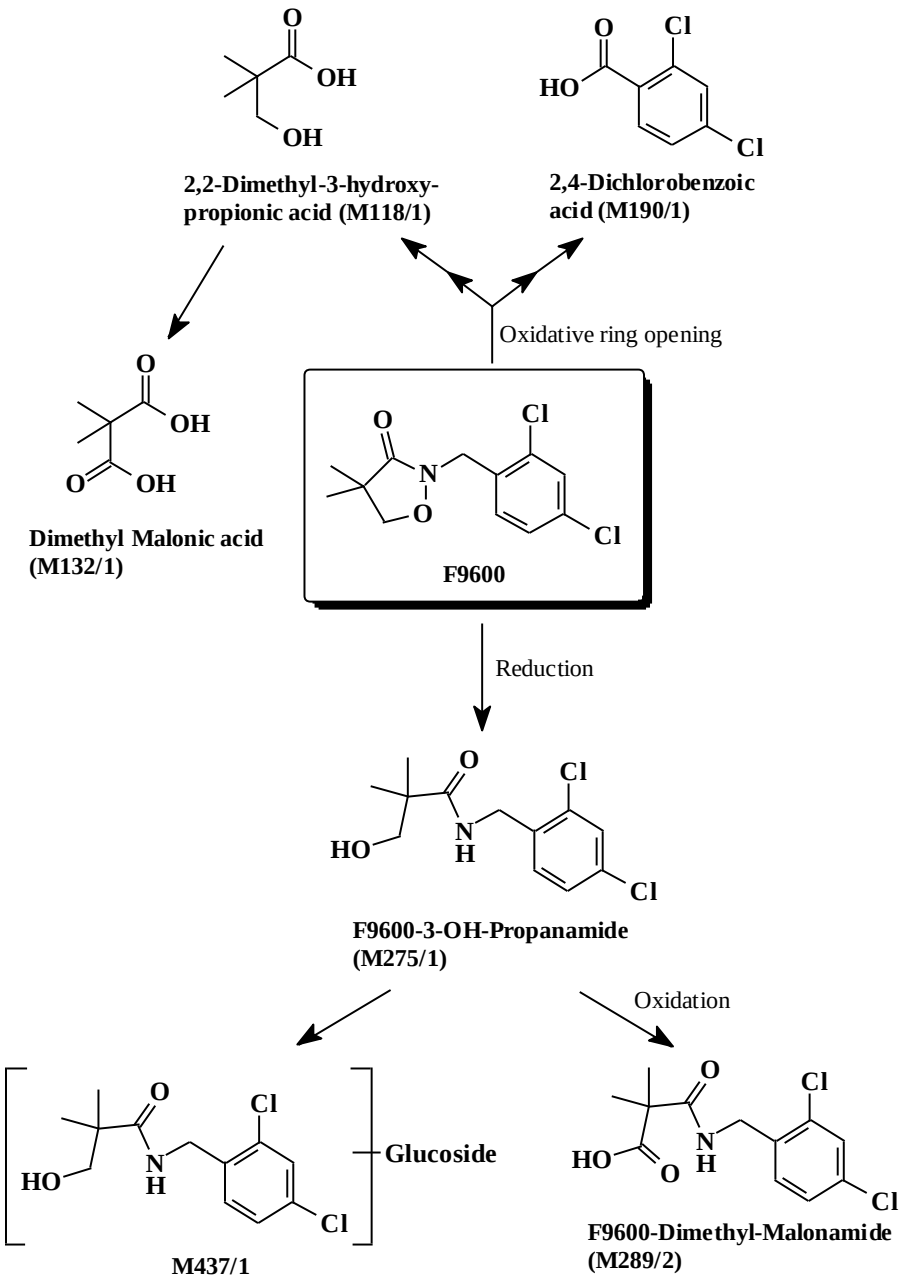
- The methodology for the further extraction of post extraction solids in the study report was not fully clear (in the study text). The results tables were used by HSE as a guide as to the sequential steps that were taken. For example, 6N acid and 6N base treatments were included in the tables that were not explained in the methods section.

- As per other primary crop metabolism studies, the overall (low) amounts of radioactivity identified in the grain (39 to 59%TRR, and up to 0.05 mg/kg) were to an extent perhaps due to the low level of residues found in grain. The unextracted radioactivity was well characterised by numerous sequential extraction steps, and each 'released' fraction in grain was small. The extracted unknown regions were given a measured residue level, the largest of which in grain was 0.008 mg/kg (most were much smaller). There were similar challenges in straw, although a markedly greater degree of initial extractability of residues was observed in straw compared to grain. As per other metabolism studies, some of the residues, at least in grain, might possibly be incorporated with natural plant constituents (as supported by the sequential enzymatic work which included use of cellulase).

- The metabolite distribution tables presented in this evaluation were (as per the heading tables) for the profile of metabolites in the acid hydrolysed extract when the initial solvent extraction had been treated with acid (1N acid hydrolysis). The integration of the peaks in the organic extract was also presented in the study report figures but data not prepared into a metabolite distribution table. The applicant stated that separate tables for peaks in the organic extracts could be prepared, however, due to the complexity of multiple conjugates of the same aglycone, it would prohibit assigning specific metabolite designation and accurate values (mg/kg). HSE agrees that the metabolite distribution data presented in the rice report from acid hydrolysis of the entire organic extract presents a detailed assessment of metabolites in rice.

- Further data on the stability of incurred primary crop residues in the metabolism context is presented at the end of section B.7.2.1 and a discussion of storage stability of residues is presented in Vol 1, Section 2.7.2.

Figure 7-6 Applicant's proposed metabolic pathway for bixlozone (F9600) in rice



Storage stability of residues - additional studies (2021 reports) on storage stability of incurred residues for samples obtained in the metabolism context

The main metabolism studies, on primary crops, rotational crops, and livestock (hen and goat), all involved undertaking the studies over a longer period than is desirable for the conduct of radiolabelled metabolism studies. OECD test guidelines on metabolism studies state that "Storage stability data are not normally necessary for samples analysed within 6 months of collection, provided evidence is given that attempts were made to limit degradation of residues by appropriate storage of matrices and extracts during the analytical portion of the study". In the case of all the bixlozone metabolism studies, the samples were stored in the freezer typically (as frozen samples, homogenised powder, or extracts) and the storage details and dates of storage were provided in each of the studies.

The OECD test guidelines on metabolism studies state that where storage stability does need to be addressed:

> "Determinations as to whether sample integrity was maintained during collection, sample preparation, and storage should be made. Such analyses should show that the basic profile of radiolabelled residues has not changed throughout the duration of the study. It is impossible to spike samples before the identity of the residue and the length of time needed for metabolism studies are known."
>
> "In those cases where a metabolism study cannot be completed within six months of sample collection, evidence should be provided that the identity of residues did not change during the period between collection and final analysis. This can be done by analyses of representative substrates early in the study and at its completion. The substrate should be the item stored, i.e., if the matrix extract is used throughout the study and the matrix is not extracted later in the study, the stability of the extract should be shown."
>
> "If changes are observed (e.g., disappearance of a particular HPLC peak or TLC spot), additional analyses or another metabolism study with a shorter collection to analysis interval may be necessary."

Time period aspects for each of the metabolism studies is as follows in Table 7-36 (see sections B.7.2 and B.7.6.1 for further information).

Table 7-36 Summary of time period aspects for metabolism studies

	Sampling dates	Time in which analysis on the matrices were completed in this study (post sampling).	Range of dates for example chromatograms available in the metabolism study reports	Times of frozen storage of samples prior to 'initial analysis' stated in the metabolism reports	Dates for signed Reference standards in the metabolism reports
Wheat	June to July 2014	All matrices (grain forage, hay straw) up to 3.4 years [confirmed in applicant email dated 19th August 2019].	Aug 2014 to Nov 2016	Wheat grain analysed at 8.5 months (12 days as raw samples, then as a fine homogenised powder for a further ca. two months, and a further 6 months storage as extract). All other matrices (forage, hay straw) analysed within 6 months.	Various up to Jan 2018, most with dates in 2017, including that for M118/1 (metabolite 2,2-dimethyl-3-hydroxy-propionic acid) – August 2017. The applicant advised that it is possible that some reference standards had standards COA* expire in 2015 and had to be replaced with updated COAs; this detail was not reflected in the reports.
Canola/oilseed rape	June to July 2014	All matrices (straw, forage and seed) up to 2.7 years [confirmed in applicant email dated 19th August 2019].	Sept 2014 to August 2015 For the oilseed plant part there was only one chromatogram available dated Aug 2015	Canola straw was analysed after frozen storage for 8.5 months. The plant material was stored in different forms over this period, for straw - stored frozen as raw agricultural commodity for 16 days, as homogenised powder for a further three days, and the remainder of time stored as extract.	

	Sampling dates	Time in which analysis on the matrices were completed in this study (post sampling).	Range of dates for example chromatograms available in the metabolism study reports	Times of frozen storage of samples prior to 'initial analysis' stated in the metabolism reports	Dates for signed Reference standards in the metabolism reports
				All other matrices (forage, seed) analysed within 6 months.	
Sugar beet	June to October 2014	All matrices (immature tops, mature tops, mature roots) up to 3.3 years [confirmed in applicant email dated 19th August 2019].	Sept 2014 to Jan 2017	All matrices (immature tops, mature tops, mature roots) analysed within 6 months.	Various up to Jan 2018, most with dates in 2017, including that for M118/1 (metabolite 2,2-dimethyl-3-hydroxy-propionic acid) – August 2017. The applicant advised that it is possible that some reference standards had standards COA* expire in 2015 and had to be replaced with updated COAs; this detail was not reflected in the reports.
Rice	October 2015	All matrices (Dryland straw, Dryland grain, Paddy straw, Paddy grain) up to 2.1 years [confirmed in applicant email dated 19th August 2019].	Nov 2015 to March 2017	All matrices (Dryland straw, Dryland grain, Paddy straw, Paddy grain) analysed within 1 month.	
Hen	July 2016	Eggs up to 1.5 years All matrices (fat, muscle and liver) up to 1.6 years	Sept 2016 to March 2018	Eggs analysed at 6.8 months (21 days as raw egg samples, then as a fine homogenised powder for a further ca. two months, and a further 4 months storage as extract). All other matrices (fat, muscle, liver) analysed at 2 months.	
Goat	October 2015	Milk up to 1.5 years	Nov 2015 to March 2017	Milk analysed at 3 months	

	Sampling dates	Time in which analysis on the matrices were completed in this study (post sampling).	Range of dates for example chromatograms available in the metabolism study reports	Times of frozen storage of samples prior to 'initial analysis' stated in the metabolism reports	Dates for signed Reference standards in the metabolism reports
		All matrices (fat, muscle and liver) up to 1.4 years [confirmed in applicant email dated 19th June 2020].		All other matrices (fat, muscle, liver, kidney) analysed at 1 month.	
Rotational crops	April to July 2015	Within 2.4 years – see below this table for further details [confirmed in applicant email dated 19th June 2020	Jan 2015 to Oct 2017	Within 9 months - See below this table for further details	Various up to Jan 2018, most with dates in 2017, including that for M118/1 (metabolite 2,2-dimethyl-3-hydroxy-propionic acid) – August 2017. The applicant advised that it is possible that some reference standards had standards COA* expire in 2015 and had to be replaced with updated COAs; this detail was not reflected in the reports.

*COA = certificates of analysis

Rotational crop metabolism study details are given in Table 7-37.

Table 7-37 Time of frozen storage of samples in rotational crop metabolism study (initial analysis)

Sample	Time of frozen storage of samples (either as frozen harvest samples, homogenised powder, or extracts) between RAC harvest and 'initial analysis'.
30 DAT wheat grain (phenyl)	9 months
120 DAT wheat grain (phenyl)	9 months
30 DAT wheat forage (phenyl)	3 months
120 DAT mature lettuce (phenyl)	4.5 months
30 DAT mature lettuce (carbonyl)	4.5 months
120 DAT mature lettuce (carbonyl)	4.5 months
Other rotational crop matrices for which metabolite identification is reported (for an overview, please see metabolism summary tables in Vol 1)	Within 6 months

Frozen storage up to the time in which analysis on the matrices were completed in this rotational crop metabolism study are given in Table 7-38.

Table 7-38 Time of frozen storage of samples in rotational crop metabolism study (complete analysis)

Sample	Time of frozen storage of samples between RAC harvest and complete analysis
30 DAT wheat forage (phenyl)	2.4 years
120 DAT mature lettuce (phenyl)	2.2 years
30 DAT mature lettuce (carbonyl)	2.2 years
120 DAT mature lettuce (carbonyl)	2.2 years
Other rotational crop matrices for which metabolite identification is reported.	Not specified (applicant did not confirm in their letter response to HSE).

All the metabolism studies stated the dates until 'initial analyses'. HSE sought to obtain more specific information from the applicant about timings of various aspects of the work in each metabolism study to determine whether the main pathways of metabolism had been elucidated in good time and to support the validity of the results of the studies. The response from the applicant lead to some further uncertainties in the understanding of the data.

The enquiry to the applicant also sought to find out whether there were further chromatographic data to inform the situation and that could help support storage stability comparisons. The applicant responded that the analytical contractor had supplied mostly detector raw data to the applicant, and it seemed that determining the extent of the work near to the 'initial HPLC analyses' versus later on in the study from chromatographic raw data was not possible. The attempts to provide support for stability from the existing data had proved challenging. The applicant stated that dates on the chromatograms in the reports matched with dates recorded in laboratory notebooks, but that chromatograms selected for inclusion in the final reports were done to highlight chromatographic separation (rather than selection according to run dates).

The range of dates of chromatograms in the metabolism reports seem to indicate that the analytical work was conducted over a range of timing rather than all close to the time of the 'initial analyses'. For example, the available chromatogram for the oilseed crop part (canola/oilseed rape metabolism study) was for analysis over a year after sampling. For the wheat study - cereal grain, the carbonyl grain example chromatograms were close to the time of 'initial analysis', 8.5 months after samples were harvested, and the phenyl grain example chromatograms were for analysis 1 year and 8 months after the time of 'initial analyses'.

Some limited storage stability investigations were conducted within the scope of some of the metabolism reports : wheat, sugar beet, and hens. See 'storage stability investigations -results' in sections B.7.2.1.1 (wheat), B.7.2.1.3 (sugar beet), and B.7.2.2 (hens). HSE concluded from these that it was difficult to draw firm conclusions on whether the stability of samples is sufficiently maintained over the long course of the studies.

In order to further address this issue, the applicant provided some new GLP reports providing retrospective stability analysis of residues in commodities of wheat, canola, sugar beet and rice. Based on the data submitted overall, the applicant is of the viewpoint that the residues metabolism package as a whole can be relied upon to understand the metabolic pathway of bixlozone and to demonstrate the lack of exposure clearly excluding consumer and livestock health risk.

These new (retrospective) storage stability reports (2021) are evaluated by HSE below.

Stability of Incurred 14C-F9600 Residues in Wheat. FMC No. 53485, Frontage Document No. 037955-1. Frontage Laboratories, Inc., Concord, OH.

Author: McClanahan R. 2021.

GLP- yes

The study considered plant sample material that had been obtained from the original metabolism wheat study (homogenised and frozen from the original wheat metabolism study) and these were analysed in a new laboratory (samples were kept frozen until processing and analysis). Samples of forage, grain, straw and hay for both radiolabels (carbonyl and phenyl) were included.

In this new study the samples (6 to 15 g sample) were re-extracted using essentially the same extraction procedures employed in the original metabolism study. A combined extract was produced from the various solvent extractions. A portion of combined solvent extract was concentrated and analysed by HPLC with radioactivity detection (HPLC/RAD). The recovery for this concentration step was determined and shown to be 72 and 74% for grain, and others > 87%. The HPLC/RAD profiles of the samples were compared to the original profiles to evaluate the storage stability of the residues. These results were obtained in this new study in August 2020, determined 4- 6 years after the analyses that were done at the time of the original metabolism study (conducted by the different laboratory). A number of reference standards were run by the new laboratory in this new study.

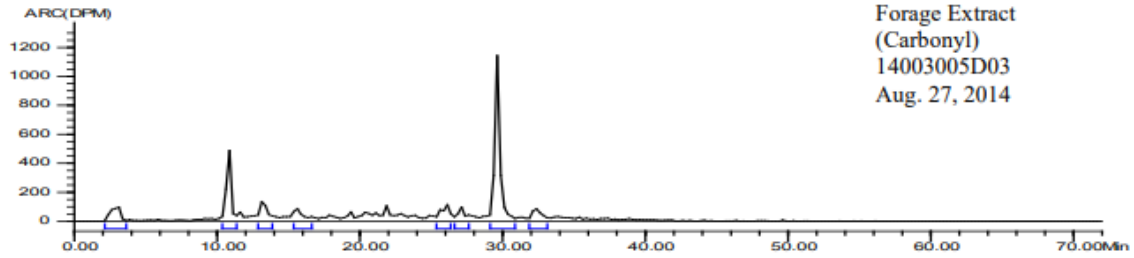
Two examples of comparative chromatograms are presented below. In the top pane of the Figures there is an HPLC/RAD chromatogram from the original laboratory (metabolism study) and in the bottom pane there is an HPLC/RAD chromatogram obtained in this study (different laboratory).

The results are considered variable, although it is not possible to draw conclusions beyond general statements. The below results presented for wheat forage (carbonyl label) are better than the comparison for wheat grain (phenyl label). Chromatograms were not labelled to name metabolite peaks. Absolute amounts of residues in the samples are not known (in the new study, a determination of the samples by combustion was not made nor were the mg/kg levels of

radioactivity in the extracts determined). As such, the analysis was not comparing mg/kg amounts of radioactive residues for 'before' and 'after'. However the chromatograms for the grain (phenyl) example suggests (from the ratio of the main peak to the baseline variation) that there has been a considerable loss of amount of radioactive residue analysed. In the top frame (grain, phenyl label) there were a number of peaks that stood out from the baseline (6), and in the bottom frame (new analysis) there were two it seems.

Figure 3: HPLC/RAD Chromatograms of Treated Forage Extracts (CA Label)

From Figure H-9, HPLC radio-chromatograms of Treated Forage Extracts from XBL: 14003 – RPT03661 Sponsor Tracking Number: 2013MET-ISX1098 (page 198)



HPLC of Wheat Forage Extract (CA label): Sample TRT-CA-HR-forage-(X4171:16A) 9_1_20_wheat_forage 01-14c-004

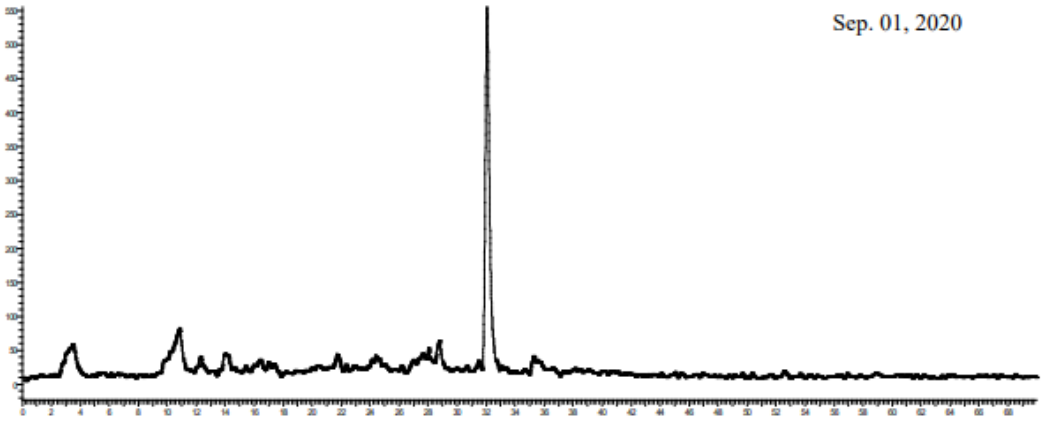
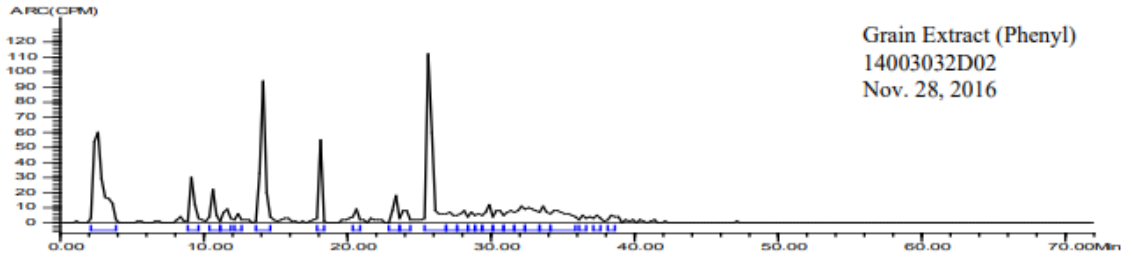
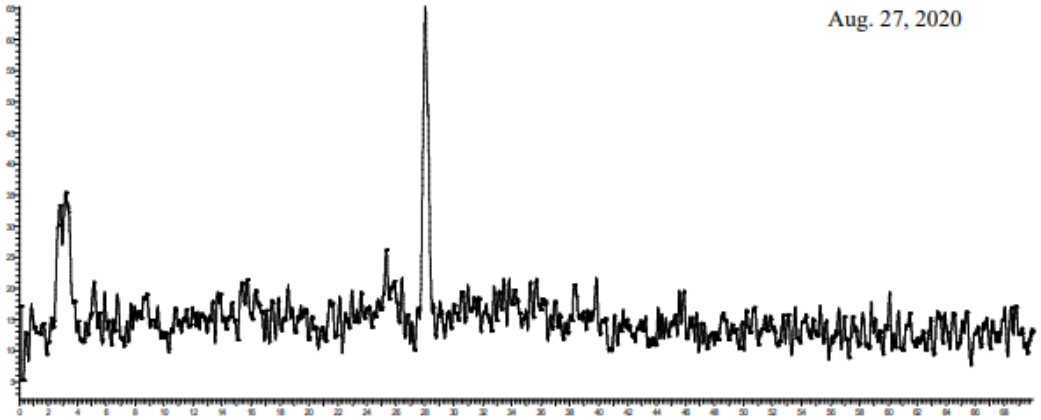


Figure 8: HPLC/RAD Chromatograms of Treated Grain Extracts (PH Label)

From Figure H-8, HPLC radio-chromatograms of Treated Grain Extracts from XBL: 14003 – RPT03661 Sponsor Tracking Number: 2013MET-ISX1098 (page 197)



HPLC of Wheat Grain Extract (PH label): Sample TRT-PH-HR-grain-(X4171:39B) 8_27_20_wheat_grain 01-14c-007



Stability of Incurred 14C-F9600 Residues in Canola. FMC No. 53483, Frontage Document No. 037967-1. Frontage Laboratories, Inc., Concord, OH.

Author: McClanahan R. 2021.

GLP-yes

The study considered plant sample material (forage and straw, both carbonyl and phenyl labels) that had been obtained from the original metabolism canola/oilseed rape study (homogenised and frozen from the original canola/oilseed rape metabolism study) and these were analysed in a new laboratory (samples were kept frozen until processing and analysis).

In this new study the samples (15 g straw sample, 40 g forage) were re-extracted using essentially the same extraction procedures employed in the original metabolism study. A combined extract was produced from the various solvent extractions. A portion of combined solvent extract was concentrated and analysed by HPLC with radioactivity detection (HPLC/RAD). The recovery for this concentration step was determined and shown to be 71 - 85%. The HPLC/RAD profiles of the samples were compared to the original profiles to evaluate the storage stability of the residues. These results were obtained in this new study in September 2020, determined 5 - 6 years after the analyses that were done at the time of the original metabolism study (conducted by the different laboratory). A number of reference standards were run by the new laboratory in this new study.

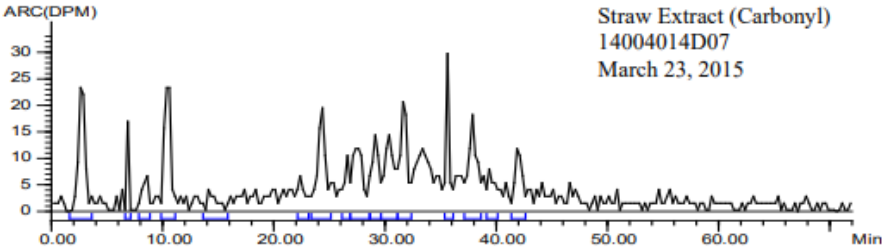
Two examples of comparative chromatograms (phenyl and carbonyl) are presented below. In the top pane of the Figures there is an HPLC/RAD chromatogram from the original laboratory (metabolism study) and in the bottom pane there is an HPLC/RAD chromatogram obtained in this study (different laboratory).

It is not possible to draw conclusions beyond general statements. Chromatograms were not labelled to name metabolite peaks. Absolute amounts of residues in the samples are not known (in the new study, a determination of the samples by combustion was not made nor were the mg/kg levels of radioactivity in the extracts determined). As

such, the analysis was not comparing mg/kg amounts of radioactive residues for 'before' and 'after'. However, the chromatograms for forage and straw show that there are a series of peaks that can be seen to remain in the 'new' compared to the original profiles. Seed was not analysed in this study (TRRs in seed were low in the original study).

Figure 5: HPLC/RAD Chromatograms of Treated Straw Extracts (CA Label)

From Figure H-8, HPLC radio-chromatograms of Treated Canola Extracts from XBL: 14004 - RPT04034 Sponsor Tracking Number: 2013MET-ISX1096 (page 157)



Canola Straw (CA label): Sample TRT-CA/HR-Straw-(X4172:44C)

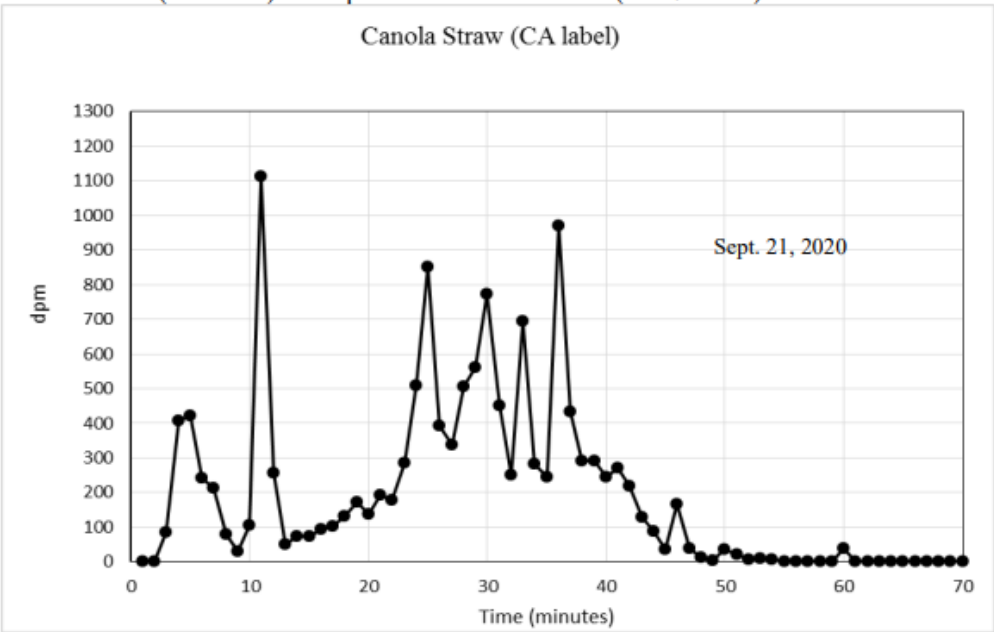
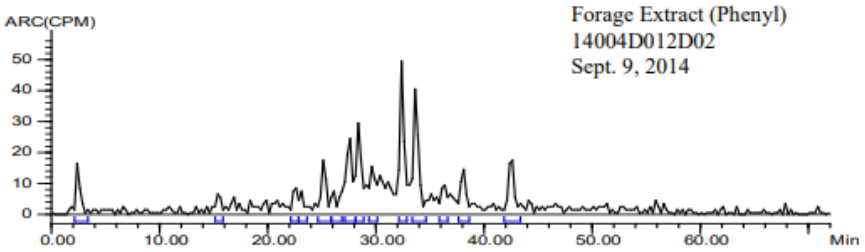
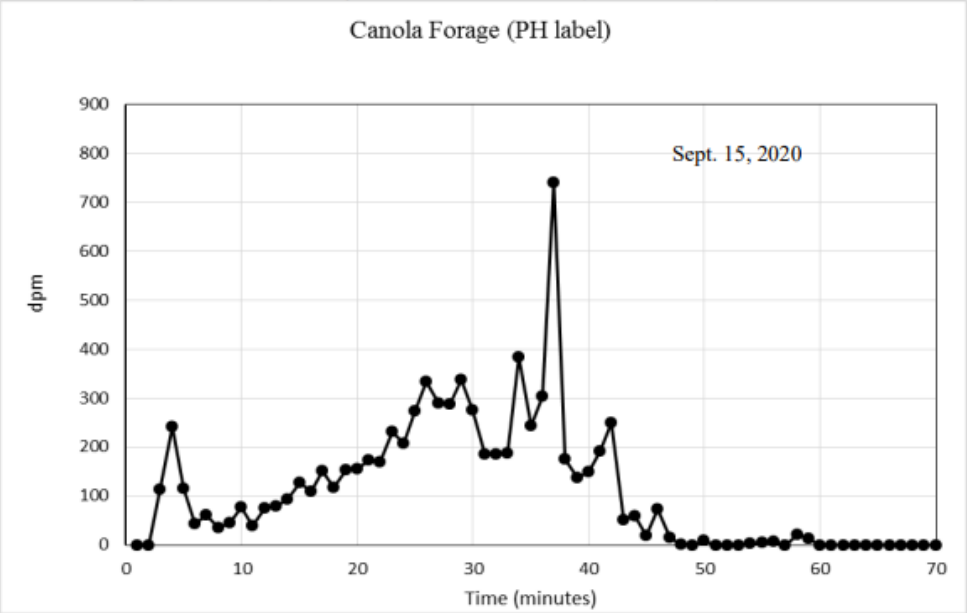


Figure 2: HPLC/RAD Chromatograms of Treated Forage Extracts (PH Label)

From Figure H-1, HPLC radio-chromatograms of Treated Canola Extracts from XBL: 14004 – RPT04034 Sponsor Tracking Number: 2013MET-ISX1096 (page 150)



Canola Forage (PH label): Sample TRT-PH/HR-For-(X4172:32A)



Stability of Incurred 14C-F9600 Residues in Sugar Beets. FMC No. 54930, Frontage Document No. 037968-1. Frontage Laboratories, Inc., Concord, OH.

Author: Miner P, 2021.

GLP-yes

The study considered plant sample material (mature tops and roots, both carbonyl and phenyl labels) that had been obtained from the original metabolism sugar beet study (homogenised and frozen from the original sugar beet metabolism study) and these were analysed in a new laboratory (samples were kept frozen until processing and analysis).

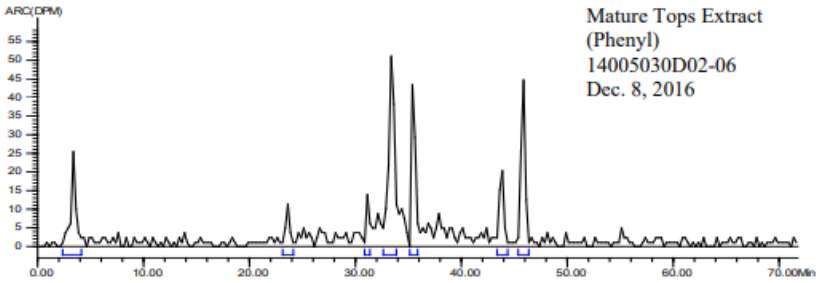
In this new study the samples (20-40 g roots, 50-80 g tops) were re-extracted using essentially the same extraction procedures employed in the original metabolism study. A combined extract was produced from the various solvent extractions. A portion of combined solvent extract was concentrated and analysed by HPLC with radioactivity detection (HPLC/RAD). The recovery for this concentration step was determined and shown to be 95-100%. The HPLC/RAD profiles of the samples were compared to the original profiles to evaluate the storage stability of the residues. These results were obtained in this new study during August to October 2020, determined 4 - 6 years after the analyses that were done at the time of the original metabolism study (conducted by the different laboratory). A number of reference standards were run by the new laboratory in this new study.

Two examples of comparative chromatograms (phenyl and carbonyl) are presented below. In the top pane of the Figures there is an HPLC/RAD chromatogram from the original laboratory (metabolism study) and in the bottom pane there is an HPLC/RAD chromatogram obtained in this study (different laboratory).

It is not possible to draw conclusions beyond general statements. Chromatograms were not labelled to name metabolite peaks. Absolute amounts of residues in the samples are not known (in the new study, a determination of the samples by combustion was not made nor were the mg/kg levels of radioactivity in the extracts determined). As such, the analysis was not comparing mg/kg amounts of radioactive residues for 'before' and 'after'. However, the chromatograms for roots and tops show that there are a series of peaks that can be seen to remain in the 'new' compared to the original profiles, although qualitatively and considering retention times there does not seem to be a good match of all the peaks.

Figure 2: HPLC/RAD Chromatograms of Treated Sugar Beet Mature Tops Extracts (PH Label)

From Figure H-6, HPLC radio-chromatograms of Treated Sugar Beet Mature Tops Extracts from XBL: 14005 – RPT03978 Sponsor Tracking Number: 2013MET-ISX1097 (page 181)



HPLC of Sugar Beet Top Extract (PH label)- TRT-PH/HR-Top (X4173:52A-1,2)

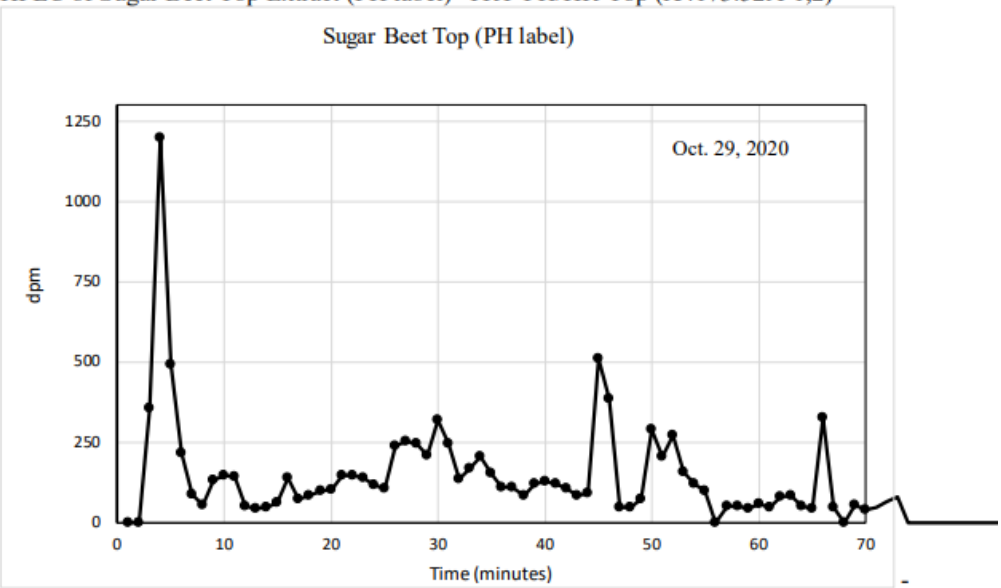
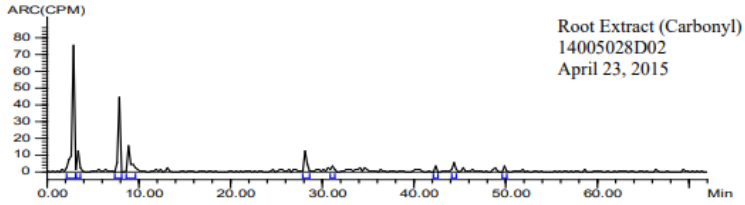
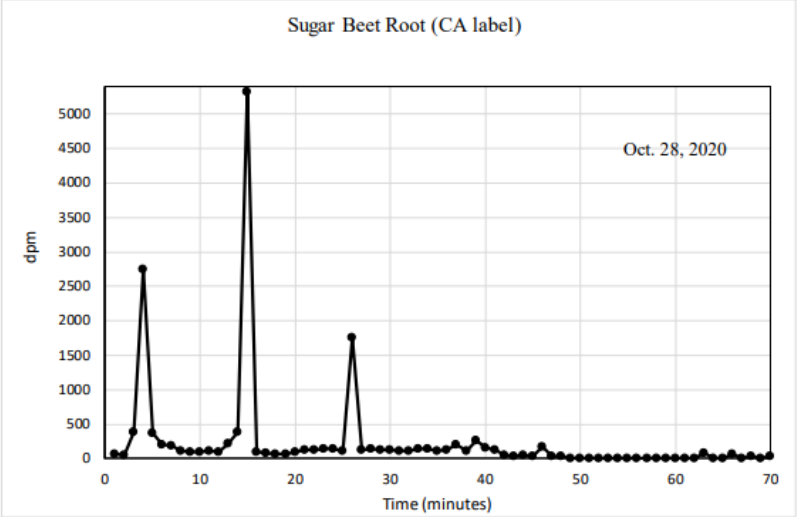


Figure 5: HPLC/RAD Chromatograms of Treated Sugar Beet Mature Roots Extracts (CA Label)

From Figure H-15, HPLC radio-chromatograms of Treated Sugar Beet Roots Extracts from XBL: 14005 – RPT03978 Sponsor Tracking Number: 2013MET-ISX1097 (page 190)



HPLC of Sugar Beet Root Extract (CA label) - TRT-CA/HR-Root (X4173:48A-1,4)



Stability of Incurred 14C- F9600 Residues in Rice. FMC No. 54931, Frontage Document No. 037969-1. Frontage Laboratories, Inc., Concord, OH

Author: Miner P, 2021.

GLP- yes

The study considered plant sample material (grain and straw, both carbonyl and phenyl labels) that had been obtained from the original metabolism rice study (homogenised and frozen from the original rice metabolism study) and these were analysed in a new laboratory (samples were kept frozen until processing and analysis).

In this new study the samples (5 g straw, 20-30 g grain) were re-extracted using essentially the same extraction procedures employed in the original metabolism study. A combined extract was produced from the various solvent extractions. A portion of combined solvent extract was concentrated and analysed by HPLC with radioactivity detection (HPLC/RAD). The recovery for this concentration step was determined and shown to be 95-100%. The HPLC/RAD profiles of the samples were compared to the original profiles to evaluate the storage stability of the residues. These results were obtained in this new study during August to October 2020, determined approximately 5 years after the analyses that were done at the time of the original metabolism study (conducted by the different laboratory). A number of reference standards were run by the new laboratory in this new study.

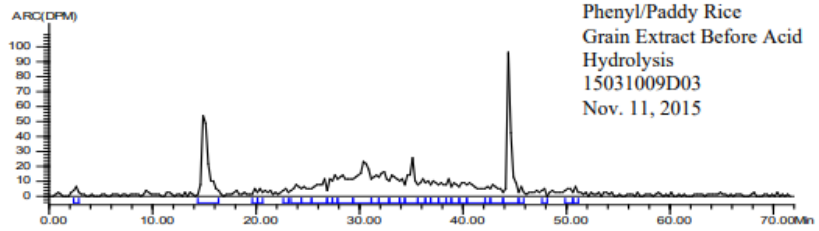
Two examples of comparative chromatograms (phenyl- grain and carbonyl-straw) are presented below. In the top pane of the Figures there is an HPLC/RAD chromatogram from the original laboratory (metabolism study) and in the bottom pane there is an HPLC/RAD chromatogram obtained in this study (different laboratory).

It is not possible to draw conclusions beyond general statements. Chromatograms were not labelled to name metabolite peaks. Absolute amounts of residues in the samples are not known (in the new study, a determination of the samples by combustion was not made nor were the mg/kg levels of radioactivity in the extracts determined). As

such, the analysis was not comparing mg/kg amounts of radioactive residues for 'before' and 'after'. However, the chromatograms for rice grain and straw show that there are main peaks that can be seen to remain in the 'new' compared to the original profiles.

Figure 2: HPLC/RAD Chromatograms of Treated Grain Extracts (PH Label)

From Figure K-16, HPLC radio-chromatograms of Extracted Rice Grain Extracts from XBL: 15031 – RPT03653 Sponsor Tracking Number: 2015MET-ISX1892 (page 236)



Rice Grain (PH label)- TRT-PH/PR/HR-Grain X4625:42A

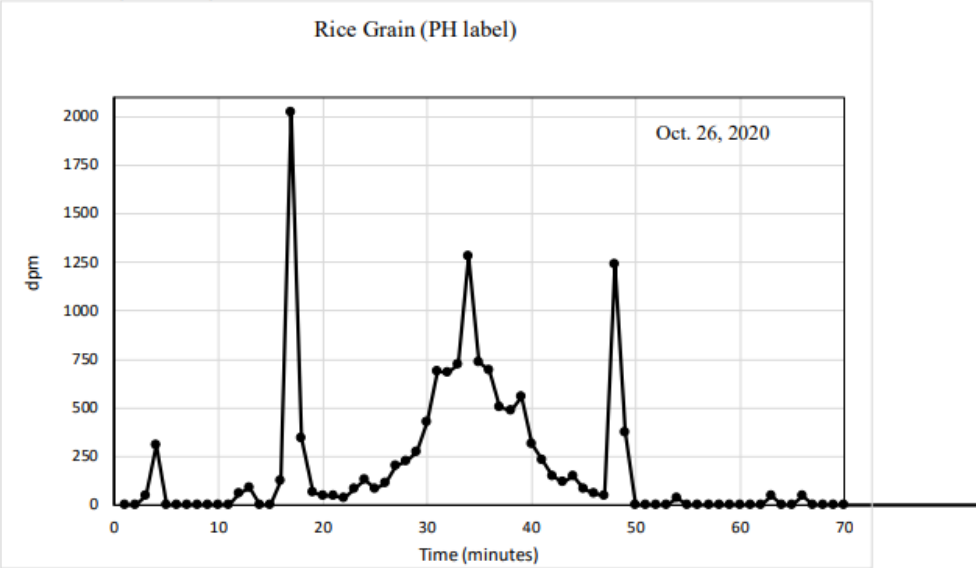
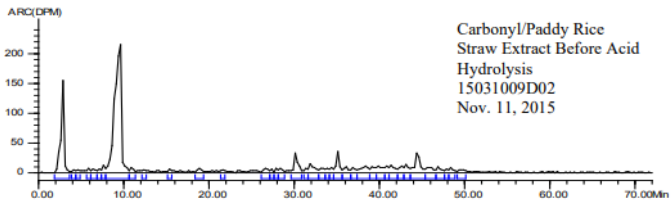
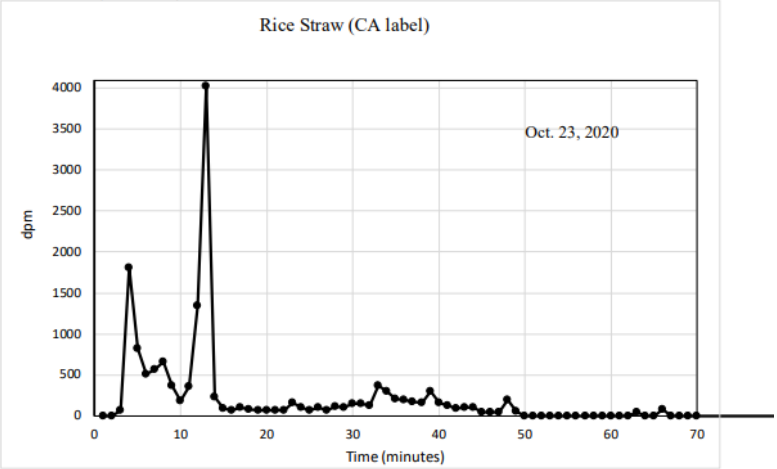


Figure 5: HPLC/RAD Chromatograms of Treated Straw Extracts (CA Label)

From Figure K-18, HPLC radio-chromatograms of Extracted Rice Straw Extracts from XBL: 15031 – RPT03653 Sponsor Tracking Number: 2015MET-ISX1892 (page 240)



Rice Straw (CA label) TRT-CA/PR/HR-Straw X4625:47A



Conclusions regarding the retrospective extraction and analysis of plant samples from the primary crop metabolism studies:

The applicant concluded that "The re-extraction and chromatographic analysis of the incurred radioactive commodities following approximately 4-6 years of the initial analyses showed no significant changes in the metabolic profiles, indicating the residues were stable during the storage.". HSE considers that there are certainly radioactive residues that remain after this long period, 4- 6 years, of frozen storage and there is also some evidence of marked loss of residues (e.g. wheat grain, where the chromatograms suggests [from the ratio of the main peak to the baseline variation] that there has been a considerable loss of amount of radioactive residue analysed). Quantitative analysis is not available, and to a varying extent there are some qualitative differences in metabolite peaks (chromatograms show patterns though are not labelled to identify specific peaks). Some chromatographic comparisons are better than others. As such HSE considers it is not possible to conclude that these data demonstrate good stability of residues in the radiolabelled metabolism context, when comparing these samples re-extracted and analysed much later on in time after frozen storage.

Please see Vol 1 for a further discussion on stability of bixlozone residues taking all storage stability information together (metabolism context- above) and 'cold' (non-radiolabelled) freezer storage stability of residues [presented in section B.7.1].

B.7.2.2. Poultry

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.2.2-01, [redacted], 2019
Title:	[^{14}C]F9600: Nature of the residue in livestock: Metabolism of F9600 in laying hens
Report No.:	15171 – RPT04349 (Report amendment date: December 23, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 503 Metabolism in Livestock, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996)
GLP	yes

Materials and methods

Materials

1. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42475
Radiochemical Purity:	99.9% (nominal); 99.9% (certified)
Specific Activity:	(bulk) 60 mCi/mmol; 8.04 MBq/mg (nominal) 10 mCi/mmol; 1.34 MBq/mg Day 1-9 dosing (actual): 9.84 mCi/mmol; Day 10-13 dosing (actual): 9.96 mCi/mmol;

2. Test Material

Test Material:	[Carbonyl –C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42476
Radiochemical Purity:	99.9% (nominal); 99.9% (certified)
Specific Activity:	(bulk) 59 mCi/mmol; 7.91 MBq/mg (nominal) 10 mCi/mmol; 1.34 MBq/mg Day 1-9 dosing (actual): 9.66 mCi/mmol; Day 10-13 dosing (actual): 9.98 mCi/mmol;

Methods

Test System

The metabolism of bixlozone was investigated in laying hens (breed *Hy-Line Brown*) following repeated oral administration of ^{14}C-bixlozone, labelled either in the dichloro-phenyl ring (phenyl-label) or on the carbonyl carbon of the dimethyl-isoxazolidin-3-one ring (carbonyl-label) (see Figure 7-2). Hens were housed individually in metabolism cages and a lighting cycle was provided (17 hours light, 7 hours darkness). The hens were provided with daily feed (19 Layer A-19-503 custom mix layer ration) and water was given *ad libitum*.

During the study, the hens were in good general health throughout the dosing and acclimatisation period, and body weights were maintained through the study.

Dosing

20 hens were dosed with radiolabelled bixlozone for 13 consecutive days (10 hens were dosed with the phenyl-label and 10 with the carbonyl-label) following an acclimatisation period of 18 days. The test items were prepared in gelatine capsules containing 2.25 mg of radiolabelled bixlozone, orally administered (once daily in the morning after egg collection). A further five control hens were dosed with a single daily blank capsule containing lactose. The average weight of the hens was 1.95 (phenyl group) to 1.98 kg (carbonyl group). Feed intake varied, but on average was 0.109 kg dry weight feed per day.

The dose regime was as follows:

Phenyl labelled: daily dose of 19.56 mg/kg food consumed (dry weight equivalent)

corresponding to 1.15 mg/kg bw/d

corresponding to 575 N (considering animal dietary burden, see Volume 1, section 2.7.5)

Carbonyl labelled: daily dose of 19.36 mg/kg food consumed (dry weight equivalent)

corresponding to 1.08 mg/kg bw/d.

corresponding to 540 N (considering animal dietary burden, see Volume 1, section 2.7.5)

Sampling

Eggs were collected twice daily (evening and prior to the morning dosing). The whole eggs were pooled by group and collection time, cracked and the samples weighed. Eggs were homogenised and stored frozen until analysis.

Excreta samples (and cage wash) were collected daily, prior to the morning dosing.

The hens were sacrificed 6-8 hours after the final dose and tissue samples of skin with attached fat, omental fat, subcutaneous fat, breast muscle, leg/thigh muscle, liver were collected.

All samples were stored at ca -20 °C until shipment and at *ca* -20 °C between frozen shipment and processing (tissue samples were processed into homogenized powder form under liquid nitrogen) and analysis.

Although samples of gastrointestinal (GI) tract with contents, whole blood and the remaining carcass were also collected, results for these were not provided.

Prior to onward analysis, the fat samples (skin fat, omental fat, and subcutaneous fat) were combined (in equal weight proportions), so the overall results of the characterisation and metabolite analysis are for the pooled fat. The TRRs were also determined for both pooled fat and also the separate fat types (skin, omental, and subcutaneous).

Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 6 months of samples being taken (ca. 6.8 months for egg samples, and 2 months for other samples taken). The latest analyses of samples was conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Excreta and egg samples were assayed by combustion analysis using an oxidiser. Cage wash was assayed by LSC. Combustion analysis for the determination of the TRR in tissue samples was performed using finely homogenized/ground tissues (liver, egg, fat and muscle). Triplicate aliquots (*ca*. 50-200 mg) of each representative tissue sample were used for combustion. The TRR was determined using biological sample oxidizer and measured evolved $^{14}CO_2$ by LSC.

Extraction

Samples for both the phenyl- and the carbonyl-label were extracted in the same manner, as outlined below for each commodity type:

Tissues (fat, muscle, liver): Tissue samples were first extracted by blending in water and then methanol. The resulting mixture was centrifuged, and the remaining solids were then re-blended with a solution of 1:1 water/methanol and centrifuged. All extracts were combined, assigned Ext-1 fraction, and assayed by LSC and analysed by HPLC. The remaining PES was combusted and analysed for radioactivity by LSC.

Microwave extraction of PES (fat, muscle, liver): PES samples were subjected to microwave extraction by using Mars 6.01. PES samples were mixed with 25-40 mL IPA: 1N HCl in water (1:1, v/v) and placed in the microwave with the processing conditions as 10 min ramp to 130 °C and hold for 15 mins. The extraction mixture was cooled and centrifuged and extraction was repeated once more. Duplicate aliquots were taken for LSC counting. The supernatant was concentrated to dryness and reconstituted in acetonitrile: water. The remaining PES were air dried and combusted in biological oxidizer.

Microwave extraction released the majority of unextracted residues, and liver (microwave extracts) were used (as well as the initial solvent extracts) for metabolite analysing and profiling. However, the HPLC analysis of microwave extracts from breast muscle, thigh muscle and fat samples did not produce metabolite profile data due to the lower mg/kg levels involved.

Eggs: Egg samples were first extracted with nanopure water and then acetonitrile and sonicated and centrifuged. The remaining solids were re-extracted twice more with water: acetonitrile (4:1), centrifuged and combined with the first extract. Combined extracts were assigned Ext-1 fraction and assayed by LSC. Ext-1 fraction was concentrated and partitioned with DCM. The DCM fraction was separated from the aqueous fraction and concentrated to dryness. The residues were reconstituted in acetonitrile: water (50:50) and assayed by HPLC/LC-MS/MS analysis. An aqueous fraction was stored for further acid hydrolysis (paragraph below). The remaining PES was combusted and analysed for radioactivity by LSC (egg PES was not subject to microwave extraction as residues remaining were low (<10%TRR/<0.01 mg/kg).

Aqueous fractions of eggs were individually subjected to acid hydrolysis (2N HCl) under reflux at 100 °C for ca. 2 hours. After acid hydrolysis, the mixture was cooled down, neutralized, concentrated under nitrogen evaporation and reconstituted in acetonitrile: water (1:1) for further analysis.

The analysis of the egg DCM extract and the acid hydrolysed extract (from the aqueous radioactivity) was used for metabolic identification and profiling.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, dimethyl malonic acid, 2,2-dimethyl-3-hydroxypropionic acid, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, 5-keto-hydrate bixlozone, bixlozone dimethyl malonamide, N-(2,4-Dichlorobenzyl)-2-hydroxy-2-methylpropanamide, 3'-hydroxy-bixlozone, bixlozone 'ring open acid', 2,4-dichlorobenzyl alcohol, 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 2,3-dichlorobenzaldoxime, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report (tabular 'Summary of F9600 reference standards assayed by LC/MS') is as follows: bixlozone (F9600), dimethyl malonic acid (M132/1), 2,4-dichlorobenzaldoxime (M189/1), 5-hydroxy-bixlozone (M289/1), and 4-hydroxymethyl-bixlozone (M289/4). These proposed structures were supported by HCD-MS[7]. Metabolites were characterised by LC/MS and further confirmed by comparison of the HPLC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards.

A list of metabolites confirmed by LC-MS/MS stated in the metabolism study report ('Summary of metabolites of F9600 identified by LC-MS/MS') is as follows: dimethyl malonic acid (M132/1), 2,4-dichlorobenzaldoxime (M189/1), 5-hydroxy-bixlozone (M289/1), and 4-hydroxymethyl-bixlozone (M289/4). These were metabolites identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

The characterization/tentative identification of some metabolites and conjugates of metabolites was performed by TLC and HPLC retention time comparison with reference standards.

Storage stability

As stated above, samples were not all analysed within a period of 6 months since sampling. Samples were stored frozen until processing and analysis. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses':

> Eggs analysed at 6.8 months (21 days as raw egg samples, then as a fine homogenised powder for a further ca. two months, and a further 4 months storage as extract).
>
> All other matrices (fat, muscle, liver) analysed at 2 months.

Frozen storage up to the time in which analysis on the matrices were completed in this study:

> Eggs up to 1.5 years
>
> All matrices (fat, muscle and liver) up to 1.6 years

[7] Higher-energy collisional dissociation with Mass Spectroscopy detection

Storage stability investigations

A comparison was made between chromatograms from the initial and subsequent analyses of the phenyl and carbonyl egg extracts having been stored in the freezer at -20ºC in the interim over a period of about 11 months. See information provided below under '*Storage stability investigations- results'.*

Results and discussion

Total radioactive residue

Levels of ^{14}C-bixlozone recoveries were generally similar for both labels. The recovered radioactivity expressed as % administered dose and mg/kg amounts of radioactivity are summarised for each label in Table 7-39.

For both labels ~93% of the total dose was recovered. It is noted that GI tract (with contents) and carcass and blood were samples taken but not analysed for radioactivity, so these could contribute to the shortfall in the % of the total dose recovered.

The majority of the dosed radioactivity was recovered in excreta for both labels (86/87%). Radioactivity in edible tissues was relatively low with a maximum level of 0.092% and 0.054% of the dose recovered from pooled fat tissue for the phenyl and the liver tissue for the carbonyl labels respectively. In egg samples 0.175% and 0.204% of the total dose was recovered for the phenyl and carbonyl labels respectively.

Table 7-39 Distribution of radioactivity of ^{14}C-bixlozone in hens. (mg/kg = mg parent eq./kg)

Commodity	**% of dosed radioactivity recovered from phenyl label**		**% of dosed radioactivity recovered from carbonyl label**	
	% of dosed	mg/kg (TRR)	% of dosed	mg/kg (TRR)
Excreta	85.9	-	87.2	-
Pooled fat (fat samples taken forward for further analysis)[£]	0.092	0.263	0.021	0.058
Thigh leg muscle	0.016	0.027	0.036	0.058
Breast muscle	0.010	0.019	0.032	0.057
Liver	0.074	0.609	0.054	0.491
Eggs	0.175		0.204	
Cage rinse	6.6	-	5.6	-
Total	***92.9***	**-**	***93.1***	**-**
Eggs (TRR for samples day 10 taken forward for further analysis)		0.089		0.103
Skin fat[£]		0.196		0.062
Omental fat[£]		0.220		0.094
Subcutaneous fat[£]		0.364		0.049

[£] Fat samples- TRRs was determined for fat of the various types sampled. Samples were then pooled (in equal proportions of the different types of fat), TRR determined for the pooled sample, and then taken forward for further analysis, as a pooled sample. The amounts of radioactivity recovered in the fat were low (<0.1% of total dose administered).

Overall, considering both labels, low levels of radioactivity were found in muscle (<0.058 mg/kg). Higher levels were observed for liver (0.609 mg/kg and 0.491 mg/kg for the phenyl and carbonyl labels respectively). In fat samples, significantly higher levels were observed in the phenyl labelled samples (0.263 mg/kg) compared to the carbonyl-labelled samples (0.058 mg/kg). These levels are based on the pooled fat sample (which was taken forward for further analysis). Levels varied slightly according to the fat type sampled, but the pattern of residues being higher in the phenyl label fat samples compared to the carbonyl label samples was the main observation for fat.

Low proportions of the administered dose were recovered in eggs for both labels (up to 0.2%). Plateau levels of radioactive residues in eggs were reached within 7-9 days of dosing, as shown in Table 7-40. Similar amounts were observed for both labels.

Table 7-40 TRR in eggs after administration of ^{14}C-bixlozone to hens. (mg/kg = mg parent eq./kg)

Study day [1)]	TRR from phenyl label		TRR from carbonyl label	
	%TRR	mg/kg	%TRR	mg/kg
1	0.003	0.0138	0.004	0.0191
2	0.005	0.0250	0.005	0.0320
3	0.008	0.0393	0.009	0.0462
4	0.009	0.0512	0.012	0.0619
5	0.013	0.0663	0.015	0.0806
6	0.014	0.0760	0.017	0.0922
7	0.019	0.0846	0.017	0.0994
8	0.018	0.0910	0.018	0.1074
9	0.018	0.0916	0.020	0.1057
10	0.018	0.0887	0.021	0.1033
11	0.018	0.0910	0.020	0.1053
12	0.018	0.0911	0.019	0.1102
13	0.002	0.0833	0.007	0.1300
Necropsy	0.012	0.1014	0.020	0.1305
Total recovery	0.175	-	0.204	-

1) Day 1-12 data represent averages of AM and PM collections. Day 13 data was from PM collection prior to sacrifice

Extractability of residues:

The characterisation in terms of the extractability of the radioactivity is presented in the following tables (Table 7-41 and Table 7-42).

Tissue and egg samples were extracted with methanol/water solvent mixtures from all commodities. High extractability of the ^{14}C residue was seen in egg samples (>90% TRR for both labels). The extractability in tissue samples was lower, 71-76% TRR for the pooled fat, thigh leg muscle and breast muscle. Whereas extractability of liver was lowest at 44.4% and 40.4% for the phenyl and carbonyl labels respectively. Up to 60% TRR (liver) remained as unextracted residues in tissue samples and the PES was subjected to further microwave extraction. Microwave extraction (of PES) was also performed on breast muscle, thigh muscle and fat. Microwave extraction was able to release most of the remaining radioactivity of the PES for all sample types (where the methodology was applied). In liver this microwave extracted radioactivity was significant at circa 50%TRR and metabolic profiling was performed with these liver extracts. Due to lower mg/kg amounts for the microwave extracted radioactivity for these matrices (compared to the liver) metabolic profiling was not successfully performed with breast muscle, thigh muscle and fat. The PES in eggs was attributed to 9.4% TRR in the phenyl-label and 3.1% TRR in the carbonyl-label and no further extractions (e.g. microwave extractions) were performed on the PES.

Table 7-41 Extractability of radioactive residues from poultry tissues. (mg/kg = mg parent eq./kg)

Commodity	**Pooled fat**		**Thigh leg muscle**		**Breast muscle**		**Liver**	
Residue Levels	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Phenyl-label								
TRR		0.263		0.027		0.019		0.609
Extracted residue (methanol/ water)	74.9	0.197	73.5	0.020	71.1	0.014	44.4	0.270
Non-extracted PES following organic extraction- PES-1	25.1	0.066	26.5	0.007	28.9	0.005	55.6	0.339

Microwave extraction of PES-1	29.8	0.078	27.5	0.007	31.5	0.006	51.1	0.311
Carbonyl-label								
TRR		0.058		0.058		0.057		0.491
Extracted residue (methanol/ water)	74.6	0.043	76.2	0.044	74.6	0.043	40.4	0.198
Non-extracted PES following organic extraction- PES-1	25.4	0.015	23.8	0.014	25.4	0.014	59.6	0.293
Microwave extraction of PES-1	27.5	0.016	23.7	0.014	21.8	0.012	49.2	0.242
Final PES	-	-	0.4	<0.001	0.1	<0.001	-	-

Table 7-42 Extractability of radioactive residues from poultry eggs

Commodity	**Label**	**TRR (mg/kg)**	**Organic/ aqueous extract** *(MeOH:H_2O)*		**PES**	
			% TRR	**mg/kg**	**% TRR**	**mg/kg**
Egg	Phenyl	0.089	90.6	0.080	9.4	0.008
	Carbonyl	0.103	96.9	0.100	3.1	0.003

Characterisation and Identification

For all matrices similar results were observed with both radiolabels. Unchanged parent bixlozone was only detected at low levels in pooled fat and eggs samples (<7.5% TRR). The characterisation of the radioactivity is presented in the following tables (Table 7-43-Table 7-47). Metabolites found at >10% TRR are discussed in detail for each matrix below. Structures of the metabolites are outlined in the appendices to Volume 1.

Liver

The most significant metabolite identified after initial organic extraction was M189/1 (10.4% TRR, 0.063 mg/kg) for the phenyl-label. Six other metabolites were detected in the organic extract at a maximum level of 9.5% (0.058 mg/kg). The most significant peak detected after microwave extraction of the PES was postulated to be M175/1 at a level of 12.2% TRR (0.074 mg/kg) and 6 further metabolites were detected at a maximum level of 2.1% TRR (0.013 mg/kg). The unknown regions were presented as a combined total from both extractions. A total of 22.5% TRR of unknown regions were detected, including 9 regions with a maximum individual level of 4.8% TRR (0.029 mg/kg).

In the carbonyl label the most significant metabolite detected after initial organic extraction was M132/1 at 21.2% TRR (0.104 mg/kg). 3 other metabolites were detected at a maximum level of 6.4% TRR (0.031 mg/kg). The microwave extraction of the PES extracted M132/1 at a level of 39.9% TRR (0.196 mg/kg) and a region characterised as M118/1 at 9.3% TRR (0.046 mg/kg). The unknown regions were presented as a combined total from both extractions. The total of all unknowns within the carbonyl label sample were associated with 12.0% TRR (0.059 mg/kg). A total of 2 unknown regions were present, the largest of which contained 9.4% TRR (0.046 mg/kg).

Some of the peaks accounting for <10%TRR included residues tentatively identified as conjugates (M465/1 [conjugate of M289/1], M465/2 and M451/3).

Breast muscle

Poultry breast muscle samples were subjected to organic extraction only. The most significant metabolite after initial organic extraction was characterised as M289/2 (22.3% TRR, 0.004 mg/kg) for the phenyl-label. 6 other metabolites were identified in the organic extract at a maximum level of 6.7% TRR (0.001 mg/kg). A total of 11.2% TRR of unknown regions were detected, including 5 regions with a maximum individual level of 4.1% TRR (0.001 mg/kg).

In the carbonyl label the most significant metabolite identified after initial organic extraction was M132/1 at 59.2% TRR (0.034 mg/kg). 4 other metabolites were detected with a maximum level of 2.6% TRR (0.001 mg/kg). No unknown regions were detected.

Some of the peaks accounting for <10%TRR included residues tentatively identified as conjugates (M465/1 [conjugate of M289/1], M465/2 and M451/3).

Thigh leg muscle

Poultry thigh leg muscle samples were subjected to organic extraction only. The most significant metabolite after initial organic extraction was characterised as M289/2 (26.5% TRR, 0.007 mg/kg) for the phenyl-label. M465/2 was a postulated conjugate at 14.4% TRR (0.004 mg/kg) and M369/1 was a postulated sulphate derivative of M289/1 at 11.3% TRR (0.003 mg/kg). 3 other metabolites were detected in the organic extract at a maximum level of 6.1% TRR (0.001 mg/kg). A total of 9.0% TRR of unknown regions were detected, including 2 regions with a maximum individual level of 2.0% TRR (0.001 mg/kg).

In the carbonyl label the most significant metabolite detected after initial organic extraction was M132/1 at 58.4% TRR (0.034 mg/kg). 3 other metabolites were detected with a maximum individual level of 3.1% TRR (0.002 mg/kg). One unknown region was detected at 3.4% TRR (0.002 mg/kg).

Fat

It is noted that the fat samples that were analysed are pooled samples of skin fat, subcutaneous fat and omental fat. Poultry fat samples were subjected to organic extraction only. The most significant metabolite identified after initial organic extraction was M289/1 (29.3% TRR, 0.077 mg/kg) for the phenyl-label. M189/1 was identified at 12.4% TRR (0.033 mg/kg). Parent bixlozone was detected at a level of 7.2% TRR (0.019 mg/kg). 2 other metabolites were detected in the organic extract with a maximum level of 1.5% TRR (0.004 mg/kg). One unknown region was detected at 7.2% TRR (0.019 mg/kg).

In the carbonyl label the most significant metabolite detected after initial organic extraction was M132/1 at 45.0% TRR (0.026 mg/kg). 3 other metabolites were detected at a maximum level of 2.2% TRR (0.001 mg/kg). A total of 1.3% TRR of unknown regions were detected, including 2 regions with a maximum individual level of 0.8% TRR (<0.001 mg/kg).

Eggs

The most significant metabolite identified after DCM extraction was M289/1 (30.9% TRR, 0.028 mg/kg) for the phenyl-label. 7 other metabolites were detected in the DCM extract at a maximum level of 4.4% (0.004 mg/kg). The acid hydrolysis of the aqueous extracts enabled M175/1 to be characterised at a level of 11.0% TRR (0.010 mg/kg), M451/3 (a conjugate) to be characterised at 10.5% TRR (0.009 mg/kg) and 2 further metabolites with a maximum individual level of 4.7% TRR (0.004 mg/kg). The unknown regions were presented as a combined total from both extraction and acid hydrolysis. A total of 9.9% TRR of unknown regions were detected, including 4 regions with a maximum individual level of 6.8% TRR (0.008 mg/kg).

In the carbonyl label the most significant metabolite detected after DCM extraction was postulated to be M289/4 at 3.8% TRR (0.004 mg/kg). M369/1 (sulphate derivative of M289/1) was also characterised from a peak at 0.6% TRR (0.001 mg/kg) and parent bixlozone was detected at 0.1% TRR (<0.001 mg/kg). The acid hydrolysis of the aqueous extracts showed a peak which was identified M132/1 at a level of 25.8% TRR (0.026 mg/kg) and a peak that was characterised as M118/1 at 10.3% TRR (0.011 mg/kg). The unknown regions were presented as a combined total from both extraction and acid hydrolysis. One unknown region was detected (RT 5.13 to 5.18) which contained 36.5% TRR (0.037 mg/kg). In accordance with OECD 503, significant attempts to identify this region should have been made. The applicant explained their attempts with working with this and other such early eluting polar metabolites, indicating that identification had proved very difficult:

> The applicant referred to the polar nature of carbonyl related metabolic degradation products, and the challenges in identification of low molecular weight polar metabolites resulting from this moiety (including 2,2-dimethyl-3-hydroxymethypivalic acid and dimethyl malonic acid). Additionally, there was very poor MS ionization of that moiety as well as for its smaller (<M118/1) molecular weight metabolic degradants. TLC analyses were attempted with no conclusive results. Additionally, the polar metabolites were isolated and the isolated residues were subjected to derivatization and LC/MS/MS analysis with no conclusive results.

Table 7-43 Distribution of [^{14}C]bixlozone and its metabolites in poultry liver in the organic extracts, and following microwave extraction of the PES (post extraction solids) . (mg/kg = mg parent eq./kg)

Metabolite	Phenyl						Carbonyl					
	Initial Identification				Total (combined organic and microwave extracts)		Initial Identification				Total (combined organic and microwave extracts)	
	Organic extract *(MeOH:H$_2$O)*		Microwave extraction of PES				Organic extract *(MeOH:H$_2$O)*		Microwave extraction of PES			
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total TRR by combustion (mg/kg)	**0.609**						**0.491**					
%TRR	44.4	0.270	51.1	0.311	95.5	0.581	40.4	0.198	49.2	0.242	89.6	0.440
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	-	-	-	-	-	-	9.3	0.046	9.3	0.046
2,4-Dichlorobenzoic acid (M190/1)	5.4	0.033	-	-	5.4	0.033	-	-	-	-	-	-
2,4-dichlorobenzylamine (M175/1)	1.5	0.009	12.2	0.074	13.7	0.083	-	-	-	-	-	-
5-Hydroxy-bixlozone- Glucuronide (M465/1)	9.5	0.058	2.1	0.013	11.6	0.071	6.4	0.031	-	-	6.4	0.031
4-Hydroxy-bixlozone- Glucuronide (M465/2)	3.6	0.022	0.9	0.005	4.5	0.027	-	-	-	-	-	-
bixlozone-3-OH-propanamide-Glucuronide (M451/3)*	-	-	1.1	0.007	1.1	0.007	-	-	-	-	-	-
5-Hydroxy-bixlozone- Sulfate (M369/1)	2.7	0.016	1.9	0.012	4.6	0.028	1.1	0.006	-	-	1.1	0.005
bixlozone-3-OH-Propanamide (M275/1)	-	-	0.5	0.003	0.5	0.003	-	-	-	-	-	-
4-Hydroxy-methyl-bixlozone (M289/4)	-	-	-	-	-	-	-	-	-	-	-	-
5-Hydroxy-bixlozone (M289/1)	-	-	0.3	0.002	0.3	0.002	-	-	-	-	-	-
2,4-dichlorobenzaldoxime (M189/1)	10.4	0.063	-	-	10.4	0.063	-	-	-	-	-	-
Dimethyl malonic acid (M132/1)	-	-	-	-	-	-	21.2	0.104	39.9	0.196	61.1	0.300

bixlozone-dimethyl-malonamide (M289/2)	3.9	0.024	-	-	3.9	0.024	2.1	0.010	-	-	2.1	0.010
Unknowns	-	-	-	-	22.5[a]	0.137[a]					12.0[b]	0.059[b]
Total identified:	**37.0**	**0.225**	**19.0**	**0.116**	**52.1**	**0.317**	**30.8**	**0.241**	**49.2**	**0.242**	**70.7**	**0.346**

*note- this metabolite is referred to as M451/1 in the study report, however as this code has been used in duplicate (sugar beet for a different structure), therefore the metabolite bixlozone-3-OH-propanamide-gluc has been referred to as M451/3 throughout this DAR This was referred to as M451/3 in the updated GLP amendment report for goat, but it had not been updated in the updated GLP amendment report for poultry.

a – Includes 9 unidentified regions, the largest of which contains 4.8% TRR, 0.029 mg/kg b – Includes 2 unidentified region, the largest of which contained 9.4% TRR, 0.046 mg/kg

Table 7-44 Distribution of [^{14}C]bixlozone and its metabolites in poultry breast muscle in the organic extracts. (mg/kg = mg parent eq./kg)

Metabolite	**Phenyl**		**Carbonyl**	
	Organic extract *(MeOH:H_2O)*		**Organic extract** *(MeOH:H_2O)*	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.019**		**0.057**	
%TRR	71.1	0.014	74.6	0.043
bixlozone	-	-	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	2.6	0.001
2,4-Dichlorobenzoic acid (M190/1)	4.2	0.001	-	-
2,4-dichlorobenzylamine (M175/1)	2.4	<0.001	-	-
5-Hydroxy-bixlozone- Gluc (M465/1)	1.4	<0.001	2.1	0.001
4-Hydroxy-bixlozone- Gluc (M465/2)	3.6	0.001	-	-
5-Hydroxy-bixlozone- Sulfate (M369/1)	6.7	0.001	0.3	0.001
5-Hydroxy-bixlozone (M289/1)	0.7	<0.001	-	-
Dimethyl malonic acid (M132/1)	-	-	59.2	0.034
bixlozone-dimethyl-malonamide (M289/2)	22.3	0.004	0.6	<0.001
Unknowns	11.2[a]	0.002[a]	-	-
Total identified:	**41.3**	**0.010**	**64.8**	**0.038**

a – Includes 5 unidentified regions, the largest of which contains 4.1% TRR, 0.001 mg/kg

Table 7-45 Distribution of [^{14}C]bixlozone and its metabolites in poultry thigh leg muscle in the organic extracts. (mg/kg = mg parent eq./kg)

Metabolite	**Phenyl**		**Carbonyl**	
	Organic extract *(MeOH:H_2O)*		**Organic extract** *(MeOH:H_2O)*	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.027**		**0.058**	
%TRR	73.5	0.020	76.2	0.044
bixlozone	-	-	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	3.1	0.002
2,4-Dichlorobenzoic acid (M190/1)	6.1	0.002	-	-
2,4-dichlorobenzylamine (M175/1)	0.9	<0.001	-	-
4-Hydroxy-bixlozone- Gluc (M465/2)	14.4	0.004	-	-
5-Hydroxy-bixlozone- Sulfate (M369/1)	11.3	0.003	1.0	0.001
5-Hydroxy-bixlozone (M289/1)	2.0	0.001	-	-
Dimethyl malonic acid (M132/1)	-	-	58.4	0.034
bixlozone-dimethyl-malonamide (M289/2)	26.5	0.007	1.8	0.001
Unknowns	9.0[a]	0.003[a]	3.4[b]	0.002[b]
Total identified:	**61.2**	**0.018**	**64.3**	**0.038**

a – Includes 2 unidentified regions, the largest of which contains 2.0% TRR, 0.001 mg/kg
b – Includes 1 unidentified region

Table 7-46 Distribution of [^{14}C]bixlozone and its metabolites in poultry fat (pooled sample) in the organic extracts. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl		Carbonyl	
	Organic extract *(MeOH:H_2O)*		**Organic extract** *(MeOH:H_2O)*	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**
Total TRR by combustion (mg/kg)	**0.263**		**0.058**	
%TRR	74.9	0.197	74.6	0.043
bixlozone	7.2	0.019	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	0.6	<0.001
2,4-Dichlorobenzoic acid (M190/1)	-	-	-	-
2,4-dichlorobenzylamine (M175/1)	-	-	-	-
5-Hydroxy-bixlozone- Gluc (M465/1)	-	-	-	-
4-Hydroxy-bixlozone- Gluc (M465/2)	-	-	-	-
bixlozone-3-OH-propanamide-glu (M451/3)*	-	-	-	-
5-Hydroxy-bixlozone- Sulfate (M369/1)	1.5	0.004	0.6	<0.001
bixlozone-3-OH-Propanamide (M275/1)	-	-	-	-
4-Hydroxy-methyl-bixlozone (M289/4)	-	-	-	-
5-Hydroxy-bixlozone (M289/1)	29.3	0.077	-	-
2,4-dichlorobenzaldoxime (M189/1)	12.4	0.033	-	-
Dimethyl malonic acid (M132/1)	-	-	45.0	0.026
bixlozone-dimethyl-malonamide (M289/2)	1.2	0.003	2.2	0.001
Unknowns	7.2[a]	0.019[a]	1.3[b]	<0.001[b]
Total identified:	**51.6**	**0.136**	**48.4**	**0.027**

*note- this metabolite is referred to as M451/1 in the study report, however as this code has been used in duplicate (sugar beet for a different structure), therefore the metabolite bixlozone-3-OH-propanamide-gluc has been referred to as M451/3 throughout this DAR This was referred to as M451/3 in the updated GLP amendment report for goat, but it had not been updated in the updated GLP amendment report for poultry

a – Includes 1 unidentified regions

b – Includes 2 unidentified region, the largest of which contained 0.8% TRR, <0.001 mg/kg

Table 7-47 Distribution of [^{14}C]bixlozone and its metabolites in poultry whole eggs. (mg/kg = mg parent eq./kg) Initial solvent extraction (with water then acetonitrile) released high amounts of radioactivity 91% in phenyl and 97% in carbonyl. DCM extraction (of the initial solvent extract) resulted in DCM and aqueous fraction; the aqueous fraction was subject to acid hydrolysis and analysed (as well as the DCM fraction).

Metabolite	Phenyl						Carbonyl					
	DCM extract (following initial organic solvent extraction)		Acid hydrolysis of the aqueous extracts (separated away from the DCM fraction)		Total (combined DCM and hydrolysis extracts)		DCM extraction		Acid hydrolysis of the aqueous extracts		Total (combined DCM and hydrolysis extracts)	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total TRR by combustion (mg/kg)	**0.089**						**0.103**					
%TRR	**45.9**	**0.041**	**44.7**	**0.039**	**90.6**	**0.080**	**5.4**	**0.006**	**91.5**	**0.094**	**96.9**	**0.100**
bixlozone	-	-	-	-	-	-	0.1	<0.001	-	-	0.1	<0.001
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	-	-	-	-	-	-	10.3	0.011	10.3	0.011
2,4-Dichlorobenzoic acid (M190/1)	0.5	<0.001	4.7	0.004	5.2	0.004	-	-	-	-	-	-
2,4-dichlorobenzylamine (M175/1)	-	-	11.0	0.010	11.0	0.010	-	-	-	-	-	-
4-Hydroxy-bixlozone- Gluc (M465/2)	4.4	0.004	4.7	0.004	9.1	0.008	-	-	-	-	-	-
bixlozone-3-OH-propanamide-glu (M451/3)*	1.4	0.001	10.5	0.009	11.9	0.010	-	-	-	-	-	-
5-Hydroxy-bixlozone- Sulfate (M369/1)	0.4	<0.001	2.1	0.002	2.1	0.002	0.6	0.001	-	-	-	-
bixlozone-3-OH-Propanamide (M275/1)	1.5	0.001	-	-	1.5	0.001	-	-	-	-	-	-
4-Hydroxy-methyl-bixlozone (M289/4)	2.5	0.002	-	-	2.5	0.002	3.8	0.004	-	-	3.8	0.004
5-Hydroxy-bixlozone (M289/1)	30.9	0.028	-	-	30.9	0.028	-	-	-	-	-	-
2,4-dichlorobenzaldoxime (M189/1)	0.3	<0.001	-	-	0.03	<0.001	-	-	-	-	-	-
Dimethyl malonic acid (M132/1)	-	-	-	-	-	-	-	-	25.8	0.026	25.8	0.027
Unknowns					9.9[a]	0.008[a]					36.5[b]	0.037[b]
Total identified:	**41.9**	**0.039**	**33**	**0.029**	**74.5**	**0.065**	**4.5**	**0.006**	**36.1**	**0.037**	**40.0**	**0.042**

*note- this metabolite is referred to as M451/1 in the study report, however as this code has been used in duplicate (sugar beet for a different structure), therefore the metabolite bixlozone-3-OH-propanamide-gluc has been referred to as M451/3 throughout this DAR This was referred to as M451/3 in the updated GLP amendment report for goat, but it had not been updated in the updated GLP amendment report for poultry

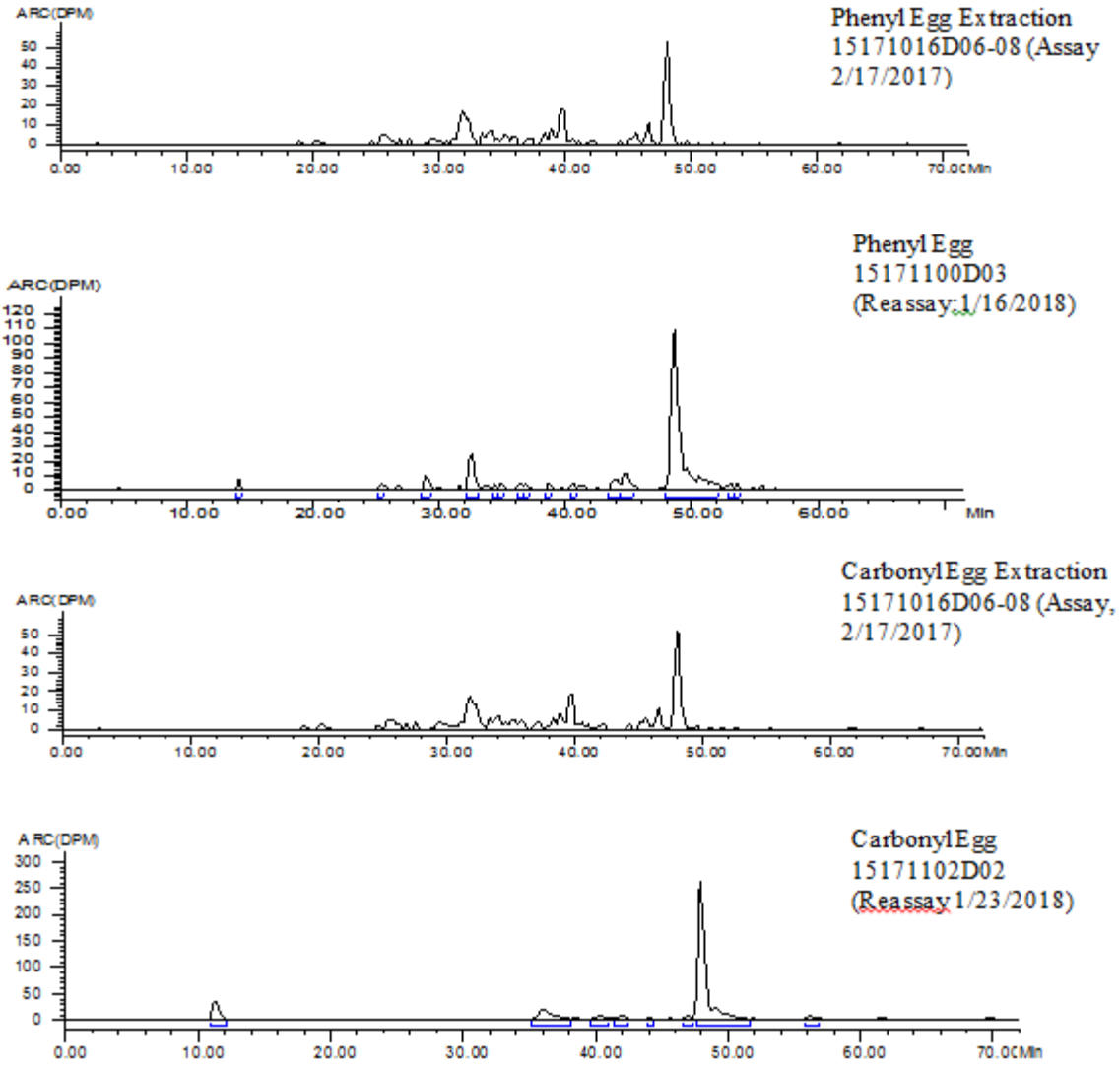
a – Includes 4 unidentified regions, the largest of which contains 6.8% TRR, 0.005 mg/kg
b – Includes 1 unidentified region (0.037 mg/kg)

Storage stability investigations- results

The applicant considers that the data provided (some example chromatograms) lend some support to the view that samples remained stable over frozen storage for a period of about a year. HSE considers that the information provided on whether samples/extracts are unchanged over a storage period in the context of the metabolism study is limited as explained below.

A comparison was made between chromatograms from the initial and subsequent analyses of phenyl and carbonyl egg extracts, with the extracts having been stored in the freezer in the interim period (at -20ºC for around 11 months) prior to re-analysis.

HSE finds it difficult to draw firm conclusions from the chromatography which the applicant provided to make the comparison (inserted below). In all chromatograms the main peak is 'maintained'. The 'initial' chromatogram is at the top, and the 'after' chromatogram is below for both the phenyl and the carbonyl extracts (these were provided to HSE in a letter response dated 19th August 2019). The chromatogram is unlabelled (metabolite peaks are not labelled), and the y axis scale is different in the two chromatograms. It is expected that the main peak is metabolite M289/1 and that these are dichloromethane extracts (considering the code numbers below and comparing to egg example chromatograms that were presented in the study report). Aside from saying that the main peak remained present in the 'after' compared to the 'initial' chromatograms, it is not possible to draw comparisons about storage stability of the extracts with regard to the range of metabolites. For both the phenyl and carbonyl extracts there seem to be more peaks in the initially analysed extracts, although the different scale (y axis) does not aid this type of comparison. It is difficult to make any quantitative or qualitative conclusions (aside from the main peak remaining present).



It is difficult to draw firm conclusions from the chromatograms compared that suggest the stability of samples is sufficiently maintained over the long course of the study.

Translocation and proposed metabolic pathway

The unchanged parent bixlozone was only found at low levels in samples of pooled fat and eggs samples (<7.5% TRR), showing that the compound is readily metabolised into various metabolite compounds. The majority of the radioactive residue 'take up' into tissues of poultry was into liver, which amounted to 0.609 mg/kg and 0.491 mg/kg for the phenyl and carbonyl labels respectively. Levels of radioactivity in eggs were up to about 0.11 mg/kg, with a plateau being reached in 7-9 days. Levels in muscle were lower at up to 0.06 mg/kg (carbonyl label) and levels in fat were up to 0.36mg/kg (subcutaneous fat- phenyl label).

When the results from both labels and across matrices are considered together, they provide sufficient data for the applicant to be able to propose a metabolic pathway for hens (poultry). The applicant's proposed metabolic pathway is outlined in Figure 7-7.

In eggs, the most significant metabolic conversion of the parent compound was hydroxylation at the 5 position of the isoxalidin-3-one ring to form 5-hydroxy-bixlozone (M289/1) and acid hydrolysis released unextracted residues of the metabolite dimethyl malonic acid (M132/1) formed via oxidative ring opening of the isoxazolidin-3-one ring in the parent compound. The TRR level in carbonyl-labelled egg (0.103 mg/kg) is slightly higher than the TRR in phenyl-labelled egg (0.089 mg/kg). A low level of the radioactivity remained unextracted (~10% in the phenyl label and ~3% in the carbonyl label).

In poultry liver samples, the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound to form the metabolite dimethyl malonic acid (M132/1). This metabolite was observed both before and after microwave extraction of the PES suggesting that it might be 'semi bound' into liver matrix. A significant level of the radioactivity remained unextracted (~55% in the phenyl label and ~60% in the carbonyl label) in the PES of liver, suggesting possible natural incorporation into the liver matrix. This PES was subjected to microwave extraction and the majority of the radioactivity was released in the microwave extract. The PES was only worked on by microwave extraction (which might be harsh technique) and not by other approaches (such as use of enzymes or use of acid/base).

In breast muscle, thigh leg muscle and pooled fat samples, the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound to form the metabolite dimethyl malonic acid (M132/1). Hydroxylation at the 5 position of the isoxazolidin-3-one ring was also observed with significant levels of 5-hydroxy-bixlozone (M289/1) observed for fat samples only. Approximately 20-30% TRR was retained in the PES for both labels in all samples, and this was all virtually released by microwave extraction. This suggests there might possibly be some level of incorporation of bixlozone into poultry tissues.

Conclusion

The metabolism of bixlozone was investigated in poultry by dosing laying hens with phenyl-labelled or carbonyl-labelled bixlozone. The overall residue levels (TRR) in the phenyl-labelled pooled fat, thigh muscle, breast muscle, liver and eggs were 0.263, 0.027, 0.019, 0.608 and 0.089 mg/kg respectively. For carbonyl-labelled pooled fat, thigh muscle, breast muscle, liver and eggs the levels were 0.058, 0.058, 0.057, 0.491, 0.103 mg/kg. Hence, TRR for thigh muscle, breast muscle, liver and eggs were similar for both labels. In pooled fat samples significantly higher TRR was observed in the phenyl-labelled sample.

For both labels, solvent extractability was high for egg, pooled fat, thigh and breast muscles (at least 70% TRR, and >90% for egg). For liver, solvent extraction retrieved ca. 45% TRR and ca. 40% TRR for the phenyl- and carbonyl-labels respectively. In the both labelled liver samples microwave extractions released a further 50-52% TRR in total.

Metabolism of bixlozone includes primarily, the hydroxylation at the 5-position and oxidative ring opening. Unchanged parent bixlozone was only detected at fairly low levels in pooled fat and eggs samples (<7.5% TRR). Dimethyl malonic acid (M132/1) accounted for a high proportion of the TRR in all matrices (accounting for ~25-60% TRR). In eggs and fat, the metabolite M289/1 was also detected at high levels of the radioactive residue (accounting for ~30% TRR). A significant unidentified region was detected in the carbonyl-labelled egg sample (36.5% TRR, 0.037 mg/kg). Identification of this region would have been beneficial to the overall metabolic pattern in egg matrices. The applicant indicated that that this component was a small molecular weight polar molecule, for which identification had proved very difficult.

Additional conjugates were also observed in all matrices, individually found at a maximum TRR of 14.4% in thigh muscle samples, and at a maximum relative level of 0.074 mg/kg (12.2% TRR) in liver samples. Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in laying poultry. Overall,

this study has enabled metabolism of bixlozone in poultry to be reasonably well-elucidated (see the applicants proposed pathway in Figure 7-7). HSE has the following evaluation remarks/observations based on this poultry (hen) metabolism study.

- Unextracted residues (following initial solvent extraction) have been worked on using quite a harsh extraction method, microwave extraction, which was successful at releasing the PES (post extraction solid) residues. Whilst it is suggested that some of these residues might be naturally incorporated with poultry tissues other analyses using different experimental approaches (such as enzyme treatments) were not used.
- At times, there has not been full clarity in the report (e.g. flow charts and raw data in Appendix G of the report) do not indicate acid hydrolysis was carried out on egg but data are presented (Table S-6 and Table S-7 in the report) for after acid hydrolysis of the extracts). Chromatograms for before and after acid hydrolysis of extracts seem to be available for liver, muscle, fat and eggs. The report only explains the acid treatment of aqueous extracts of egg following dichloromethane extraction.
- A significant unidentified region was detected in the carbonyl-labelled egg sample (36.5% TRR, 0.037 mg/kg). Ideally this component would have been identified. The applicant indicated that that this component was a small molecular weight polar molecule, for which identification had proved very difficult. The applicant referred to the polar nature of carbonyl related metabolic degradation products, and the challenges in identification of polar metabolites resulting from this moiety (a further explanation is provided in the text for the results of metabolite identification for egg).
- A discussion of storage stability of residues is presented in Vol 1, Section 2.7.2.

Figure 7-7 Applicant's proposed metabolic pathway for bixlozone (F9600) in hens

Names and metabolite codes to support the pathway diagram:

F9600 = bixlozone

5-OH-F9600 = 5-hydroxy bixlozone (M289/1)

4-OH-Me-F9600 = 4-hydroxy methyl bixlozone (M289/4)

2,4-Dichlorobenzoic acid (M190/1)

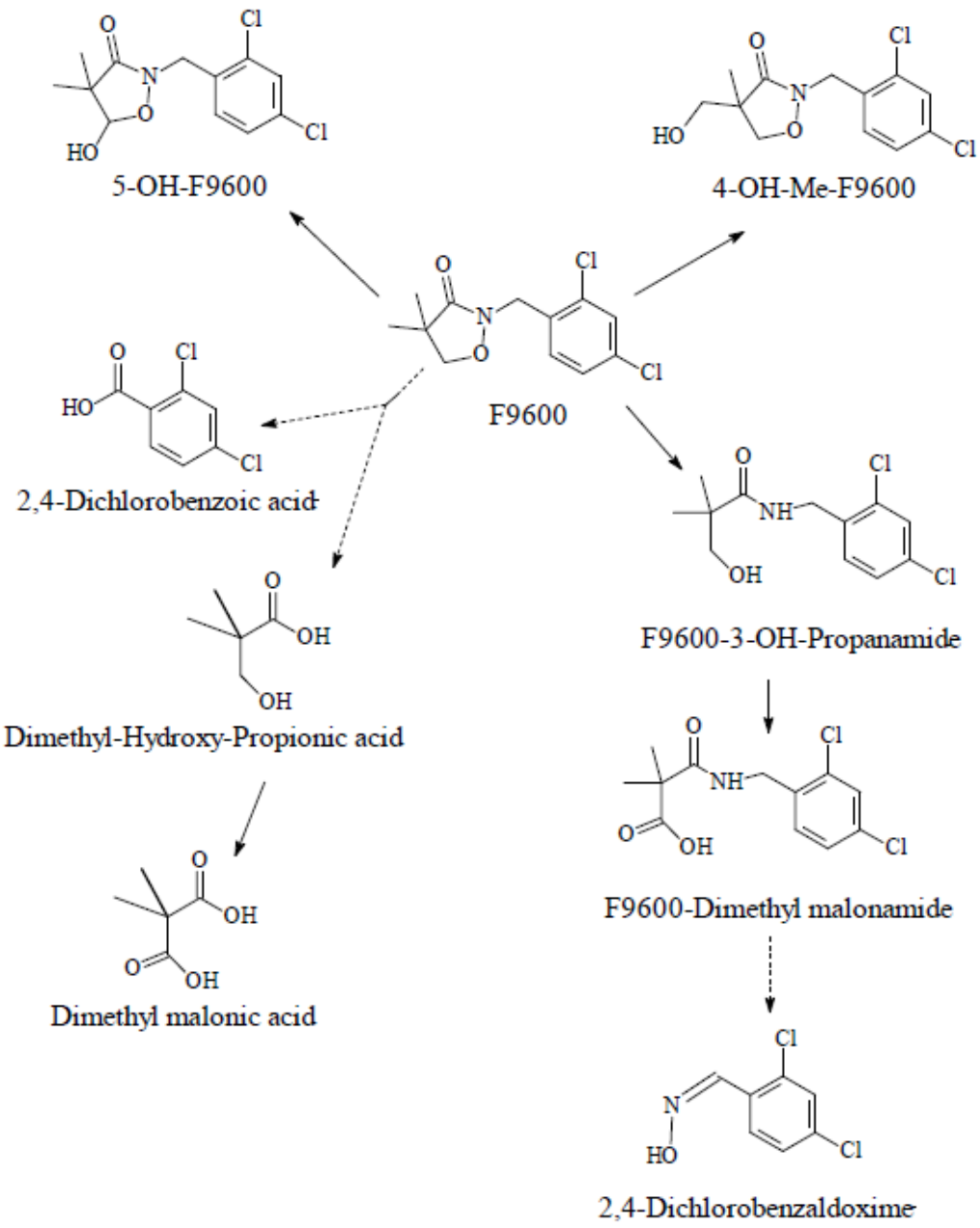
F9600-3-OH-Propanamide (M275/1)

Dimethyl-hydroxy-propionic acid (M118/1)

F9600-Dimethyl malonamide (M289/2)

Dimethyl malonic acid (M132/1)

2,4-Dichlorobenzaldoxime (M189/1)


5-OH-F9600
4-OH-Me-F9600
F9600
2,4-Dichlorobenzoic acid
F9600-3-OH-Propanamide
Dimethyl-Hydroxy-Propionic acid
F9600-Dimethyl malonamide
Dimethyl malonic acid
2,4-Dichlorobenzaldoxime

B.7.2.3. Lactating ruminants

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.2.3-01, [redacted], 2019
Title:	[^{14}C]F9600: Nature of the residue in livestock: Metabolism of F9600 in lactating goats
Report No.:	15086 – RPT04035 (Report amendment date: October 30, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 503 Metabolism in Livestock, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996)
GLP	yes

Materials and methods

Materials

3. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42475
Radiochemical Purity:	99.9% (nominal); 99.9% (certified)
Specific Activity:	(bulk) 60 mCi/mmol; 8.04 MBq/mg (nominal) 10 mCi/mmol (nominal); 9.62 mCi/mmol (certified) (nominal) 1.34 MBq/mg (nominal); 1.29 MBq/mg (certified)

4. Test Material

Test Material:	[Carbonyl –C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42476
Radiochemical Purity:	99.9% (nominal); 99.9% (certified)
Specific Activity:	(bulk) 59 mCi/mmol; 7.91 MBq/mg (nominal) 10 mCi/mmol (nominal); 10.22 mCi/mmol (certified) (nominal) 1.34 MBq/mg (nominal); 1.37 MBq/mg (certified)

Methods

Test System

The metabolism of bixlozone was investigated in lactating goats (breed *Nubian*) following repeated oral administration of ^{14}C-bixlozone, labelled either in the dichloro-phenyl ring (phenyl-label) or on the carbonyl carbon of the dimethyl-isoxazolidin-3-one ring (carbonyl-label) (see Figure 7-2). Goats were housed individually in pens and a lighting cycle was provided (10 hours light, 14 hours darkness). The goats were provided with daily feed (alfalfa hay and rolled barley) and water was given *ad libitum*.

During the study, the goats were in good general health throughout the dosing and acclimatisation period, and body weights were maintained through the study. The food intake of the treated and control goats did drop by a small amount (up to 6%) over the dosing period compared to pre-dosing; this is thought likely to the goats being caged, and the observations between the controls and treated goats were similar. Milk production for the 'phenyl' goat was reduced by a small amount (by 10% compared to the pre-dosing acclimatisation period). This is explained by the 'phenyl' goat being 1 months pregnant at sacrifice. The control goat was 3 months at sacrifice and milk production was significantly reduced in this animal. The milk production for the 'carbonyl' label goat was not reduced and this animal was not pregnant.

Dosing

Two goats were dosed with radiolabelled bixlozone for 7 consecutive days (one goat dosed with the phenyl-label and one goat with the carbonyl-label) following an acclimatisation period of 11 days. The test items were prepared in gelatine capsules containing 30 mg of radiolabelled bixlozone, orally administered (once daily in the morning after milking). A further control goat was dosed with a single daily blank capsule containing lactose. The weight of the

goats was 74 kg (phenyl) and 61 kg (carbonyl). Feed intake varied, but on average was 1.462 kg (phenyl) and 1.434 kg (carbonyl) dry weight feed per day.

The dose regime was as follows:

Phenyl labelled: daily dose of 20.52 mg/kg food consumed (dry weight equivalent)

corresponding to 0.405 mg/kg bw/d

corresponding to 101 N (considering animal dietary burden, see Volume 1, section 2.7.5)

Carbonyl labelled: daily dose of 20.92 mg/kg food consumed (dry weight equivalent)

corresponding to 0.492 mg/kg bw/d.

corresponding to 123 N (considering animal dietary burden, see Volume 1, section 2.7.5)

Sampling

Each goat was milked twice daily (AM and PM) from day 1 - 6 of the study. The morning milk was collected prior to dosing each morning. One milk collection was made on day 7 prior to sacrifice. Milk was also collected in the acclimatisation period (so -11 to 7 days). An aliquot of each daily milk sample was separated into cream and skimmed milk samples by centrifugation. PM samples (as well as a pooled AM/PM sample) were used for the metabolite profiling analysis of the study. The TRRs reported in the study for milk, cream and skimmed milk, were results averaged across AM and PM.

Urine, faeces and cage wash were collected daily, prior to the morning dosing.

The goats were sacrificed 6-8 hours after the final dose and tissue samples of liver, kidney, muscle (flank and loin), and fat (omental fat, subcutaneous fat, and perirenal fat) were collected.

All samples were stored at ca -15 °C until shipment and at *ca* -20 °C between frozen shipment and processing (tissue samples were processed into homogenized powder form under liquid nitrogen) and analysis.

Although samples of gastrointestinal (GI) tract, GI tract contents, whole blood and the remaining carcass were also collected, results for these were not provided.

In terms of pooling of samples: separate TRRs were analysed and reported for loin muscle and flank muscle. As residues were a bit higher in flank muscle, only flank muscle was used for the extractability and onward metabolic profiling work (referred to thereafter in this evaluation as just 'muscle'. For the fat, separate TRRs were analysed and reported for perirenal fat, omental fat, and subcutaneous fat. For the carbonyl label, as residues were a bit lower in the perirenal fat, only omental and subcutaneous fat samples were combined to make the pooled sample, referred to thereafter in this evaluation as 'fat'. For the phenyl label, perirenal, omental and subcutaneous fat samples were combined (equal volumes) to make the pooled sample, referred to thereafter in this evaluation as 'fat'.

Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 3 months of samples being taken. The latest analyses of samples was conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Combustion analysis for the determination of the TRR in tissue samples was performed using finely homogenized/ground tissues (liver, kidney, fat and muscle). Triplicate aliquots (*ca*. 50-200 mg) of each representative tissue sample were used for combustion. The TRR was determined using biological sample oxidizer and measured evolved ^{14}Carbon dioxide ($^{14}CO_2$) by LSC. The TRR is faeces was also determined by combustion and followed by LSC. TRRs for urine, cage wash, milk, cream milk and skim milk (also assumed for bile [not stated in the report], which had reported TRR results) were determined directly by LSC.

Extraction

Samples for both the phenyl- and the carbonyl-label were extracted in the same manner, as outlined below for each commodity type:

Tissues: Tissue samples were first extracted by blending in water (three times) then methanol (three times) for about three minutes each for each extraction. The resulting mixture was centrifuged and filtered under vacuum to separate solids. The remaining solids were then re-blended with a solution of 1:1 water/methanol, centrifuged, and filtered under vacuum. All extracts were combined, assigned Ext-1 fraction, and assayed by LSC. Ext-1 fraction was concentrated when necessary and analysed by HPLC. A fraction of Ext-1 was also concentrated to dryness and reconstituted in aqueous solution (AQ-1). The concentrated extracts were stored for further enzyme and acid hydrolysis. Fat samples were not further worked on in terms of characterising residues in the fat extract (Ext-1 fraction) and enzyme and acid hydrolysis of Ext-1.

Tissues - enzyme hydrolysis of organic extracts (where relevant, milk, liver and kidney): Aliquots of Ext-1 were concentrated to remove organic solvent. The remaining aqueous solution was then incubated in 1M ammonium acetate in water (pH 5.5; pH adjusted with acetic acid) with a mixture of β-glucuronidase/arylsulfatase enzymes. After the 24-hr incubation, acetonitrile was added to the incubation mixture to stop the enzymatic reaction. The incubation mixtures were concentrated down by nitrogen evaporation and then directly analysed by HPLC.

Tissues- acid hydrolysis of organic extracts (where relevant, milk, liver, kidney, muscle): Aliquots of Ext-1 were concentrated to remove organic solvent. The remaining aqueous solutions were individually subjected to acid hydrolysis (2N HCl) under reflux at 100 °C for ca. 2 hours. After acid hydrolysis, the mixture was cooled down, neutralized, concentrated under nitrogen evaporation and reconstituted in acetonitrile: water for further analysis.

It is noted that the acid and enzyme treatments were not done sequentially, but different Ext-1 aliquots were used for these treatments.

Milk: Milk samples were extracted using acetonitrile. The extracts were mixed, and the resulting mixture was centrifuged for *ca*. 10 minutes at 4ºC. The supernatant was decanted into a suitable container. Aliquots were radio-assayed by LSC. The Milk PES (post extraction solids) was dissolved in 1N HCl and directly analysed by LSC.

Post extraction solids (PES): The remaining PES samples (tissues, see above for milk) following organic extraction (PES-1) were air-dried and combusted using an oxidizer, followed by LSC to determine remaining residual ^{14}C-unextracted material. In all cases, the PES residues were low, therefore further extractions were not carried out.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, dimethyl malonic acid, 2,2-dimethyl-3-hydroxypropionic acid, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, 3'-hydroxy-bixlozone, bixlozone 'ring open acid derivative', 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report (tabular 'Summary of F9600 reference standards assayed by LC/MS') is as follows: bixlozone (F9600), 2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-3-OH-Propanamide (M275/1), 5-hydroxy-bixlozone (M289/1), bixlozone-Dimethyl Malonamide (M289/2), 4-hydroxymethyl-bixlozone (M289/4) and bixlozone-3-OH-Propanamide-Sulfate (M355/1). These proposed structures were supported by HCD-MS[8]. Metabolites were characterised by LC/MS and further confirmed by comparison of the HPLC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards.

A list of metabolites generally* confirmed by LC-MS/MS stated in the metabolism study report ('Summary of metabolites of F9600 identified by LC-MS/MS') is as follows: 2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-3-OH-Propanamide (M275/1), 5-hydroxy-bixlozone (M289/1), bixlozone-Dimethyl Malonamide (M289/2), 4-hydroxymethyl-bixlozone (M289/4) and bixlozone-3-OH-Propanamide-Sulfate (M355/1), 5-hydroxy-bixlozone-sulfate (M369/1) , bixlozone-3-OH-propanamide-Glucuronide (M451/3), 5-hydroxy-bixlozone-Glucuronide (M465/1) and 4-hydroxy-bixlozone-Glucuronide (M465/2). These were metabolites generally* identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

*it is noted that the conjugate M369/1 in milk was identified/confirmed in milk (the conjugates in goat milk (M369/1 was found in milk) were unequivocally identified by synthesizing the reference standards and matching them with the metabolites in the samples), whereas the conjugates of metabolites M465/1, M465/2, M451/3 and M369/1 were

[8] Higher-energy collisional dissociation with Mass Spectroscopy detection

characterised/tentatively identified in kidney, muscle and liver according to their hydrolysis (de-conjugation behaviour).

Storage stability

As stated above, samples were not all analysed within a period of 6 months since sampling. Samples were stored frozen until processing and analysis. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses':

Milk analysed at 3 months

All other matrices (fat, muscle, liver, kidney) analysed at 1 month.

Frozen storage up to the time in which analysis on the matrices were completed in this study:

Milk up to 1.5 years

All matrices (fat, muscle and liver) up to 1.4 years

Storage stability investigations

No storage stability investigations performed within the context of this metabolism study (e.g. a comparison between chromatograms from the initial and subsequent analyses of extracts). A limited investigation was presented for poultry metabolism- eggs (see section B.7.2.2).

Results and discussion

Total radioactive residue

Levels of ^{14}C-bixlozone recoveries were generally similar for both labels. The recovered radioactivity, expressed as % administered dose, are summarised for each label in

Table 7-48. Total radioactive residues, TRRs, are reported in Table 7-49 and Table 7-53 (timecourse for milk).

For both labels ~83-87% of the total dose was recovered. It is noted that GI tract (and GI contents), bile, carcass and blood were samples taken. However, of these, only TRR results were determined and reported in the study for 'GI contents', but the GI contents residues were not included in the estimates of % recovered radioactivity, so this could contribute to the shortfall in the % of the total dose recovered.

The majority of the dosed radioactivity was recovered in excreta for both labels (in urine 76% and 74% for phenyl and carbonyl labels respectively, and in faeces 9.5% and 7.7% for phenyl and carbonyl labels respectively). Radioactivity in edible tissues was relatively low with a maximum level of 0.074% and 0.064% of the dose recovered from liver tissue for the phenyl and carbonyl labels respectively.

In milk samples low percentages, 0.117% and 0.096%, of the total administered dose was recovered for the phenyl and carbonyl labels respectively. The potential for a plateau levels of radioactive residues in milk to have been reached were considered using the data shown in Table 7-50. The study report did not report separate TRR data for AM and PM, and it is noted that the data for the final day (7) are AM results only. Plateau levels of radioactive residues in milk might have been reached within 2 days of dosing. However variable results at day 7 (higher) compared to day 6, give a possible impression that residue levels could be increasing. However the day 7 collection was just from afternoon collection, whereas the earlier data points were averages from the morning/afternoon collections, so it is difficult to conclude. Also the phenyl label residues were especially variable, with the highest residue (aside from day 7) was seen at day 5, with a lower residue at day 6. Based on the carbonyl data it seems more likely was reached at around day 2.

Overall, considering both labels, low levels of radioactivity were found in muscle, milk and fat (<0.072 mg/kg). Higher levels were observed for liver (0.133 mg/kg and 0.120 mg/kg for the phenyl and carbonyl labels respectively), whilst the highest levels of radioactivity were observed in kidney tissue for both labels (0.343 mg/kg in the phenyl label and 0.369 mg/kg in the carbonyl label).

Table 7-48 Distribution of radioactivity of ^{14}C-bixlozone in goats

Commodity	% of dosed radioactivity recovered from phenyl label	% of dosed radioactivity recovered from carbonyl label
Urine	75.854	74.457
Faeces	9.596	7.68
Milk	0.117	0.096
Cage wash	1.685	1.266
Loin muscle	0.004	0.005
Flank muscle	0.005	0.005
Liver	0.074	0.064
Kidney	0.030	0.029
Peritoneal fat	0.011	0.004
Subcutaneous fat	0.005	0.006
Omental fat	0.005	0.006
Total [£]	***87.39***	***83.62***

[£]- not including GI contents, GI tract, carcass and blood.

Table 7-49 TRR in tissues, milk and cream. E denotes the samples that were taken for onward extraction.

Commodity	TRR from phenyl label *[mg parent eq./kg]*	TRR from carbonyl label *[mg parent eq./kg]*
Liver - E	0.133	0.120
Kidney-E	0.343	0.369
Muscle-loin	0.009	0.008
Muscle-flank -E	0.010	0.011
Milk [1)] -E	0.072	0.069
Skim milk[1)] -E	0.037	0.026
Milk cream[1)] -E	0.053	0.041
Omental fat	0.011	0.013
Perirenal fat	0.022	0.008
Subcutaneous fat	0.010	0.013
Fat-E	0.022	0.013

1) Milk samples (including skim and cream) from Day 5 (pm sample) for phenyl label and Day 3 (pm sample) for carbonyl label at steady state were selected for TRR analysis and onward extraction (E).

Table 7-50 TRR in milk after administration of ^{14}C-bixlozone to goats

Study day *1)*	**TRR from phenyl label** *[mg parent eq./kg]*	**TRR from carbonyl label** *[mg parent eq./kg]*
1	0.033	0.027
2	0.039	0.029
3	0.043	0.029
4	0.030	0.021
5	0.047	0.021
6	0.040	0.021
7	0.075	0.029

1) Day 1-6 data represent averages of AM and PM collections. Day 7 data was from PM collection prior to sacrifice

Extractability

The extractability of ^{14}C residues from ruminant commodities is summarized in Table 7-51. Tissue samples were extracted with methanol and water solvent mixtures and milk was extracted using acetonitrile. High extractability of the ^{14}C residue was seen in liver, kidney, muscle, milk, skim milk and milk cream samples (>90% TRR for both labels). The extractability in fat samples was lower, *ca*. 67% and 54% TRR for the phenyl- and carbonyl-label respectively. Portions of the organic extracts (Ext-1), were further subjected to both (separate) acid and enzyme hydrolysis. This was not done for fat, and muscle was not subject to enzyme treatment.

The PES remaining after the initial solvent analysis (to yield Ext-1) was not further worked on. In fat, although this was attributed to 32.6% TRR in the phenyl-label and 46.3% TRR in the carbonyl-label, the mg/kg levels of radioactivity were low (<0.007 mg/kg).

Table 7-51 Extractability of radioactive residues from ruminant commodities. (mg/kg = mg parent eq./kg)

Commodity	**TRR (mg/kg)**	**Organic/ aqueous extract** *(MeOH:H_2O)*		**PES**	
		% TRR	**mg/kg**	**% TRR**	**mg/kg**
Phenyl-label					
Liver	0.133	90.6	0.120	9.4	0.013
Kidney	0.343	95.5	0.328	4.5	0.015
Muscle (flank)	0.010	96.6	0.010	3.4	<0.001
Milk *1)*	0.072	95.3	0.069	4.7	0.003
Skim milk*1)*	0.037	97.8	0.036	2.2	0.001
Milk cream*1)*	0.053	98.3	0.052	1.7	0.001
Fat	0.022	67.4	0.015	32.6	0.007
Carbonyl-label					
Liver	0.120	92.9	0.111	7.1	0.009
Kidney	0.369	94.1	0.347	5.9	0.022
Muscle (flank)	0.011	96.3	0.011	3.7	<0.001
Milk *1)*	0.069	95.3	0.066	4.7	0.003
Skim milk*1)*	0.026	97.2	0.025	2.8	0.001
Milk cream*1)*	0.041	94.1	0.038	5.9	0.002
Fat	0.013	53.7	0.007	46.3	0.006

1) Milk samples (including skim and cream) from Day 5 (pm) for phenyl label and Day 3 (pm) for carbonyl label at steady state were selected for extraction.

Characterisation and Identification

For all matrices similar results were observed with both radiolabels. No unchanged parent was detected for any matrix using either label. The characterisation of the radioactivity is presented in the following tables (Table 7-52, Table 7-53 and Table 7-54). Metabolites found at >10% TRR are discussed in detail for each matrix below. Structures of the metabolites are outlined in the appendices to Volume 1.

Fat

Following the determination of extractability of residues into the organic solvent extract (Ext1), no further characterisation/metabolic profiling of residues in fat was undertaken.

Kidney

The most significant metabolite fraction after initial organic solvent extraction was identified as the conjugate M465/1 for both labels, with 30.4% TRR (0.104 mg/kg) and 50.1% TRR (0.185 mg/kg) for the phenyl-label and the carbonyl-label respectively. A further 5 metabolites were identified in the organic extracts for both labels. In the phenyl-label, M289/2 (24.3% TRR, 0.083 mg/kg) and M355/1 (13.2% TRR, 0.045 mg/kg)) were identified, a fraction at 13.2% TRR, 0.045 mg/kg was postulated as conjugate M369/1, with two other metabolites at a maximum level of 7.1% TRR (0.024 mg/kg). In the carbonyl-label, M289/2 (19.7% TRR, 0.073 mg/kg) and M355/1 (10.0% TRR, 0.037mg/kg) were identified. Three other metabolites were present with a maximum individual level of 6.0% TRR (0.022 mg/kg).

The total of all unknowns within the organic extracts were accounted for by 5.8% TRR (0.020 mg/kg) for the phenyl-labels which included a total of 2 unknown regions, the largest of which contained 3.7% TRR (0.013 mg/kg). For the carbonyl-label no unknowns were detected.

For the phenyl-label, the acid hydrolysis of the organic extracts led to the identification of 46.0% TRR (0.158 mg/kg) of the residue as metabolite M289/1 and 29.7% TRR (0.102 mg/kg) of the residue as metabolite M275/1. A further three metabolites were detected at a maximum level of 9.7% TRR (0.033 mg/kg). One unknown region was detected (1.7% TRR, 0.006 mg/kg).

After acid hydrolysis of the carbonyl-label, M289/1 was present at 31.2% TRR (0.115 mg/kg), M275/1 at 19.1% TRR (0.070 mg/kg), M118/1 at 17.4% TRR (0.064 mg/kg) and M289/2 at 15.8% TRR (0.058 mg/kg). Two other metabolites were found at a maximum level of 2.8% TRR (0.010 mg/kg). A further 6.0% TRR (0.022 mg/kg) was assigned as unknown radioactivity, which includes 5 unidentified regions, the largest of which contained 1.5% TRR (0.001 mg/kg).

The enzyme hydrolysis of the organic extracts for the phenyl-label led to the identification of 49.5% TRR (0.17 mg/kg) as the metabolite M289/1, as well as M289/2 at 13.8% TRR (0.047 mg/kg) , M190/1 at 12.3% TRR (0.042 mg/kg) and M355/1 at 10.8% TRR (0.037mg/kg). M275/1 was identified at a level of 9.1% TRR (0.031 mg/kg). No further unknown regions were detected.

For the carbonyl-label, a similar pattern was observed after the enzyme hydrolysis as in the phenyl-label. The metabolite M289/1 was identified at 53.1% TRR (0.196 mg/kg), as well as M289/2 at 16.5% TRR (0.061 mg/kg), and the conjugate M355/1 at 12.6% TRR (0.047 mg/kg). Three other metabolites were identified with a maximum individual level of 5.0% TRR (0.018 mg/kg). No further unknown regions were detected.

Liver

The most significant metabolite identified after initial organic solvent extraction was M289/2 (27.6% TRR, 0.037 mg/kg) for the phenyl-label and for the carbonyl-label the most significant region was postulated to be the conjugate M465/2 (33.2% TRR, 0.040 mg/kg). In the phenyl-label the following conjugates were also tentatively identified M465/1 (20.3% TRR, 0.027 mg/kg) and M369/1 (10.2% TRR, 0.014 mg/kg). Three other metabolites at a maximum level of 7.4% TRR (0.010 mg/kg) were found. In the carbonyl-label M289/2 was also identified at 24.8% TRR (0.030 mg/kg), with four other metabolites present with a maximum individual level of 7.2% TRR (0.009 mg/kg).

The total of all unknowns within the organic extracts was attributed to 17.3% TRR (0.024 mg/kg) for the phenyl-label. A total of four unknowns were present, the largest of which contained 5.9% TRR (0.008 mg/kg). For the carbonyl-label the total of all unknowns was attributed to 10.8% TRR (0.013 mg/kg); two unknowns were present, the largest of which contained 7.8% TRR (0.009 mg/kg).

For the phenyl-label, the acid hydrolysis of the organic extracts led to the identification of M289/2 at 21.4% TRR (0.029 mg/kg), M289/1 at 19.0% TRR (0.026 mg/kg) and M275/1 at 16.0% TRR (0.022 mg/kg). M289/4 was detected

in low amounts (4.1% TRR, 0.006 mg/kg). A region of unknowns was attributed to 27.3% TRR of radioactivity, consisting of 8 unidentified regions, the largest of which contained 7.8% TRR (0.010 mg/kg).

For the carbonyl-label, after acid hydrolysis M289/1 was present at 17.7% TRR (0.021 mg/kg), and M118/1 at 17.2% TRR (0.021 mg/kg). Four other metabolites were found at a maximum level of 9.5% TRR (0.011 mg/kg). A total of 25.0% TRR (0.030 mg/kg) was attributed as unknown material, which included 4 unidentified regions, the largest of which contained 14.9% TRR (0.018 mg/kg); this unknown peak had an early RT of 4.13 mins. In accordance with OECD 503, it would have been desirable if this component had been identified (the guidelines indicate that attempts should be made to identify a metabolite at this level). In this evaluation (see poultry- egg section B.7.2.2) the applicant's difficulties in trying to identify some carbonyl related residues, including early eluting polar metabolites has been explained.

The enzyme hydrolysis of the organic extracts for the phenyl-label led to the identification of a further 33.4% TRR (0.045 mg/kg) as the metabolite M289/1, as well as M289/2 at 14.4% TRR (0.019 mg/kg). Six other metabolites were identified with a maximum individual level of 9.4% TRR (0.013 mg/kg). No further unknown regions were detected.

For the carbonyl-label, a similar pattern was observed after the enzyme hydrolysis as in the phenyl-label. The metabolite M289/1 was identified at 32.2% TRR (0.039 mg/kg), and M289/2 at 17.9% TRR (0.021 mg/kg). Five other metabolites were identified with a maximum individual level of 6.9% TRR (0.008 mg/kg). One unknown region was detected at 8.8% TRR (0.010 mg/kg).

Muscle

The most significant metabolite identified after initial organic solvent extraction was M289/2 (24.0% TRR, 0.002 mg/kg) for the phenyl-label and for the carbonyl-label the most significant peak (35.3% TRR, 0.004 mg/kg) was postulated to be the conjugate M465/1. In the phenyl-label, peaks were also postulated to be the conjugates M465/1 (22.9% TRR, 0.002 mg/kg) and M369/1 (13.6% TRR, 0.001 mg/kg). Three other metabolites were found at a maximum level of 6.3% TRR (0.001 mg/kg). In the carbonyl-label 17.7% TRR (0.002 mg/kg) was identified as M289/2 and a peak (which was postulated to be M451/3) was present at 3.0% TRR (<0.001 mg/kg).

The total of all unknowns within the organic extracts was attributed to 15.4% TRR (0.001 mg/kg) for the phenyl-label. A total of three unknowns were present, the largest of which contained 10.4% TRR (0.001 mg/kg). For the carbonyl-label no unknown regions were detected.

For the phenyl-label, the acid hydrolysis of the organic extracts led to the identification of M289/1 at 43.5% TRR (0.004 mg/kg) and M275/1 at 15.0% TRR (0.002 mg/kg). Four other metabolites were detected at a maximum level of 9.5% TRR (0.001 mg/kg). 7.5% TRR was attributed to unknown material, consisting of two unidentified regions, the largest of which contained 5.1% TRR (0.001 mg/kg).

In the carbonyl-label, after acid hydrolysis M118/1 was present at 29.3% TRR (0.003 mg/kg), and M289/1 at 12.1% TRR (0.001 mg/kg). Three other metabolites were found at a maximum level of 7.0% TRR (0.001 mg/kg). A total of 39.1% TRR (0.004 mg/kg) was attributed to unknown radioactivity, which included three unidentified regions, the largest of which contained 19.6% TRR (0.002 mg/kg).

The enzyme hydrolysis of the organic extracts was not carried out in muscle samples.

Milk

The most significant metabolite after initial organic solvent extraction was identified as the conjugate M369/1 for both radio labels, with 81.8% TRR (0.059 mg/kg) for the phenyl-label and 72.8% TRR (0.050 mg/kg) for the carbonyl-label. M355/1 was found in both radio labels, identified at levels of 13.6% TRR (0.01 mg/kg) and 22.0% TRR (0.016 mg/kg) for the phenyl and carbonyl-labels respectively. This distribution of metabolites was similar in cream and skim milk for both labels and no significant accumulation of residues in cream (fat), therefore the residues are not considered to be particularly fat-soluble. (Table 7-55 and Table 7-56).

For the phenyl-label, the acid hydrolysis of the organic extracts enabled M289/1 to be identified at 73.0% TRR (0.053 mg/kg) and M275/1 at 11.6% TRR (0.008 mg/kg). M289/4 was identified at 0.7% TRR (0.001 mg/kg). A total of 10.0% TRR was assigned to unknown radioactive material, consisting of four unidentified regions, the largest of which contained 5.0% TRR (0.004 mg/kg).

For the carbonyl-label the acid hydrolysis led to M289/1 found and identified at 63.6% TRR (0.044 mg/kg), and M275/1 at 26.5% TRR (0.018 mg/kg). One unknown region was detected at a level of 5.1% TRR (0.004 mg/kg).

The enzyme hydrolysis of the organic extracts for the phenyl-label enabled 79.5% TRR (0.057 mg/kg) to be identified as the metabolite M289/1, as well as 16.0% TRR (0.012 mg/kg) as M355/1. M275/1 was identified at 0.5% TRR (<0.001 mg/kg). No further unknown regions were detected.

For the carbonyl-label, a similar pattern was observed after the enzyme hydrolysis as in the phenyl-label. The metabolite M289/1 was identified at 93.6% TRR (0.068 mg/kg) and M275/1 was identified at 1.6% TRR (0.001 mg/kg). No further unknown regions were detected.

Table 7-52 Distribution of [^{14}C]bixlozone and its metabolites in goat kidney based on analysis of the organic extract (Ext-1) . (mg/kg = mg parent eq./kg)

Metabolite	Phenyl						Carbonyl					
	Organic extract *(MeOH:H_2O)*		**Acid hydrolysis of organic extract**		**Enzyme hydrolysis of the organic extract**		**Organic extract** *(MeOH:H_2O)*		**Acid hydrolysis of organic extract**		**Enzyme hydrolysis of the organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total %TRR by combustion	**95.5**	**0.328**	**95.5**	**0.328**	**95.5**	**0.328**	**94.1**	**0.347**	**94.1**	**0.347**	**94.1**	**0.347**
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	-	-	-	-	-	-	17.4	0.064	-	-
2,4-Dichlorobenzoic acid (M190/1)	-	-	-	-	12.3	0.042	-	-	-	-	-	-
bixlozone-3-OH-Propanamide-Glucuronide (M451/3)	7.1	0.024	-	-	-	-	5.2	0.019	-	-	-	-
bixlozone-3-OH-Propanamide-Sulfate (M355/1)	13.2	0.045	-	-	10.8	0.037	10.0	0.037	-	-	12.6	0.047
bixlozone-Dimethyl-Malonamide (M289/2)	24.3	0.083	9.7	0.033	13.8	0.047	19.7	0.073	15.8	0.058	16.5	0.061
5-Hydroxy-bixlozone- Sulfate (M369/1)	13.2	0.045	-	-	-	-	6.0	0.022	-	-	2.7	0.010
5-Hydroxy-bixlozone- Glucuronide (M465/1)	30.4	0.104	4.3	0.015	-	-	50.1	0.185	1.9	0.007	-	-
4-Hydroxy-bixlozone- Glucuronide (M465/2)	1.5	0.005	-	-	-	-	3.1	0.012	-	-	-	-
bixlozone-3-OH-Propanamide (M275/1)	-	-	29.7	0.102	9.1	0.031	-	-	19.1	0.070	5.0	0.018
4-Hydroxy-methyl-bixlozone (M289/4)	-	-	4.1	0.014	-	-	-	-	2.8	0.010	2.4	0.009
5-Hydroxy-bixlozone (M289/1)	-	-	46.0	0.158	49.5	0.17	-	-	31.2	0.115	53.1	0.196
Unknowns	5.8[a]	0.020[a]	1.7[b]	0.006[b]	-	-	-	-	6.0[e]	0.022[e]	-	-
Total positively identified:	**89.7**	**0.306**	**93.8**	**0.322**	**95.5**	**0.327**	**94.1**	**0.348**	**88.2**	**0.324**	**92.3**	**0.341**

a – Includes 2 unidentified regions, the largest of which contains 3.7% TRR, 0.013 mg/kg
b – Includes 1 unidentified region
c – Includes 5 unidentified regions, the largest of which contained 1.5% TRR, 0.006 mg/kg

Table 7-53 Distribution of [^{14}C]bixlozone and its metabolites in goat liver based on analysis of the organic extract (Ext-1) . (mg/kg = mg parent eq./kg)

Metabolite	Phenyl						Carbonyl					
	Organic extract *(MeOH:H_2O)*		Acid hydrolysis of organic extract		Enzyme hydrolysis of the organic extract		Organic extract *(MeOH:H_2O)*		Acid hydrolysis of organic extract		Enzyme hydrolysis of the organic extract	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total %TRR by combustion†	**90.6**	**0.122**	**90.6**	**0.122**	**84.7**	**0.122**	**92.9**	**0.111**	**92.9**	**0.111**	**92.9**	**0.111**
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	-	-	-	-	-	-	17.2	0.021	-	-
2,4-Dichlorobenzoic acid (M190/1)	-	-	-	-	3.6	0.005	-	-	-	-		
bixlozone-3-OH-Propanamide-Glucuronide (M451/3)	5.5	0.007	-	-	-	-	6.9	0.008	-	-		
bixlozone-3-OH-Propanamide-Sulfate (M355/1)	7.4	0.01	-	-	9.4	0.013	4.9	0.006	-	-	4.0	0.005
bixlozone-Dimethyl-Malonamide (M289/2)	27.6	0.037	21.4	0.029	14.4	0.019	24.8	0.03	8.1	0.010	17.9	0.021
5-Hydroxy-bixlozone- Sulfate (M369/1)	10.2	0.014	-	-	9.0	0.012	5.2	0.006	-	-	3.7	0.004
5-Hydroxy-bixlozone- Glucuronide (M465/1)	20.3	0.027	-	-	-	-	33.2	0.04	9.5	0.011		
4-Hydroxy-bixlozone- Glucuronide (M465/2)	2.4	0.003	-	-	3.8	0.005	7.2	0.009	-	-	4.2	0.005
bixlozone-3-OH-Propanamide (M275/1)	-	-	16.0	0.022	6.2	0.008	-	-	9.2	0.011	6.9	0.008
4-Hydroxy-methyl-bixlozone (M289/4)	-	-	4.1	0.006	4.9	0.007	-	-	3.9	0.005	3.9	0.005
5-Hydroxy-bixlozone (M289/1)	-	-	19.0	0.026	33.4	0.045	-	-	17.7	0.021	32.2	0.039
Unknowns	17.3[a]	0.024[a]	27.3[b]	0.036[b]	-	-	10.8[c]	0.013[c]	25.0[d]	0.030[d]	8.8[e]	0.010[e]
Total positively identified:	**73.4**	**0.098**	**60.5**	**0.083**	**84.7**	**0.114**	**82.1**	**0.099**	**65.6**	**0.079**	**72.8**	**0.087**

† For liver samples, the %TRR presented is the total amount from organic and microwave extraction
a – Includes 4 unidentified regions, the largest of which contains 5.9% TRR, 0.008 mg/kg
b – Includes 8 unidentified regions, the largest of which contained 7.8% TRR, 0.010 mg/kg

c – Includes 2 unidentified regions, the largest of which contains 7.8% TRR, 0.009 mg/kg
d – Includes 4 unidentified regions, the largest of which contained 14.9% TRR, 0.018 mg/kg
e – Includes 1 unidentified region.

Table 7-54 Distribution of [^{14}C]bixlozone and its metabolites in goat muscle based on analysis of the organic extract (Ext-1) . (mg/kg = mg parent eq./kg)

Metabolite	Phenyl				Carbonyl			
	Organic extract *(MeOH:H_2O)*		Acid hydrolysis of organic extract		Organic extract *(MeOH:H_2O)*		Acid hydrolysis of organic extract	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total %TRR by combustion	**96.6**	**0.010**	**96.6**	**0.010**	**96.3**	**0.011**	**96.3**	**0.011**
bixlozone	-	-	-	-	-	-	-	-
2-2 dimethyl-3-OH –Propionic acid (M118/1)	-	-	-	-	-	-	29.3	0.003
2,4-Dichlorobenzoic acid (M190/1)	-	-	9.0	0.001	-	-	-	-
bixlozone-3-OH-Propanamide- Glucuronide (M451/3)	5.2	0.001	-	-	3.0	<0.001	-	-
bixlozone-3-OH-Propanamide-Sulfate (M355/1)	6.3	0.001	-	-	-	-	-	-
bixlozone-Dimethyl-Malonamide (M289/2)	24.0	0.002	9.5	0.001	17.4	0.002	5.9	0.001
5-Hydroxy-bixlozone- Sulfate (M369/1)	13.6	0.001	-	-	-	-	-	-
5-Hydroxy-bixlozone- Glucuronide (M465/1)	22.9	0.002	8.2	0.001	35.3	0.004	-	-
4-Hydroxy-bixlozone- Glucuronide (M465/2)	1.7	<0.001	-	-	-	-	-	-
bixlozone-3-OH-Propanamide (M275/1)	-	-	15.0	0.002	-	-	7.0	0.001
4-Hydroxy-methyl-bixlozone (M289/4)	-	-	4.0	<0.001	-	-	3.0	<0.001
5-Hydroxy-bixlozone (M289/1)	-	-	43.5	0.004	-	-	12.1	0.001
Unknowns	15.4[a]	0.001[a]	7.5[b]	0.001[b]	-	-	39.1[c]	0.004[c]
Total positively identified:	**73.7**	**0.007**	**89.2**	**0.009**	**55.7**	**0.006**	**57.3**	**0.006**

a – Includes 3 unidentified regions, the largest of which contains 10.4% TRR, 0.001 mg/kg
b - Includes 2 unidentified regions, the largest of which contained 5.1% TRR, 0.001 mg/kg
c - Includes 3 unidentified regions, the largest of which contained 19.6% TRR, 0.002 mg/kg

Table 7-55 Distribution of [^{14}C]bixlozone and its metabolites in goat milk based on analysis of the organic extract (Ext-1) . (mg/kg = mg parent eq./kg)

Metabolite	Phenyl						Carbonyl					
	Organic extract *(MeOH:H_2O)*		**Acid hydrolysis of organic extract**		**Enzyme hydrolysis of the organic extract**		**Organic extract** *(MeOH:H_2O)*		**Acid hydrolysis of organic extract**		**Enzyme hydrolysis of the organic extract**	
	%TRR	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**	**%TRR**	**mg/kg**
Total %TRR by combustion	**95.3**	**0.069**	**95.3**	**0.069**	**96.0**	**0.069**	**95.3**	**0.066**	**95.3**	**0.066**	**95.3**	**0.066**
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
bixlozone-3-OH-Propanamide-Sulfate (M355/1)	13.6	0.01	-	-	16.0	0.012	22.5	0.016	-	-	-	-
5-Hydroxy-bixlozone- Sulfate (M369/1)	81.8	0.059	-	-	-	-	72.8	0.050	-	-	-	-
bixlozone-3-OH-Propanamide (M275/1)	-	-	11.6	0.008	0.5	<0.001	-	-	26.5	0.018	1.6	0.001
4-Hydroxy-methyl-bixlozone (M289/4)	-	-	0.7	0.001	-	-	-	-	-	-	-	-
5-Hydroxy-bixlozone (M289/1)	-	-	73.0	0.053	79.5	0.057	-	-	63.6	0.044	93.6	0.068
Unknowns	-	-	10.0[a]	0.007[a]	-	-	-	-	5.1[b]	0.004[b]	-	-
Total positively identified:	**95.4**	**0.069**	**85.3**	**0.062**	**96.0**	**0.069**	**95.3**	**0.066**	**90.1**	**0.062**	**95.2**	**0.069**

a – Includes 4 unidentified regions, the largest of which contained 5.0% TRR, 0.004 mg/kg
b – Includes 1 unidentified region.

Table 7-56 Characterisation of total residues (bixlozone equivalents) in milk, cream and skim milk from goats dosed with [^{14}C]bixlozone. (mg/kg = mg parent eq./kg)

Metabolite	Phenyl [^{14}C]bixlozone						Carbonyl [^{14}C]bixlozone					
	Milk		Cream		Skim milk		Milk		Cream		Skim milk	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Total %TRR by combustion	*95.3*	*0.069*	*98.4*	*0.052*	*97.8*	*0.036*	*95.3*	*0.069*	*94.1*	*0.038*	*97.2*	*0.025*
bixlozone-3-OH-Propanamide-Sulfate (M355/1)	13.5	0.010	12.3	0.007	15.8	0.006	22.0	0.016	22.6	0.009	24.9	0.006
5-Hydroxy-bixlozone- Sulfate (M369/1)	81.8	0.059	85.1	0.045	74.6	0.027	71.1	0.052	67.7	0.027	67.2	0.017
Unknown	-	-	1.0	0.001	7.4	0.003	-	-	3.8	0.002	5.1	0.001
Total positively identified	**95.3**	**0.069**	**97.4**	**0.052**	**90.4**	**0.033**	**93.1**	**0.068**	**90.3**	**0.036**	**92.1**	**0.023**

Storage stability investigations- results

No storage stability investigations were performed in the context of metabolism within this goat metabolism study. See section B.7.2.2, some limited information was provided for eggs (comparison of extracts analysed before and after storage). However from this, it is difficult to draw firm conclusions from the chromatograms compared that suggest the stability of samples is sufficiently maintained over the long course of the study.

Translocation and proposed metabolic pathway

The unchanged parent bixlozone was not found in any matrices, showing that the compound is readily metabolised into various metabolite compounds. [In poultry the parent compound was detected, but also shown to have been readily metabolised. In poultry, parent bixlozone was only found at low levels in samples of pooled fat and eggs samples (<7.5% TRR)].

The majority of the radioactive residue 'take up' into tissues of goats was into liver and kidney, which amounted to 0.13 mg/kg and 0.12 mg/kg for the phenyl and carbonyl labels respectively for liver and 0.34 mg/kg and 0.37 mg/kg for the phenyl and carbonyl labels respectively for kidney. Levels were lower in muscle and fat- up to 0.011 mg/kg in muscle, and up to 0.022 mg/kg in fat (perirenal fat). Levels in milk (average from AM and PM collections of milk) reached around 0.05 mg/kg (phenyl) and 0.03 mg/kg (carbonyl label), with a plateau likely reached by about day 2 (variable data).

When the results from both labels and across matrices are considered together, they provide sufficient data for the applicant to be able to propose a metabolic pathway for goats (ruminants). The applicant's proposed metabolic pathway is outlined in Figure 7-8.

In goats, the most significant metabolic conversion of the parent compound was hydroxylation at the 5 position of the isoxalidin-3-one ring to form 5-hydroxy-bixlozone (M289/1) and subsequent conjugation of this metabolite. In milk M369/1 (sulfate conjugate of 5-hydroxy-bixlozone) was present in very high amounts in the initial solvent extract, and the glucuronide conjugate of this metabolite (5-hydroxy-bixlozone) was also found in substantial amounts in kidney (in the initial solvent extract).

The carbonyl labelled metabolite M118/1, indicative of oxidative ring opening of the isoxazolidin-3-one ring was formed in appreciable appoints > 10%TRR in kidney, liver and muscle. It is not fully clear how well the metabolite M132/1 was sought in the goat (see below bullet points). [In poultry the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound to form the metabolite dimethyl malonic acid (M132/1)]. In the ruminant the (phenyl specific) metabolite (M190/1) was also found.

A further significant route of metabolism in the goat was reduction to M275/1 (and onwards conjugation, M355/1 sulfate conjugate) and also a further metabolic route was to the formation of M289/2 (bixlozone (bixlozone)-Dimethyl-malonamide).

Conclusion

The metabolism of bixlozone was investigated in ruminants by dosing goats with phenyl-labelled or carbonyl-labelled bixlozone. The overall residue levels (TRR) in the phenyl-labelled pooled fat, muscle, kidney, liver and milk (average of AM and PM collections) were 0.022, 0.010, 0.34, 0.13 and 0.05 mg/kg respectively. For carbonyl-labelled pooled fat, thigh muscle, breast muscle, liver and eggs the levels were 0.013, 0.011, 0.37, 0.12, 0.03 mg/kg. Hence, TRRs were similar for both labels (with residues in pooled fat being a little higher in the phenyl-labelled sample compared to the carbonyl labelled sample.

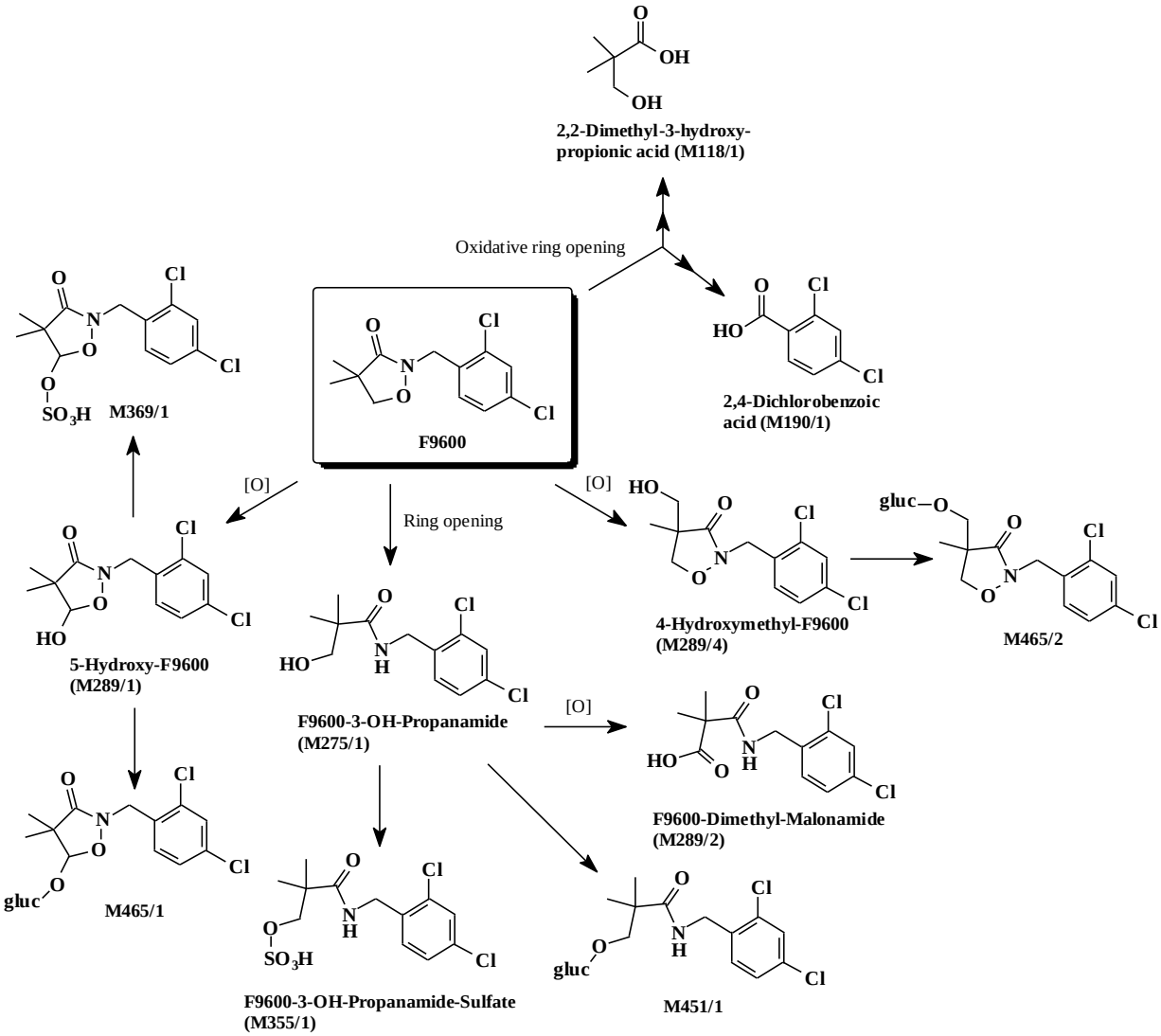
For both labels, solvent extractability was high for all matrices (at least 90% TRR extracted), aside from fat, where the recoveries were lower (67% phenyl label and 54% in the carbonyl label, %TRR extracted). The unextracted residues (in the PES) were not worked on further as the mg/kg level of the radioactive residue in fat was up to 0.007 mg/kg.

Unchanged parent was not found indicating metabolism occurs readily. Metabolism of bixlozone includes, the hydroxylation at the 5-position (M289/1), with subsequent conjugation) and oxidative ring opening, together with other metabolic routes (oxidative ring opening of the dimethyl-oxazolidone ring to form M289/2 and hydroxylation at the 4 position of the 5-membered ring), and further conjugation of residues. In milk the sulfonated conjugate (M369/1) of M289/1 accounted for the majority of the radioactivity >73 %TRR in the initial organic extract. Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism.

Overall, this study has enabled metabolism of bixlozone in goats to be reasonably well-elucidated (see the applicants proposed pathway in Figure 7-8). HSE has the following evaluation remarks/observations based on this ruminant (goat) metabolism study.

- A main route of metabolism found in the poultry study was formation of the metabolite dimethyl malonic acid (M132/1) formed via oxidative ring opening of the isoxazolidin-3-one ring in the parent compound. In poultry this metabolite was found across all matrices at up to 59%TRR (carbonyl labelled study only). The goat metabolism study included this metabolite in the list of reference standards was used for initial scoping by HPLC, however it is not clear how comprehensively this reference standard was used to check against major metabolite fractions, since this component (M132/1) was not designated as an identified residue, and this metabolite (and its identification) was not discussed in the goat metabolism study report.
- A discussion of storage stability of residues is presented in Vol 1, Section 2.7.2.

Figure 7-9 Applicant's proposed metabolic pathway for bixlozone (F9600) in lactating goats



B.7.2.4. Pigs

As the metabolic pathway observed in hens and goats is similar to the metabolism observed in rats (Vol 3 CA B6, Section 6.1), an assessment of the metabolism in pigs is not required.

The broad comparability in metabolite pathways of animal species and the generally non-fat soluble nature of the residues in rats and livestock studies is noted also at the end of section 2.7.2 (Volume 1).

B.7.2.5. Fish

At present there is no agreed guidance on how to conduct fish metabolism studies to determine the residue definition for risk assessment and monitoring and there are no agreed guidance documents on how then to conduct a fish feeding study. It is also the case that there is no agreed diet for farmed fish. The EU guidance: SANCO/10181/2013– rev. 5, 12 June 2019, Guidance Document For Applicants On Preparing Dossiers For The Approval Of A Chemical New Active Substance And For The Renewal Of Approval Of A Chemical Active Substance According To Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 states:

"In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, the non-submission of particular studies required by the EU legislation should be thoroughly justified and statements (often referred to as 'position papers') must be substantiated with data or information provided by the applicant in the dossier. Applicants should follow on a routine basis the current developments, e.g. activities of the European Food Safety Authority for guidance documents and in particular publications in the Official Journal and the updates of the Commission Communications 2013/C 95/01 and 2013/C 95/02"

It is noted that the data requirements under Regulation (EU) No 283/2013 make it clear that bioaccumulation studies can be considered to address this data requirement. However, at the PRAS meeting in December 2017, for the expert discussion on Spinosad, it was agreed by the experts and EFSA that the use of such a study could not be considered at this time as it was not clear how the study design was applicable to assessing residues for consumer exposure and agreement on the approach to the dietary assessment for fish was required.

Guidance on residues in fish (metabolism studies and feeding studies) has been under development in the EU. The OECD programme on residue guidelines has not yet considered guidelines applicable to fish.

Since no agreed guidance is available, and there is no agreed data on the diets of fish (to address fish dietary burden) at this time, it is considered that the above requirements do not need to be addressed in the current evaluation.

The applicant did not submit any more detailed position paper since they considered that residues in crops were insignificant, and the dietary intake for fish would be very low.

B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

The GAPs proposed for the approval of bixlozone are outlined in the GAP table in the list of endpoints as well as in section 1.5.1 in Volume 1 of the DAR.

A summary of the trials submitted to support the proposed GB GAPs are given in Table 7-59, Table 7-61, Table 7-63, Table 7-65, Table 7-70, Table 7-72, Table 7-76, Table 7-77, Table 7-79, Table 7-80 and Table 7-82.

Trials supporting the GB GAPs are single underlined, e.g. <u><0.01</u>. It should be noted that the relevant results considering the proposed GAP conditions (other than geographic location) from SEU trials are also underlined, but with a dotted underlining format, e.g. <u><0.01</u>. The SEU trials have been reported for completeness, but they do not support the GB GAP. Some of the SEU data has been used to assist with a range of considerations with regards to field trials data, summarised in Volume 1 (e.g. formulation comparison across field trials, residues observed after pre-emergence application compared to early post-emergence application and to aid with decisions in relation to positive residues found in untreated control samples). However, the SEU trials have not been relied upon in support of the GB GAP (please refer to the summaries in Volume 1, section 2.7.4). The purpose of underlining the residue results of the metabolite 2,2-dimethyl-3-hydroxy propionic acid in all the residue field trials is to show the derivation of the residue levels used in the exposure assessment and TTC consideration of this metabolite (please refer to Volume 1). The underlining used to indicate the relevant results is a double underlining format, e.g. <u><0.01.</u>

The basic criteria for acceptability are listed below:

Trials details:

- crop variety
- location, position and year of trial - acceptable spread of location/season

- formulations used - formulation reported/as proposed
- application/dilution rate - reported/as specified on label
- maximum number of treatments - reported/applicable
- method of application - reported/applicable
- growth stage of the crop at treatment or
- pre-harvest interval - appropriate to proposed GAP
- geo-climate information - relevant to the GB climate
- residue level (control and treated)

Analytical aspects:

- method specified and submitted
- storage of samples prior to analysis (conditions and time period)
- limit of determination at an acceptable level
- acceptable recovery (means 70 - 110%).

Note:

GLP: All trials were conducted in accordance with GLP and meet the requirements of Regulation (EC) No 1107/2009 with regards to GLP.

Analytical methods: Under Regulation (EC) No 1107/2009, as outlined in Regulation (EC) No 283/2013, analytical methods used to generate data should be validated. The formal validation data supporting methods used in studies submitted to support the approval are outlined in section B.5.1 of the DAR.

MRLs: The MRLs required for the proposed GAPs have been outlined on the basis of the OECD calculator.

Residue definitions:

As a result of the assessment the following residues definitions are being considered for the intended uses, see Volume 1, section 2.7.3 for further discussion:

Residue definition for risk assessment purposes:

Sum of residues of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone.

[the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid].

Please refer to Volume 1, section 2.7.3 for an exposure assessment of the metabolite 2,2-dimethyl-3-hydroxy propionic acid (plus dimethyl malonic acid) versus the TTC (Threshold of Toxicological Concern). The underlining of the residue results of the metabolite 2,2-dimethyl-3-hydroxy propionic acid in all the trials in this section, is to show the derivation of the residue levels used in this exposure assessment and TTC consideration.

Residue definition for monitoring purposes: bixlozone

Further notes:

Clomazone: The applicant detailed in the study plans to note where the active substance clomazone had been used as a maintenance product or in previous seasons at the trial sites. This was planned due to concerns over ‘competing chemistry’ given the similar structures of bixlozone and clomazone. However, the use of clomazone was not avoided in all cases; where applicable this has been noted in the study summaries. Now the metabolism evaluation is complete, there is no particular concern regarding ‘competing chemistry’ or common major metabolites; the di-chloro ring structure within bixlozone appears to remain intact during metabolism (note: clomazone contains a single chlorine atom on the same ring structure).

Extract storage stability: In both the residues trials and the storage stability studies, the samples extracts were not analysed immediately. This is not ideal as Regulation (EC) 283/2013 states that: "the stability of extracts shall be investigated if extracts are not analysed immediately." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required.

B.7.3.1. Wheat and Barley

Representative GAPs

The uses on wheat and barley detailed in Table 7-57 are proposed within this application for approval of bixlozone.

Table 7-57 Representative GAP for use on wheat and barley

Crop and/or situation (a)	For G (b)	Pest or group of pests controlled (c)	Formulation rate per treatment		Application			Application rate per treatment			PHI (days) (l)
			Type (d-f)	Conc of a.s. (i)	method, kind, if other than spray (f-h)	growth stage (j)	number (range) (k)	g a.s./ha, where appropriate	water L/ha	g a.s./hL, where appropriate	
Winter wheat Winter barley	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 00-09	1	200	150 – 400	50 - 133	N/A
Winter wheat	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 11-13	1	200	150 - 400	50 - 133	N/A

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure).
(b) Outdoor or field use (F), glasshouse application (G) or indoor application.
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds.
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR).
(e) GCPF Codes – GIFAP Technical Monograph No 2, 1989.
(f) All abbreviations used must be explained.
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench.
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated.
(i) g/kg or g/L.
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application.
(k) Indicate the minimum and maximum number of application possible under practical conditions of use.
(l) PHI – minimum pre-harvest interval.

A total of 5 trials on barley and 8 trials on wheat, conducted in the Northern European zone, were submitted to support the proposed GAPs. The Northern European trials are considered relevant to the GB climate. 4 trials on barley and 8 trials on wheat conducted in the Southern European zone were submitted and have been summarised for completeness. For this UK only application, these Southern European zone trials have not been considered further.

Given the proposed application timing is before the forming the edible part of the plant (BBCH 51 for cereals), extrapolation of residue trials data from barley to wheat is acceptable for trials supporting the proposed GAPs. It should be noted that trials on spring wheat and barley are considered supportive of uses on winter wheat and barley also.

OECD 509 describes the commodity to be analysed for barley as 'grain – whole commodity', 'hay – whole commodity' and 'straw – whole commodity', and for wheat as 'grain – whole commodity', 'forage – whole commodity', 'hay – whole commodity' and 'straw – whole commodity'. As a specific GAP for use on forage has not been proposed, analysis of the 'hay' commodity is not considered necessary but has been reported for completeness.

Residue field trials supporting both the pre emergence and post emergence GAPs have been summarised within the same study reports. Upon consideration in Volume 1 (section 2.7.4), it is clear that there is no significant difference between the results determined using both the proposed GAPs. Therefore, the results from trials considered relevant to either proposed GAP (pre or post emergence, relevant results indicted by underlining) can be combined and considered supportive of both GAPs. This is further discussed in Volume 1.

The proposed GAPs being considered include uses on barley and wheat (latest time of application BBCH 09). In accordance with the extrapolations guidance document (SANCO 7525/VI/95, Rev. 10.3, a minimum of eight trials conducted on barley are sufficient to support of uses on wheat and vice versa, when the application is made before forming the edible part of the plant (BBCH 51 for cereals). Therefore, as the application is made in all available trials before forming the edible part of the plant, extrapolation between wheat and barley is possible.

Therefore, a total of 5 trials on barley and 8 trials on wheat conducted in the Northern EU zone are available to support the uses on barley and wheat in the Northern EU zone. This is a sufficient number of relevant trials to support the proposed uses on wheat and barley. A full consideration of the relevant trials is given in Vol 1 (section 2.7.4).

Trials performed on Barley

The proposed GAP on barley is: 200 g a.s./ha x 1, BBCH 00-09.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.2-01, Gemrot, F. 2017
Title:	Magnitude of the Residues of F9600 in Barley (Raw Agricultural Commodity) after one pre emergence or post emergence application of F9600-4 SC in Northern and Southern Europe – 2015 and 2016
Report No.:	15SGS110; 2015RES-ISX2150
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Three trials on barley were conducted outdoors during 2015-2016. A fourth trial was begun as part of this study report however due to heavy rainfall at this site during winter, the soil saturated with water and consequently the crop did not grow correctly. Therefore, this trial was cancelled. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones.

Trial site 'SP04' has been tested in another trial reported in the DAR (SP05, study 15 SGS109). This trial site was tested in the same season at the same time (application on 12/11/15 in both trials) on different crops in different plots (barley and wheat). The trials were conducted on different crops with a similar morphology. The application was made before the edible part of the crop had formed (before BBCH 51). At an early growth stage such as this, extrapolation between these crops is possible (in accordance with the extrapolations document SANCO 7525/VI/95, Rev. 10.3). The crops are not significantly different. Therefore, these trials are not considered truly independent. In accordance with the OECD Guidance on Crop Field Trials, the mean of the results from these two trials has been reported below. The two trials can only be considered a single trial when considering the overall number of trials supporting the proposed uses.

All three trials were residue decline trials. The formulation 'F9600-4 SC' containing 37.4 % w/w active substance was applied to the trial sites. One application was made to barley at BBCH 00 (pre-emergence) in trials FR01 and SP04, and BBCH 11-13 (post-emergence) in trial HU02.

The product tested in the trials was 'F9600-4 SC' which is the representative product being considered within this application for approval. These trials can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was not applied either in the previous three seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-59 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices (whole plant, straw and grain) from the three trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.05 mg/kg). Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 20m apart.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 0.5 kg of straw and forage, at least 1 kg of grain). No adverse weather was observed in the trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in barley forage, straw and grain. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were stored under frozen (< -18°C) conditions for up to 448 days prior to extraction. There are sufficient storage stability data reported under section 7.1 and summarised in Volume 1 to support this time period of storage for barley forage, straw and grains, for each of the analytes analysed. Samples were stored for up to 7 days under refrigerated conditions (~4°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. A summary of the procedural recovery results is given in Table 7-58. The procedural recoveries reported are all within the acceptable range (70 – 110%).

Only two fortified control samples of whole plant and one fortified control sample of straw was available for each fortification level. Ideally, separate procedural recovery data should be reported for each matrix individually. As the same analytical method was used to analyse whole plant and straw, the results were combined. As sufficient and acceptable method recoveries were demonstrated for cereal straw in the method validation data (see Vol 3CA B5), acceptable procedural recoveries for barley whole plant and straw were demonstrated in the other barley field trial (CA 6.3.2-03) where the same analytical method was used and the limited data are not adverse, this is not considered to significantly affect the validity of the study.

A summary of the residue trial results are given in Table 7-59. Mean values considering the replicate trial on wheat ('SP04') have been reported in italics in the table.

Table 7-58 Summary of procedural recovery data

Matrix	**Fortification level (mg/kg)**	**bixlozone**		**5'-hydroxy-bixlozone**		**2,4-dichloro benzoic acid**		**2,2-dimethyl-3-hydroxy propionic acid**	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)
Barley grain	0.01	82, 84, 92	83 (8.6)	73, 78, 88	88 (12.3)	92, 97, 99	97, (3.0)	-	86 (9.6)
	0.05	-		-		-		78, 80, 83	
	0.1	71, 82, 88		92, 97, 101		95, 99, 99		-	
	0.5	-		-		-		81, 92, 99	
Whole plant and straw	0.01	83, 84, 86	80 (6.4)	80, 83, 92	83 (6.9)	92, 99, 101	97 (3.4)	-	97 (8.9)
	0.05	-		-		-		99, 102, 108	
	0.1	72, 77, 80		76, 79, 86		94, 96, 97		-	
	0.5	-		-		-		82, 96, 97	

Table 7-59 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
Northern France 41800 Villedieu le Chateau (Centre) 15SGS110 FR01	Winter Barley, ETINCEL	1) 19/10/15 2) 12/05/16 to 20/05/16 3) 10/07/16 to 15/07/16	Foliar application, boom sprayer	0.256	203.3	0.126	BBCH 00	BBCH 37-39	192	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: 15SGS101 Frozen storage Max. 370 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+28%). Proportionality principle applied (**bold** font). Proposed application rate:
								BBCH 51-55	203	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 65-69	211	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 75	219	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	259	Grain	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	0.097 [UTC 0.092] **<u>0.076</u> [UTC 0.072]**	
								BBCH	259	Straw	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.05</u>	

Table 7-59 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
								89							200 g a.s./ha Scaling factor: 0.78 (1.28x overdosed)
Hungary H-3397 Makl ár-Andornaktály a 15SGS110 HU02	Winter Barley, LAVERDA	1) 25/09/15 2) 06/05/16 to 13/05/16 3) 20/06/16 to 21/06/16	Foliar application, boom sprayer	0.260	206.7	0.126	BBCH 11-13	BBCH 39	203	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: 15SGS101 Frozen storage Max. 372 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+30%). Proportionality principle
								BBCH 47	208	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 65	215	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 75	223	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	257	Grain	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	257	Straw	<0.01	<0.01	<0.01	0.089 [UTC 0.083] **0.069**	

Table 7-59 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
														[UTC 0.064]	applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.77 (1.30x overdosed)

Table 7-59 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
SOUTHERN EUROPE															

Spain 41410 Carmona (Andalusia) 15SGS110SP04	Winter Barley, TRAVELER	1) 09/11/15 2) 01/03/16 to 21/03/16 3) 26/05/16	Foliar application, boom sprayer	0.245	291.9	0.084	BBCH 00	BBCH 39	95	Whole plant	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.05 *(<0.05)*	Ref: 15SGS101 Frozen storage Max. 448 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Mean of results from replicate trials (SP05, study 15SGS109 and SP04, study 15SGS110) reported in *italics.* Trial within ± 25% but scaled in line with other trials. Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.82 (1.23x overdosed)
								BBCH 45	105	Whole plant	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.05 *(<0.05)*	
								BBCH 65	117	Whole plant	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.05 *(<0.05)*	
								BBCH 77	148	Whole plant	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.05 *(0.093)* ***(0.08)*** [UTC <0.05, *(0.076)* ***(0.066)***]	
								BBCH 89	196	Grain	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	0.183 *(0.117)* [UTC 0.118 *(0.084)*] **0.150** ***(0.10)*** **[UTC 0.097** ***(0.074)]***	
								BBCH 89	196	Straw	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	0.108 *(0.105)* [UTC 0.101 *(0.113)*] **0.089**	

Table 7-59 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
														(0.086) **[UTC 0.083** ***(0.092)*** **]**	

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

The trial in the Southern EU zone was conducted with an application rate within ± 25% of the proposed rate in terms of kg a.s./ha. The two trials in the Northern EU zone were conducted with an application rate slightly higher than ± 25% (up to 30% overdosed) of the proposed rate in terms of kg a.s./ha. Given the discussion in Volume 1 (section 2.7.4) regarding the data supporting both pre-emergence and post-emergence GAPs and the significant time between application and harvest observed in the trials, the timing of application in these trials, although different, can be considered representative of the cGAP. Therefore, in this case the proportionality principle has been applied to trials. In accordance with the OECD guidance on crop field trials, appropriate scaling has been applied to all trials, including those within 25% of the cGAP, to prevent bias. Scaling of positive results has been denoted in Table 7-59 in **bold** text. Results reported as <LOQ at the overdosed application rate will remain <LOQ considering scaling, therefore these results have not been repeated.

Although the proposed GAP for use on barley does not include application up to BBCH 13, given the possible extrapolations described in the introduction and Volume 1 (Section 2.7.4), these results have been considered relevant to the proposed GAP, indicated by underlining.

A full consideration of the trials considered supportive of the proposed GAP for 'F9600-4 SC' on wheat and barley is given in Volume 1.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.2-03, Semrau J. 2018
Title:	Determination of residues of F9600 after one pre-emergence or post-emergence application of F9600-4 SC in Barley at 6 sites in Northern and Southern Europe 2016
Report No.:	S16-01154 / 2016RES-ISX2486
Guidelines:	Reg. (EU) 283/2013 and 284/2013; OECD 2009 Guidance Document on Overview of Residue Chemistry Studies (Series on Testing and Assessment No. 64); OECD Test Guideline 509; OECD Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164); EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4; OECD (1998) Principles of Good Laboratory Practice
GLP	Yes

Six trials on barley were conducted outdoors during 2016. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones.

Trial site '03' has been tested in another trial reported in the DAR ('03', study S16-01153). This trial site was tested in the same season at the same time (application on 19/03/16 in both trials) for trials on different crops (barley and wheat). The trials were conducted on different crops with a similar morphology. The application was made before the edible part of the crop had formed (before BBCH 51). At an early growth stage such as this, extrapolation between these crops is possible (in accordance with the extrapolations document SANCO 7525/VI/95, Rev. 10.3). The crops are not significantly different. Therefore, these trials are not considered truly independent. In accordance with the OECD Guidance on Crop Field Trials, the mean of the results from these two trials has been reported in italicised text. The two trials can only be considered a single trial when considering the overall number of trials supporting the proposed uses.

Two of the trials were residue decline trials. The formulation 'F9600-4 SC' containing 36.4 % w/w active substance was applied to the trial sites. One application was made to barley between BBCH 00 (pre-emergence) to BBCH 13.

The product tested in the trials was 'F9600-4 SC' which is the representative product being considered within this application for approval. Therefore, these trials can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. The similar active substance clomazone was also not applied either in the previous seasons or during the trials except during the 2013/14 season, clomazone was applied to trial site 02. Please refer to the introduction to section 7.3 for a full consideration.

Residues of 5'-hydroxy bixlozone were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-61 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices (whole plant, straw and grain) from the trials did not contain residues above the respective LOQs. Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in Section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 10 m apart.

The application was made using a boom sprayer. No adverse weather conditions were observed in the field trials. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 0.5 kg of straw and at least 1 kg of grain) except in trial 04. In trial 04, an insufficient sample size was collected for both grain and straw at harvest. Samples of both untreated and treated grain were 0.55 kg when a minimum sample size of 1 kg is stated in OECD Guideline 509. Similarly, samples of both untreated and treated straw were 0.4 kg when a minimum sample size of 0.5 kg is stated in OECD Guideline 509. This insufficient sample size may have affected the representativeness of the results reported from this trial.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in barley forage, straw and grain. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

For some samples the results determined using the quantification and confirmation transition have been reported. Although it is routine to determine both results, only the quantifier is normally reported. Normally, the qualifier and quantifier mass transitions would lead to the same result, but in this case with complex chromatograms, it was considered necessary to consider the confirmation transition. The more intense mass transition seems to be influenced by an interference which has the identical retention time as bixlozone. This is only visible from the qualifier/quantifier peak area ratio which is shifted towards the quantifier peak area for the false positive samples. The applicant confirmed that the second mass transition seems to be more selective and was used for confirmation if residues were above the LOQ. The confirmatory mass transition for bixlozone has been considered fully validated in accordance with SANCO/3029/99 rev. 4 in DAR CA B5 Section B.5.1.2.5 considering the matrices wheat grain, straw and hay, which are considered supportive of data generated in barley crop fractions. As both methods are considered validated (quantification and confirmation transitions), the mean of the two results reported has been calculated and taken forward.

Samples were stored under frozen (< -18°C) conditions for up to 445 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for barley whole plant, straw and grains, for each of the analytes analysed. Samples were stored for up to 7 days under refrigerated conditions (1-10°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. A summary of the procedural recovery results is given in Table 7-60. The quantification transition has been considered when determining the procedural recoveries. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). Some individual recoveries were outside of this range; however the mean recoveries are all within the acceptable range therefore no further consideration is required.

A summary of the residue trial results is given in Table 7-61.

Table 7-60 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)
Whole plant	0.01	73, 85, 88	80 (7.9)	72, 75, 80	78 (6)	78, 98, 103	89 (11)	-	79 (7.6)
	0.05	-		-		-		76, 82, 89	
	0.1	73, 77, 82		75, 82 84		84, 86, 86		-	
	0.5	-		-		-		73, 74, 79	
Grain (barley)	0.01	105, 106, 110	105 (3.5)	108, 109, 110	108 (2.1)	104, 106, 117	109 (4.3)	-	101 (7.7)
	0.05	-		-		-		99, 106, 112	
	0.1	99, 103, 106		104, 107, 110		107, 110, 112		-	
	0.5	-		-		-		91, 94, 101	
Straw (barley)	0.01	60, 72, 87	75 (12)	79, 83, 91	85 (4.7)	72, 72, 76	83 (13)	-	87 (14)
	0.05	-		-		-		80, 97, 107	
	0.1	73, 76, 79		83, 85, 86		89, 94, 95		-	
	0.5	-		-		-		78, 78, 80	

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
S16-01154-0164-600. Uścikowo. Wielkopolska. Poland	Spring Barley, HORVS skald	1) 01/04/16 2) 05/06/16 to 10/06/16 3) 27/07/16	Broadcast spray with boom sprayer	0.254	305	0.083	BBCH 01	BBCH 77	79	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S16-01154 Frozen storage Max. 418 days.
								BBCH 89	114	Grain	<0.01	<0.01	<0.01	0.11 [UTC 0.093] **0.09 [UTC 0.073]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	114	Straw	<0.01	<0.01	<0.01	0.073 [UTC 0.063] **0.058 [UTC 0.050]**	Trial overdosed (+27%). Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.79 (1.27x overdosed)
S16-	Spring	1) 13/03/16	Broadc	0.248	297	0.084	BBCH	BBCH 51	64	Whole	0.015[$]	<0.01	<0.01	<0.05	Ref: S16-01154

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36.4 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
01154-0216356, Ahrensfelde-Eiche, Brandenburg, Germany	Barley, HORVS Grace	2) 02/06/16 to 07/06/16 3) 19/07/16	ast spray with boom sprayer				05			plant	[UTC 0.013][$] **0.012 [UTC 0.011]**				Frozen storage Max. 445 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% but scaled in line with other trials. Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.81 (1.24x overdosed)
								BBCH 69	76	Whole plant	<0.01	<0.01	<0.01	0.057 [UTC 0.05] **<0.05 [UTC <0.05]**	
								BBCH 75	82	Whole plant	<0.01	<0.01	<0.01	0.056 [UTC 0.063] **<0.05 [UTC 0.051]**	

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
								BBCH 83	100	Whole plant	<0.01	<0.01	<0.01	0.063 [UTC 0.071] **0.051 [UTC 0.058]**	
								BBCH 89	118	Grain	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	0.093 [UTC 0.096] **<u>0.075</u> [UTC 0.078]**	
								BBCH 89	118	Straw	0.011[$] **<u>≤0.01</u>**	<u>≤0.01</u>	<u>≤0.01</u>	0.17 [UTC 0.14] **<u>0.14</u>**	

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
														[UTC 0.11]	
S16-01154-05 H-8522, Nemesgörzsöny, Veszprém, Hungary	Spring Barley HORVS Scarlett	1) 01/04/16 2) n/a 3) 09/07/16	Broadcast spray with boom sprayer	0.259	310	0.084	BBCH 13	BBCH 77	63	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S16-01154 Frozen storage Max. 421 days.
								BBCH 89	83	Grain	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	0.10 [UTC 0.084] **<u>0.077</u> [UTC 0.065]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	83	Straw	<u><0.01</u>	<u><0.01</u>	0.012 **<u><0.01</u>**	0.10 [UTC 0.081] **<u>0.077</u> [UTC 0.062]**	Trial overdosed (+30%). Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.77 (1.30x overdosed)

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
SOUTHERN EUROPE															
S16-01154-03 GR-63080, Krimni, Chalkidiki Greece	Spring Barley, HORVS Kares	1) 17/03/16 2) 17/05/16 to 28/05/16 3) 27/06/16	Broadcast spray with boom sprayer	0.261	417	0.063	BBCH 00	BBCH 75	80	Whole plant	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	0.058 *(0.204)* [UTC 0.076 *(0.158)*] **<0.05 *(0.16)* [UTC 0.059 *(0.12)*]**	Ref: S16-01154 Frozen storage Max. 427 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Mean of results from replicate trials ('03', study S16-01153 and S16-01154-03, GR-63080) reported in *italics*. Trial overdosed (+31%). Proportionality principle applied
								BBCH 89	100	Grain	0.011[$] *(0.01)* **<0.01 *(<0.01)***	<0.01 *(<0.01)*	<0.01 *(<0.01)*	0.11 *(0.16)* [UTC 0.12 *(0.16)*] **0.085 *(0.14)* [UTC**	

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
								BBCH 89	100	Straw	<0.01 *(<0.01)*	<0.01 *(<0.01)*	0.033 *(0.022)* [UTC 0.035 *(0.023)*] **0.025 *(0.018)* [UTC 0.027 *(0.019)*]**	**0.092 *(0.15)*]** 0.15 *(0.20)* [UTC 0.12 *(0.18)*] **0.12 *(0.16)* [UTC 0.092 *(0.13)*]**	(**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.77 (1.31x overdosed)
S16-01154-0450059, Montanana, Aragon,	Spring Barley HORVS Unia	1) 04/04/16 2) n/a 3) 19/08/16	Broadcast spray with boom sprayer	0.246	197	0.125	BBCH 00	BBCH 37-39 BBCH 45-55	61 70	Whole plant Whole plant	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.05 0.058 [UTC 0.062]	Ref: S16-01154 Frozen storage Max. 432 days. Unless a residue value is stated, untreated

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
Spain								BBCH 65-69						**<0.05 [UTC 0.050]**	control samples contained residues <LOQ.
									90	Whole plant	<0.01	<0.01	<0.01	0.13 [UTC 0.14]	Trial within ± 25% but scaled in line with other trials.
								BBCH 89						**0.11 [UTC 0.11]**	Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.81 (1.23x overdosed)
									133	Grain	<0.01	<0.01	<0.01	0.13 [UTC 0.14]	
								BBCH 89						**0.11 [UTC 0.11]**	A small sample size of 0.55 kg grain and 0.4 kg of straw was taken. However as positive results are observed, the trial has not been
									133	Straw	<0.01	<0.01	<0.01	0.27	

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
														[UTC 0.21] **0.22 [UTC 0.17]**	excluded.
S16-01154-0640057, Granarolo Emilia, Bologna, Italy	Spring Barley HORVS Meseta	1) 30/01/16 2) 30/04/16 to 08/05/16 3) 22/06/16	Broadcast spray with boom sprayer	0.274	327	0.084	BBCH 12	BBCH 77	72	Whole plant	<0.01	<0.01	0.01 **<0.01**	<0.05	Ref: S16-01154 Frozen storage Max. 445 days.
								BBCH 89	99	Grain	<0.01	<0.01	<0.01	0.098 [UTC 0.081] **0.072 [UTC 0.059]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	99	Straw	<0.01	0.018 **0.013**	<0.01	0.12 **0.088**	Trial overdosed (+37%). Proportionality principle applied (**bold** font). Proposed application rate: 200 g

Table 7-61 Residues in barley following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36.4 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
															a.s./ha Scaling factor: 0.73 (1.37x overdosed)

[$] Mean result for bixlozone determined using the confirmation transition results and positive results which were observed using the quantification transition, given that both methods have been considered fully validated.

Trial 02: Whole plant (BBCH 51) UTC: Quant: 0.016, Conf: <0.01 (mean 0.013 mg/kg); Treated: Quant: 0.019, Conf <0.01 (mean 0.015 mg/kg). Straw Treated: Quant: 0.011, Conf: <0.01 (mean 0.011 mg/kg)

Trial 03: Grain Treated: Quant: 0.011, Conf: <0.01 (mean 0.011 mg/kg)

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

Trials 02 and 04 were conducted with an application rate within ± 25% of the proposed rate in terms of kg a.s./ha. Trials 01, 03, 05 and 06 were conducted with application rates slightly higher than ± 25% of the proposed rate in terms of kg a.s./ha. Given the discussion in Volume 1 (section 2.7.4) regarding the data supporting both pre-emergence and post-emergence GAPs and the significant time between application and harvest observed in the trials, the timing of application in these trials, although different, can be considered representative of the cGAP. Therefore, in this case the proportionality principle has been applied to trials. In accordance with the OECD guidance on crop field trials, appropriate scaling has been applied to all trials, including those within 25% of the cGAP, to prevent bias. Scaling of positive results has been denoted in Table 7-61 in **bold** text. Results reported as <LOQ at the overdosed application rate will remain <LOQ considering scaling, therefore these results have not been repeated.

Although the proposed GAP for use on barley does not include application up to BBCH 13, given the possible extrapolations described in the introduction and Volume 1 (Section 2.7.4), these results have been considered relevant to the proposed GAP, indicated by underlining.

During trial '04', an insufficient sample size was taken. The results in barley grain and straw from this trial appear slightly higher than the results reported in other representative trials. This may be due to the sample analysed being skewed due to a smaller sample being taken. As the results show some positive residues, these data have not been excluded from the evaluation.

A full consideration of the trials considered supportive of the proposed GAP for 'F9600-4 SC' on wheat and barley is given in Volume 1.

Trials performed on wheat

The proposed GAPs on wheat are: 200 g a.s./ha x 1, BBCH 00-09; and 200 g a.s./ha x 1, BBCH 11-13.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.2-02, Gemrot, F. 2017
Title:	Magnitude of the Residues of F9600 in Wheat (Raw Agricultural Commodity) after one pre emergence or post emergence application of F9600-4 SC in Northern and Southern Europe – 2015 and 2016
Report No.:	15SGS109; 2015RES-ISX2149
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Six trials on wheat were conducted outdoors during 2016. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones. Two other trials were begun as part of this study report however these trials were lost due to phytotoxicity of the formulated product at the post emergence stage application. These trials were cancelled and removed from the study.

Another trial was conducted at the same location as trial site 'SP05' (SP04, study 15 SGS110). This trial site was tested in the same season at the same time (application on 12/11/15 in both trials) for trials on different crops on different plots (barley and wheat). The trials were conducted on different crops with a similar morphology. The application was made before the edible part of the crop had formed (before BBCH 51). At an early growth stage such as this, extrapolation between these crops is possible (in accordance with the extrapolations document SANCO 7525/VI/95, Rev. 10.3). The crops are not significantly different. Therefore, these trials are not considered truly independent. In accordance with the OECD Guidance on Crop Field Trials, the mean of the results from these two trials has been reported below in italicised text. The two trials can only be considered a single trial when considering the overall number of trials supporting the proposed uses. It should be noted that there is no corresponding sample for hay in the replicate trial performed on barley. Therefore it was not possible to report a mean result.

Another trial was conducted at the same location as trial site 'FR08' (FR03, study 15 SGS072). This trial site was tested in the same season but at a sufficiently different time (application on 20/11/15 in wheat trial, 30/04/15 for maize) for trials on different crops on different plots (wheat and maize). The trials were conducted on different crops with a different morphology. In accordance with the extrapolations document SANCO 7525/VI/95, Rev. 10.3, wheat is significantly different to maize (i.e. extrapolation is not supported). Therefore, the crops are considered sufficiently different and there is sufficient time between applications that trials are considered independent.

Three of the trials were residue decline trials. The formulation 'F9600-4 SC' containing 37.4 % w/w active substance was applied to the trial sites. One application was made to wheat between BBCH 00 (pre-emergence) to BBCH 12.

The product tested in the trials was 'F9600-4 SC' which is representative product being considered within this application for approval. Therefore, these trials can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in the previous seasons or during the trials.

Residues of bixlozone and 5'-hydroxy bixlozone were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-63Table 7-59 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices (whole plant, straw and grain) from the three trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.05 mg/kg). Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 20 m apart.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 0.5 kg of straw and forage, at least 1 kg of grain). No adverse weather was observed in the trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in wheat whole plant, straw and grain. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were stored under frozen (< -18°C) conditions for up to 611 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for wheat forage, hay, straw and grains, for each of the analytes analysed. Samples were stored for up to 20 days under refrigerated conditions (~4°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. A summary of the procedural recovery results is given in Table 7-62. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). Some individual recoveries were outside of this range; however, the mean recoveries are all within the acceptable range therefore no further consideration is required. A high %RSD (>20%) was reported for the procedural recoveries of 2.2-dimethyl-3-hydroxy propionic acid in wheat grain. This appears to be due to a single unusually high recovery reported (135%), when considering the range of recoveries in this study report (64 – 120%). The use of the Grubbs or Dixons test to identify this as an outlier is not fully supported given only three recovery determinations have been made at each fortification level (SANCO 3029/99 rev. 4 states that five recovery determinations should be made at each fortification level).

A summary of the residue trial results are given in Table 7-63.

Table 7-62 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)
Whole plant	0.01	81 – 100 (n=12)	92 (9.2)	81 – 106 (n=12)	95 (7.1)	83 – 120 (n=12)	103 (9.5)	-	96 (10.1)
	0.05	-		-		-		83 – 108 (n=12)	
	0.1	84 – 106 (n=12)		89 – 110 (n=12)		99 – 113 (n=12)		-	
	0.5	-		-		-		72 – 109 (n=12)	
Hay	0.01	84, 85, 86	88 (4.6)	65, 72, 81	81 (13.4)	87, 91, 103	97 (6.9)	-	94 (19.7)
	0.05	-		-		-		64, 107, 111	
	0.1	88, 90, 95		85, 89, 94		100, 100, 103		-	
	0.5	-		-		-		81, 91, 107	
Grain (Wheat)	0.01	87, 87, 93	88 (4.5)	70, 81, 92	84 (9.7)	101, 102, 102	100 (2.8)	-	92 (24.6)
	0.05	-		-		-		77, 87, 135	
	0.1	82, 87, 92		84, 84, 92		96, 97, 102		-	
	0.5	-		-		-		72, 85, 94	
Straw (Wheat)	0.01	66, 83, 83	83 (10.9)	71, 72, 77	82 (13.8)	75, 78, 80	83 (7.7)	-	100 (7.9)
	0.05	-		-		-		90, 101, 110	
	0.1	86, 90, 91		86, 86, 101		86, 89, 91		-	
	0.5	-		-		-		91, 104, 104	

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
Northern France 51450 Betheny (Champagne-Ardenne) 15SGS109 FR01	Winter Wheat, LEAR	1) 08/10/15 2) 01/06/16 to 20/06/16 3) 25/07/16	Foliar application, boom sprayer	0.253	201.1	0.126	BBCH 01	BBCH 37	209	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: 15SGS109 Frozen storage Max. 423 days.
								BBCH 47	226	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 65	240	Whole plant	<0.01	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 75	253	Whole plant	<0.01	<0.01	<0.01	0.064 [UTC 0.064]	
														0.051 [UTC 0.051]	Trial overdosed (+27%). Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.79 (1.27x overdosed)
								BBCH 75	258	Hay (5 days drying)	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	286	Grain	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	0.081 [UTC 0.176]	

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
								BBCH 89	286	Straw	<0.01	<0.01	<0.01	**0.064 [UTC 0.139]** 0.091 [UTC 0.100] **0.072 [UTC 0.079]**	
Hungary 4461 Nyirtelek-Ferenctanya (Szabolcs-Szatmar-Bereg) 15SGS109 HU02	Winter Wheat, HYXTRA	1) 16/10/15 2) 19/06/16 to 25/06/16 3) 05/07/16 to 06/07/16	Foliar application, boom sprayer	0.255	202.8	0.126	BBCH 11-12	BBCH 39 BBCH 49 BBCH 67 BBCH 75	175 182 192 207	Whole plant	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 0.025 [UTC 0.020] **0.020**	<0.05 <0.05 <0.05 0.059 [UTC 0.060] **<0.05**	Ref: 15SGS109 Frozen storage Max. 530 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+28%). Proportionality principle

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
													[UTC 0.016]	**[UTC <0.05]**	applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.78 (1.28x overdosed)
								BBCH 75	215	Hay (8 days drying)	<0.01	<0.01	0.045 [UTC 0.033] **0.035 [UTC 0.026]**	0.083 **0.065**	
								BBCH 89	235	Grain	<0.01	<0.01	0.013 [UTC 0.012] **0.010 [UTC <0.01]**	0.123 [UTC 0.090] **0.096 [UTC 0.070]**	
								BBCH 89	235	Straw	<0.01	<0.01	0.036 [UTC 0.033]	0.334 [UTC 0.294]	

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
													<u>0.028</u> [UTC 0.026]	**<u>0.261</u> [UTC 0.229]**	
Germany 49685 Bühren (Lower Saxony) 15SGS109 GE03	Wheat, SMAR AGD	1) 24/10/15 2) 10/06/16 to 17/06/16 3) 25/07/16 to 27/07/16	Foliar application, boom sprayer	0.243	193.3	0.126	BBCH 06	BBCH 75	241	Whole plant	<0.01	<0.01	<0.01	<0.01	Ref: 15SGS109 Frozen storage Max. 490 days.
								BBCH 75	252	Hay (11 day drying)	<0.01	<0.01	<0.01	<0.01	
								BBCH 89	275	Grain	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	0.088 [UTC 0.106]	Unless a residue value is stated, untreated control samples contained residues <LOQ.
														<u>0.072</u> [UTC 0.087]	Trial within ± 25% but scaled in line with other trials.
								BBCH 89	275	Straw	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	0.241 [UTC 0.183]	Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.82 (1.22x overdosed)

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
														<u>0.198</u> [UTC 0.150]	
SOUTHERN EUROPE															
Spain 41410 Carmona (Andalusia) 15SGS109 SP05	Winter Wheat, ARTUR NICK	1) 10/11/15 2) 19/02/16 to 09/03/16 3) 31/05/16	Foliar application, boom sprayer	0.247	294.9	0.084	BBCH 00	BBCH 39 BBCH 55 BBCH 65 BBCH 75	83 95 105 137	Whole plant Whole plant Whole plant Whole plant	<0.01 *(<0.01)* <0.01 *(<0.01)* <0.01 *(<0.01)* <0.01 *(<0.01)*	<0.01 *(<0.01)* <0.01 *(<0.01)* <0.01 *(<0.01)* <0.01 *(<0.01)*	<0.01 *(<0.01)* <0.01 *(<0.01)* <0.01 *(<0.01)* <0.01 *(<0.01)*	<0.05 *(<0.05)* <0.05 *(<0.05)* <0.05 *(<0.05)* 0.136 *(0.093)* [UTC 0.101 *(0.076)*] **0.110 *(0.08))* [UTC 0.082 *(0.066)*]**	Ref: 15SGS109 Frozen storage Max. 661 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Mean of results from replicate trials (SP04, study 15SGS101) reported in *italics*. GAP relevant results not underlined here, underlining has been done in the summary of the replicate trial.

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
								BBCH 75	174	Hay (37 day drying) Grain	<0.01	<0.01	<0.01	<0.05	Trial within ± 25% but scaled in line with other trials.
								BBCH 89	201		<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.05 *(0.117)* [UTC <0.05 *(0.084)*] **<0.05 *(0.10)* [UTC <0.05 *(0.074)*]**	Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.81 (1.24x overdosed)
								BBCH 89	201	Straw	<0.01 *(<0.01)*	<0.01 *(<0.01)*	<0.01 *(<0.01)*	0.101 *(0.105)* [UTC 0.125 *(0.113)*]	

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
														0.082 ***(0.086)*** **[UTC 0.101** ***(0.092)*** **]**	
Southern France 24230 Saint Antoine de Breuilh (Aquitaine) 15SGS109 FR07	Wheat, BOLOGNA	1) 21/10/15 2) 25/05/16 to 05/06/16 3) 05/07/16	Foliar application, boom sprayer	0.252	200.0	0.126	BBCH 01	BBCH 77	243	Whole plant	<0.01	<0.01	<0.01	0.084 [UTC 0.075]	Ref: 15SGS109 Frozen storage Max. 491 days.
														0.066 [UTC 0.059]	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 77	255	Hay (12 day drying) Grain	<0.01	<0.01	<0.01	<0.05	Trial overdosed (+26%). Proportionality principle applied (**bold** font).
								BBCH 89	256		<0.01	<0.01	<0.01	0.083 [UTC 0.080]	Proposed application rate: 200 g a.s./ha Scaling factor: 0.79 (1.26x overdosed)

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
								BBCH 89	256	Straw	<0.01	<0.01	<0.01	**0.066 [UTC 0.063]** 0.119 [UTC 0.136] **0.094 [UTC 0.107]**	
Southern France 84210 Pernes les Fontaines (PACA) 15SGS109 FR08	Wheat, MIRA DOUX	1) 25/10/15 2) 04/05/16 to 13/05/16 3) 28/06/16	Foliar application, boom sprayer	0.255	202.2	0.126	BBCH 12	BBCH 75-77	194	Whole plant	<0.01	<0.01	<0.01	0.084 [UTC 0.086] **0.066 [UTC 0.067]**	Ref: 15SGS109 Frozen storage Max. 512 days. Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 75-77	213	Hay (19 day	<0.01	<0.01	<0.01	0.249 [UTC	Trial overdosed (+28%).

Table 7-63 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
										drying)				0.222] **0.194 [UTC 0.173]**	Proportionality principle applied (**bold** font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.78 (1.28x overdosed)
								BBCH 89	220	Grain	<0.01	<0.01	<0.01	0.060 [UTC 0.111] **<0.05 [UTC 0.087]**	
								BBCH 89	220	Straw	0.014 **0.011**	<0.01	<0.01	0.247 [UTC 0.270] **0.193 [UTC 0.211]**	

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

Trials 'GE03' and 'SP04' were conducted with application rates within ± 25% of the proposed rate in terms of kg a.s./ha. Trials 'FR01', 'HU02', 'FR07' and 'FR08' were conducted with application rates slightly higher than ± 25% of the proposed rate in terms of kg a.s./ha. Given the discussion in Volume 1 (section 2.7.4) regarding the data supporting both pre-emergence and post-emergence GAPs and the significant time between application and harvest observed in the trials, the timing of application in these trials, although different, can be considered representative of the cGAP. Therefore, in this case the proportionality principle has been applied to trials. In accordance with the OECD guidance on crop field trials, appropriate scaling has been applied to all trials, including those within 25% of the cGAP, to prevent bias. Scaling of positive results has been denoted in Table 7-63 in **bold** text. Results reported as <LOQ at the overdosed application rate will remain <LOQ considering scaling, therefore these results have not been repeated.

A full consideration of the trials considered supportive of the proposed GAP for 'F9600-4 SC' on wheat and barley is given in Volume 1.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.2-04, Semrau J, 2018
Title:	Determination of residues of F9600 after one pre-emergence or post-emergence application of F9600-4 SC in Wheat at 10 sites in Northern and Southern Europe 2016
Report No.:	S16-01153; 2016RES-ISX2485
Guidelines:	Reg. (EU) 283/2013 and 284/2013; OECD 2009 Guidance Document on Overview of Residue Chemistry Studies (Series on Testing and Assessment No. 64); OECD Test Guideline 509; OECD Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164); EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4; OECD (1998) Principles of Good Laboratory Practice
GLP	Yes

Ten trials on wheat were conducted outdoors during 2016. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones.

Another trial was conducted at the same location as trial site '03' (03, study S16-01154). This trial site was tested in the same season at the same time (application on 19/03/16 in both trials) for trials on different crops (barley and wheat). The trials were conducted on different crops with a similar morphology. The application was made before the edible part of the crop had formed (before BBCH 51). At an early growth stage such as this, extrapolation between these crops is possible (in accordance with the extrapolations document SANCO 7525/VI/95, Rev. 10.3). The crops are not significantly different. Therefore, these trials are not considered truly independent. In accordance with the OECD Guidance on Crop Field Trials, the mean of the results from these two trials has been reported below in italicised text. The two trials can only be considered a single trial when considering the overall number of trials supporting the proposed uses.

Four of the trials were residue decline trials. The formulation 'F9600-4 SC' containing 36.4 % w/w active substance was applied to the trial sites. One application was made to wheat between BBCH 00 (pre-emergence) to BBCH 13.

The product tested in the trials was 'F9600-4 SC' which is representative product being considered within this application for approval. Therefore, these trials can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in the previous seasons or during the trials except for trial sites 01 and 05 where clomazone was applied to these trial sites during 2014. Please refer to the introduction to section 7.3 for a full consideration.

Additionally, the active substance propiconazole was applied to trial site 05 during 2013 and trial site 07 during 2015; this active substance contains the same 2,4-dichlorobenzoic acid moiety. However, considering the similarity of the results to other trials where this active substance has not been applied, and the lack of positive residues of 2.4-dichlorobenzoic acid in the untreated control samples, this is not considered to significantly affect the validity of the trial.

Residues of bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 5'-hydroxy bixlozone and 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-65 (denoted as 'UTC' with the corresponding treated results). Only a single untreated sample had positive residues of 5'-hydroxy bixlozone (trial 10, wheat hay). The result was positive but low (0.02 mg/kg). All other untreated samples of other matrices (whole plant, straw and grain) from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.05 mg/kg). Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 10 m (trial sites 01, 02, 04, 05, 07, 08, 09), ≥ 30 m (trial site 10) or ≥ 45 m apart (trial sites 03, 06).

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 0.5 kg of straw and forage, at least 1 kg of grain) except in trial 01 where an insufficient sample of grain was taken (between 0.44 and 0.82 kg). This insufficient sample size may have affected the representativeness of the results reported from this trial. No adverse weather was observed in the trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in wheat whole plant, straw and grain. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.2 mg/kg for wheat grain and hay and 0.05 mg/kg in wheat straw and whole plant, was supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were stored under frozen (< -18°C) conditions for up to 614 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for wheat forage, hay, straw and grains, for each of the analytes analysed. Samples were stored for up to 14 days under refrigerated conditions (1-10°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. A summary of the procedural recovery results is given in Table 7-64. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). Some individual recoveries were outside of this range; however, the mean recoveries are all within the acceptable range therefore no further consideration is required. Only single recovery determinations were made at each fortification level in straw however as these results were within the acceptable range and the method has been fully validated in this matrix, this is not considered to affect the validity of the study.

A summary of the residue trial results are given in Table 7-65.

Table 7-64 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)
Whole plant	0.01	82, 86, 93	83 (7.1)	71, 86, 103	89 (12)	73, 94, 101	97 (13)	-	87 (14)
	0.05	-		-		-		73, 78, 105	
	0.1	75, 82, 82		87, 93, 96		102, 104, 110		-	
	0.5	-		-		-		82, 88, 98	
Hay	0.01	71, 80, 81	77 (4.6)	72, 73, 81	74 (5.2)	71, 80, 94	78 (11)	-	95 (9.3)
	0.05	-		-		-		99, 101, 108	
	0.1	76, 77, 78		70, 72, 73		72, 75, 78		-	
	0.5	-		-		-		85, 89, 89	
Grain (Wheat)	0.01	77, 93, 107	96 (14)	82, 107, 110	100 (12)	79, 91, 95	97 (11)	-	77 (9.4)
	0.05	-		-		-		66, 71, 75	
	0.1	86, 104, 111		86, 102, 110		100, 106, 109		-	
	0.5	-		-		-		82, 83, 83	
Straw (Wheat)	0.01	85	83 (4.3)	107	102 (7.7)	91	97 (8.7)	-	98 (13)
	0.05	-		-		-		110	
	0.1	80		96		103		-	
	0.5	-		-		-		100	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36.4 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
S16-01153-018181, Unterfladnitz, Styria, Austria	Spring Wheat TRZAS Trappe	1) 28/03/16 2) mid June to end of June 16 3) 03/08/16	Broadcast spray using boom sprayer	0.252	404	0.062	BBCH 01	BBCH 75	96	Whole plant	<0.01	<0.01	<0.01	0.08 [UTC 0.08] **0.06 [UTC 0.06]**	Ref: S1601153 Frozen storage max. 574 days. Insufficient sample of grain was taken (between 0.44 and 0.82 kg), however the trial has not been excluded. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+26%). Proportionality
								BBCH 75	103	Hay	<0.01	<0.01	<0.01	0.20 [UTC 0.22] **<0.2 [UTC <0.2]**	
								BBCH 89	126	Grain	<0.01	<0.01	<0.01	<0.20 [UTC 0.23] **[UTC <0.2]**	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w		
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
								BBCH 89	126	Straw	<0.01	<0.01	<0.01	<0.05	principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.79 (1.26x overdosed)

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w		
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
S16-01153-0227449, Mulsum, Lower Saxony, Germany	Spring Wheat TRZAS KWS chasmin	1) 24/03/16 2) 14/06/16 to 21/06/16 3) 16/08/16	Broadcast spray using boom sprayer	0.259	259	0.10	BBCH 03	BBCH 37	56	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S1601153 Frozen storage max. 572 days.
								BBCH 51	66	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 65	77	Whole plant	<0.01	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 76	96	Whole plant	<0.01	<0.01	<0.01	0.05 **<0.05**	
								BBCH 76	103	Hay	<0.01	<0.01	<0.01	<0.2	
								BBCH 89	137	Grain	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.2</u>	Trial overdosed (+30%). Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.77 (1.30x overdosed)
								BBCH 89	137	Straw	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.05</u>	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w		
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
S16-01153-0591150, Mespuits, Essone, France	Spring Wheat TRZAS Lennox	1) 24/03/16 2) 15/06/16 to 27/06/16 3) 12/08/16	Broadcast spray using boom sprayer	0.265	212	0.125	BBCH 12	BBCH 75	82	Hay	0.01 **<0.01**	<0.01	<0.01	0.21 [UTC 0.21] **<0.2 [UTC <0.2]**	Ref: S1601153 Frozen storage max. 573 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+33%). Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.75 (1.33x overdosed)
								BBCH 89	113	Grain	<0.01	<0.01	<0.01	<0.2	
								BBCH 89	113	Straw	<0.01	0.02 **0.015**	<0.01	0.13 [UTC 0.17] **0.10 [UTC 0.13]**	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
S16-01153-06 DE73 8BB, Isley Walton, Leicestershire, UK	Spring Wheat TRZAS Mulika	1) 25/03/16 2) 30/04/16 to 08/05/16 3) 08/09/16	Broadcast spray using boom sprayer	0.239	192	0.124	BBCH 12	BBCH 75-77	73	Whole plant	<0.01	<0.01	<0.01	<0.05 [UTC 0.06] **[UTC 0.05]**	Ref: S1601153 Frozen storage max. 560 days. Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% but scaled in line with other trials. Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.84 (1.20x overdosed)
								BBCH 75-77	84	Hay	<0.01	<0.01	<0.01	<0.2	
								BBCH 89	129	Grain	<0.01	<0.01	<0.01	<0.2	
								BBCH 89	129	Straw	<0.01	<0.01	<0.01	<0.05	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
S16-01153-0771679, Baden Würtemberg, Germany	Spring Wheat TRZAS Cornetto	1) 15/03/16 2) 20/06/16 to 25/06/16 3) 01/08/16	Broadcast spray using boom sprayer	0.250	300	0.083	BBCH 12-13	BBCH 37-39	44	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S1601153 Frozen storage max. 555 days.
								BBCH 49-51	48	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 67	62	Whole plant	<0.01	<0.01	<0.01	<0.05 [UTC 0.05] **[UTC <0.05]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 77	78	Whole plant	<0.01	<0.01	<0.01	0.06 [UTC 0.07] **<0.05 [UTC 0.06]**	Trial within ± 25% but scaled in line with other trials. Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.80 (1.25x overdosed)
								BBCH 77	105	Hay	<0.01	0.03 **0.024**	<0.01	0.29 [UTC 0.22] **0.23**	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36.4 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
								BBCH 89	104	Grain	<0.01	<0.01	<0.01	**[UTC <0.2]** <0.2	
								BBCH 89	104	Straw	<0.01	<0.01	<0.01	0.08 [UTC 0.08] **0.064** **[UTC 0.064]**	
SOUTHERN EUROPE															
S16-01153-03 GR-63080, Krimni, Chalkidiki Greece	Spring Wheat TRZAS Athos	1) 18/03/16 2) 13/05/16 to 23/05/16 3) 27/06/16	Broadcast spray using boom sprayer	0.262	419	0.063	BBCH 00	BBCH 75	80	Whole plant	0.01 **<0.01** *<0.01*	<0.01 *<0.01*	<0.01 *<0.01*	0.35 *0.204* [UTC 0.24 (*0.158*)] **0.27** ***(0.16)*** ***[UTC 0.18***	Ref: S1601153 Frozen storage max. 603 days. Unless a residue value is stated, untreated control samples contained residues <LOQ.

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
								BBCH 75	85	Hay	0.02 **0.015**	<0.01	<0.01	***(0.12)]*** 0.68 [UTC 0.76] **0.52** **[UTC 0.58]**	Trial overdosed (+31%). Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.76 (1.31x overdosed) Relevant results from replicate trial 00154-03 on barley underlined.
								BBCH 89	100	Grain	<0.01 *0.01* ***<0.01***	<0.01 *<0.01*	<0.01 *<0.01*	<0.2 *0.16* ***0.14*** [UTC <0.2 *(0.16)* ***(0.15)***]	
								BBCH 89	100	Straw	<0.01 *<0.01*	<0.01 *<0.01*	<0.01 *<0.01*	0.25 *0.20* [UTC 0.23 *(0.18)*)]	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
														0.19 (0.16) [UTC 0.17 (0.13)]	
S16-01153-04 40068, Idice, Bologna, Italy.	Spring Wheat TRZAS Palesio	1) 22/03/16 2) 30/05/16 to 09/06/16 3) 11/07/16	Broadcast spray using boom sprayer	0.254	203	0.125	BBCH 00	BBCH 39	56	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S1601153 Frozen storage max. 519 days.
								BBCH 53	62	Whole plant	0.01 **<0.01**	<0.01	<0.01	<0.05	
								BBCH 69	77	Whole plant	<0.01	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 77	84	Whole plant	<0.01	<0.01	<0.01	0.06 [UTC 0.06] **<0.05 [UTC <0.05]**	Trial overdosed (+27%). Proportionality principle applied (bold font).
								BBCH 77	92	Hay	0.03 **0.02**	<0.01	<0.01	0.22 [UTC	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
														0.25] **<0.2 [UTC <0.2]**	Proposed application rate: 200 g a.s./ha Scaling factor: 0.79 (1.27x overdosed)
								BBCH 89	109	Grain	<0.01	<0.01	<0.01	<0.2	
								BBCH 89	109	Straw	<0.01	0.02 **0.016**	<0.01	0.19 [UTC 0.22] **0.15 [UTC 0.17]**	
S16-01153-08 82700, Saint Porquier, Tarn et Garonne, France	Spring Wheat TRZAS Triso	1) 22/03/16 2) 10/06/16 to 17/06/16 3) 27/07/16	Broadcast spray using boom sprayer	0.267	213	0.125	BBCH 11-12	BBCH 75	89	Whole plant	<0.01	<0.01	<0.01	0.10 [UTC 0.10] **0.075 [UTC 0.075]**	Ref: S1601153 Frozen storage max. 581 days. Unless a residue value is stated, untreated control samples contained
								BBCH 75	94	Hay	0.01	0.04	<0.01	0.28	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
											<0.01	**0.03**		[UTC 0.31] **0.21 [UTC 0.23]**	residues <LOQ. Trial overdosed (+34%). Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.75 (1.34x overdosed)
								BBCH 89	117	Grain	<0.01	<0.01	<0.01	<0.2 [UTC 0.22] **[UTC <0.2]**	
								BBCH 89	117	Straw	<0.01	0.02 **0.015**	<0.01	0.13 [UTC 0.06] **0.098 [UTC <0.05]**	
S16-01153-09 40054,	Spring Wheat TRZAS	1) 21/02/16 2) 10/05/16 to 20/05/16	Broadcast spray	0.266	317	0.084	BBCH 13	BBCH 77	73	Whole plant	<0.01	<0.01	<0.01	0.13 [UTC 0.14]	Ref: S1601153 Frozen storage max. 614 days.

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
Dugliola, Bologna, Italy	Michael	3) 22/06/16	using boom sprayer					BBCH 77	78	Hay	0.01 **<0.01**	<0.01	<0.01	**0.098 [UTC 0.11]** 0.29 [UTC 0.27] **0.22 [UTC 0.20]**	Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+33%). Proportionality principle applied (bold font). Proposed application rate: 200 g a.s./ha Scaling factor: 0.75 (1.33x overdosed)
								BBCH 89 BBCH 89	99 99	Grain Straw	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.2 0.31 [UTC 0.38] **0.23 [UTC 0.29]**	
S16-01153-10 50059,	Spring Wheat TRZAS	1) 30/12/15 2) 16/05/16 to 25/05/16	Broadcast spray	0.267	213	0.125	BBCH 11-13	BBCH 39 BBCH 55-	40 45	Whole plant Whole	0.01 **<0.01** <0.01	<0.01 <0.01	<0.01 <0.01	<0.05 <0.05	Ref: S1601153 Frozen storage max. 602 days.

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
Montana, Zaragoza, Spain	Kilo Pondio	3) 01/07/16	using boom sprayer					59 BBCH 67-69	54	plant Whole plant	0.01 **<0.01**	<0.01	<0.01	0.07 [UTC 0.06] **0.05 [UTC <0.05]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 75-77	69	Whole plant	<0.01	<0.01	<0.01	0.09 [UTC 0.07]	Trial overdosed (+34%). Proportionality principle applied (bold font).
								BBCH 75-77	73	Hay	0.01 **<0.01**	<0.01 [UTC 0.02] **[UTC 0.015]**	<0.01	0.21 **<0.2** [UTC <0.2]	Proposed application rate: 200 g a.s./ha Scaling factor: 0.75 (1.34x overdosed)
								BBCH 89	91	Grain	<0.01	<0.01	<0.01	<0.2	
								BBCH 89	91	Straw	<0.01	0.02	<0.01	0.21	

Table 7-65 Residues in wheat following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w		
Formulation (*e.g.* WP)	: SC	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl -3-hydroxy propionic acid (d)

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of application	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
												0.015		[UTC 0.20] **0.16 [UTC 0.15]**	

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

Trials 01, 02, 03, 04, 05, 08 and 09 were conducted with application rates slightly higher than ± 25% of the proposed rate in terms of kg a.s./ha. Trials 06, 07 and 10 were conducted with application rates within ± 25% of the proposed GAPs on wheat. Given the discussion in Volume 1 (section 2.7.4) regarding the data supporting both pre-emergence and post-emergence GAPs and the significant time between application and harvest observed in the trials, the timing of application in these trials, although different, can be considered representative of the cGAP. Therefore, in this case the proportionality principle has been applied to trials. In accordance with the OECD guidance on crop field trials, appropriate scaling has been applied to all trials, including those within 25% of the cGAP, to prevent bias. Scaling of positive results has been denoted in Table 7-65 in bold text. Results reported as <LOQ at the overdosed application rate will remain <LOQ considering scaling, therefore these results have not been repeated.

A full consideration of the trials considered supportive of the proposed GAP for 'F9600-4 SC' on wheat and barley is given in Volume 1.

Additional supporting information from processing studies

Please see section B.7.5.3 for a full consideration of these data with regards to processing. During each field trial performed to generate samples for the subsequent processing studies, a field trial was performed in accordance with the proposed GAP on wheat and barley. Therefore, these data may be considered supportive information and are summarised below.

Wheat

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-01, Semrau, J., 2018
Title:	Determination of residue of F9600 after one pre-emergence application of F9600-4 SC in wheat and in processed fractions of wheat at 2 sites in Northern and Southern Europe 2016
Report No.:	S16-05487
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

For a full consideration of this study please refer to section B.7.5.3. A single foliar application was made via boom sprayer of F9600-4 SC to treated plot 2 at BBCH 00-08, in both field trials (NEU and SEU). These trial conditions are in line with the proposed GAP for use on wheat. Therefore, the results from these trials can be considered supportive of the use on primary crops. A full consideration of the conduct of these trials has not been made given that treated plot 3 was at the same location therefore may not be considered an independent trial. However, these results can be considered supportive information when considering the proposed use on wheat as a primary crop. A summary of the results from treated plot 2 are presented in Table 7-66.

The results are in line with those reported for the primary crop field trials: bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid <0.01 mg/kg in grain. Positive residues of 2,2-dimethyl-3-hydroxy propionic acid were observed in grain. It should be noted that straw was not analysed as part of these studies.

Barley

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-02, Semrau, J., 2018
Title:	Determination of residue of F9600 after one pre-emergence application of F9600-4 SC in barley and in processed fractions of barley at 2 sites in Northern and Southern Europe 2016
Report No.:	S16-05488
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

For a full consideration of this study please refer to section B.7.5.3. A single foliar application was made via boom sprayer of F9600-4 SC to treated plot 2 at BBCH 00-08, in both field trials (NEU and SEU). These trial conditions are in line with the proposed GAP for use on barley. Therefore, the results from these trials can be considered supportive of the use on primary crops. A full consideration of the conduct of these trials has not been made. It should be noted that treated plot 3, which processing samples were taken from, was at the same location and treated at the same time, therefore may not be considered an independent trial. However, these results can be considered supportive information when considering the proposed use on wheat as a primary crop. A summary of the results from treated plot 2 are presented in Table 7-67.

The results are in line with those reported for the primary crop field trials: bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid <0.01 mg/kg and 2,2-dimethyl-3-hydroxy propionic acid <0.2 mg/kg in grain. It should be noted that straw was not analysed as part of these studies.

Table 7-66 Summary of results from barley field trials, treated plot 2 with GAP relevant application rate and timing

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
S16-05488-01: 71706, Markgröningen, Baden-Württemberg, Germany	Winter barley/ HORVW / California	1) 01/10/16 2) 28/05/17 – 11/06/17 3) 17/07/17	Foliar application, boom sprayer	0.247	247	0.100	BBCH 00-07	BBCH 89	272	Grain	<0.01	<0.01	<0.01	<0.2	Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+24%). Proposed application rate: 200 g a.s./ha
SOUTHERN EUROPE															
S16-	Winter	1) 21/11/16	Foliar	0.251	201	0.125	BBCH		174	Whole	<0.01	<0.01	<0.01	<0.2	Unless a residue

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Barley	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
05488-02: 82290, Barry d'Islemade, Tarnet Garonne, France	barley/ HORVW / Isocel	2) N/R 3) 19/06/17	application, boom sprayer				00	BBCH 89	207	plant Grain	<0.01	<0.01	<0.01	<0.2	value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+26%). Proposed application rate: 200 g a.s./ha

Table 7-67 Summary of results from wheat field trials, treated plot 2 with GAP relevant application rate and timing

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
S16-05487-01: 21726, Oldendorf, Niedersachsen, Germany	Winter wheat/ TRZAW / Anapolis	1) 14/10/15 2) N.R 3) 07/08/17	Foliar application, boom sprayer	0.254	305	0.083	BBCH 07	BBCH 75-77 BBCH 89	249 291	Whole plant Grain	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.2 0.27	Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial overdosed (+27%). Proposed application rate: 200 g a.s./ha
SOUTHERN EUROPE															
S16-05487-02: 50180	Winter wheat/ TRZAW /	1) 11/11/16 2) Early-mid May 2017	Foliar application, boom	0.252	201	0.125	BBCH 00-08	BBCH 89	225	Grain	<0.01	<0.01	<0.01	<0.2	Unless a residue value is stated, untreated control samples

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Wheat	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
, Utebo , Aragon, Spain	Nogal	3) 29/06/17	sprayer												contained residues <LOQ. Trial overdosed (+26%). Proposed application rate: 200 g a.s./ha

B.7.3.2. Oilseed rape

Representative GAPs

The uses on oilseed rape detailed in Table 7-68 Representative GAP for use on oilseed rapeTable 7-68 are proposed within this application for approval of bixlozone.

Table 7-68 Representative GAP for use on oilseed rape

Crop and/or situation	For	Pest or group of pests	Formulation rate per treatment		Application			Application rate per treatment			PHI (days)
(a)	G (b)	controlled (c)	Type (d-f)	Conc of a.s. (i)	method, kind , if other than spray (f-h)	growth stage (j)	number (range) (k)	g a.s./ha, where appropriate	water L/ha	g a.s./hL, where appropriate	(l)
Winter oilseed rape	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 00-09	1	200 - 300	150 – 400	50 - 200	N/A

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure).
(b) Outdoor or field use (F), glasshouse application (G) or indoor application.
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds.
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR).
(e) GCPF Codes – GIFAP Technical Monograph No 2, 1989.
(f) All abbreviations used must be explained.
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench.
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated.
(i) g/kg or g/L.
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application.
(k) Indicate the minimum and maximum number of application possible under practical conditions of use.
(l) PHI – minimum pre-harvest interval.

A total of 7 trials on oilseed rape, conducted in the Northern European zone, were submitted to support the proposed GAP. The Northern European trials are considered relevant to the GB climate. 5 trials on oilseed rape conducted in the Southern European zone were submitted and have been summarised for completeness. For this UK only application, these Southern European zone trials have not been considered further.

All trials analysed the raw agricultural commodity ‘seeds’ at growth stage BBCH 89. OECD 509 describes the commodity to be analysed for oilseed rape as ‘seed – whole commodity’ and ‘fodder and straw – whole commodity’. Not all of the trials analysed a ‘fodder/straw’ fraction. However, as a specific GAP for use as a forage crop has not been proposed, analysis of the ‘fodder and straw’ commodity is not considered necessary. The analysis of seeds is considered sufficient to support the GAP being considered. It should be noted that where forage uses have not been proposed, the only input relevant to oilseed rape in the most recent OECD animal dietary burden calculator is ‘seeds’.

Trials performed on Oilseed rape

The proposed GAP on oilseed rape is: 300 g a.s./ha x 1, BBCH 00-09.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.1-01, Gemrot, F. 2017
Title:	Magnitude of the Residues of F9600 in Oil Seed Rape (Raw Agricultural Commodity) after one pre emergence or post emergence application of F9600-4 SC in Northern Europe (Northern France and Germany) and Southern Europe (Italy and Spain) – 2015 and 2016
Report No.:	15SGS108; 2015RES-ISX2146
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Four trials on oilseed rape were conducted outdoors during 2015-2016. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones. Four other trials were begun as part of this study report however these were lost to phytotoxicity of the formulated product applied at post emergence stage. These trials were cancelled and removed from the study.

Other trials on wheat and barley were performed at the same time (application on 12/11/15 in wheat and barley trials, 29/10/15 for oilseed rape) at the same location as trials site 'SP07'. Given extrapolation is not possible between these crops, the oilseed rape trial can be considered independent. No issues regarding possible cross contamination have been reported.

One of the trials was a residue decline trial. The formulation 'F9600-4 SC' containing 36 % w/w active substance was applied to the trial sites. One application was made to oilseed rape at BBCH 00-08 (pre-emergence) in the trials.

The product tested in the trials was 'F9600-4 SC' which is representative product being considered within this application for approval. Therefore, these trials can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in previous seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid were not found (<LOQ, <0.01 mg/kg or 0.05 mg/kg for 2,2-dimethyl-3-hydroxy-propionic acid) in any of the untreated control samples. Further discussion on the occurrence of residues in untreated control samples can be found in section 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 20 m apart.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 0.5 kg of seed). No adverse weather conditions were reported during the trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in oilseed rape crop fractions. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were stored under frozen (< -18°C) conditions for up to 527 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for oilseed rape crop fractions for each of the analytes analysed. Samples were stored for up to 4 days under refrigerated conditions (~4°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. A summary of the procedural recovery results is given in

Table 7-69. The %RSD was not calculated as only one recovery determination was made at each fortification level. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). However, the data set available to calculate these mean values is limited (2 determinations). Considering the proposed use (not a forage use), the relevant results are residues in seed, for which the recoveries are all within the range 70-110%.

A summary of the residue trial results are given in Table 7-70.

Table 7-69 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%)	Recovery (%)	Mean recovery (%)	Recovery (%)	Mean recovery (%)	Recovery (%)	Mean recovery (%)
Whole plant	0.01	90	90	95	92	81	89	-	109
	0.05	-		-		-		92	
	0.1	89		89		96		-	
	0.5	-		-		-		125	
Whole plant w/o pod	0.01	91	90	92	90	96	98	-	109
	0.05	-		-		-		102	
	0.1	89		88		99		-	
	0.5	-		-		-		115	
Pods	0.01	85	87	94	95	86	91	-	96
	0.05	-		-		-		96	
	0.1	89		96		96		-	
	0.5	-		-		-		95	
Seeds	0.01	71	74	99	95	104	94	-	102
	0.05	-		-		-		99	
	0.1	77		90		84		-	
	0.5	-		-		-		105	
Silage	0.01	104	100	95	97	99	100	-	93
	0.05	-		-		-		94	
	0.1	96		99		101		-	
	0.5	-		-		-		91	

Table 7-70 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
Northern France 41 500 Suevres (Centre) 15SGS108 FR01	Oilseed rape DK EXSTORM	1) 07/09/15 2) 18/04/16 to 03/05/16 3) 18/07/16	Foliar application, boom sprayer	0.254	201.8	0.126	BBCH 00	BBCH 19-20	89	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: 15SGS108 Frozen storage Max. 527 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha
								BBCH 35	181	Whole plant (silage)	<0.01	<0.01	<0.01	<0.05	
								BBCH 50-55	205	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 80	261	WP w/o pods	<0.01	<0.01	<0.01	<0.05	
								BBCH 80	261	Pods	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	313	Seeds	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.05</u>	

Table 7-70 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
Germany 49681 Beverbruch / Garrel (Lower Saxony) 15SGS108 GE03	Oilseed Rape PX108	1) 05/09/15 2) 21/04/16 to 18/05/16 3) 22/07/16 to 25/07/16	Foliar application, boom sprayer	0.262	208.3	0.126	BBCH 08	BBCH 39 BBCH 89	212 320	Whole plant (silage) Seeds	<0.01 <u><0.01</u>	<0.01 <u><0.01</u>	<0.01 <u><0.01</u>	<0.05 <u><0.05</u>	Ref: 15SGS108 Frozen storage Max. 399 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-70 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
SOUTHERN EUROPE															
Italy 06052 Cerqueto, Marsciano (Umbria) 15SGS108 IT06	Oilseed Rape KODIAK KWS	1) 09/10/15 2) 15/04/16 to 25/04/16 3) 10/06/16 to 20/06/16	Foliar application, boom sprayer	0.246	391	0.063	BBCH 00	BBCH 89	252	Seeds	<0.01	<0.01	<0.01	<0.05	Ref: 15SGS108 Frozen storage Max. 329 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-70 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
Spain 41 410 Carmona (Andalusia) 15SGS108 SP07	Oilseed Rape OMEGA 9	1) 25/10/15 2) 16/02/16 to 04/04/16 3) 27/05/16	Foliar application, boom sprayer	0.248	296	0.084	BBCH 00	BBCH 39 BBCH 89	97 211	Whole plant (silage) Seeds	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.05 <0.05	Ref: 15SGS108 Frozen storage Max. 464 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

All four trials were conducted with an application rate within ± 25% of the proposed rate in terms of kg a.s./ha. The results from all trials are <LOQ for all chemical components analysed for.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.1-02, Semrau, J. 2018
Title:	Determination of residues of F9600 after one pre-emergence application of F9600-4 SC in Oilseed Rape at 8 sites in Northern and Southern Europe 2016
Report No.:	S16-010155; 2016RES-ISX2487
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Eight trials on oilseed rape were conducted outdoors during 2016. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones. Two trials in Spain were originally planned as part of this study but were cancelled due to late drilling of the crop leading to plants not reaching maturity.

Other trials on wheat and barley were performed at the same time (application on 19/03/16 for barley and wheat, 24/03/16 for oilseed rape) at the same location as trials site '08'. Given extrapolation is not possible between these crops, the oilseed rape trial can be considered independent. No issues regarding possible cross contamination have been reported. Trial site '04' appears to have a similar address to a trial site tested in the wheat field trials in study 'S16-01153-07', however the plots are in sufficiently different locations, approximately 20 km apart.

Three of the trials were residue decline trials. The formulation 'F9600-4 SC' containing 36.4 % w/w active substance was applied to the trial sites. One application was made to oilseed rape at BBCH 00-05 (pre-emergence) in the trials.

The product tested in the trials was 'F9600-4 SC' which is representative product being considered within this application for approval. Therefore, these trials can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in previous seasons or during the trials. The active substance propiconazole which contains the 2,4-dichlorobenzoic acid moiety was applied to trial site 04 during 2014. However, considering the similarity of the results to other trials where this active substance has not been applied, and the lack of positive residues of 2.4-dichlorobenzoic acid in the untreated control samples, this is not considered to significantly affect the validity of the trial.

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid were not found (<LOQ, <0.01 mg/kg or 0.05 mg/kg for 2,2-dimethyl-3-hydroxy-propionic acid) in any of the untreated control samples. Further discussion on the occurrence of residues in untreated control samples can be found in section 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 10 m apart.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The treated samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 0.5 kg of seed).

The environmental conditions observed did not alter the normal growth, development and maturity of the crop at the trial sites to such a degree to have a negative impact on the validity of the study. Generally, a higher level of rainfall was observed, compared to the average, in May/July 2016 at the trial sites 02, 04 and 05. The air temperatures are similar to the averages reported for the trial sites. Overall, the change in weather patterns is not expected to affect the validity of the study with three field trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in oilseed rape crop fractions. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. A reduced data set was provided to support

the oilseed rape crop fractions other than seed (whole plant, flowers, plant without pods, pods) within the field trial study report. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.3 and 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data) for all oilseed rape crop fractions.

Samples were stored under frozen (< -18°C) conditions for up to 650 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for oilseed rape crop fractions for each of the analytes analysed. Samples were stored for up to 5 days under refrigerated conditions (1-10°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. A summary of the procedural recovery results is given in Table 7-71. The %RSD was not calculated for recoveries determined in seeds as only one recovery determination was made at each fortification level. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). However, the data set available to calculate these mean values is limited and some recoveries are outside of the acceptable range. Considering the proposed use (not a forage use), the relevant results are residues in seed, for which the recoveries are all within the range 70-110%.

A summary of the residue trial results are given in Table 7-72.

Table 7-71 Summary of procedural recovery data

Matrix	**Fortification level (mg/kg)**	**bixlozone**		**5'-hydroxy-bixlozone**		**2,4-dichloro benzoic acid**		**2,2-dimethyl-3-hydroxy propionic acid**	
		Recovery (%)	**Mean recovery (%) (%RSD)**	**Recovery (%)**	**Mean recovery (%) (%RSD)**	**Recovery (%)**	**Mean recovery (%) (%RSD)**	**Recovery (%)**	**Mean recovery (%) (%RSD)**
Whole plant	0.01	105, 106, 115	102 (17)	89, 103, 105	100 (7.0)	102, 109, 119	108 (6.1)	-	86 (11)
	0.05	-		-		-		71, 86, 95	
	0.1	83, 111, 120		102, 103, 105		101, 110, 112		-	
	0.5	-		-		-		87, 92, 96	
	1.0	72		90		102		-	
	5.0	-		-		-		78	
Flowers	0.01	86, 104, 104	103 (8.9)	73, 76, 84	74 (7.4)	102, 106, 109	105 (2.5)	-	97 (10)
	0.05	-		-		-		88, 97, 115	
	0.1	103, 106, 114		69, 70, 72		103, 104, 107		-	
	0.5	-		-		-		90, 91, 99	
Plants w/o pods	0.01	104, 105, 112	103 (5.6)	82, 92, 115	101 (12)	99, 110, 118	109 (5.7)	-	109 (7.2)
	0.05	-		-		-		103, 105, 120	
	0.1	96, 98, 100		101, 105, 108		107, 107, 110		-	
	0.5	-		-		-		100, 112, 116	
Pods	0.01	79, 81, 82	84 (5.4)	71, 71, 73	76 (5.3)	87, 97, 100	99 (6.6)	-	90 (6.5)
	0.05	-		-		-		80, 93, 98	
	0.1	83, 85, 92		77, 77, 78		103, 103, 105		-	
	0.5	-		-		-		90, 90, 90	

Matrix	**Fortification level (mg/kg)**	**bixlozone**		**5'-hydroxy-bixlozone**		**2,4-dichloro benzoic acid**		**2,2-dimethyl-3-hydroxy propionic acid**	
		Recovery (%)	**Mean recovery (%) (%RSD)**	**Recovery (%)**	**Mean recovery (%) (%RSD)**	**Recovery (%)**	**Mean recovery (%) (%RSD)**	**Recovery (%)**	**Mean recovery (%) (%RSD)**
Seeds	0.01	83	82 (-)	97	97 (-)	108	103 (-)	-	82 (-)
	0.05	-		-		-		78	
	0.1	81		97		97		-	
	0.5	-		-		-		86	

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
5500 Middelfart, South Denmark, Denmark S16-01155-01	Oilseed Rape BRSNS Ability	1) 20/04/16 2) 05/06/16 to 28/06/16 3) 08/09/16	Broadcast Spray with boom sprayer	0.253	202	0.125	BBCH 00	BBCH 39 BBCH 65 BBCH 89	43 55 141	Whole plant Flowers Seeds	0.01 0.012 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 <0.05 <0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
H-8127 Aba, Fejer, Hungary S16-01155-02	Oilseed Rape BRSNS Ability	1) 25/04/16 2) n/r 3) 16/09/16	Broadcast Spray with boom sprayer	0.247	346	0.071	BBCH 00	BBCH 35 BBCH 65 BBCH 89	46 77 140	Whole plant Flowers Seeds	<0.01 <0.01 <u><0.01</u>	<0.01 <0.01 <u><0.01</u>	<0.01 <0.01 <u><0.01</u>	<0.05 <0.05 <u><0.05</u>	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
21726 Oldendorf, Lower Saxony, Germany S16-01155-03	Oilseed Rape BRSNS Ability	1) 08/04/16 2) 18/06/16 to 30/06/16 3) 07/09/16	Broadcast Spray with boom sprayer	0.252	302	0.083	BBCH 05	BBCH 35 BBCH 65 BBCH 89	45 72 148	Whole plant Flowers Seeds	0.011 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.05 <0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
71723, Großbottwar, Baden-Württemberg, Germany S16-01155-04	Oilseed Rape BRSNS Heros	1) 26/03/16 2) 27/06/16 to 11/07/16 3) 08/09/16	Broadcast Spray with boom sprayer	0.257	309	0.083	BBCH 03	BBCH 20-22	64	Whole plant	0.018	<0.01	<0.01	<0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha
								BBCH 36	67	Whole plant	0.015	<0.01	<0.01	<0.05	
								BBCH 55	70	Whole plant	0.013	<0.01	<0.01	<0.05	
								BBCH 65	96	Flowers	<0.01	<0.01	<0.01	0.06	
								BBCH 80	112	Plants without pods	<0.01	<0.01	<0.01	0.51	
								BBCH 80	112	Pods	<0.01	<0.01	<0.01	1.3	
								BBCH 89	161	Seeds	<0.01	<0.01	<0.01	<0.05	

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
45300, Audeville (Carbouville), Loiret, France (N EU) S16-01155-05	Oilseed Rape BRSNS Mozaik	1) 06/04/16 2) 20/06/16 to 15/07/16 3) 08/09/16	Broadcast Spray with boom sprayer	0.273	219	0.125	BBCH 05	BBCH 21	42	Whole plant	0.024	<0.01	<0.01	<0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha
								BBCH 31	50	Whole plant	0.015	<0.01	<0.01	<0.05	
								BBCH 55	66	Whole plant	0.013	<0.01	<0.01	<0.05	
								BBCH 65	76	Flowers	<0.01	<0.01	<0.01	<0.05	
								BBCH 80	100	Plants without pods	0.013	<0.01	<0.01	0.59	
								BBCH 80	100	Pods	<0.01	<0.01	<0.01	2.2	
								BBCH 89	149	Seeds	<0.01	<0.01	<0.01	<0.05	

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
SOUTHERN EUROPE															
5570, Letnitsa, Lovech, Bulgaria S16-01155-07	Oilseed Rape BRSNS Doktrin	1) 21/03/16 2) 27/05/16 to 20/06/16 3) 26/07/16	Broadcast spray with boom sprayer	0.244	391	0.062	BBCH 00	BBCH 35-39 BBCH 65 BBCH 89	56 67 122	Whole plant Flowers Seeds	<0.01 0.011 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.06 <0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
GR-63080, Krimni, Chalkidiki, Greece S16-01155-08	Oilseed Rape BRSNS Smilla	1) 23/03/16 2) 07/05/16 to 01/06/16 3) 02/07/16	Broadcast spray with boom sprayer	0.260	416	0.063	BBCH 00	BBCH 35 BBCH 65 BBCH 89	40 54 100	Whole plant Flowers Seeds	0.017 0.048 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 <0.05 <0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha

Table 7-72 Residues in oilseed rape following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
40054, Mezzolara, Emilia Romagna, Italy S16-01155-10	Oilseed Rape BRSNS Mirakel	1) 31/03/16 2) 25/05/16 to 14/06/16 3) 15/07/16	Broadcast spray with boom sprayer	0.252	302	0.083	BBCH 00	BBCH 20	53	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S1601155 Frozen storage Max. 650 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 300 g a.s./ha
								BBCH 50-55	57	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 73-75	76	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 63-65	62	Flowers	0.012	<0.01	<0.01	<0.05	
								BBCH 80	85	Plants without pods	<0.01	<0.01	<0.01	0.56	
								BBCH 80	85	Pods	<0.01	<0.01	<0.01	1.1	
								BBCH 89	106	Seeds	<0.01	<0.01	<0.01	<0.05	

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

All eight trials were conducted with an application rate within ± 25% of the proposed rate in terms of kg a.s./ha. The residues found in seeds at harvest from all trials are <LOQ for all chemical components analysed for.

A full consideration of the trials considered supportive of the proposed GAP for 'F9600-4 SC' on oilseed rape is given in Volume 1.

Additional supporting information from processing studies

Please see section B.7.5.3 for a full consideration of these data with regards to processing. During each field trial performed to generate samples for the subsequent processing studies, a field trial was performed in accordance with the proposed GAP on oilseed rape. Therefore these data may be considered supportive information and are summarised below.

Oilseed rape

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-03, Semrau, J., 2018
Title:	Determination of residue of F9600 after one pre-emergence application of F9600-4 SC in oilseed rape and in processed fractions of oilseed rape at 4 sites in Northern and Southern Europe 2016
Report No.:	S16-05489
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

For a full consideration of this study please refer to section B.7.5.3. A single foliar application was made via boom sprayer of F9600-4 SC to treated plot 2 at BBCH 00-01, in all four field trials (NEU and SEU). These trial conditions are in line with the proposed GAP for use on oilseed rape. Therefore, the results from these trials can be considered supportive of the use on primary crops. A full consideration of the conduct of these trials has not been made. It should be noted that treated plot 3, which processing samples were taken from, was at the same location and treated at the same time, therefore may not be considered an independent trial. However, these results from plot 2 can be considered supportive information when considering the proposed use on oilseed rape as a primary crop. A summary of the results from treated plot 2 are presented in Table *7-73*.

The results are in line with those reported for the primary crop field trials: bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid <0.01 mg/kg and 2,2-dimethyl-3-hydroxy propionic acid <0.05 mg/kg in seed.

Table 7-73 Summary of results from oilseed rape field trials, treated plot 2 with GAP relevant application rate and timing

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
S16-05489-01: 27449, Mulsum, Niedersachsen, Germany	Winter oilseed rape/ BRSNW / DK Imperial CL	1) 31/08/16 2) 29/04 – 14/05/17 3) 05/08/17	Foliar application, boom sprayer	0.249	299	0.083	BBCH 01	BBCH 20-25	80	Whole plant	0.013	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ. Within ±25% of GAP Proposed application rate: 300 g a.s./ha
								BBCH 35-39	206	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 50-55	209	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 63-65	243	Flowers	<0.01	<0.01	<0.01	<0.05	
								BBCH 80	292	Plant w/o pods	<0.01	<0.01	<0.01	<0.05	
								BBCH 80	292	Pods	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	337	Seeds	<0.01	<0.01	<0.01	<0.05	
SOUTHERN EUROPE															
S16-	Winter	1) 27/09/16	Foliar	0.258	304	0.083	BBCH	BBCH	167	Whole	<0.01	<0.01	<0.01	<0.05	Unless a residue

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
05489-02: 40016, S. Giorgio di Piano, Bologna, Italy	oilseed rape/ BRSNW / Excalibur	2) 04/04 – 25/04/17 3) 27/06/17	application, boom sprayer				01	35-39 BBCH 63-65 BBCH 89	187 271	plant Flowers Seeds	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.05 <0.05	value is stated, untreated control samples contained residues <LOQ. Within ±25% of GAP Proposed application rate: 300 g a.s./ha
S16-05489-03: 22193, Arascues, Arago	Winter oilseed rape/ BRSNW / Hydromel	1) 30 Sep 2016 2) n/r 3) 19 Jun 2017	Foliar application, boom sprayer	0.249	299	0.083	BBCH 00	BBCH 20-25 BBCH 35-39 BBCH 50-55 BBCH 63-65	143 155 161 178	Whole plant Whole plant Whole plant Flowers	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.05 <0.05 <0.05 <0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ. Within ±25% of

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Oilseed rape	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
n, Spain								BBCH 80	226	Plant w/o pods	<0.01	<0.01	<0.01	<0.05	GAP Proposed application rate: 300 g a.s./ha
								BBCH 80	226	Pods	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	252	Seeds	<0.01	<0.01	<0.01	<0.05	
S16-05489-04: 22280, Gurrca de Gallego, Aragon, Spain	Winter oilseed rape/ BRSNW / Albatros	1) 26 Sep 2016 2) n/r 3) 15 Jun 2017	Foliar application, boom sprayer	0.255	306	0.083	BBCH 00	BBCH 35-39	165	Whole plant	<0.01	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ. Within ±25% of GAP Proposed application rate: 300 g a.s./ha
								BBCH 63-65	181	Flowers	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	258	Seeds	<0.01	<0.01	<0.01	<0.05	

B.7.3.3. Maize

Representative GAPs

The uses on maize detailed in Table 7-74 are proposed within this application for approval of bixlozone.

Table 7-74 Representative GAP for use on maize

Crop and/or situation (a)	For G (b)	Pest or group of pests controlled (c)	Formulation rate per treatment		Application			Application rate per treatment			PHI (days) (l)
			Type (d-f)	Conc of a.s. (i)	method, kind , if other than spray (f-h)	growth stage (j)	number (range) (k)	g a.s./ha, where appropriate	water L/ha	g a.s./hL, where appropriate	
Maize	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 00-09	1	250 - 375	150 – 400	62.5 - 250	N/A

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure).
(b) Outdoor or field use (F), glasshouse application (G) or indoor application.
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds.
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR).
(e) GCPF Codes – GIFAP Technical Monograph No 2, 1989.
(f) All abbreviations used must be explained.
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench.
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated.
(i) g/kg or g/L.
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application.
(k) Indicate the minimum and maximum number of application possible under practical conditions of use.
(l) PHI – minimum pre-harvest interval.

A total of 8 trials on maize, conducted in the Northern European zone, were submitted to support the proposed GAP. The Northern European trials are considered relevant to the GB climate. 8 trials on maize conducted in the Southern European zone were submitted and have been summarised for completeness. For this UK only application, these Southern European zone trials have not been considered further.

All trials analysed the raw agricultural commodities ‘grain’ and ‘straw’ at growth stage BBCH 89. OECD 509 describes the commodity to be analysed for maize, relevant to the EU uses, as ‘grain – whole commodity, grain without husk or cob’, ‘fodder/stover – whole commodity’ and ‘forage’. As a specific GAP for use as a forage crop has not been proposed, analysis of the ‘forage’ is not considered necessary; the results for the ‘whole plant’ crop fraction have not been considered further. The analysis of ‘grain’ and ‘straw’ (also referred to as stover) is considered sufficient to support the GAP being considered.

Trials performed on Maize

The proposed GAP on maize is: 375 g a.s./ha x 1, BBCH 00-09.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.3-01, Gemrot, F. 2017
Title:	Magnitude of the Residues of F9600 in maize (Raw Agricultural Commodity) after one pre emergence application of F9600-4 SC or F9600-28 CS in Northern Europe (Northern France and Germany) and Southern Europe (Southern France and Spain) – 2015
Report No.:	15SGS072; 2015RES-ISX1939
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Four trials on maize were conducted outdoors during 2015. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones.

Another trial on wheat was performed at the same time (application on 20/11/15 in wheat trial, 30/04/15 for maize) at the same location as trials site 'FR03'. Given extrapolation is not possible between these crops, the maize trial can be considered independent. No concerns regarding cross contamination have been reported.

Trial site 'SP04' has been tested in another trial reported in the DAR (SP04, study 15SGS073). This trial site was tested in the same season at a similar time (application on 30/04/15 in study 15SGS072, 13/05/15 in study 15SGS073) on the same crop (maize), on different plots. However, as the trial in study 15SGS073 is significantly under-dosed and scaling is not possible due to <LOQ residues, as discussed in the summary of study 15SGS073, no further consideration of the independence of these trails has been made at this time.

None of the trials were residue decline trials. The formulations 'F9600-4 SC' containing 37.4 % w/w active substance and 'F9600-28 CS' containing 34.3 % w/w active substance were applied to different plots within the trial sites. One application was made to maize at BBCH 00-08 (pre-emergence) in the trials.

'F9600-4 SC' is the representative product being considered within this application for approval. Therefore, trials performed with this product can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested. 'F9600-28 CS' is not the representative product and is a different formulation type. The formulations are both diluted in water prior to application therefore should give comparable results given the long time between application and harvest. A full consideration of the comparability of results from trials at the same site performed using the 'SC' and 'CS' formulation has been made in Volume 1, section 2.7.4. The values taken forward for MRL calculation and consideration of consumer risk should be independent, therefore only one value has been selected from each trial (same experimental location). In this case, the highest value has been selected from these two plots. Please see further discussion in Volume 1, section 2.7.4.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in previous seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone, 5-hydroxy-bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-76 and Table 7-77 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.05 mg/kg). Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 20 m apart.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 1 kg of grain, 12 plants of fodder/stover and 12 plants or at least 1 kg of forage). No adverse weather conditions were observed during the field trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 5-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in maize crop fractions. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference

above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were analysed using two analytical methods. Samples analysed using both methods 'CAM-0154' and 'CAM-0180' have been considered. Method 'CAM-0180' includes a hydrolysis step therefore considers conjugated residues and analyses the additional metabolite 5-hydroxy bixlozone. When considering the results in samples determined using method 'CAM-0154', the only positive residues reported were of 2,2-dimethyl-3-hydroxy-propionic acid in maize straw (trial FR03): 0.011 – 0.013 mg/kg. These results are <0.05 mg/kg, the LOQ of method 'CAM-0180'. Therefore, the results determined using method 'CAM-0180', where more critical higher positive residues have been reported, have been taken forward.

Samples were stored under frozen (< -18°C) conditions for up to 704 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for maize crop fractions for each of the analytes analysed. Samples were stored for up to 8 days under refrigerated conditions (~4°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. The data reported was generated using method 'CAM-0180'. A summary of the procedural recovery results is given in Table 7-75. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). Some individual recoveries were outside of this range; however, the mean recoveries within this range, this is acceptable. The overall mean recovery for maize whole plant (silage) and the component 5'-hydroxy-bixlozone was 111% which slightly exceeds the acceptable range. As this exceedance is only small and mostly due to two individual recoveries which are above the acceptable range, there is good precision across the other matrices for this analyte, and the results in forage are less relevant as a GAP specifically for use on forage has not been proposed, this is considered acceptable.

Quinoa grain was used for the 2,2-dimethyl-3-hydroxy propionic acid procedural recoveries due to high background levels seen in untreated maize grain samples. Untreated wheat grain was also analysed but contained residues of 2,2-dimethyl-3-hydroxy propionic acid also therefore was not suitable. Following the completion of the maize studies, untreated barley grains containing no 2,2-dimethyl-3-hydroxy propionic acid were used to generated acceptable procedural recoveries in support of barley and wheat studies. Maize, barley and quinoa belong to the cereals group and are grown for their seeds, therefore in this case, this is considered sufficient to rely on data from these alternative crops to demonstrate method performance.

A summary of the residue trial results are given in Table 7-76 and Table 7-77.

Table 7-75 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		5-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%)(%RSD)
Whole maize plant (silage)	0.01	100, 102, 105	104 (2.7)	102, 106, 110	111 (5.8)	99, 102, 107	106 (4.1)	97, 98, 100	107 (8.5)	-	87 (13.1)
	0.05	-		-		-		-		71, 74, 93	
	0.1	105, 107, 107		110, 118, 118		108, 109, 110		113, 115, 116		-	
	0.5	-		-		-		-		92, 95, 97	
Maize grain (except for 2,2-dimethyl-3-hydroxy propionic acid where quinoa grain was used as an alternative to maize grain)	0.01	107, 109, 110	109 (1.0)	90, 90, 98	96 (5.1)	102, 109, 118	109 (4.9)	97, 103, 105	106 (5.3)	-	81 (7.9)
	0.05	-		-		-		-		71, 83, 86	
	0.1	108, 108, 109		97, 100, 101		106, 107, 109		109, 109, 113		-	
	0.5	-		-		-		-		76, 80, 88	
Maize straw (also referred to as stover)	0.01	94, 100, 104	102 (5.8)	88, 90, 94	92 (4.1)	101, 103, 105	107 (5.7)	85, 87, 95	94 (6.4)	-	93 (1.2)
	0.05	-		-		-		-		75, 76, 91	
	0.1	101, 101, 112		89, 96, 97		105, 109, 118		97, 98, 99		-	
	0.5	-				-		-		92, 94, 94	

Table 7-76 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d), 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
NORTHERN EUROPE																

Northern France 41190 Santenay FR01 Plot T1	Maize GIRESS	1) 23/04/15 2) 15/07/15 to 21/07/15 3) 06/10/15 to 10/10/15	Foliar application Boom sprayer	0.384	306.7	0.125	BBCH 00	BBCH 85 BBCH 89 BBCH 89	120 160 160	Whole plant Grain Straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.10 [UTC 0.12] <0.05	<0.01 <0.01 <0.01	Ref: 15SGS072 Frozen storage Max. 642 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 375 g a.s./ha Highest result from replicate trial with 'CS' formulation underlined.
Germany 16816 Kranzlin (Brandenburg) GE02 Plot T1	Maize ZOEY	1) 28/04/15 2) 25/07/15 to 03/08/15 3) 30/10/15 to 31/10/15	Foliar application Boom sprayer	0.370	196.7	0.188	BBCH 00	BBCH 85 BBCH 89 BBCH 89	134 185 185	Whole plant Grain Straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.12 [UTC 0.09] <0.05	<0.01 <0.01 <0.01	Ref: 15SGS072 Frozen storage Max. 619 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 375 g a.s./ha Highest result from replicate trial with 'CS' formulation

Table 7-76 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d), 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
																underlined.
SOUTHERN EUROPE																
Southern France 84210 Pernes les Fontaines (PACA) FR03 Plot T1	Maize CORETTA	1) 20/04/15 2) 21/07/15 to 28/07/15 3) 27/10/15	Foliar application Boom sprayer	0.383	305.6	0.125	BBCH 05	BBCH 85	134	Whole plant	<0.01	<0.01	<0.01	0.06 [UTC 0.05]	<0.01	Ref: 15SGS072 Frozen storage Max. 623 days
								BBCH 89	179	Grain	<0.01	<0.01	<0.01	0.33 [UTC 0.29] **0.32 [UTC 0.28]**	<0.01	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH	179	Straw	<0.01	<0.01	<0.01		<0.01	Trial within ± 25% of proposed application rate: 375

Table 7-76 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d), 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
								89						0.05 **<0.05**		g a.s./ha Highest result from replicate trial with 'CS' formulation underlined. Trial within ± 25% of GAP application rate. Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 0.98 (1.02x overdosed)

Table 7-76 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d), 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
Spain 29 700 Velez Malaga (Andalusia) SP04 Plot T1	Maize SCANDI CS	1) 28/04/15 2) 24/06/15 to 09/07/15 3) 06/08/15	Foliar application Boom sprayer	0.372	297.2	0.125	BBCH 01	BBCH 85	82	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS072 Frozen storage Max. 704 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 375
								BBCH 89	98	Grain	<0.01	<0.01	<0.01	0.19 [UTC 0.2]	<0.01	
								BBCH 89	98	Straw	<0.01	<0.01	<0.01	<0.05	<0.01	

Table 7-76 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d), 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
																g a.s./ha Replicate trial. Highest result from replicate trial with 'CS' formulation underlined.

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
NORTHERN EUROPE																
Northern France 41190 Santenay FR01 Plot T2	Maize GIRESS	1) 23/04/15 2) 15/07/15 to 21/07/15 3) 06/10/15 to 10/10/15	Foliar application Boom sprayer	0.379	303.3	0.125	BBCH 00	BBCH 85	120	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS072 Frozen storage Max. 642 days Unless a residue value is stated, untreated control samples contained residues
								BBCH 89	160	Grain	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	<u>0.13</u> [UTC 0.12] **0.13 [UTC 0.12]**	<u>≤0.01</u>	
								BBCH 89	160	Straw	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.01</u>	<u>≤0.05</u>	<u>≤0.01</u>	

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																<LOQ. Trial within ± 25% of proposed application rate: 375 g a.s./ha Highest result from replicate trial with 'SC' formulation underlined.

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 0.99 (1.01x overdosed)
Germany 16816	Maize ZOEY	1) 28/04/15	Foliar application	0.384	205.0	0.187	BBCH 00	BBCH 85 BBCH	134 185	Whole plant Grain	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.05 0.17	<0.01 <0.01	Ref: 15SGS072 Frozen storage

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
Kranzlin (Brandenburg) GE02 Plot T2		2) 25/07/15 to 03/08/15 3) 30/10/15 to 31/10/15	Boom sprayer					89 BBCH 89	185	Straw	<0.01	<0.01	<0.01	[UTC 0.09] **0.17 [UTC 0.09]** <0.05	<0.01	Max. 619 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36.3 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: CS	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																proposed application rate: 375 g a.s./ha Highest result from replicate trial with 'SC' formulation underlined. Proportionality principle applied (**bold** font) in line with

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																underdosed trials. Scaling factor: 0.98 (1.02x overdosed)
SOUTHERN EUROPE																
Southern France 84210 Pernes les Fontaines	Maize CORETTA	1) 20/04/15 2) 21/07/15 to 28/07/1	Foliar application Boom sprayer	0.380	303.7	0.125	BBCH 05	BBCH 85	134	Whole plant	<0.01	<0.01	<0.01	0.06 [UTC 0.05]	<0.01	Ref: 15SGS072 Frozen storage Max. 619 days
								BBCH 89	179	Grain	<0.01	<0.01	<0.01	0.33 [UTC 0.29]	<0.01	Unless a residue value is

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
(PACA) FR03 Plot T2		5 3) 27/10/15						BBCH 89	179	Straw	<0.01	<0.01	<0.01	<0.05	<0.01	stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 375 g

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the	
Content of active substance (g/kg or g/L)	: 36.3 % w/w	formulation (common name and content)	: None
Formulation (*e.g.* WP)	: CS	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																a.s./ha Highest result from replicate trial with 'SC' formulation underlined.

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
Spain 29 700 Velez Malaga (Andalusia) SP04 Plot T2	Maize SCANDI CS	1) 28/04/15 2) 24/06/15 to 09/07/15 3) 06/08/15	Foliar application Boom sprayer	0.385	308.3	0.125	BBCH 01	BBCH 85	82	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS072 Frozen storage Max. 704 days Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	98	Grain	<0.01	<0.01	<0.01	0.25 [UTC 0.20] **0.24 [UTC 0.19]**	<0.01	
								BBCH 89	98	Straw	<0.01	<0.01	<0.01	<0.05	<0.01	

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																Trial within ± 25% of proposed application rate (375 g a.s./ha). Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling

Table 7-77 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																factor: 0.98 (1.02x overdosed) Replicate trial. Highest result from replicate trial with 'SC' formulation underlined.

Residues of bixlozone, 5'-hydroxy bixlozone, 5-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

All trials were conducted with an application rate within ± 25% of the proposed rate in terms of kg a.s./ha. The results from all trials are <LOQ for the following chemical components: bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid. Positive residues were observed for the metabolite 2,2-dimethyl-3-hydroxy propionic acid. Further discussion on the relevance of these trials data to the proposed GAP is presented in Volume 1, section 2.7.4.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.3-02, Gemrot, F. 2017
Title:	Magnitude of the Residues of F9600 in maize (Raw Agricultural Commodity) after one post emergence application of F9600-4 SC or F9600-28 CS in Northern Europe (Northern France and Germany) and Southern Europe (Southern France and Spain) – 2015
Report No.:	15SGS073; 2015RES-ISX1943
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Four trials on maize were conducted outdoors during 2015. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones.

Trial site 'SP04' has been tested in another trial reported in the DAR (SP04, study 15SGS072). This trial site was tested in the same season at a similar time (application on 30/04/15 in study 15SGS072, 13/05/15 in study 15SGS073) on the same crop (maize), on different plots. Trial site 'GE02' has been tested in another trial reported in the DAR (GE02, study 15SGS072). The trial sites have a different post code and appear to be at least 5 km apart. The application was made in the same season at a similar time (application on 28/04/15 in study 15SGS072, 19/05/15 in study 15SGS073) to the same crop (maize). However, as the trials in study 15SGS073 are significantly under-dosed and scaling is not possible due to <LOQ residues, as discussed after the trial results, no further consideration of the independence of these trails has been made at this time.

None of the trials were residue decline trials. The formulations 'F9600-4 SC' containing 37.4 % w/w active substance and 'F9600-28 CS' containing 34.3 % w/w active substance were applied to different plots within the trial sites. One application was made to maize at BBCH 12-13 (post-emergence) in the trials.

'F9600-4 SC' is the representative product being considered within this application for approval. Therefore trials performed with this product can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested. 'F9600-28 CS' is not the representative product and is a different formulation type. The formulations are both diluted in water prior to application therefore should give comparable results given the long time between application and harvest. A full consideration of the comparability of results from trials at the same site performed using the 'SC' and 'CS' formulation has been made in Volume 1, section 2.7.4. The values taken forward for MRL calculation and consideration of consumer risk should be independent, therefore only one value has been selected from each trial (same experimental location). In this case, the highest value has been selected from these two plots. Please see further discussion in Volume 1, section 2.7.4.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in previous seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone, 5-hydroxy-bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-79 and Table 7-80 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.05 mg/kg). Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 20 m apart.

Samples were analysed using two analytical methods. Samples analysed using both methods 'CAM-0154' and 'CAM-0180' have been considered. Method 'CAM-0180' includes a hydrolysis step therefore considers conjugated residues and analyses the additional metabolite 5-hydroxy bixlozone. When considering the results in samples determined using method 'CAM-0154', the only positive residues reported were of 2,2-dimethyl-3-hydroxy-propionic acid in maize straw (trial FR03): 0.016 – 0.019 mg/kg. These results are <0.05 mg/kg, the LOQ of method 'CAM-0180'. Therefore, the results determined using method 'CAM-0180', where more critical higher positive residues have been reported, have been taken forward.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 1 kg of grain, 12 plants of fodder/stover and 12 plants or at least 1 kg of forage). No adverse weather conditions were observed during the field trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 5-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in maize crop fractions. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were stored under frozen (< -18°C) conditions for up to 708 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for maize crop fractions for each of the analytes analysed. Samples were stored for up to 9 days under refrigerated conditions (~4°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. The data reported was generated using method 'CAM-0180'. A summary of the procedural recovery results is given in Table 7-78.

The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). Some individual recoveries were outside of this range; however, the mean recoveries within this range, this is acceptable.

A summary of the residue trial results are given in Table 7-79 and Table 7-80.

Table 7-78 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		5-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%)(%RSD)
Maize Whole plant (silage)	0.01	108, 109, 110	110 (0)	96, 97, 98	103 (6.8)	97, 97, 106	107 (8.0)	97, 102, 103	107 (6.4)	-	90 (6.0)
	0.05	-		-		-		-		83, 84, 90	
	0.1	110, 110, 110		104, 109, 113		111, 112, 118		110, 112, 115		-	
	0.5	-		-		-		-		90, 95, 96	
Maize grain	0.01	98, 106, 111	107 (4.7)	91, 105, 108	102 (6.5)	103, 114, 124	109 (8.5)	105, 108, 111	110 (4.0)	-	86 (13.0)
	0.05	-		-		-		-		91, 92, 100	
	0.1	106, 106, 112		98, 103, 108		100, 102, 113		108, 111, 118		-	
	0.5	-		-		-		-		69, 78, 83	
Maize straw (also referred to as stover)	0.01	88, 88, 89	96 (10.4)	84, 94, 99	94 (6.2)	83, 86, 90	98 (13.6)	90, 92, 96	96 (4.0)	-	101 (17.8)
	0.05	-		-		-		-		107, 109, 120	
	0.1	94, 108, 109		91, 97, 99		106, 106, 116		98, 99, 99		-	
	0.5	-		-		-		-		68, 96, 108	

Table 7-79 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
NORTHERN EUROPE																
Northern France 02 190 Amifontaine (Picardie) FR01 Plot T1	Maize MILL ESIM	1) 21/04/15 2) 15/07/15 to 15/08/15 3) 15/10/15	Foliar application Boom sprayer	0.128	206.7	0.062	BBCH 13	BBCH 85	106	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS073 Frozen storage Max. 639 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial underdosed by 66%, 34% of proposed application rate
								BBCH 89	148	Grain	<0.01	<0.01	<0.01	0.30 [UTC 0.349]	<0.01	
								BBCH 89	148	Straw	<0.01	<0.01	<0.01	0.055 [UTC 0.059]	<0.01	

Table 7-79 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
																(375 g a.s./ha) applied (scaling factor 2.93). This is within the proportionality scaling range however results <LOQ cannot be scaled up. Highest result from replicate trial with 'CS' formulation underlined.
Germany	Maize ZOEY	1) 28/04/15	Foliar applic	0.126	203.3	0.062	BBCH 12	BBCH 85	114	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS073 Frozen storage

Table 7-79 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
16818 Kranzlin (Brandenburg) GE02 Plot T1		2) 25/07/15 to 03/08/15 3) 30/10/15 to 31/10/15	Boom sprayer					BBCH 89	164	Grain	<0.01	<0.01	<0.01	0.091 [UTC 0.157]	<0.01	Max. 623 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial underdosed by 66%, 34% of proposed application rate (375 g a.s./ha) applied (scaling factor 2.98). This
								BBCH 89	164	Straw	<0.01	<0.01	<0.01	<0.05	<0.01	

Table 7-79 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
																is within the proportionality scaling range however results <LOQ cannot be scaled up. Highest result from replicate trial with 'CS' formulation underlined.

Table 7-79 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
SOUTHERN EUROPE																
Southern France 40 320 Miramont Sensacq (Aquitaine) FR03 Plot T1	Maize ES ARC HIPEL	1) 26/05/15 2) 29/07/15 to 14/08/15 3) 27/10/15 to 30/10/15	Foliar application Boom sprayer	0.119	286.7	0.041	BBCH 13	BBCH 85	85	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS073 Frozen storage Max. 623 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial underdosed by 68%, 32% of proposed application rate
								BBCH 89	135	Grain	<0.01	<0.01	<0.01	0.259 [UTC 0.384]	<0.01	
								BBCH 89	135	Straw	<0.01	<0.01	<0.01	0.072 [UTC 0.075] **0.23 [UTC 0.24]**	<0.01	

																(375 g a.s./ha) applied (scaling factor 3.15). This is within the proportionality scaling range however results <LOQ cannot be scaled up. Proportionality principle applied (**bold** font) to positive results. Highest result from replicate trial with 'CS' formulation underlined.
Spain 29 700 Velez Malaga (Andalusia) SP04 Plot T1	Maize SCANDI CS	1) 28/04/15 2) 24/06/15 to 09/07/15 3) 06/08/15	Foliar application Boom sprayer	0.124	300.0	0.041	BBCH 13	BBCH 85 BBCH 89 BBCH 89	69 85 85	Whole plant Grain Straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.599 [UTC <0.05] **1.81** <0.05 [UTC 0.055]	<0.01 <0.01 <0.01	Ref: 15SGS073 Frozen storage Max. 708 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial underdosed by 67%, 33% of proposed application rate (375 g a.s./ha) applied (scaling factor 3.02). This is within the proportionality scaling range

Table 7-79 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy bixlozone (e)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	e	
																however results <LOQ cannot be scaled up. Proportionality principle applied (**bold** font) to positive results. Replicate trial Highest result from replicate trial with 'CS' formulation underlined.

Table 7-80 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
NORTHERN EUROPE																
Northern France 02 190 Amifontaine (Picardie) FR01 Plot T1	Maize MILL ESIM	1) 21/04/15 2) 15/07/15 to 15/08/15 3) 15/10/15	Foliar application Boom sprayer	0.126	200.0	0.063	BBCH 13	BBCH 85	106	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS073 Frozen storage Max. 639 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial underdosed by
								BBCH 89	148	Grain	<0.01	<0.01	<0.01	0.396 [UTC 0.349] **<u>1.18</u> [UTC 1.04]**	<0.01	
								BBCH 89	148	Straw	<0.01	<0.01	<0.01	0.059 [UTC 0.059] **<u>0.18</u> [UTC 0.18]**	<0.01	

																66%, 34% of proposed application rate (375 g a.s./ha) applied (scaling factor 2.98). This is within the proportionality scaling range however results <LOQ cannot be scaled up. Proportionality principle applied (**bold** font) to positive results. Highest result from replicate trial with 'SC' formulation underlined.
Germany 16818 Kranzlin (Brandenburg) GE02 Plot T1	Maize ZOEY	1) 28/04/15 2) 25/07/15 to 03/08/15 3) 30/10/15 to 31/10/15	Foliar application Boom sprayer	0.128	201.7	0.063	BBCH 12	BBCH 85 BBCH 89 BBCH 89	114 164 164	Whole plant Grain Straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.168 [UTC 0.157] **<u>0.49</u> [UTC 0.46]** <u><0.05</u>	<0.01 <0.01 <0.01	Ref: 15SGS073 Frozen storage Max. 623 days Unless a residue value is stated, untreated control samples contained

Table 7-80 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																residues <LOQ. Trial underdosed by 66%, 34% of proposed application rate (375 g a.s./ha) applied (scaling factor 2.93). This is within the proportionality scaling range however results <LOQ cannot be

Table 7-80 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																scaled up. Proportionality principle applied (**bold** font) to positive results. Highest result from replicate trial with 'SC' formulation underlined.

Table 7-80 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
SOUTHERN EUROPE																
Southern France 40 320 Miramont Sensacq (Aquitaine) FR03 Plot T1	Maize ES ARCHIPEL	1) 26/05/15 2) 29/07/15 to 14/08/15 3) 27/10/15 to 30/10/15	Foliar application Boom sprayer	0.124	293.3	0.042	BBCH 13	BBCH 85	85	Whole plant	<0.01	<0.01	<0.01	<0.05	<0.01	Ref: 15SGS073 Frozen storage Max. 623 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial underdosed by
								BBCH 89	135	Grain	<0.01	<0.01	<0.01	0.336 [UTC 0.384] **1.06 [UTC 1.21]**	<0.01	
								BBCH 89	135	Straw	<0.01	<0.01	<0.01	<0.05 [UTC 0.075]	<0.01	

																67%, 33% of proposed application rate (375 g a.s./ha) applied (scaling factor 3.02). This is within the proportionality scaling range however results <LOQ cannot be scaled up. Proportionality principle applied (**bold** font) to positive results. Highest result from replicate trial with 'SC' formulation underlined.
Spain 29 700 Velez Malaga (Andalusia) SP04 Plot T1	Maize SCANDI CS	1) 28/04/15 2) 24/06/15 to 09/07/15 3) 06/08/15	Foliar application Boom sprayer	0.124	294.4	0.042	BBCH 13	BBCH 85 BBCH 89 BBCH 89	69 85 85	Whole plant Grain Straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.232 [UTC <0.05] <0.05 [UTC 0.055]	<0.01 <0.01 <0.01	Ref: 15SGS073 Frozen storage Max. 708 days Unless a residue value is stated, untreated control samples contained

Table 7-80 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																residues <LOQ. Trial underdosed by 67%, 33% of proposed application rate (375 g a.s./ha) applied (scaling factor 3.02). This is within the proportionality scaling range however results <LOQ cannot be

Table 7-80 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-28 CS
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 36.3 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d) 5-hydroxy-bixlozone (e)
Formulation (*e.g.* WP)	: CS		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)					Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	e	
																scaled up. Replicate trial Highest result from replicate trial with 'SC' formulation underlined.

Residues of bixlozone, 5'-hydroxy bixlozone, 5-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

The results from all trials are <LOQ for the following chemical components: bixlozone, 5'-hydroxy bixlozone, 5-hydroxy bixlozone and 2,4-dichlorobenzoic acid. Positive residues were observed for the metabolite 2,2-dimethyl-3-hydroxy propionic acid. The trials were conducted with an application rate significantly lower (0.118 – 0.128 kg a.s./ha) than the proposed rate in terms of kg a.s./ha (0.375 kg a.s./ha). These low application rates are within the possible scaling range of the proportionality approach, however, as the majority of results were <LOQ, scaling up is not possible in this case. Further discussion on the relevance of these trials data to the proposed GAP is presented in Volume 1, section 2.7.4.

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.3.3-03, Semrau J, 2018
Title:	Determination of residues of F9600 after one pre-emergence or post emergence application of F9600-4 SC in Maize in Northern and Southern Europe 2016
Report No.:	S16-01152; 2015RES-ISX2484
Guidelines:	OECD Test Guideline 509; EU Guidance 7029/VI/95 rev. 5; EU Guidance SANCO/3029/99 rev. 4
GLP	Yes

Eight trials on maize were conducted outdoors during 2016. Trials were conducted in sufficiently different geographic locations within the Northern and Southern European zones.

Trial site '02' has been tested in another trial reported in the DAR (S16-01153-02). This trial site was tested in the same season but at a sufficiently different time (application on 01/04/16 in study S16-01153, 18/05/16 in study S16-01152) on a different crop (maize and wheat) on different plots. As the crops are sufficiently different (extrapolation not possible between these crops) and there is a significant time (48 days) between applications, the trials are considered independent.

Similarly, trial site '03' has been tested in another trial reported in the DAR (S16-01153-08). This trial site was tested in the same season at a different time (application on 01/04/16 in study S16-01153, 20/04/16 in study S16-01152) on a different crop (maize and wheat) on different plots. As the crops are sufficiently different (extrapolation not possible between these crops) and there is time (20 days) between applications, the trials are considered independent.

Four of the trials were residue decline trials. The formulations 'F9600-4 SC' containing 36.4 % w/w active substance was applied to the trial sites. One application was made to maize at BBCH 00-13 (pre and post-emergence) in the trials.

'F9600-4 SC' is the representative product being considered within this application for approval. Therefore, trials performed with this product can be considered representative of the proposed GAP for 'F9600-4 SC' with regards to the formulation tested.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in previous seasons or during the trials. The active substance propiconazole was applied to trial site 05 during 2013; this active substance contains the 2,4-dichlorobenzoic acid moiety. However, considering the similarity of the results to other trials where this active substance has not been applied, and the lack of positive residues of 2,4-dichlorobenzoic acid in the untreated control samples, this is not considered to significantly affect the validity of the trial.

Residues of bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples as listed in Table 7-82 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.05 mg/kg). Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.4 of Volume 1. Treated and untreated plots were situated ≥ 10 m apart.

The application was made using a boom sprayer. Samples were taken from a minimum of 12 areas within the plot. The samples taken were of a sufficient size in accordance with OECD 509 Crop field trial guidance (at least 1 kg of grain, 12 plants of fodder/stover and 12 plants or at least 1 kg of forage). No adverse weather conditions were observed during the field trials.

The analytical method 'CAM-0180/002' was used to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy-propionic acid in maize crop fractions. This method has been considered fully validated in DAR CA B5 Section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid, and 0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid. In some field trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.05 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

Samples were stored under frozen (< -18°C) conditions for up to 609 days prior to extraction. There are sufficient storage stability data reported under section 7.1 to support this time period of storage for maize crop fractions for each of the analytes analysed. Samples were stored for up to 4 days under refrigerated conditions (1-10°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

During the analysis of field samples, fortified specimens were analysed also to confirm the acceptability of the analytical procedure. The data reported was generated using method 'CAM-0180'. A summary of the procedural recovery results is given in Table 7-81. The quantification transition has been considered when determining the procedural recoveries. The overall mean procedural recoveries are all within the acceptable range (SANCO 3029/99 rev. 4 states that mean recoveries for each level should be in the range 70-110%, ideally with the mean in the range 80-100%). Some individual recoveries were outside of this range; however, the mean recoveries within this range, this is acceptable.

A summary of the residue trial results are given in Table 7-82.

Table 7-81 Summary of procedural recovery data

Matrix	Fortification level (mg/kg)	bixlozone		5'-hydroxy-bixlozone		2,4-dichloro benzoic acid		2,2-dimethyl-3-hydroxy propionic acid	
		Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%) (%RSD)	Recovery (%)	Mean recovery (%)(%RSD)
Maize Whole plant	0.01	103, 108, 118	102 (9.7)	90, 93, 107	94 (7.1)	96, 101, 117	107 (7.0)	-	101 (4.4)
	0.05	-		-		-		100, 103, 103	
	0.1	93, 93, 96		88, 93, 93		108, 110, 111		-	
	0.5	-		-		-		93, 100, 106	
Maize Whole plant (silage)	0.01	73, 75, 79	74 (6.3)	71, 92, 92	81 (12)	94, 107, 114	101 (8.2)	-	81 (6.0)
	0.05	-		-		-		74, 75, 86	
	0.1	67, 70, 78		72, 75, 85		83, 84, 96		-	
	0.5	-		-		-		82, 83, 83	
Maize grain	0.01	95, 98, 100	94 (5.2)	71, 85, 89	88 (12)	106, 107, 108	109 (2.9)	-	80 (13)
	0.05	-		-		-		70, 71, 74	
	0.1	88, 90, 90		87, 93, 104		106, 111, 114		-	
	0.5	-		-		-		83, 90, 94	
Maize straw	0.01	88, 98, 98	93 (5.5)	72, 81, 81	94 (19)	81, 91, 100	93 (7.8)	-	80 (7.8)
	0.05	-		-		-		72, 75, 87	
	0.1	87, 89, 95		108, 110, 111		92, 95, 101		-	
	0.5	-		-		-		76, 84, 85	

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															

S16-01152-0167140, Stozheim, Bas-Rhin, Alsace, France	Maize/ ZEAMX DKC 4795	1) 06/05/16 2) 15/07/16 to 25/07/16 3) 12/10/16	Broadcast spray with boom sprayer	0.325	270	0.120	BBCH 05	BBCH 85	122	Whole plant (silage)	<0.01	<0.01	<0.01	<0.05	Ref: S16-01152 Frozen storage max. 609 days
								BBCH 89	156	Grain	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	0.11 [UTC 0.15] **<u>0.13</u> [UTC 0.12]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	156	Straw	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.05</u>	Trial within ± 25% of proposed application rate: 375 g a.s./ha Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 1.15 (0.87x underdosed)
S16-01152-0227449, Mulsum, Lower Saxony, Germany	Maize/ ZEAMX Toninio	1) 14/05/16 2) 25/07/16 to 10/08/16 3) 29/09/16	Broadcast spray with boom sprayer	0.293	244	0.120	BBCH 00	BBCH 36	49	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S16-01152 Frozen storage max. 609 days
								BBCH 65	75	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 75	97	Whole plant	<0.01	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 85	126	Whole plant (silage)	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	134	Grain	<u><0.01</u>	<u><0.01</u>	<u><0.01</u>	<u><0.05</u>	

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
								BBCH 89	134	Straw	<0.01	<0.01	<0.01	≤0.05	Trial within ± 25% of proposed application rate: 375 g a.s./ha

S16-01152-055492, Vissenbjerg, South Denmark, Denmark	Maize/ ZEAM X Fieldstar	1) 13/05/16 2) n/r 3) 17/10/16	Broadcast spray with boom sprayer	0.310	207	0.150	BBCH 13	BBCH 85 BBCH 89 BBCH 89	116 137 137	Whole plant (silage) Grain Straw	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.05 0.057 [UTC 0.055] **<u>0.069</u> [UTC 0.067]** <u>≤0.05</u>	Ref: S16-01152 Frozen storage max. 609 days Unless a residue value is stated, untreated control samples contained residues <LOQ. Trial within ± 25% of proposed application rate: 375 g a.s./ha Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 1.21 (0.83x underdosed)
S16-01152-0616230, Tempelfelde, Brandenburg, Germany	Maize ZEAM X Monumental	1) 03/05/16 2) 10/07/16 to 25/07/16 3) 21/09/16	Broadcast spray with boom sprayer	0.299	299	0.100	BBCH 12	BBCH 36 BBCH 65 BBCH 75 BBCH 85 BBCH	40 61 89 114 126	Whole plant Whole plant Whole plant Whole plant (silage) Grain	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.05 <0.05 <0.05 0.071 [UTC 0.068] <u>0.32</u>	Ref: S16-01152 Frozen storage max. 609 days Unless a residue value is stated, untreated control samples contained residues <LOQ.

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
								89						[UTC 0.22] **<u>0.40</u> [UTC 0.28]**	Trial within ± 25% of proposed application rate: 375 g a.s./ha Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 1.25 (0.80x underdosed)
								BBCH 89	126	Straw	<0.01	<0.01	<0.01	<u><0.05</u>	

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
SOUTHERN EUROPE															
S16-01152-03 82700, St Porquier, Tarn et Garonne, France	Maize ZEAM X DKC5830	1) 20/04/16 2) 25/07/16 to 03/08/16 3) 06/10/16	Broadcast spray with boom sprayer	0.305	203	0.150	BBCH 00	BBCH 85	134	Whole plant (silage)	<0.01	<0.01	<0.01	<0.05	Ref: S16-01152 Frozen storage max. 609 days
								BBCH 89	169	Grain	<0.01	<0.01	<0.01	0.24 [UTC 0.31] **0.30 [UTC 0.38]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	169	Straw	<0.01	<0.01	<0.01	<0.05	Trial within ± 25% of proposed application rate: 375 g a.s./ha Proportionality principle

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
															applied (**bold** font) in line with underdosed trials. Scaling factor: 1.23 (0.81x underdosed)
S16-01152-0450180, Utebo, Aragon, Spain	Maize ZEAMX DAS03700	1) 05/05/16 2) 15/07/16 to 05/08/16 3) 07/11/16	Broadcast spray with boom sprayer	0.325	324	0.100	BBCH 00-08	BBCH 35	63	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S16-01152 Frozen storage max. 609 days Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 63-65	84	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 75	102	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 85	115	Whole plant (silage)	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	185	Grain	≤0.01	≤0.01	≤0.01	0.27 [UTC	

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
														0.31] **0.32 [UTC 0.33]**	Trial within ± 25% of proposed application rate: 375 g a.s./ha Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 1.23 (0.81x underdosed)
								BBCH 89	185	Straw	<0.01	<0.01	<0.01	<0.05	
S16-01152-07 GR-57008,	Maize ZEAM X DKC67	1) 28/04/16 2) 17/06/16 to 06/07/16 3) 12/10/16	Broadcast spray with	0.320	427	0.075	BBCH 11	BBCH 85 BBCH	99 154	Whole plant (silage) Grain	0.021 0.035	<0.01 <0.01	<0.01 <0.01	<0.05 0.27	Ref: S16-01152 Frozen storage max. 609 days

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
Nea Magnisiq, Thessaloniki, Greece	95		boom sprayer					89			**0.041**			[UTC 0.28] **0.32 [UTC 0.33]**	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 89	154	Straw	0.013 **0.016**	<0.01	<0.01	<0.05 [UTC 0.064] **[UTC 0.08]**	Trial within ± 25% of proposed application rate: 375 g a.s./ha Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		
UTC: Untreated Control sample			

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
															factor: 1.17 (0.85x underdosed)
S16-01152-0840016, San Giorgio di Piano, Bologna, Italy	Maize ZEAMX KIM905	1) 30/04/16 2) 27/06/16 to 07/07/16 3) 07/09/16	Broadcast spray with motorised knapsack sprayer linked to a boom	0.297	297	0.100	BBCH 13	BBCH 38	59	Whole plant	<0.01	<0.01	<0.01	<0.05	Ref: S16-01152 Frozen storage max. 609 days
								BBCH 63	73	Whole plant	<0.01	<0.01	<0.01	<0.05	
								BBCH 73	85	Whole plant	<0.01	<0.01	<0.01	<0.05	Unless a residue value is stated, untreated control samples contained residues <LOQ.
								BBCH 85	107	Whole plant (silage)	<0.01	<0.01	<0.01	<0.05	
								BBCH 89	142	Grain	<0.01	<0.01	<0.01	0.22 [UTC 0.31] **0.28 [UTC**	Trial within ± 25% of proposed application rate: 375 g a.s./ha

Table 7-82 Residues in maize following treatment with bixlozone (Northern and Southern Europe)

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 37.4 % w/w	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comment
				kg a.s./ha	Water (L/ha)	Kg a.s./hL					a	b	c	d	
								BBCH 89	142	Straw	<0.01	<0.01	<0.01	**0.39]** <0.05 [UTC 0.051]	Proportionality principle applied (**bold** font) in line with underdosed trials. Scaling factor: 1.26 (0.79x underdosed)

Residues of bixlozone, 5'-hydroxy bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid have been determined in the trials. Please refer to the discussion relating to the proposal of the residue definition in Volume 1, section 2.7.3.

The results from all trials are <LOQ for the following chemical components: 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid. Positive residues were observed for bixlozone, in only one trial (06) in whole plant (silage), grain and straw, and the metabolite 2,2-dimethyl-3-hydroxy propionic acid, in 7 trials in grain and one trial (07) in whole plant (silage). The trials were all conducted with an application rate within ± 25% of the proposed rate in terms of kg a.s./ha (0.375 kg a.s./ha). All of the application rates tested were below the highest proposed rate. These lower application rates are within the possible scaling range of the proportionality approach, however, as the majority of results were <LOQ, scaling up is not possible in this case. Please refer to Volume 1 for a full consideration of the supporting trials.

Additional supporting information from processing studies

Please see section B.7.5.3 for a full consideration of these data with regards to processing. During each field trial performed to generate samples for the subsequent processing studies, a field trial was performed in accordance with the proposed GAP on maize. Therefore these data may be considered supportive information and are summarised below.

Maize

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-04, Semrau, J., 2018
Title:	Determination of residue of F9600 after one application of F9600-4 SC in maize and in processed fractions of maize at 2 sites in Northern and Southern Europe 2016
Report No.:	S16-05486
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

For a full consideration of this study please refer to section B.7.5.3. A single foliar application was made via boom sprayer of F9600-4 SC to treated plot 2 at BBCH 12, in both field trials (NEU and SEU). These trial conditions are in line with the proposed GAP for use on maize. Therefore, the results from these trials can be considered supportive of the use on primary crops. A full consideration of the conduct of these trials has not been made. It should be noted that treated plot 3, which processing samples were taken from, was at the same location and treated at the same time, therefore may not be considered an independent trial. However, these results from plot 2 can be considered supportive information when considering the proposed use on maize as a primary crop. A summary of the results from treated plot 2 are presented in Table 7-83.

The results are in line with those reported for the primary crop field trials: bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid <0.01 mg/kg in grain. Positive residues of 2,2-dimethyl-3-hydroxy propionic acid were observed in grain. It should be noted that straw/stover was not analysed as part of these studies.

Table 7-83 Summary of results from maize field trials, treated plot 2 with GAP relevant application rate and timing

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
NORTHERN EUROPE															
S16-05486-01: 71706 Markgröningen, Baden-Württemberg, Germany	Maize/ ZEAMX / ES Ewrojet	1) 24 Apr 2017 2) 20 Jul – 04 Aug 2017 3) 18 Oct 2017	Foliar application, boom sprayer	0.307	307	0.100	BBCH 12	BBCH 89	155	Grain	<0.01	<0.01	<0.01	<0.2	Unless a residue value is stated, untreated control samples contained residues <LOQ. Within ±25% of GAP Proposed application rate: 375 g a.s./ha
SOUTHERN EUROPE															
S16-05486	Maize/ ZEAM	1) 20 Mar 2017	Foliar applicat	0.303	403	0.075	BBCH 12	BBCH 89	145	Grain	<0.01	<0.01	<0.01	0.51 [UTC	Unless a residue value is stated,

Active substance (common name)	: bixlozone	Commercial Product (name)	: F9600-4 SC
Crop/crop group	: Maize	Producer of commercial product	: FMC
Responsible body for reporting (name, address)	: HSE	Indoor/Outdoor	: Outdoor
Country	: UK	Other active substance in the formulation (common name and content)	: None
Content of active substance (g/kg or g/L)	: 396 g/L	Residues calculated as	: bixlozone (a), 5'-hydroxy bixlozone (b), 2,4-dichlorobenzoic acid (c), 2,2-dimethyl-3-hydroxy propionic acid (d)
Formulation (*e.g.* WP)	: SC		

UTC: Untreated Control sample

Country (Region)	Crop (Variety)	Date of: 1) Sowing or Planting 2) Flowering 3) Harvest	Method of treatment	Application Rate			Growth stage at application	Growth stage at sampling	PHI (days)	Crop part	Residue found (mg/kg)				Comments
				kg a.s./ha	Water (L/ha)	kg a.s./hL					a	b	c	d	
-02: 40016, S. Giorgio di Piano, Bologna, Italy	X / Kamil	2) 10 – 25 Jun 2017 3) 30 Aug 2017	ion, boom sprayer											0.31]	untreated control samples contained residues <LOQ. Within ±25% of GAP Proposed application rate: 300 g a.s./ha

B.7.4. FEEDING STUDIES

Animal intake calculations presented in Volume 1 show intakes are less than 0.004 mg/kg bw/day considering exposure to bixlozone and 2,4-dichlorobenzoic acid. Feeding studies have not been submitted. For a full discussion of expected animal intakes please refer to Volume 1, section 2.7.5.

B.7.4.1. Poultry

Animal intake calculations presented in Volume 1 show intakes are less than 0.004 mg/kg bw/day considering exposure to bixlozone and 2,4-dichlorobenzoic acid, from the proposed uses on oilseed rape, barley, wheat and maize, and any exposure resulting from possible residues in rotational crops. Hen metabolism studies indicate that residues expected in products of poultry origin as a result of the representative GAPs will be <0.01 mg/kg, considering parent bixlozone and the range of metabolites analysed in the metabolism study. Feeding studies have not been submitted. For a full discussion of expected animal intakes please refer to Volume 1, section 2.7.5.

B.7.4.2. Ruminants

Animal intake calculations presented in Volume 1 show intakes are less than 0.004 mg/kg bw/day considering exposure to bixlozone and 2,4-dichlorobenzoic acid, from the proposed uses on oilseed rape, barley, wheat and maize, and any exposure resulting from possible residues in rotational crops. Ruminant metabolism studies indicate that residues expected in products of ruminant origin as a result of the representative GAPs will be <0.01 mg/kg, considering parent bixlozone and the range of metabolites analysed in the metabolism study. Feeding studies have not been submitted. For a full discussion of expected animal intakes please refer to Volume 1, section 2.7.5.

B.7.4.3. Pigs

Animal intake calculations presented in Volume 1 show intakes are less than 0.004 mg/kg bw/day considering exposure to bixlozone and 2,4-dichlorobenzoic acid, from the proposed uses on oilseed rape, barley, wheat and maize, and any exposure resulting from possible residues in rotational crops. Ruminant metabolism studies indicate that residues expected in products of ruminant origin as a result of the representative GAPs will be <0.01 mg/kg, considering parent bixlozone and the range of metabolites analysed in the metabolism study. Feeding studies have not been submitted. For a full discussion of expected animal intakes please refer to Volume 1, section 2.7.5.

B.7.4.4. Fish

Please refer to section B.7.2.5 for further information relating to residues in fish. The requirement for fish feeding studies can only be considered once metabolism in fish has been addressed.

B.7.5. EFFECTS OF PROCESSING

B.7.5.1. Nature of the residue

Please refer to Vol 1 for a discussion on residue definition (section 2.7.3) and section 2.7.6 (processing) which discusses the requirement for all processing studies.

The hydrolysis study addresses parent bixlozone but does not consider the hydrolysis of 2,4-dichlorobenzoic acid which is also proposed for consideration in the RD-RA, and is found (infrequent finding) at a low level in cereal grain but not oilseed rape seed. Whilst according to the intended uses on oilseed rape and cereals, residues of parent bixlozone are not expected to be found as a primary crop residue, there is the potential for parent bixlozone to occasionally be found in rotational crops (e.g. leafy vegetables).

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.1-01, Mende, P., 2017
Title:	[^{14}C]F9600: Hydrolysis under typical conditions (pH, temperature and time) of processing
Report No.:	S16-05475
Guidelines:	EC working document 7035/VI/95 rev.5, EC working document 1607/VI/97 rev.2, OECD 507
GLP	yes

Materials and methods

Materials

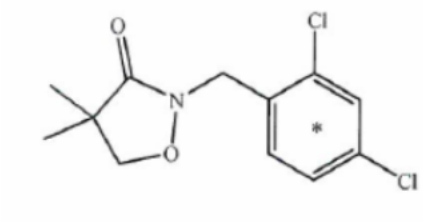
1. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42475
Radiochemical Purity:	99.9%
Specific Activity:	60 mCi/mmol; 8.04 MBq/mg

2. Test Material

Test Material:	[Carbonyl -C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42476
Radiochemical Purity:	99.9%
Specific Activity:	59 mCi/mmol; 7.91 MBq/mg

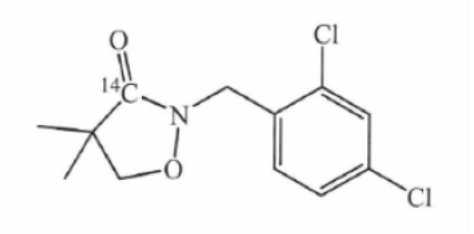
Figure 7-10 Structures of radiolabelled bixlozone



[Phenyl -U-^{14}C]bixlozone

(*denotes ^{14}C position)

"Phenyl label"



[Carbonyl -C5-^{14}C]bixlozone

"Carbonyl label"

Methods

A standard hydrolysis study was performed with ^{14}C-bixlozone labelled either at the dichloro-phenyl ring (phenyl-label) or on the carbonyl carbon of the dimethyl-isoxazolidin-3-one ring (carbonyl-label). Different processes (pasteurisation, baking, brewing, boiling, and sterilisation) were simulated in order to investigate any potential degradation of bixlozone during industrial processing or household preparation.

The ^{14}C-labeled test substance was dissolved in aqueous buffer solutions of different pH values (citric acid monohydrate in water, pH adjusted with 1M NaOH) and heated according to the parameters given in Table 7-84. Samples were prepared by pipetting 92-94 µL of ^{14}C-labelled bixlozone in 20 mL of the appropriate pH buffer solution. An oil bath was used to maintain the test temperatures. The concentration of the test item was within the range 3.67-4.23 mg/L. this is above the recommended concentration of 1 mg/L as stated in OECD 507. However, as the

concentration used in the study is higher than the recommended level, it is likely to have minimal effect on the validity of the study and can be considered acceptable. In each case the pH was checked before and after processing and found to be constant. Samples were stored frozen for a maximum of 16 days before analysis and therefore no additional extraction was conducted for storage stability evaluation.

Table 7-84 Conditions tested in the standard hydrolysis study

Temperature (°C)	pH	Time (min)	Simulated processing procedure
90	4	20	pasteurisation
100	5	60	baking, brewing, boiling
120	6	20	sterilisation (in the dark)

Total radioactivity was determined by liquid scintillation counting (LSC), composition of the radioactive residue was analysed directly by HPLC (radio detection/ MS detection).

Results and Discussion

Total radioactivity before and after incubation were similar, indicating absence of major loss of radioactivity (see Table 7-85). Before and after incubation, bixlozone accounted for almost all of the radioactivity, indicating absence of any degradation product at levels of 1.2% TRR or higher (see Table 7-86).

Table 7-85 Levels of radioactivity before and after incubation

	Phenyl-label			**Carbonyl-label**		
	pH 4, 90°C (20 min)	**pH 5, 100°C (60 min)**	**pH 6, 120°C (20 min)**	**pH 4, 90°C (20 min)**	**pH 5, 100°C (60 min)**	**pH 6, 120°C (20 min)**
	Total Radioactivity (TRR)					
	% TRR (mean)	**% TRR (mean)**	**% TRR (mean)**	**% TRR (mean)**	**% TRR (mean)**	**% TRR (mean)**
total (prior to treatment) [1)]	100.0	100.0	100.0	100.0	100.0	100.0
total (post treatment)	99.2, 100.7 (99.9)	107.2, 106.8 (107.0)	98.5, 97.8 (98.2)	104.5, 105.2 (104.9)	117.7, 98.0 (104.8)	97.3, 101.3 (99.3)
bixlozone (post treatment)	98.4, 97.1 (97.8)	91.2, 91.8 (91.5)	98.3, 98.7 (98.4)	92.9, 92.3 (92.6)	87.0, 98.9 (92.6)	98.7, 95.2 (96.9)

[1)] *actual concentration of each sample was determined by LSC before each incubation to use as value for 100% TRR.*

Table 7-86 Distribution before and after incubation

	Phenyl-label						Carbonyl-label					
	Prior to treatment			Post treatment			Prior to treatment			Post treatment		
	pH 4, 90°C, 20min	pH 5, 100°C, 60 min	pH 6, 120°C, 20 min	pH 4, 90°C, 20min	pH 5, 100°C, 60 min	pH 6, 120°C, 20 min	pH 4, 90°C, 20min	pH 5, 100°C, 60 min	pH 6, 120°C, 20 min	pH 4, 90°C, 20min	pH 5, 100°C, 60 min	pH 6, 120°C, 20 min
	% TRR (mg/L)											
bixlozone	97.9 (3.59)	97.6 (3.60)	97.6 (3.68)	97.8 (3.59)	91.5 (3.87)	98.4 (3.58)	96.9 (3.86)	96.9 (3.91)	97.1 (3.86)	92.6 (4.05)	92.6 (4.11)	96.9 (3.80)
D1	-	-	-	-	-	-	-	-	-	-	-	0.33 (0.01)
D2	-	-	0.29 (0.01)	-	-	-	-	-	-	-	-	-
D3	0.27 (0.01)	0.39 (0.01)	0.35 (0.01)	0.35 (0.01)	0.29 (0.01)	0.39 (0.01)	0.42 (0.02)	0.46 (0.02)	0.37 (0.01)	0.38 (0.02)	0.44 (0.02)	0.51 (0.01)
D4	0.39 (0.01)	0.34 (0.01)	0.42 (0.02)	0.41 (0.01)	0.41 (0.02)	0.51 (0.02)	0.36 (0.01)	0.42 (0.01)	0.35 (0.01)	0.37 (0.02)	0.31 (0.01)	0.44 (0.02)
D5	1.02 (0.04)	1.04 (0.04)	1.09 (0.04)	0.94 (0.03)	0.91 (0.04)	0.97 (0.04)	1.00 (0.04)	1.02 (0.04)	0.95 (0.04)	0.85 (0.04)	0.81 (0.04)	0.90 (0.04)
D6	-	-	-	0.26 (0.01)	-	0.50 (0.02)	0.37 (0.01)	0.38 (0.02)	0.37 (0.01)	0.35 (0.02)	0.36 (0.02)	0.49 (0.02)
D7	0.39 (0.01)	0.37 (0.01)	0.29 (0.01)	0.34 (0.01)	0.33 (0.01)	0.59 (0.02)	0.57 (0.02)	0.51 (0.02)	0.57 (0.02)	0.49 (0.02)	0.50 (0.02)	0.67 (0.03)
D8	-	0.29 (0.01)	-	-	-	0.44 (0.02)	0.35 (0.01)	0.33 (0.01)	0.32 (0.01)	0.34 (0.01)	0.37 (0.02)	0.45 (0.02)

Mean values from duplicate determinations. (mg/L in brackets)

Characterisation and Identification

The identity of bixlozone was verified by comparing retention time, accurate mass spectra and MS^2 product ion spectra with unlabelled reference item. Unknown fractions were also isolated; however, no formal characterisation was carried out due to the low relative amounts detected (shown in the table above as degrades, D1-D8). Many of these minor components are present both before and after the processing treatment, so they might be test item impurities. It is stated in the report that retention time matching to the previous metabolism studies can be used to characterise the residues, however this data was not presented. In accordance with OECD 507, characterisation of peaks at this level (<10%, 0.01-0.05) need only be attempted if straightforward and therefore characterisation is considered sufficiently addressed.

Conclusion

Under conditions representative of pasteurisation (pH 4, 90 °C, 20 min), baking, boiling, brewing (pH 5, 100 °C, 60 min) and sterilisation (pH 6, 120 °C, 20 min) bixlozone is stable. Notably, no degradation product at or exceeding 1.2% of total radioactivity was detected. In conclusion, as bixlozone can be regarded as stable to hydrolysis, the nature of the residue is not affected by processing operations.

No conclusions can be taken from this study about the nature of residues of 2,4-dichlorobenzoic acid over processing. Please refer to Vol 1 (section 2.7.6) for a further discussion regarding this primary crop plant metabolite.

B.7.5.2. Distribution of the residue in peel and pulp

The representative uses to be evaluated in this dossier (cereals and oilseeds) are crops with no peel or edible peel only. Therefore, studies on the distribution between peel and pulp are not required.

B.7.5.3. Magnitude of residues in processed commodities

Based on the proposed RD-RA including bixlozone and 2,4-dichlorobenzoic acid, the residue levels in the oilseeds and cereal grains RACs are below 0.1 mg/kg, the theoretical maximum daily intake is <10% of the ADI and the estimated daily intake is <10% of the ARfD, and therefore magnitude of residues studies are not strictly required for the intended uses.

Studies were submitted on the magnitude of residues (of parent bixlozone, 2,4-dichlorobenzoic acid, 5'hydroxy bixlozone, and 2,2-dimethyl-3-hydroxy propionic acid) over processing in wheat (*production of various processed*

fractions, including flour (white and wholemeal), bread, (white and wholemeal) germ, bran, starch/gluten), barley (*pearl barley, malt and beer*), oilseed rape (*crude oil and refined oil, and press cake used as animal feed*), and maize (*bran, flour, starch, protein, meal and oil*) and are presented below. The studies used field trial derived residues (field incurred residues) and the processing operations followed detailed simulated industrial practices conducted in the laboratory. These processing studies aimed to use shortened harvest intervals of around 28 to 30 days (compared to the intended GAPs of pre-emergence or early post-emergence) in order to increase the chances of there being positive field incurred residues to study in these processing studies; the GAP compliant trials had resulted in low residues of most analytes (see section B.7.3). The applicant has been concerned that use of overdosed trials might result in phytotoxicity. Whilst an acceptable strategy (to shorten the PHI), these processing trials lead to generally low residues which is not ideal for setting processing factors. For a discussion on processing factors for the proposed residue definition, based on these processing studies please see Volume 1, section 2.7.6.

B.7.5.3.1. *Wheat*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-01, Semrau, J., 2018
Title:	Determination of residue of F9600 after one pre-emergence application of F9600-4 SC in wheat and in processed fractions of wheat at 2 sites in Northern and Southern Europe 2016
Report No.:	S16-05487
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

Materials and methods

A processing study on wheat (varieties: *Anapolis*, *Nogal* Wheat winter), was conducted outdoors at a location in Germany (S16-05487-01 – N EU zone) and a location in Spain (S16-05487-02 – S EU zone) during the 2016/2017 growing season. Normal agricultural procedures were followed, and no unusual weather events were recorded. At each field trial site there was two treated plots (plot 2 and 3) and one untreated control plot (plot 1). Treated processing samples were taken from plot 3 only. A single foliar application was made via boom sprayer of F9600-4 SC (an SC formulation containing 396 g/L bixlozone) to treated plot 2 at BBCH 00-08 (the timing in the proposed cGAP), at a target rate of 0.631 L product/ha (equivalent to 250 g a.s./ha). In treated plot 3, a single application of F9600-4 SC was made, the application was made at 30±3 days before harvest at a nominal rate of 0.631 L/ha (equivalent to 250 g a.s./ha). Applications in plot 3 were made closer to harvest (an alternative timing to the proposed cGAP), in an attempt to achieve measurable residues at harvest, as an increased application rate at an earlier application timing might lead to phytotoxicity. All applications were made using a spray volume of 200-400 L/ha. In trial S16-05487-01, samples for residue analysis of the crop were taken from all plots at growth stages BBCH 75-77 and BBCH 89 (normal commercial harvest). In trial S16-05487-02, sufficiently sized samples for the residue analysis of the crop were taken from all plots at growth stage BBCH 89 (normal commercial harvest). In both trials samples for processing were taken from the untreated control and from plot 3 at BBCH 89 (normal commercial harvest).

The application to plot 2 was made broadly in accordance with the proposed GAP for use on wheat (which is a single application at 200 g a.s./ha on wheat). Therefore, the results from this trial can be considered supportive of the use on primary crops. These results can be considered supportive information when considering the proposed use on wheat and barley as a primary crop. A summary of these results is presented in section 7.3.1.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in the previous three seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples for some limited processed fractions as listed in Table 7-95 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices (whole plant, straw and grain) from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.2 mg/kg). It is

difficult to fully understand or explain the reasoning for residues in the untreated controls in this study as the residues in untreated controls were for wholemeal flour and bran, where the residues in grain (RAC) were not reported to contain residues in controls. Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.6 of Volume 1. In this processing trial, treated and untreated plots were situated ≥ 10 m apart.

Samples were stored under frozen (< -18°C) conditions prior to extraction. Samples of the RAC were stored frozen (<-18°C) for up to 301 days. Samples of the processing fractions were stored frozen (<-18°C) for up to 288 days. No storage stability data was available for the processed fractions of wheat. Please refer to discussion of the storage stability in Vol 1 (section 2.7.1). Samples of the extracts were stored (between 1 °C and 10 °C) for up to 9 days between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

The processing of fresh wheat grain samples started with a cleaning step, impurities (debris) were removed and the grain was sorted by size, and the grain with a size of >2.0mm was kept. The sample 'cleaned grains' was taken. This grain was then processed, simulating industrial practice at a laboratory scale, into bran, white flour type 550, whole meal flour, white bread, wholemeal bread, wet starch, wet gluten and wheat germs. The processing was carried out in the following stages:

Milling of wheat grain into white flour (type 550):

The grain was milled using a "Bühler Mahlautomat" mill type into eight fractions. Two of the fractions are bran fractions ('coarse bran' and 'fine bran'), two are 'shorts' fractions and the remaining four are the 'flour fractions without shorts'. 'Fine bran', 'coarse bran' and 'shorts' were sampled. Flour fraction 'flour without shorts' was mixed with the remaining 'shorts' fraction. 'Fine bran' and 'coarse bran' were mixed in the ratio of 1:1 and the sample 'total bran' was taken.

The remaining bran was used to make the 'toppings' and the 'middlings'. 'Coarse bran' was cleaned with a bran duster to get 'toppings 1'. 'Fine bran' was cleaned with a bran duster to get 'toppings 2' and 'middlings'. Toppings 1 and 2 were combined to give 'toppings'. Samples of 'toppings' and 'middlings' were taken.

The ash content of the 'flour with shorts' was taken using a muffle furnace. 'toppings' were added to the flour fraction of sample L16-05487-02-022A to give 'refined flour (type 550)'. For the other samples the ash content was such that the additions of 'toppings' was not required. A sample of 'refined flour type 550' was taken.

White bread processing:

The following ingredients were placed in a kneading machine:

Ingredients	**Dough**
White flour	0.32-1.5 kg
Salt	2%
Bread yeast (fresh)	2%
Water	50-60%

Basis of percentage values is the weight of white flour (type 550) used. Weight of water was adapted to the properties of the flour.

The dough was placed with moistened towels covering the bowl in environmental cabinets with a controlled climate of 25 °C for a total time of 65 minutes. This time is divided into three fermentation steps. After 25 minutes, the dough was kneaded for 1 minute and returned to the cabinet for a further 15 minutes. The dough was the manually divided into loaves of comparable weight, placed into baking forms and returned to the cabinet for 25 mins.

At the end of fermentation, the sample 'dough (white bread)' was taken.

The forms were placed in a preheated oven with steam injection and baked at approximately 210 °C for 25 minutes. The bread was allowed to cool and the sample 'white bread' was taken.

Wholemeal flour:

First the grain was manually mixed while adding water to a moisture content of around 14% and then stored for between 14 and 22 hours at an ambient temperature of 20.8-22.0 °C. The sample 'grain after conditioning' was taken.

The wheat was milled using a hammer mill (grinder). Wholemeal flour was produced without waste and the sample 'wholemeal flour' was taken.

Wholemeal bread processing:

The following ingredients were placed in a kneading machine:

Ingredients	**Dough**
Wholemeal flour	1.5-2.4 kg
Salt	2%
Bread yeast (fresh)	2%
Water	50%

Basis of percentage values is the weight of wholemeal flour used.

The dough was placed with moistened towels covering the bowl in environmental cabinets with a controlled climate of 25 °C for a total time of 60 minutes. This time is divided into three fermentation steps. After 25 minutes, the dough was kneaded for 1 minute and returned to the cabinet for a further 15 minutes. The dough was the manually divided into loaves of comparable weight, placed into baking forms and returned to the cabinet for 20 mins.

At the end of fermentation, the sample 'dough' was taken.

The forms were placed in a preheated oven with steam injection and baked at approximately 210 °C for 20-25 minutes. The bread was allowed to cool and the sample 'wholemeal bread' was taken.

Starch and gluten:

Water and white flour (Type 550, with the exception of sample L16-05487-01-004A) were added to the kneading machine in the ratio of water: flour 0.4. A 3% aqueous salt solution was added in several steps (ratio of dough: salt solution = 0.09 to 0.35). During each step dough was constantly kneaded. After each step, the starch/salt solution was drained and retained for the starch/table water centrifugation. The process was stopped when no more starch dissolved into the water/salt solution and the solution stayed clear.

The starch/table salt solution was separated by centrifugation several times. Samples of 'wet gluten' and 'wet starch' were taken. The wet samples of starch and gluten were dried using a drying oven at 70 °C for between 24 hours 5 minutes and 87 hours.

The fractions dried starch and dried gluten were combined in a 1:1 and mixed thoroughly, samples of 'gluten meal' were taken.

Wheat germs:

Grains were soaked in tap water (1 kg) at a temperature of 18-23.2 °C for 1 to 3 days. After this time the germs were manually separated from the grains using a knife. Samples of 'wheat grains without germs' and 'wheat germs' were taken.

Details of how each processed fraction was produced are given in Figure 7-11 - Figure 7-17. The mass of each processed fraction is given in Table 7-87.

Figure 7-11 Division, cleaning and conditioning of wheat grains

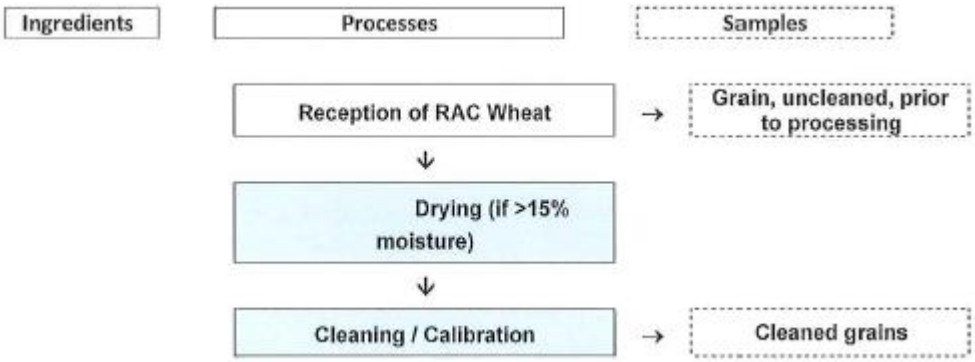


Figure 7-12 Processing of wheat grains into white flour (type 550)

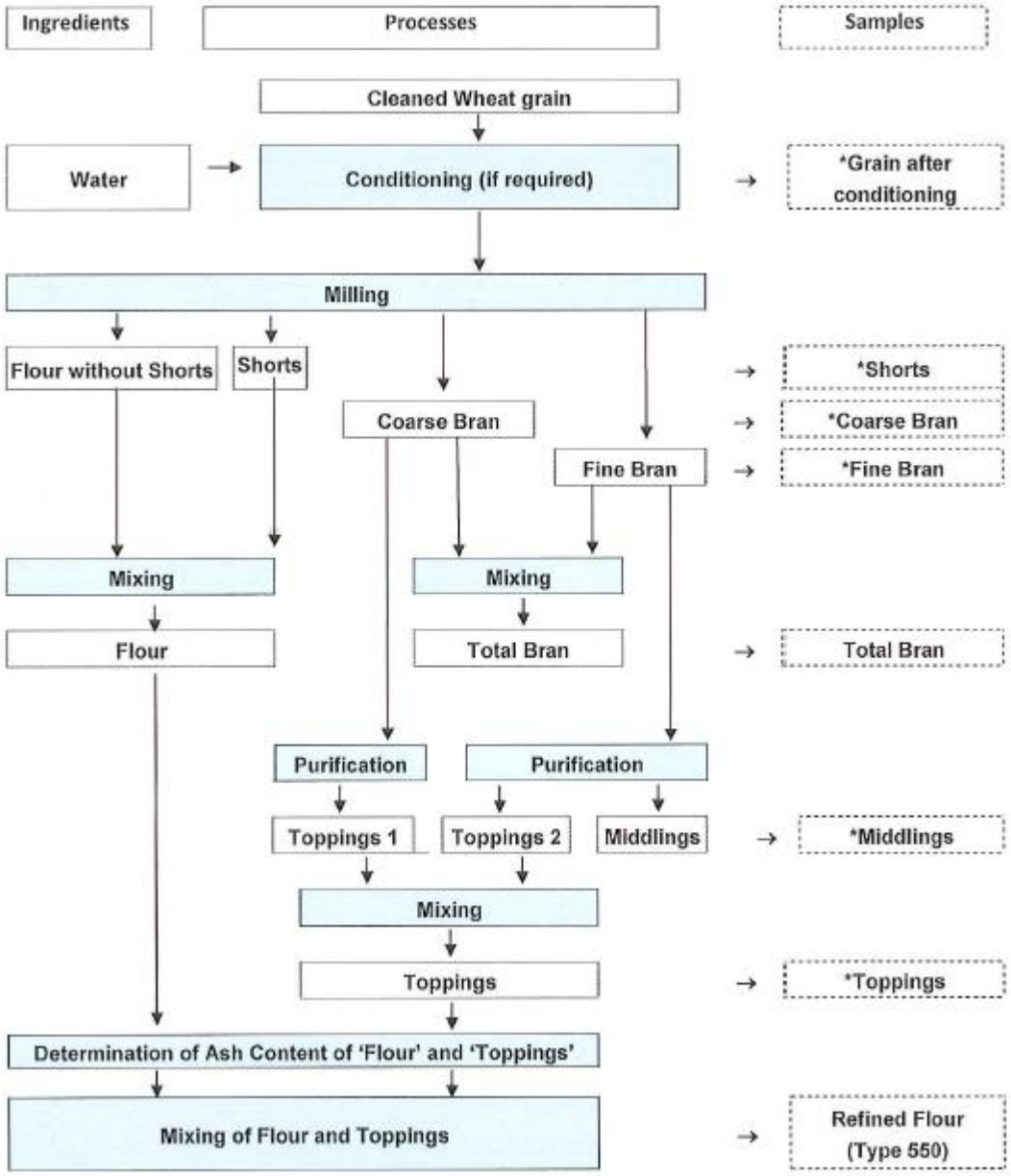


Figure 7-13 Processing of refined flour into white bread

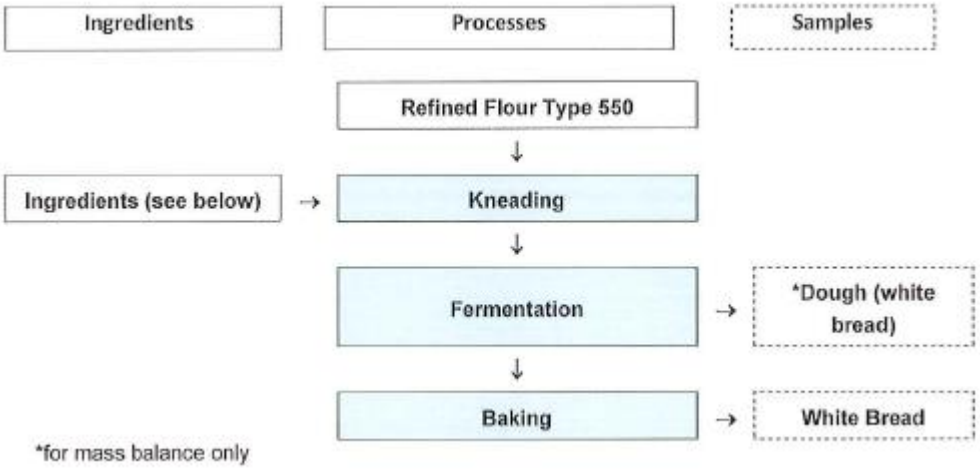


Figure 7-14 Processing of wheat grains into wholemeal flour

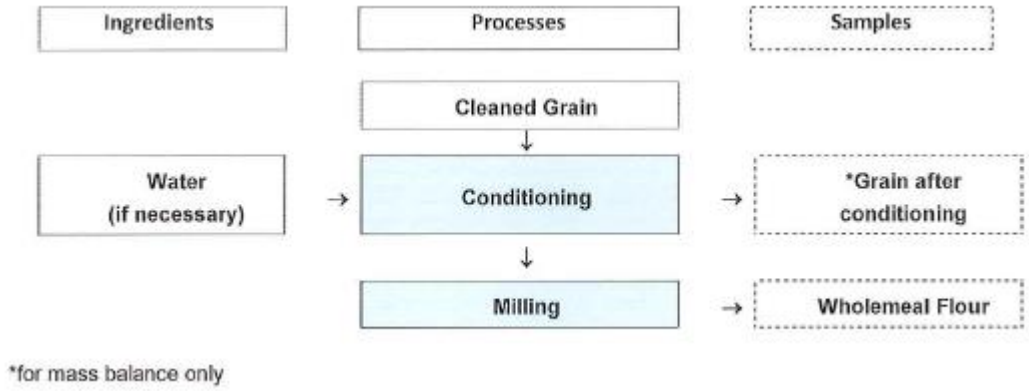


Figure 7-15 Processing of wholemeal flour into wholemeal bread

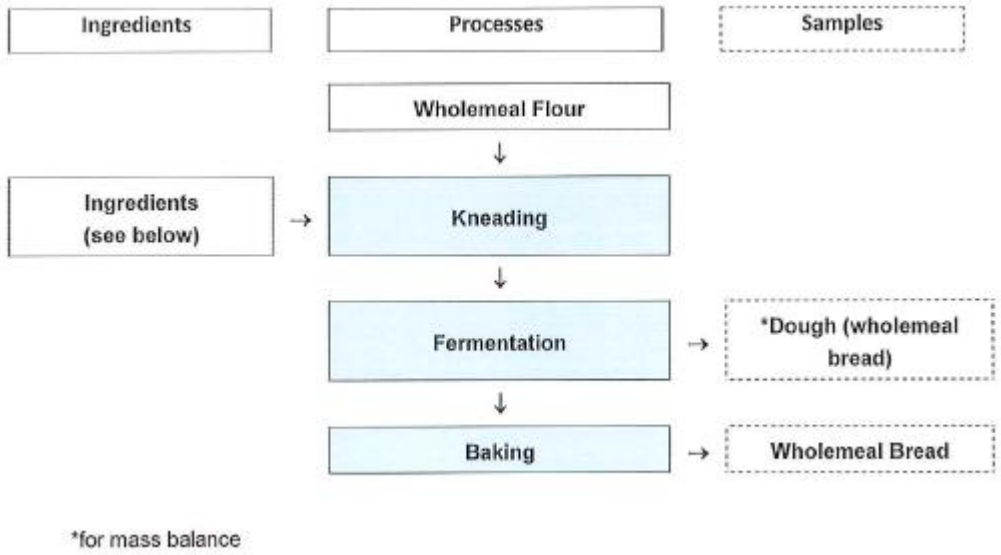


Figure 7-16 Processing of white flour into starch and gluten

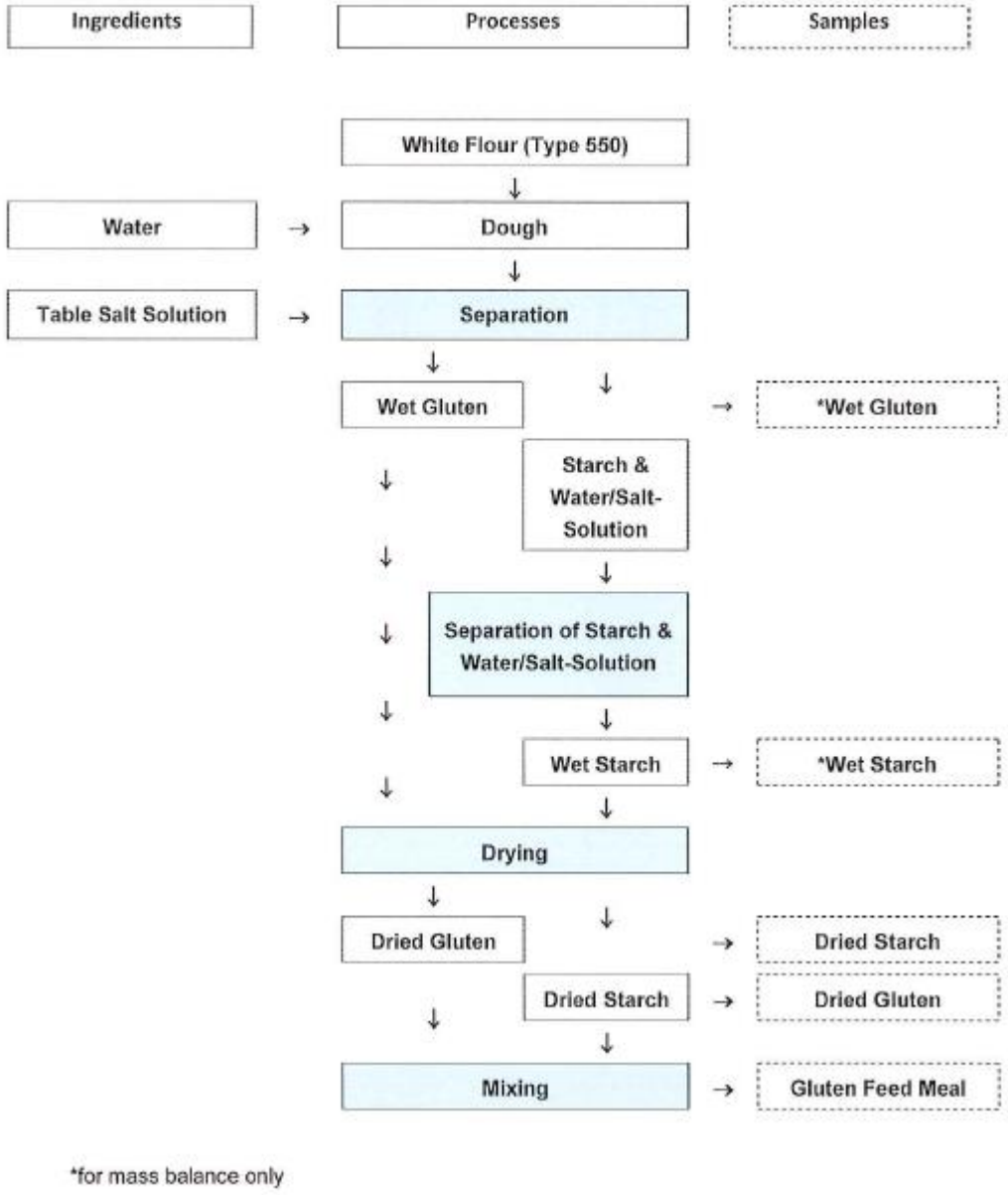


Figure 7-17 Processing of wheat grain into wheat germs

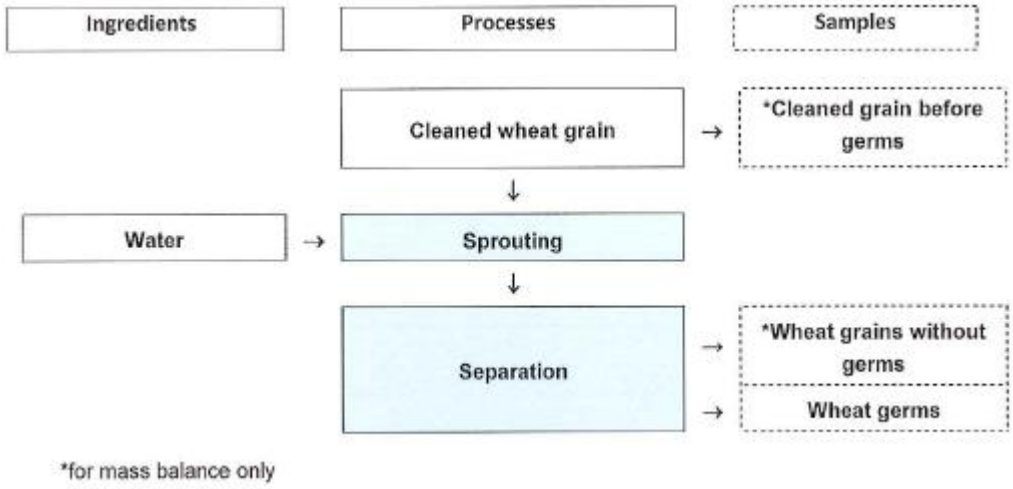


Table 7-87 Weight of processed fractions for each trial

Processed fraction	**Sample weight (kg)**			
	L16-05487-01-004A (untreated control) **NEU**	**L16-05487-01-008A (treated)** **NEU**	**L16-05487-02-002A (untreated control)** **SEU**	**L16-05487-02-006A (treated)** **SEU**
Grain	43.7	44.0	42.0	42.0
Cleaned grains	36.7	38.0	38.2	38.4
Conditioned wheat	32.0	32.1	32.9	33.5
Fine bran	2.6	8.9	11.4	11.7
Coarse bran	25.7	16.6	10.9	12.1
Total bran	0.6	0.5	0.6	0.6
Toppings 1	3.1	2.2	1.3	1.7
Middlings	0.88	3.9	5.6	6.7
Toppings 2	0.19	0.55	1.5	1.4
Toppings	3.3	0.5	2.8	3.1
Flour type 550	-	-	8.3	-
White dough	0.52	1.6	2.3	2.3
White bread	0.31	1.0	2.1	2.2
Wholemeal flour	2.7	3.1	4.5	4.7
Wholemeal dough	2.3	3.7	2.3	2.3
Wholemeal bread	1.5	2.8	2.1	2.2
Wet gluten	0.20	0.50	0.46	0.40
Wet starch	0.88	1.4	7.2	4.9
Dried gluten	0.06	0.18	0.17	0.14
Dried starch	0.28	0.49	0.60	0.60

Gluten feed meal	-	0.1	0.15	0.1
Wheat germs	0.02	0.02	0.02	0.02

The level of residues in the RAC and each processed fraction was determined for bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid (method number CAM-0180/001 and CAM-0180-version 2). The LOQ for bixlozone, 2,4-dichlorobenzoic acid and 5'-hydroxy-bixlozone is 0.01 mg/kg. In some processing trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.2 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

The method is fully validated in wheat straw, grain and whole plant fractions as well as a range of other commodity groups (including high acid, high oil and high water). Full details of the sample preparation and validation data for these methods are given in DAR CA B5 Section B.5.1.2.5. Validation in the processed fractions was carried out within the processing study reports by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the recoveries. Control material was taken from the control plots in the trials. Three (3) fortifications of untreated control samples at the level of LOQ (0.01 mg/kg) and three (3) fortifications at the level of tenfold LOQ mg/kg) were performed, representing a reduced validation data set. The recovery data for the processed fractions studied in this way (white bread, wholemeal bread, gluten, starch and germs) are considered adequately addressed even though a lower number of validations per fortification level were carried out as the results are acceptable (recovery range, mean recovery and %RSDs). Some matrices were only studied (in terms of method validations) in a more limited way, with only one recovery per fortification level: bran, middlings, shorts, toppings, flour and dough. The limited procedural recovery data for these matrices does not indicate a concern with the performance of the analytical method in these matrices. Details of the procedural recoveries are given in Table 7-88 - Table 7-91. The mean procedural recoveries are within the acceptable range (70-110%).

Table 7-88 Procedural recovery data for bixlozone in wheat commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	274 → 159 *m/z* quantification	0.01	81; 78; 83	81	3.1 (3)
		0.10	92; 102; 94	96	5.5 (3)
	274 → 123 *m/z* confirmation	0.01	101; 103; 101	102	1.1 (3)
		0.10	97; 104; 97	99	4.1 (3)
Grain	274 → 159 *m/z* quantification	0.01	88; 99; 91	93	6.1 (3)
		0.10	97; 89; 91	92	4.5 (3)
	274 → 123 *m/z* confirmation	0.01	95; 102; 100	99	3.6 (3)
		0.10	96; 89; 87	91	5.2 (3)
White bread	274 → 159 *m/z* quantification	0.01	97; 87; 96	93	5.9 (3)
		0.10	94; 90 ;94	93	2.5 (3)
	274 → 123 *m/z* confirmation	0.01	93; 90; 103	95	7.1 (3)
		0.10	95; 94; 96	95	1.1 (3)
Wholemeal bread	274 → 159 *m/z* quantification	0.01	88; 96; 92	92	4.3 (3)
		0.10	93; 89; 91	91	2.2 (3)
	274 → 123 *m/z* confirmation	0.01	93; 95; 86	91	5.2 (3)
		0.10	91; 88; 92	90	2.3 (3)
Gluten	274 → 159 *m/z* quantification	0.01	95; 85; 86	89	6.2 (3)
		0.10	87; 83; 81	84	3.7 (3)
	274 → 123 *m/z* confirmation	0.01	94; 92; 82	89	7.2 (3)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
		0.10	86; 84; 83	84	1.8 (3)
Starch	274 → 159 *m/z* quantification	0.01	97; 91; 98	95	4.0 (3)
		0.10	91; 94; 92	92	1.7 (3)
	274 → 123 *m/z* confirmation	0.01	79; 89; 87	83	6.4 (3)
		0.10	98; 96; 94	90	5.9 (3)
Germs	274 → 123 *m/z* quantification	0.01	84; 80; 87	84	4.2 (3)
		0.10	98; 96; 94	96	2.1 (3)
	274 → 125 *m/z* confirmation	0.01	77; 72; 73	74	4.2 (3)
		0.10	86; 81; 83	83	2.1 (3)
Bran	274 → 159 *m/z* quantification	0.01	92	92	-
		0.10	97	97	-
Middlings	274 → 159 *m/z* quantification	0.01	93	93	-
		0.10	104	104	-
Shorts	274 → 159 *m/z* quantification	0.01	94	94	-
		0.10	107	107	-
Toppings	274 → 159 *m/z* quantification	0.01	100	100	-
		0.10	92	92	-
Flour	274 → 159 *m/z* quantification	0.01	106	106	-
		0.10	102	102	-
Dough	274 → 159 *m/z* quantification	0.01	76	76	-
		0.10	88	88	-

Table 7-89 Procedural recovery data for 2,4-dichlorobenzoic acid in wheat commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	189 → 145 *m/z* quantification	0.01	96; 105; 98	100	4.7 (3)
		0.10	103; 102; 106	104	2.0 (3)
	189 → 145 *m/z* confirmation	0.01	104; 103; 98	102	3.2 (3)
		0.10	87; 92; 94	91	4.0 (3)
Grain	189 → 145 *m/z* quantification	0.01	92; 102; 110	101	8.9 (3)
		0.10	108; 108; 108	108	0.0 (3)
	189 → 145 *m/z* confirmation	0.01	86; 97; 100	94	7.8 (3)
		0.10	98; 103; 94	98	4.6 (3)
White bread	189 → 145 *m/z* quantification	0.01	105; 101; 107	104	2.9 (3)
		0.10	103; 106; 108	106	2.4 (3)
	189 → 145 *m/z* confirmation	0.01	103; 108; 109	107	3.0 (3)
		0.10	86; 91; 101	93	8.2 (3)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Wholemeal bread	189 → 145 *m/z* quantification	0.01	108; 99; 102	103	4.4 (3)
		0.10	108; 103; 107	106	2.5 (3)
	189 → 145 *m/z* confirmation	0.01	95; 74; 90	86	13.0 (3)
		0.10	93; 94; 110	99	7.3 (3)
Gluten	189 → 145 *m/z* quantification	0.01	102; 113; 78	98	18 (3)
		0.10	104; 99; 90	98	7.3 (3)
	189 → 145 *m/z* confirmation	0.01	82; 105; 99	95	13.0 (3)
		0.10	91; 94; 87	91	3.9 (3)
Starch	189 → 145 *m/z* quantification	0.01	84; 75; 99	86	14.0 (3)
		0.10	102; 103; 105	103	1.5 (3)
	189 → 145 *m/z* confirmation	0.01	114; 101; 113	109	6.6 (3)
		0.10	88; 95; 91	91	3.8 (3)
Germs	189 → 145 *m/z* quantification	0.01	93; 95; 104	97	6.0 (3)
		0.10	103; 109; 100	104	4.4 (3)
	189 → 145 *m/z* confirmation	0.01	101; 92; 115	103	11.0 (3)
		0.10	89; 88; 86	88	1.7 (3)
Bran	189 → 145 *m/z* quantification	0.01	96	96	-
		0.10	104	104	-
Middlings	189 → 145 *m/z* quantification	0.01	91	91	-
		0.10	110	110	-
Shorts	189 → 145 *m/z* quantification	0.01	78	78	-
		0.10	103	103	-
Toppings	189 → 145 *m/z* quantification	0.01	104	104	-
		0.10	102	102	-
Flour	189 → 145 *m/z* quantification	0.01	107	107	-
		0.10	108	108	-
Dough	189 → 145 *m/z* quantification	0.01	109	109	-
		0.10	107	107	-

Due to the fact that for 2,4-dichlorobenzoic acid only one mass transition is available, an additional method confirmation was necessary for the determination of this analyte in all representative crops. The confirmation transition was determined by repeating in another sequence with a different column.

Table 7-90 Procedural recovery data for 5'-hydroxy-bixlozone in wheat commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	290 → 175 *m/z* quantification	0.01	89; 85; 88	87	2.4 (3)
		0.10	86; 96; 93	92	5.6 (3)
	290 → 111 *m/z* confirmation	0.01	72; 88; 72	77	12.0 (3)
		0.10	90; 105; 97	97	7.7 (3)
Grain†	290 → 175 *m/z* quantification	0.01	100; 114; 101	105	7.4 (3)
		0.10	111; 99; 101	104	6.2 (3)
	290 → 111 *m/z* confirmation	0.01	108; 109; 112	110	1.9 (3)
		0.10	110; 98; 111	106	6.8 (3)
White bread	290 → 175 *m/z* quantification	0.01	79; 77; 89	82	7.9 (3)
		0.10	81; 85; 88	85	4.1 (3)
	290 → 111 *m/z* confirmation	0.01	97; 94; 92	94	2.7 (3)
		0.10	82; 86; 88	85	3.6 (3)
Wholemeal bread	290 → 175 *m/z* quantification	0.01	104; 96; 97	99	4.4 (3)
		0.10	85; 88; 92	88	4.0 (3)
	290 → 111 *m/z* confirmation	0.01	98; 91; 92	94	4.0 (3)
		0.10	88; 84; 91	88	4.0 (3)
Gluten	290 → 175 *m/z* quantification	0.01	81; 95; 84	87	8.5 (3)
		0.10	85; 72; 71	76	10.0 (3)
	290 → 111 *m/z* confirmation	0.01	73; 98; 80	84	15.0 (3)
		0.10	85; 72; 71	76	10.0 (3)
Starch	290 → 175 *m/z* quantification	0.01	103; 97; 101	100	3.0 (3)
		0.10	85; 86; 87	86	1.2 (3)
	290 → 111 *m/z* confirmation	0.01	87; 81; 94	87	7.5 (3)
		0.10	81; 91; 91	88	6.6 (3)
Germs	290 → 175 *m/z* quantification	0.01	83; 91; 74	83	10.0 (3)
		0.10	82; 90; 79	84	6.8 (3)
	290 → 111 *m/z* confirmation	0.01	96; 94; 88	93	4.5 (3)
		0.10	93; 93; 93	93	0.0 (3)
Bran	290 → 175 *m/z* quantification	0.01	90	90	-
		0.10	77	77	-
Middlings	290 → 175 *m/z* quantification	0.01	92	92	-
		0.10	89	89	-
Shorts	290 → 175 *m/z* quantification	0.01	87	87	-
		0.10	98	98	-
Toppings	290 → 175 *m/z* quantification	0.01	109	109	-

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
		0.10	104	104	-
Flour	290 → 175 *m/z* quantification	0.01 0.10	95 95	95 95	- -
Dough	290 → 175 *m/z* quantification	0.01 0.10	102 90	102 90	- -

†in one control sample of grain for procedural recoveries, 5'-hydroxy-bixlozone was detected at the LOQ (0.01 mg/kg)

Table 7-91 Procedural recovery data for 2,2-dimethyl-3-hydroxy propionic acid in wheat commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	117 → 87 *m/z* quantification	0.2 2.0	111; 106; 103 101; 98; 107	107 102	3.8 (3) 4.5 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	105; 105; 101 107; 98; 105	104 103	2.2 (3) 4.6 (3)
Grain†	117 → 87 *m/z* quantification	0.2 2.0	98; 96; 111 110; 109; 111	102 110	8.0 (3) 0.9 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	120; 102; 104 109; 105; 107	109 107	9.1 (3) 1.9 (3)
White bread	117 → 87 *m/z* quantification	0.2 2.0	95; 90; 105 91; 96; 101	97 96	7.9 (3) 5.2 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	106; 106; 117 92; 93; 89	110 94	5.8 (3) 3.4 (3)
Wholemeal bread	117 → 87 *m/z* quantification	0.2 2.0	113; 112; 106 110; 106; 112	110 109	3.4 (3) 2.8 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	102; 92; 104 97; 88; 100	99 95	6.5 (3) 6.6 (3)
Gluten	117 → 87 *m/z* quantification	0.2 2.0	80; 105; 90 91; 89; 89	92 90	14.0 (3) 1.3 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	102; 114; 110 94; 90; 84	109 89	5.6 (3) 5.6 (3)
Starch	117 → 87 *m/z* quantification	0.2 2.0	87; 84; 81 82; 86; 89	84 86	3.6 (3) 4.1 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	93; 101; 97 87; 92; 93	97 91	4.1 (3) 3.5 (3)
Germs	117 → 87 *m/z* quantification	0.2 2.0	109; 110; 107 95; 109; 107	109 104	1.4 (3) 7.3 (3)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
	117 → 87 *m/z* confirmation	0.2 2.0	95; 88; 90 87; 85; 88	91 87	4.0 (3) 1.8 (3)
Bran†	117 → 87 *m/z* quantification	0.2 2.0	102 102	102 102	- -
Middlings	117 → 87 *m/z* quantification	0.2 2.0	90 98	90 98	- -
Shorts	117 → 87 *m/z* quantification	0.2 2.0	86 97	86 97	- -
Toppings	117 → 87 *m/z* quantification	0.2 2.0	110 110	110 110	- -
Flour†	117 → 87 *m/z* quantification	0.2 2.0	102 110	102 110	- -
Dough	117 → 87 *m/z* quantification	0.2 2.0	104 97	104 97	- -

†in six control samples of grain (0.21 mg/kg, 3 x 0.22 mg/kg and 2 x 0.23 mg/kg), one control sample of bran (0.21 mg/kg) and one control sample of flour (0.21 mg/kg) for procedural recoveries, 2,2-dimethyl-3-hydroxy propionic acid was detected above the LOQ

Due to the fact that for 2,2-dimethyl-3-hydroxy propionic acid only one mass transition is available, an additional method confirmation was necessary for the determination of this analyte in all representative crops. The confirmation transition was determined by repeating in another sequence with a different column.

Results and discussion

The effect of processing on residues of bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid from wheat grain (sampled at BBCH 89) to processed fractions was determined for two trials. The hydrolysis study on bixlozone indicated that the metabolites 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid were not formed from parent bixlozone on processing (see Section B.7.5.1); however, as they could be present in the RAC, a consideration of their levels in processed commodities was conducted within the scope of this processing study. In accordance with OECD 508, the application conditions used in the trails should aim to allow for quantifiable residues to allow the study of processing and impact of processing on residues to be observed. This is why the applicant designed the trial (plot 3) with a shortened harvest interval to increase the chances of observing positive residues. Although an acceptable strategy, the residues in this study (and across processed fractions) were sometimes low and this was not ideal for the setting of robust processing factors. Where positive residues were not observed (especially in RAC prior to processing) it was not possible to derive a reliable processing factor. Therefore, from this processing study, only limited processing factors were obtained, for a discussion on processing factors for the proposed residue definition, please refer to section 2.7.6 of the Volume 1.

Bixlozone

Residues of bixlozone in wheat grain (RAC) were <0.01 and 0.017 mg/kg. Residues of bixlozone were <0.01 mg/kg in fine bran, toppings, flour type 550, white dough, white bread, wholemeal dough, wet gluten, dried gluten, dried starch, gluten feed meal and wheat germs. Residues of bixlozone were 0.012 mg/kg in coarse bran, <0.01 and 0.014 mg/kg in total bran, <0.01 and 0.013 mg/kg in wholemeal flour and <0.01 and 0.01 mg/kg in wholemeal bread.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for bixlozone are shown in Table 7-92.

2,4-dichlorobenzoic acid

Residues of 2,4-dichlorobenzoic acid in wheat grain (RAC) were 0.075 and <0.01 mg/kg. Residues of 2,4-dichlorobenzoic acid were <0.01mg/kg in fine bran, white dough, white bread and wet gluten, 0.12 mg/kg in coarse bran, 0.085 and <0.01 mg/kg in total bran, 0.017 mg/kg in toppings, 0.01 and <0.01 mg/kg in flour type 550, 0.068 and <0.01 mg/kg in wholemeal flour, 0.05 mg/kg in wholemeal dough, 0.064 and <0.01 mg/kg in wholemeal bread, 0.012 and <0.01 mg/kg in dried gluten and dried starch, 0.01 and <0.01 mg/kg in gluten feed meal and 0.03 and <0.01 mg/kg in wheat germs.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 2,4-dichlorobenzoic acid are shown in Table 7-93.

5'-hydroxy-bixlozone

Residues of 5'-hydroxy-bixlozone in wheat grain (RAC) were <0.01 mg/kg. Residues of 5'-hydroxy-bixlozone in all processed fractions were <0.01.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 5'-hydroxy-bixlozone are shown in Table 7-94.

2,2-dimethyl-3-hydroxy propionic acid

Mean residues of 2,2-dimethyl-3-hydroxy propionic acid in wheat grain (RAC) were 0.22 and <0.20 mg/kg. Residues of 2,2-dimethyl-3-hydroxy propionic acid were <0.20 mg/kg in fine bran, toppings, flour type 550, white dough, white bread, wholemeal dough, wholemeal bread, wet gluten, dried gluten, dried starch, gluten feed meal and wheat germs, 0.36 mg/kg in coarse bran, 0.25 and <0.20 mg/kg in total bran, 0.22 and <0.20 mg/kg in wholemeal flour.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results). For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 2,2-dimethyl-3-hydroxy propionic acid are shown in Table 7-95.

Table 7-92 Bixlozone results from processing trials on wheat

Product	Bixlozone residue (mg/kg)	
	Trial S16-05487-01 NEU	**Trial S16-05487-02 SEU**
Grain (RAC)	<0.01	0.017
Fine bran	<0.01	nd
Coarse bran	0.012	nd
Total bran	<0.01	0.014
Toppings	<0.01	nd
Flour type 550	<0.01	<0.01
White dough	<0.01	nd
White bread	<0.01	<0.01
Wholemeal flour	<0.01	0.013
Wholemeal dough	<0.01	nd
Wholemeal bread	<0.01	0.010
Wet gluten	<0.01	nd
Dried gluten	<0.01	<0.01
Dried starch	<0.01	<0.01
Gluten feed meal	<0.01	<0.01
Wheat germs	<0.01	<0.01

nd = not determined

Table 7-93 2,4-dichlorobenzoic acid results from processing trials on wheat

Product	2,4-dichlorobenzoic acid residue (mg/kg)	
	Trial S16-05487-01 NEU	**Trial S16-05487-02 NEU**
Grain (RAC)	0.075	<0.01
Fine bran	<0.01	nd
Coarse bran	0.120	nd
Total bran	0.085	<0.01
Toppings	0.017	nd
Flour type 550	0.010	<0.01
White dough	<0.01	nd
White bread	<0.01	<0.01
Wholemeal flour	0.068	<0.01
Wholemeal dough	0.050	nd
Wholemeal bread	0.064	<0.01

Wet gluten	<0.01	nd
Dried gluten	0.012	<0.01
Dried starch	0.012	<0.01
Gluten feed meal	0.010	<0.01
Wheat germs	0.030	<0.01

nd = not determined

Table 7-94 5'-hydroxy-bixlozone results from processing trials on wheat

Product	**5'-hydroxy-bixlozone residue (mg/kg)**	
	Trial S16-05487-01 NEU	**Trial S16-05487-02 SEU**
Grain (RAC)	<0.01	<0.01
Fine bran	<0.01	nd
Coarse bran	<0.01	nd
Total bran	<0.01	<0.01
Toppings	<0.01	nd
Flour type 550	<0.01	<0.01
White dough	<0.01	nd
White bread	<0.01	<0.01
Wholemeal flour	<0.01	<0.01
Wholemeal dough	<0.01	nd
Wholemeal bread	<0.01	<0.01
Wet gluten	<0.01	nd
Dried gluten	<0.01	<0.01
Dried starch	<0.01	<0.01
Gluten feed meal	<0.01	<0.01
Wheat germs	<0.01	<0.01

nd = not determined

Table 7-95 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on wheat

Product	2,2-dimethyl-3-hydroxy propionic acid residue (mg/kg)	
	Trial S16-05487-01 NEU	Trial S16-05487-02 SEU
Grain (RAC)	0.22	<0.20
Fine bran	<0.20	nd
Coarse bran	0.36	nd
Total bran	0.25	<0.20 [UTC: 0.21]
Toppings	<0.20	nd
Flour type 550	<0.20	<0.20
White dough	<0.20	nd
White bread	<0.20	<0.20
Wholemeal flour	0.22 [UTC: 0.21]	<0.20
Wholemeal dough	<0.20	nd
Wholemeal bread	<0.20	<0.20
Wet gluten	<0.20	nd
Dried gluten	<0.20	<0.20
Dried starch	<0.20	<0.20
Gluten feed meal	<0.20	<0.20
Wheat germs	<0.20	<0.20

nd = not determined

Conclusions

The submitted processing studies attempted to assess the impact of processing on the magnitude of residue levels of bixlozone, and other analytes (2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid) over processing of wheat and relevant fractions. For all analytes tested, <LOQ residues were observed in the RAC in at least one of the two analysed trials.

HSE Remarks

The applicant aimed to increase chances of getting positive residues by carrying out the application with a shortened PHI (30±3 days). The applicant expressed some concern that conducting studies at an exaggerated rate might lead to phytotoxicity. However, there was a high frequency of <LOQ results observed throughout the trials.

The applicant provided acceptable method validation work for the analytes in some processed fractions including white bread, wholemeal bread, gluten, starch and germs.

The submitted trials used field trials to generate incurred residues for the processing study, which was then assessed by following a scheme of practice simulating industrial practice at a laboratory scale. Full details of each of the simulated processes were provided.

Although two processing trials are available for wheat, the derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results). For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

Samples (of RAC and processed fractions) were stored in the freezer for around 300 days prior to extraction and analysis. Although a range of freezer storage stability data are available for these analytes, specific freezer storage stability data have not been provided on these analytes for grain. This is discussed further in Vol 1, section 2.7.1.

B.7.5.3.2. *Barley*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-02, Semrau, J., 2018
Title:	Determination of residue of F9600 after one pre-emergence application of F9600-4 SC in barley and in processed fractions of barley at 2 sites in Northern and Southern Europe 2016
Report No.:	S16-05487
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

Materials and methods

A processing study on barley (varieties: *California*, *Isocel* winter barley), was conducted outdoors at a location in Germany (S16-05488-01 - N EU zone) and a location in Southern France (S16-05488-02 - S EU zone) during the 2016/2017 growing season. However, only the SEU trial (S16-05488-02) was taken forward for processing. Normal agricultural procedures were followed, and no unusual weather events were recorded. In in trial S16-05488-02 there was two treated plots (plot 2 and 3) and one untreated control plot (plot 1). Treated processing samples were taken from plot 3 only. A single foliar application was made using a boom sprayer of F9600-4 SC (an SC formulation containing 396 g/L bixlozone) to treated plot 2 at BBCH 00, at a target rate of 0.631 L product/ha (equivalent to 250 g a.s./ha). In treated plot 3, a single application of F9600-4 SC was made, the application was made at 28 days before harvest, at a nominal rate of 0.631 L/ha (equivalent to 250 g a.s./ha). All applications were made using a spray volume of 200-250 L/ha. In trial S16-05488-01 all samples were taken at BBCH 89. In trial S16-05488-02, samples for residue analysis of the crop were taken from plot 2 and the untreated plot (plot 1) at growth stages BBCH 75 and BBCH 89 (normal commercial harvest). Samples (of sufficient size) for processing were taken from the untreated control and from plot 3 at BBCH 89 (normal commercial harvest) in trail S16-05488-02. Samples from trial S16-05488-01 were not taken forward for processing and are not considered further.

The application to plot 2 was made broadly in accordance with the proposed GAP for use on barley (which is a single application at 200 g a.s./ha on barley). Therefore, the results from this trial can be considered supportive of the use on primary crops. However, these results can be considered supportive information when considering the proposed use on wheat and barley as a primary crop. A summary of these results is presented in section 7.3.1.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in the previous three seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples for some limited processed fractions as listed in Table 7-104 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices (whole plant, straw and grain) from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.2 mg/kg). It is difficult to fully understand or explain the reasoning for residues in the untreated controls in this study as the residues in untreated controls were for malt sprouts and malt, where the residues in grain (RAC) were not reported to contain residues in controls. Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.6 of Volume 1. In this processing trial, treated and untreated plots were situated ≥ 10 m apart.

Samples were stored under frozen (< -18°C) conditions prior to extraction. Samples of the RAC were stored frozen (<-18°C) for up to 336 days. Samples of the processing fractions were stored frozen (<-18°C) for up to 279 days. No storage stability data was available for the processed fractions of cereals. Please refer to discussion of the storage stability in Vol 1 (section 2.7.1). Samples of the extracts were stored (between 1 °C and 10 °C) for up to 2 days between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

The processing of fresh barley grain samples started with a cleaning step, impurities (debris) were removed, and the grain was sorted by size, and the grain with a size of >2.5mm was kept. The sample 'cleaned grains' was taken. This grain was then processed, simulating industrial practice at a laboratory scale, into pearl barley, malt and beer. The processing was carried out in the following stages:

Malting:

The grain was soaked in tap water in a plastic box in an environmental test chamber at a target temperature of +16 °C. This steeping stage was carried out alternating between a sub-water period and a sub-air period three times. The gains were drained and dried in a kiln drying oven at 50 °C for 8 hours, then at 64 °C for 11 hours, followed by at 80 °C for 5 hours and finally at 30 °C for a further 2 hours. The moisture content of the dried malt was measured (1-1.2%). Directly after kiln drying the malt seedings were separated mechanically from the seeds and the sample 'malt sprouts' was taken

Brewing:

The malt, processed as outlined above, was stored in chilled conditions (≈7 °C) for 15-16 days prior to brewing. Dried and ground hops and yeast were commercially sourced.

The malt was ground coarsely immediately before mashing, to obtain 6 kg of ground malt. 18L of water were heated to 45 °C and 6 kg of the malt were filled in the malt tube and added to the water. Brewing was carried out at a pH between 5.45 and 5.55, with 90% lactic acid as follows:

Step	**Step number**	**Type of step**	**Temperature**	**Duration**
Mashing	1	Rest	≈45 °C	20 min
Brewing	2	Ramp	≈45-52 °C	10 min
	3	Ramp	≈52-64 °C	10 min
	4	Rest	≈64 ±2 °C	20 min
	5	Ramp	≈64-74 °C	10 min
	6	Rest	≈74 ±2 °C	30 min
Wort boiling	7	Ramp	≈74-100 °C	30 min
	8	Rest	≈100 ±2 °C	90 min

The mash in the malt tube was removed and rinsed with 3 x 5 L of tap water at approx. 74 °C for 10-15 minutes. The sample 'spent grain' was taken.

1.5 g of hops (extracted in CO_2 at 50% alpha) was added for 30 L. After cooling, the pH was measured and adjusted between 5.15 and 5.25 by the addition of lactic acid (90%). After cooling the wort, the deposit trub/ flocs (hops draff) were removed and the worts were transferred to the fermentation tank. The samples 'spent grain' and 'flocs' were taken.

Fermentation was started by adding brewer's yeast at 80 g/hL. Fermentation was completed when the concentration of sugars reached a <5.5° plateau. The fermentation tank was cold stored at 7 °C for 16-21, after which the beer was racked and filtered and the sample 'brewer's yeast' was taken. The beer was stored in bottles, the sample 'beer' was taken and then the bottles were deep frozen.

Pearl barley:

First the grains were moistened at room temperature for 18-22 hours. The grains were decorticated to achieve an abrasion in the range of 25-30%. The sample 'Pearl barley' was taken.

Details of how each processed fraction was produced are given in Figure 7-18 - Figure 7-21. The mass of each processed fraction is given in Table 7-96.

Figure 7-18 Division, cleaning and conditioning of barley grains

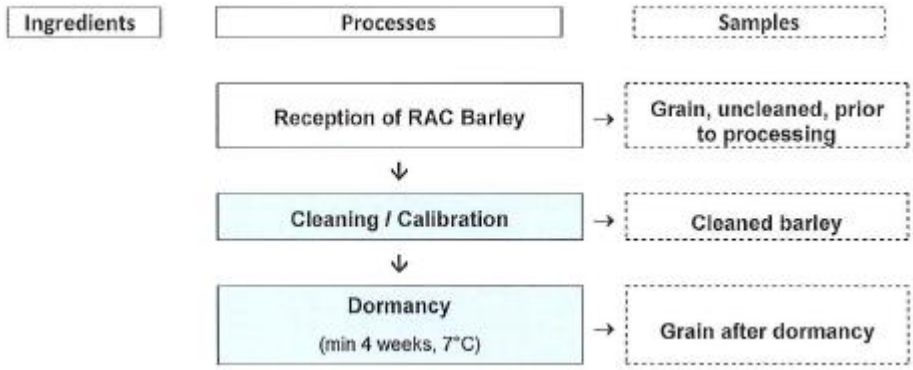


Figure 7-19 Processing of barley grains into malt

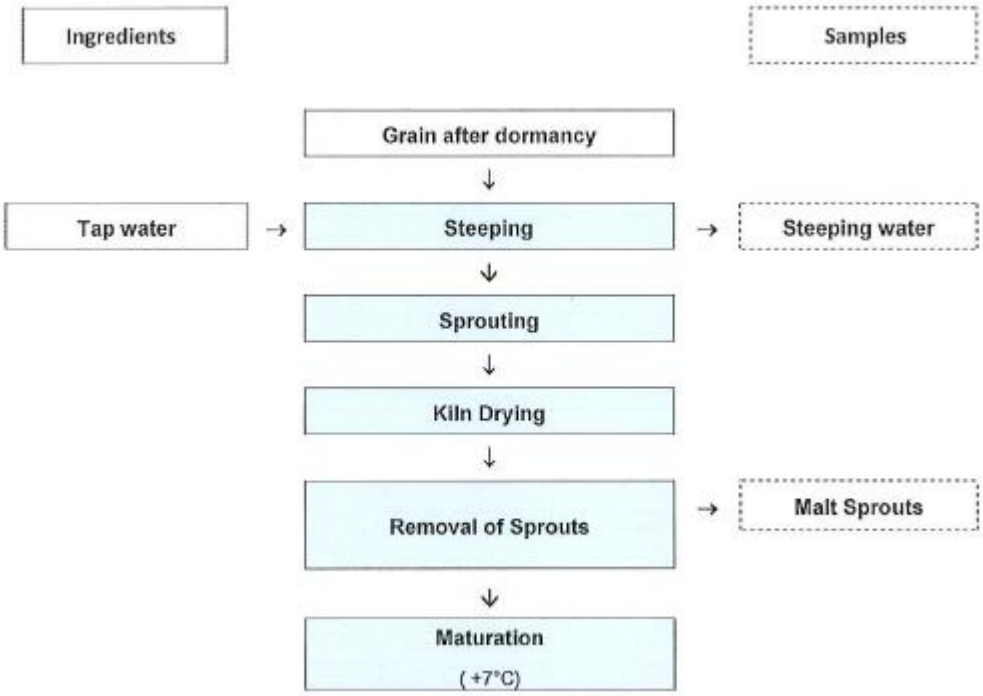


Figure 7-20 Processing of malt into beer

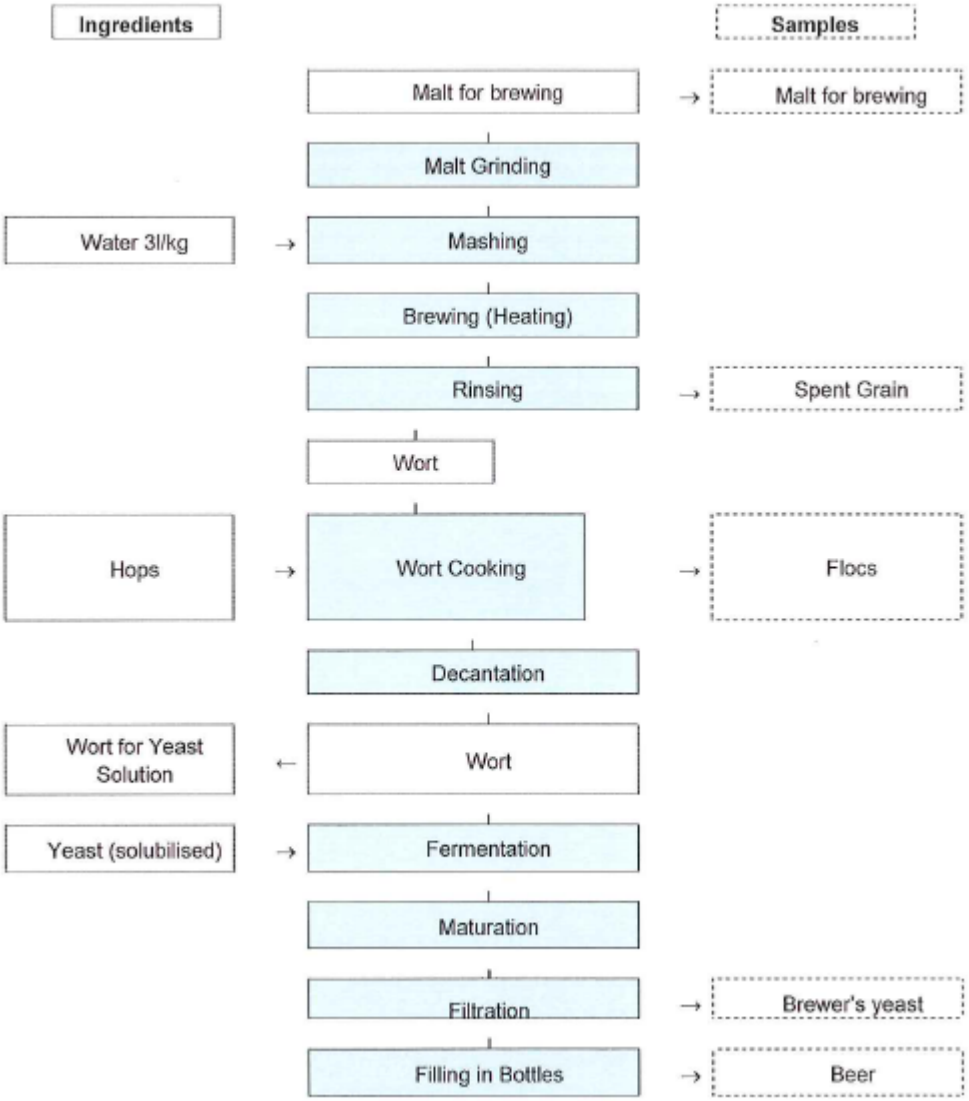


Figure 7-21 Processing of barley grains into pearl barley

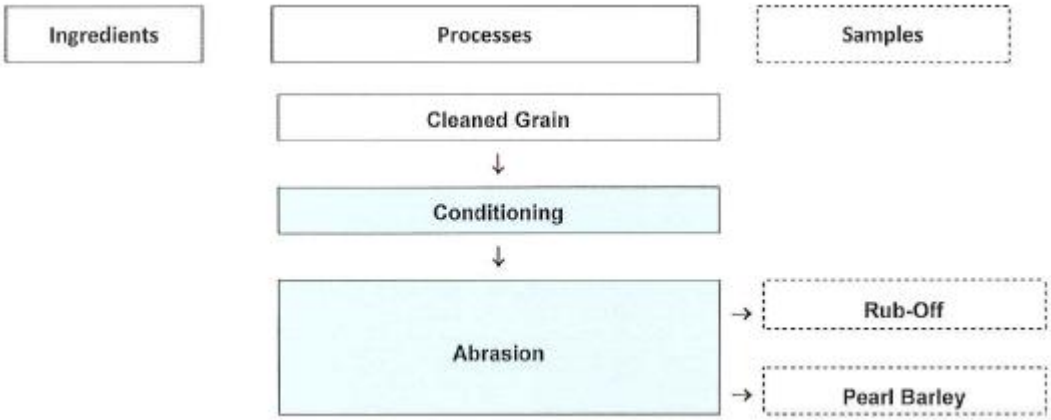


Table 7-96 Weight of processed fractions for each trial

Processed fraction	**Sample weight (kg)**	
	L16-05488-02-004A (untreated control)	**L16-05488-02-008A (treated)**
Grain	40.8	40.4
Cleaned grains	31.2	28.4
Malt	13.3	13.3
Malt sprouts	12.8	12.8
Spent grain	10.2	10.8
Flocs	1.52	1.40
Brewer's yeast	2.54	2.44
Filtered beer	19.07	19.62

The level of residues in the RAC and each processed fraction was determined for bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid (method number CAM-0180/001 and CAM-0180-version 2). The LOQ for bixlozone, 2,4-dichlorobenzoic acid and 5'-hydroxy-bixlozone is 0.01 mg/kg. In some processing trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.2 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

The method is fully validated in barley straw, grain and whole plant fractions as well as a range of other commodity groups (including high acid, high oil and high water). Full details of the sample preparation and validation data for these methods are given in DAR CA B5 Section B.5.1.2.5. Validation in the processed fractions was carried out within the processing study reports by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the recoveries. Control material was taken from the control plots in the trials. Three (3) fortifications of untreated control samples at the level of LOQ (0.01 mg/kg) and three (3) fortifications at the level of tenfold LOQ mg/kg) were performed, representing a reduced validation data set. The recovery data for the processed fractions studied in this way (steeping water, malt, brewer's yeast and beer) are considered adequately addressed even though a lower number of validations per fortification level were carried out as the results are acceptable (recovery range, mean recovery and %RSDs). Some matrices were only studied (in terms of method validations) in a more limited way, with only one recovery per fortification level: whole plant and flocs. The limited procedural recovery data does not indicate a concern with the performance of the analytical method in these matrices. Details of the procedural recoveries are given in Table 7-97 - Table 7-100. The mean procedural recoveries are within the acceptable range (70-110%).

Table 7-97 Procedural recovery data for bixlozone in barley commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	274 → 123 *m/z* quantification	0.01	90; 86; 85	87	3.0 (3)
		0.10	81; 77; 80	79	2.6 (3)
	276 → 125 *m/z* confirmation	0.01	90; 87; 77	85	8.0 (3)
		0.10	78; 74; 77	76	2.7 (3)
Steeping water	274 → 159 *m/z* quantification	0.01	105; 101; 103	103	1.9 (3)
		0.10	100; 101; 101	101	0.57 (3)
	274 → 123 *m/z* confirmation	0.01	100; 95; 101	99	3.3 (3)
		0.10	100; 99; 92	97	4.5 (3)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Malt	274 → 159 *m/z* quantification	0.01	83; 81; 86	83	3.0 (3)
		0.10	81; 82; 77	80	3.3 (3)
	274 → 123 *m/z* confirmation	0.01	87; 104; 94	95	9.0 (3)
		0.10	77; 83; 80	80	3.8 (3)
Brewer's yeast	274 → 159 *m/z* quantification	0.01	88; 91; 92	90	2.3 (3)
		0.10	100; 105; 93	99	6.1 (3)
	274 → 123 *m/z* confirmation	0.01	93; 86; 93	91	4.5 (3)
		0.10	98; 104; 92	98	6.1 (3)
Beer	274 → 159 *m/z* quantification	0.01	99; 93; 99	97	3.6 (3)
		0.10	99; 98; 100	99	1.0 (3)
	274 → 123 *m/z* confirmation	0.01	97; 98; 93	96	2.8 (3)
		0.10	106; 98; 100	101	4.1 (3)
Whole plant	274 → 123 *m/z* quantification	0.01	92	-	-
		0.10	86	-	-
Flocs	274 → 123 *m/z* quantification	0.01	95	-	-
		0.10	98	-	-

Table 7-98 Procedural recovery data for 2,4-dichlorobenzoic acid in barley commodities (method number CAM-0180)

Sample material	Mass transition*	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	189 → 145 *m/z* quantification	0.01	100; 109; 107	105	4.5 (3)
		0.10	98; 94; 101	98	3.6 (3)
	189 → 145 *m/z* confirmation	0.01	87; 100; 97	95	7.2 (3)
		0.10	93; 84; 86	88	5.4 (3)
Steeping water	189 → 145 *m/z* quantification	0.01	94; 105; 108	102	7.2 (3)
		0.10	109; 108; 106	108	1.4 (3)
	189 → 145 *m/z* confirmation	0.01	102; 114; 105	107	5.8 (3)
		0.10	87; 90; 95	91	1.4 (3)
Malt	189 → 145 *m/z* quantification	0.01	98; 106; 98	101	4.6 (3)
		0.10	105; 110; 107	107	2.3 (3)
	189 → 145 *m/z* confirmation	0.01	93; 101; 109	101	7.9 (3)
		0.10	91; 100; 94	95	4.8 (3)
Brewer's yeast	189 → 145 *m/z* quantification	0.01	90; 89; 107	95	11.0 (3)
		0.10	108; 107; 106	107	0.93 (3)
	189 → 145 *m/z* confirmation	0.01	79; 76; 84	80	5.1 (3)
		0.10	81; 87; 86	85	3.8 (3)
Beer	189 → 145 *m/z* quantification	0.01	86; 117; 108	104	15.0 (3)

Sample material	Mass transition*	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
		0.10	106; 93; 102	100	6.6 (3)
	189 → 145 *m/z* confirmation	0.01 0.10	103; 110; 93 106; 91; 95	102 97	8.4 (3) 8.0 (3)
Whole plant	189 → 145 *m/z* quantification	0.01 0.10	79 93	- -	- -
Flocs	189 → 145 *m/z* confirmation	0.01 0.10	107 106	- -	- -

*Due to the fact that for 2,4-dichlorobenzoic acid only one mass transition is available, an additional method confirmation was necessary for the determination of this analyte in all representative crops. The confirmation transition was determined by repeating in another sequence with a different column.

Table 7-99 Procedural recovery data for 5'-hydroxy-bixlozone in barley commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	290 → 175 *m/z* quantification	0.01 0.10	79; 79; 75 71; 67; 72	78 70	3.0 (3) 3.8 (3)
	290 → 111 *m/z* confirmation	0.01 0.10	74; 81; 79 71; 66; 72	78 70	4.6 (3) 4.6 (3)
Steeping water	290 → 175 *m/z* quantification	0.01 0.10	105; 100; 101 98; 104; 100	102 101	2.6 (3) 3.0 (3)
	290 → 111 *m/z* confirmation	0.01 0.10	89; 93; 97 98; 103; 97	93 99	2.6 (3) 3.0 (3)
Malt	290 → 175 *m/z* quantification	0.01 0.10	91; 97; 82 74; 84; 82	90 80	8.4 (3) 6.6 (3)
	290 → 111 *m/z* confirmation	0.01 0.10	97; 80; 88 71; 78; 81	88 77	10.0 (3) 6.7 (3)
Brewer's yeast	290 → 175 *m/z* quantification	0.01 0.10	93; 99; 94 94; 97; 106	95 99	3.4 (3) 6.3 (3)
	290 → 111 *m/z* confirmation	0.01 0.10	82; 92; 101 94; 97; 107	92 100	10.0 (3) 6.5 (3)
Beer	290 → 175 *m/z* quantification	0.01 0.10	106; 93; 93 100; 97; 100	97 99	7.7 (3) 1.7 (3)
	290 → 111 *m/z* confirmation	0.01 0.10	105; 95; 97 101; 98; 103	99 101	5.3 (3) 2.5 (3)
Whole plant	290 → 175 *m/z* quantification	0.01 0.10	103 83	- -	- -
Flocs	290 → 111 *m/z* confirmation	0.01	97	-	-

		0.10	106	-	-

Table 7-100 Procedural recovery data for 2,2-dimethyl-3-hydroxy propionic acid in barley commodities (method number CAM-0180)

Sample material	Mass transition*	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	117 → 87 *m/z* quantification	0.2	85; 92; 93	90	4.8 (3)
		2.0	85; 85; 93	88	5.3 (3)
	117 → 87 *m/z* confirmation	0.2	80; 74; 71	75	6.1 (3)
		2.0	78; 72; 77	76	4.2 (3)
Steeping water	117 → 87 *m/z* quantification	0.2	110; 106; 101	106	4.3 (3)
		2.0	106; 104; 101	104	2.4 (3)
	117 → 87 *m/z* confirmation	0.2	95; 97; 100	97	2.6 (3)
		2.0	98; 97; 87	94	6.5 (3)
Malt	117 → 87 *m/z* quantification	0.2	112; 101; 97	103	7.9 (3)
		2.0	99; 113; 105	106	5.2 (3)
	117 → 87 *m/z* confirmation	0.2	96; 74; 87	86	13.0 (3)
		2.0	91; 108; 97	99	8.7 (3)
Brewer's yeast	117 → 87 *m/z* quantification	0.2	93; 95; 93	94	1.2 (3)
		2.0	94; 97; 96	96	1.6 (3)
	117 → 87 *m/z* confirmation	0.2	90; 85; 86	87	3.0 (3)
		2.0	90; 88; 86	88	2.3 (3)
Beer	117 → 87 *m/z* quantification	0.2	103; 100; 99	101	2.1 (3)
		2.0	103; 107; 89	100	9.5 (3)
	117 → 87 *m/z* confirmation	0.2	94; 98; 95	96	2.2 (3)
		2.0	96; 105; 92	98	6.8 (3)
Whole plant	117 → 87 *m/z* quantification	0.2	110	-	-
		2.0	107	-	-
Flocs	117 → 87 *m/z* quantification	0.2	95	-	-
		2.0	98	-	-

* Due to the fact that for 2,2-dimethyl-3-hydroxy propionic acid only one mass transition is available, an additional method confirmation was necessary for the determination of this analyte in all representative crops. The confirmation transition was determined by repeating in another sequence with a different column.

Results and discussion

The effect of processing on residues of bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid from barley grain (sampled at BBCH 89) to processed fractions was determined in one trial. The samples from trial S16-05488-01 were not taken forward for processing. The hydrolysis study on bixlozone indicated that the metabolites 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid were not formed from parent bixlozone on processing (see Section B.7.5.1); however, as they could be present in the RAC, a consideration of their levels in processed commodities was conducted within the scope of this processing study. In accordance with OECD 508, the application conditions used in the trails should aim to allow for quantifiable residues to allow the study of processing and impact of processing on residues to be observed. This is why the

applicant designed the trial (plot 3) with a shortened harvest interval to increase the chances of observing positive residues. Although an acceptable strategy, the residues in this study (and across processed fractions) were sometimes low and this was not ideal for the setting of robust processing factors. Where positive residues were not observed (especially in RAC prior to processing) it was not possible to derive a reliable processing factor. Therefore, from this processing study, only limited processing factors were obtained, for a discussion on processing factors for the proposed residue definition, please refer to section 2.7.6 of the Volume 1.

Bixlozone

Residues of bixlozone in barley grain (RAC) were <0.01 mg/kg. Residues of bixlozone were 0.027 mg/kg in malt sprouts and <0.01 mg/kg in malt, spent grain, flocs, brewer's yeast, beer and pearl barley.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for bixlozone are shown in Table 7-101.

2,4-dichlorobenzoic acid

Residues of 2,4-dichlorobenzoic acid in barley grain (RAC) were 0.033 mg/kg. Residues of 2,4-dichlorobenzoic acid were <0.01mg/kg in flocs, brewer's yeast and beer, 0.045 mg/kg in malt sprouts, 0.031 mg/kg in malt, 0.016 mg/kg in spent grain and 0.019 mg/kg in pearly barley.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 2,4-dichlorobenzoic acid are shown in Table 7-102.

5'-hydroxy-bixlozone

Residues of 5'-hydroxy-bixlozone in barley grain (RAC) were 0.047 mg/kg. Residues of 5'-hydroxy-bixlozone were 0.018 mg/kg in malt sprouts, 0.140 mg/kg in malt, 0.023 mg/kg in spent grain, 0.030 mg/kg in pearl barley and <0.01 mg/kg in flocs, brewer's grain and beer.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 5'-hydroxy-bixlozone are shown in Table 7-103.

2,2-dimethyl-3-hydroxy propionic acid

Residues of 2,2-dimethyl-3-hydroxy propionic acid in barley grain (RAC) were <0.2 mg/kg. Residues of 2,2-dimethyl-3-hydroxy propionic acid were <0.2mg/kg in spent grain, flocs, brewer's yeast, beer and pearl barley, 0.42 mg/kg in malt sprouts and 0.23 mg/kg in malt.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 2,2-dimethyl-3-hydroxy propionic acid are shown in Table 7-104.

Table 7-101 Bixlozone results from processing trials on barley

Product	**Trial L16-05488-02-008A**
	Bixlozone residue (mg/kg)
Grain (RAC)	<0.01
Malt sprouts	0.027
Malt	<0.01

Product	Trial L16-05488-02-008A
	Bixlozone residue (mg/kg)
Spent grain	<0.01
Flocs	<0.01
Brewer's yeast	<0.01
Beer	<0.01
Pearl barley	<0.01

Table 7-102 2,4-dichlorobenzoic acid results from processing trials on barley

Product	Trial L16-05488-02-008A
	2,4-dichlorobenzoic acid residue (mg/kg)
Grain (RAC)	0.033
Malt sprouts	0.045
Malt	0.031
Spent grain	0.016
Flocs	<0.01
Brewer's yeast	<0.01
Beer	<0.01
Pearl barley	0.019

Table 7-103 5'-hydroxy-bixlozone results from processing trials on barley

Product	Trial L16-05488-02-008A
	5'-hydroxy-bixlozone residue (mg/kg)
Grain (RAC)	0.047
Malt sprouts	0.018
Malt	0.140
Spent grain	0.023
Flocs	<0.01
Brewer's yeast	<0.01
Beer	<0.01
Pearl barley	0.030

Table 7-104 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on barley

Product	Trial L16-05488-02-008A
	2,2-dimethyl-3-hydroxy propionic acid residue (mg/kg)
Grain (RAC)	<0.20
Malt sprouts	0.42 [UTC: 0.37]

Product	Trial L16-05488-02-008A
	2,2-dimethyl-3-hydroxy propionic acid residue (mg/kg)
Malt	0.23 [UTC: 0.23]
Spent grain	<0.20
Flocs	<0.20
Brewer's yeast	<0.20
Beer	<0.20
Pearl barley	<0.20

Conclusions

The submitted processing study attempted to assess the impact of processing on the magnitude of residue levels of bixlozone, and other analytes (2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid) over processing of barley and relevant fractions. For all analytes tested, only one trial was taken forward for processing and number of <LOQ results observed, including the unprocessed grain (RAC) for parent bixlozone.

HSE Remarks

The applicant aimed to increase chances of getting positive residues by carrying out the application with a shortened PHI (28 days). However, there were a number of <LOQ results observed throughout the trial, including the unprocessed grain (RAC) for parent bixlozone.

The applicant provided acceptable method validation work for the analytes in some processed fractions including steeping water, malt, brewer's yeast and beer.

The submitted trials used field trials to generate incurred residues for the processing study, which was then assessed by following a scheme of practice simulating industrial practice at a laboratory scale. Full details of each of the simulated processes were provided.

Only one processing trial has been conducted. The derivation of processing factors is complicated in view of the occurrence of some results below the LOQ (which was seen in both RAC and processed fraction results). For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

Samples (of RAC and processed fractions) were stored in the freezer for up to around 330 days prior to extraction and analysis. Although a range of freezer storage stability data are available for these analytes, specific freezer storage stability data have not been provided on these analytes for grain. This is discussed further in Vol 1, section 2.7.1.

B.7.5.3.3. *Oilseed rape*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-03, Semrau, J., 2018
Title:	Determination of residue of F9600 after one pre-emergence application of F9600-4 SC in oilseed rape and in processed fractions of oilseed rape at 4 sites in Northern and Southern Europe 2016
Report No.:	S16-05489
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

Materials and methods

A processing study on oilseed rape (varieties: *DK Imperial CL*, *Excalibur, Hydromel* winter oilseed rape), was conducted outdoors at a location in Germany (S16-05489-01 - N EU zone), a location in Italy (S16-05489-02 - S EU zone) and two locations in Spain (S16-05489-03 and -04 - S EU zone) during the 2016/2017 growing season. Only two trials (Germany - S16-05489-01 - N EU zone and Italy - S16-05489-02 - S EU zone) were taken forward for processing. Normal agricultural procedures were followed, and no unusual weather events were recorded. In trials S16-05489-01 and -02 there were two treated plots (plot 2 and 3) and one untreated control plot (plot 1). Treated processing samples were taken from plot 3 only. A single foliar application was made of F9600-4 SC (an SC formulation containing 396 g/L bixlozone) to treated plot 2 at BBCH 00-01 at a target rate of 0.631 L product/ha (equivalent to 250 g a.s./ha). In treated plot 3, a single application of F9600-4 SC was made, at 45-47 days before harvest at a nominal rate of 0.631 L/ha (equivalent to 250 g a.s./ha). All applications were made using a spray volume of 300-400 L/ha and a boom sprayer. In trial S16-05489-01 sample for residue analysis of the crop were taken from the control plot 1 and treated plot 2 at BBCH 10, 33-35, 51, 63, 80 and 89 (normal commercial harvest). Samples for processing were taken from the untreated control and from plot 3 at BBCH 89 (normal commercial harvest). In trial S16-05489-02 samples for residue analysis of the crop were taken from control plot 1 and treated plot 2 at BBCH 35, 65 and 87. Crop samples for processing were taken from control plot 1 and treated plot 3 at BBCH 89 (normal commercial harvest). No samples for processing were taken from trials S16-05489-03 and -04 and these were not considered further.

The application to plot 2 was made broadly in accordance with the proposed GAP for use on oilseed rape (which is a single application at 300 g a.s./ha on oilseed rape). Therefore, the results from this trial can be considered supportive of the use on primary crops. These results can be considered supportive information when considering the proposed use on oilseed rape as a primary crop. A summary of these results is presented in section 7.3.2.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in the previous three seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Residues of 2,2-dimethyl-3-hydroxy propionic acid were found in control samples at levels above 30% the LOQ of 0.05 mg/kg. No residues were found in the control samples above the LOQ. Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.6 of Volume 1. Treated and untreated plots were situated ≥ 10 m apart.

Samples were stored under frozen (< -18°C) conditions prior to extraction. All laboratory samples of the RAC and processed commodities were stored deep-frozen at ≤-18°C until analysis. Samples of the RAC were stored frozen for up to 469 days. Samples of the processed fractions were stored for up to 280 days. Freezer storage stability data are available indicating stability of these residues in oilseed rape seed over frozen storage for a period of 24 months (see Vol 1 (section 2.7.1)). Samples of the extracts were stored (between 1 °C and 10 °C) for up to 3 days between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

The processing of fresh oilseed rape seed samples started with a cleaning step and impurities (debris) were removed. The sample 'cleaned seeds' was taken. These seeds were then processed, simulating industrial practice at a laboratory scale, crude oil, refined oil and press cake (used for animal feed). The processing was carried out in the following stages:

Crude oil:

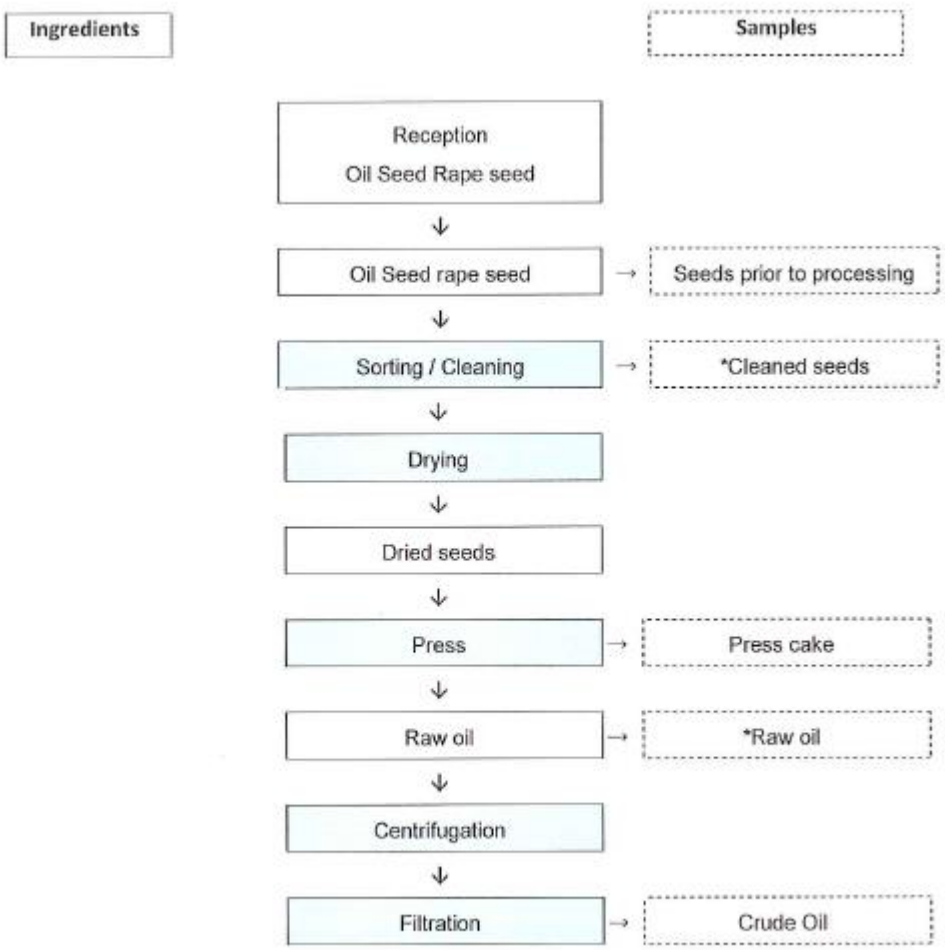
Cleaned seeds were dried in a drying oven at approx. 60 °C for 16 hours until the moisture content was between 0.9 and 2.1%. The dried seeds were pressed mechanically though an 'oil extrusion press' and the samples 'press cake' and 'raw oil' were taken. The raw oil was centrifuged for 3 minutes, filtered and the sample 'crude oil' was taken.

Refined oil:

Citric acid solution (0.4 mL at 625 g/L) was added per 100 g of crude oil. The mixture was heated to 95 °C for 35 minutes with continuous stirring. A soda solution (NaOH at 118 g/L) was added to the oil (1.8 mL per 100 g of crude oil) and the mixture was stirred for a further 50 minutes at 90 °C. The mixture was cooled, centrifuged, soap wasters were removed and then the mixture was cleaned with 10% w/w demineralised water at 95 °C for 20-30 minutes in an oven. The water was then filtered with a settle funnel twice. The oil was ten stored in cool conditions (7 °C) for 12 hours minimum. The sample 'aqueous waste' was taken. The oil was centrifuged until clear and then placed in an oven at 200 °C for 75 minutes. The sample 'refined oil' was taken.

Details of how each processed fraction was produced are given in Figure 7-22 - Figure 7-23. The mass of each processed fraction is given in Table 7-105.

Figure 7-22 Division and cleaning of oilseed rape seeds and processing to crude oil



*only for mass balance

Figure 7-23 Processing of crude oil into refined oil

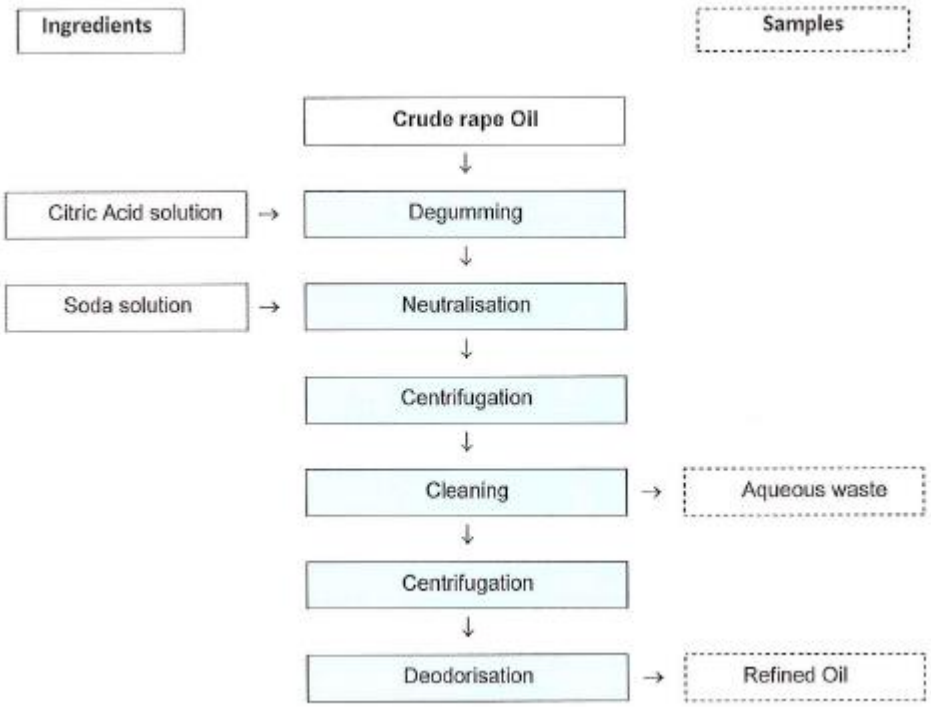


Table 7-105 Weight of processed fractions for each trial

Processed fraction	**Sample weight (kg)**			
	L16-05489-01-014A (untreated control)	**L16-05489-02-006A (untreated control)**	**L16-05489-01-014A (treated)**	**L16-05489-02-006A (treated)**
Seeds (RAC)	23.9	16.1	22.4	14.9
Cleaned seeds	21.5	13.6	20.1	12.5
Press cake	13.3	8.2	12.5	7.5
Raw oil	5.83	3.55	4.87	3.02
Crude oil	4.44	3.22	3.63	2.60
Refined oil	3.22	2.22	2.63	1.73

The level of residues in the RAC and each processed fraction was determined for bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid (method number CAM-0180/001 and CAM-0180-version 2). The LOQ for bixlozone, 2,4-dichlorobenzoic acid and 5'-hydroxy-bixlozone is 0.01 mg/kg. In some processing trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, matrix interference and residues in the control samples for 2,2-dimethyl-3-hydroxy-propionic acid supported the higher LOQ of 0.05 mg/kg.

The method is fully validated in oilseed rape seeds as well as a range of other commodity groups (including high acid, high oil and high water). Full details of the sample preparation and validation data for these methods are given in DAR CA B5 Section B.5.1.2.5. Validation in the processed fractions was carried out within the processing study reports by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the recoveries. Control material was taken from the control plots in the trials. Three (3) fortifications of untreated control samples at the level of LOQ (0.01 mg/kg) and three (3) fortifications at the level of tenfold LOQ mg/kg) were performed, representing a reduced validation data set. The recovery data for the processed fractions (aside from press

cake) are considered adequately addressed even though a lower number of validations per fortification level were carried out as the results are acceptable (recovery range, mean recovery and %RSDs). Press cake (for animal feed) was only studied (in terms of method validations) in a more limited way, with only one recovery per fortification level. The limited procedural recovery data does not indicate a concern with the performance of the analytical method in the press cake. Details of the procedural recoveries are given in Table 7-106 - Table 7-109. The mean recoveries are all within the acceptable range (70-110%).

Table 7-106 Procedural recovery data for bixlozone in oilseed rape commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	274 → 123 *m/z* quantification	0.01	107; 102; 109	106	3.4 (3)
		0.10	97; 117; 113	109	9.7 (3)
	274 → 125 *m/z* confirmation	0.01	106; 103; 120	110	8.3 (3)
		0.10	101; 112; 116	110	7.1 (3)
Flowers	274 → 123 *m/z* quantification	0.01	108; 107; 98	104	5.3 (3)
		0.10	90; 91; 89	90	1.1 (3)
	274 → 125 *m/z* confirmation	0.01	89; 85; 88	87	2.4 (3)
		0.10	96; 90; 96	94	3.7 (3)
Plant without pods	274 → 159 *m/z* quantification	0.01	112; 111; 101	108	5.6 (3)
		0.10	94; 86; 91	90	4.5 (3)
	274 → 123 *m/z* confirmation	0.01	103; 103; 101	102	1.1 (3)
		0.10	96; 87; 89	91	5.2 (3)
Pods	274 → 123 *m/z* quantification	0.01	95; 92; 107	98	8.1 (3)
		0.10	101; 102; 114	106	6.8 (3)
	274 → 125 *m/z* confirmation	0.01	98; 88; 89	92	6.0 (3)
		0.10	101; 99; 97	99	2.0 (3)
Seeds	274 → 159 *m/z* quantification	0.01	100; 82; 81	88	12.0 (3)
		0.10	73; 77; 73	74	3.1 (3)
	274 → 123 *m/z* confirmation	0.01	86; 83; 77	82	5.6 (3)
		0.10	77; 78; 79	78	1.3 (3)
Oil	274 → 159 *m/z* quantification	0.01	79; 75; 78	77	2.7 (3)
		0.10	77; 76; 80	78	2.7 (3)
	274 → 123 *m/z* confirmation	0.01	76; 81; 74	77	4.7 (3)
		0.10	77; 77; 81	78	2.9 (3)
Aqueous waste	274 → 159 *m/z* quantification	0.01	88; 88; 89	88	0.7 (3)
		0.10	93; 91; 92	92	1.1 (3)
	274 → 123 *m/z* confirmation	0.01	93; 85; 94	91	5.4 (3)
		0.10	93; 94; 98	95	2.8 (3)
Press cake	274 → 159 *m/z* quantification	0.01	92	-	-
		0.10	83	-	-

Table 7-107 Procedural recovery data for 2,4-dichlorobenzoic acid in oilseed rape commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	189 → 145 *m/z* quantification	0.01	86; 102; 105	98	10.0 (3)
		0.10	104; 97; 111	104	6.7 (3)
	189 → 147 *m/z* confirmation	0.01	97; 92; 92	94	3.1 (3)
		0.10	98; 95; 103	99	4.1 (3)
Flowers	189 → 145 *m/z* quantification	0.01	88; 100; 92	93	6.5 (3)
		0.10	95; 95; 91	94	2.5 (3)
	189 → 147 *m/z* confirmation	0.01	107; 79; 81	89	18.0 (3)
		0.10	95; 99; 103	99	4.0 (3)
Plant without pods	189 → 145 *m/z* quantification	0.01	92; 92; 101	95	5.5 (3)
		0.10	110; 103; 105	106	3.4 (3)
	189 → 147 *m/z* confirmation	0.01	107; 96; 94	99	7.1 (3)
		0.10	110; 106; 95	104	7.5 (3)
Pods	189 → 145 *m/z* quantification	0.01	82; 99; 102	94	11.0 (3)
		0.10	107; 107; 107	107	0.0 (3)
	189 → 147 *m/z* confirmation	0.01	96; 86; 110	97	12.4 (3)
		0.10	112; 110; 103	108	4.4 (3)
Seeds	189 → 145 *m/z* quantification	0.01	105; 109; 95	103	7.0 (3)
		0.10	96; 93; 94	94	1.6 (3)
	189 → 147 *m/z* confirmation	0.01	101; 108; 111	107	4.8 (3)
		0.10	101; 100; 94	98	3.9 (3)
Oil	189 → 145 *m/z* quantification	0.01	96; 81; 114	97	17.0 (3)
		0.10	107; 109; 95	104	7.3 (3)
	189 → 145 *m/z* confirmation	0.01	109; 92; 95	99	9.2 (3)
		0.10	105; 109; 96	103	6.4 (3)
Aqueous waste	189 → 145 *m/z* quantification	0.01	95; 91; 92	93	2.2 (3)
		0.10	91; 102; 99	97	5.8 (3)
	189 → 147 *m/z* confirmation	0.01	100; 87; 89	92	7.6 (3)
		0.10	88; 107; 96	97	9.8 (3)
Press cake	189 → 145 *m/z* quantification	0.01	84	-	-
		0.10	98	-	-

Table 7-108 Procedural recovery data for 5'-hydroxy-bixlozone in oilseed rape commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	290 → 175 *m/z* quantification	0.01	99; 116; 113	109	8.3 (3)
		0.10	98; 96; 95	96	1.6 (3)
	290 → 111 *m/z* confirmation	0.01	98; 101; 107	102	4.5 (3)
		0.10	86; 88; 95	90	5.3 (3)
Flowers	290 → 111 *m/z* quantification	0.01	102; 90; 95	96	6.3 (3)
		0.10	85; 86; 86	86	0.7 (3)
	290 → 111 *m/z* confirmation*	0.01	89; 102; 100	97	7.2 (3)
		0.10	92; 91; 91	91	0.63 (3)
Plant without pods	290 → 175 *m/z* quantification	0.01	84; 94; 98	92	7.8 (3)
		0.10	100; 91; 93	95	5.0 (3)
	290 → 111 *m/z* confirmation	0.01	71; 81; 97	83	16.0 (3)
		0.10	104; 88; 91	94	9.0 (3)
Pods	290 → 175 *m/z* quantification	0.01	111; 101; 116	109	7.0 (3)
		0.10	103; 98; 96	99	3.6 (3)
	290 → 111 *m/z* confirmation	0.01	93; 94; 106	98	7.4 (3)
		0.10	97; 87; 88	91	6.1 (3)
Seeds	290 → 175 *m/z* quantification	0.01	117; 100; 113	110	8.1 (3)
		0.10	115; 107; 104	109	5.2 (3)
	290 → 111 *m/z* confirmation	0.01	88; 73; 72	78	12.0 (3)
		0.10	85; 71; 70	75	11.0 (3)
Oil	290 → 175 *m/z* quantification	0.01	101; 104; 104	103	1.7 (3)
		0.10	115; 118; 96	110	11.0 (3)
	290 → 111 *m/z* confirmation	0.01	106; 108; 113	109	3.3 (3)
		0.10	102; 115; 94	104	10.0 (3)
Aqueous waste	290 → 175 *m/z* quantification	0.01	88; 93; 90	90	2.8 (3)
		0.10	96; 96; 93	95	1.8 (3)
	290 → 111 *m/z* confirmation	0.01	98; 98; 96	97	1.2 (3)
		0.10	96; 97; 95	96	1.0 (3)
Press cake	290 → 175 *m/z* quantification	0.01	78	-	-
		0.10	86	-	-

*Confirmation transition was determined by repeating in another sequence with a different column. In general, additional confirmatory analysis is not required when primary methods are shown to be specific to the analyte of interest. The confirmatory data have been presented for completeness only.

Table 7-109 Procedural recovery data for 2,2-dimethyl-3-hydroxy propionic acid in oilseed rape commodities (method number CAM-0180)

Sample material	Mass transition*	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Whole plant	117 → 87 *m/z* quantification	0.05	71; 78; 75	75	4.7 (3)
		0.50	76; 84; 73	78	7.3 (3)
	117 → 87 *m/z* confirmation	0.05	84; 81; 78	81	3.7 (3)
		0.50	91; 82; 90	88	5.6 (3)
Flowers	117 → 87 *m/z* quantification	0.05	98; 76; 99	91	14.0 (3)
		0.50	99; 97; 99	98	1.2 (3)
	117 → 87 *m/z* confirmation	0.05	107; 110; 98	105	5.9 (3)
		0.50	98; 107; 102	102	4.4 (3)
Plant without pods	117 → 87 *m/z* quantification	0.05	81; 87; 87	85	4.1 (3)
		0.50	104; 96; 98	99	4.2 (3)
	117 → 87 *m/z* confirmation	0.05	94; 96; 99	96	2.6 (3)
		0.50	96; 96; 96	96	0.0 (3)
Pods	117 → 87 *m/z* quantification	0.05	87; 91; 89	89	2.2 (3)
		0.50	105; 88; 102	98	9.2 (3)
	117 → 87 *m/z* confirmation	0.05	108; 81; 82	90	17.0 (3)
		0.50	96; 103; 104	101	4.3 (3)
Seeds	117 → 87 *m/z* quantification	0.05	98; 91; 88	92	5.6 (3)
		0.50	107; 86; 97	97	11.0 (3)
	117 → 87 *m/z* confirmation	0.05	106; 113; 110	110	3.2 (3)
		0.50	101; 85; 100	95	9.4 (3)
Oil	290 → 175 *m/z* quantification	0.05	87; 108; 107	101	12.0 (3)
		0.50	95; 115; 96	102	11.0 (3)
	290 → 111 *m/z* confirmation	0.05	87; 86; 107	93	13.0 (3)
		0.50	87; 103; 93	94	8.6 (3)
Aqueous waste	117 → 87 *m/z* quantification	0.05	109; 106; 107	107	1.4 (3)
		0.50	89; 100; 101	97	6.9 (3)
	117 → 87 *m/z* confirmation	0.05	105; 87; 97	96	9.4 (3)
		0.50	85; 88; 86	86	1.8 (3)
Press cake	117 → 87 *m/z* quantification	0.05	103	-	-
		0.50	88	-	-

*Confirmation transition was determined by repeating in another sequence with a different column. In general, additional confirmatory analysis is not required when primary methods are shown to be specific to the analyte of interest. The confirmatory data have been presented for completeness only.

Results and discussion

The effect of processing on residues of bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid from oilseed rape seeds (sampled at BBCH 89) to processed fractions was determined in two trials. The hydrolysis study on bixlozone indicated that the metabolites 2,4-dichlorobenzoic acid, 5'-hydroxy-

bixlozone and 2,2-dimethyl-3-hydroxy propionic acid were not formed from parent bixlozone on processing (see Section B.7.5.1); however, as they could be present in the RAC, a consideration of their levels in processed commodities was conducted within the scope of this processing study. In accordance with OECD 508, the application conditions used in the trails should aim to allow for quantifiable residues to allow the study of processing and impact of processing on residues to be observed. This is why the applicant designed the trial (plot 3) with a shortened harvest interval to increase the chances of observing positive residues. Although an acceptable strategy, the residues in this study (and across processed fractions) were sometimes low and this was not ideal for the setting of robust processing factors. Where positive residues were not observed (especially in RAC prior to processing) it was not possible to derive a reliable processing factor. Therefore, from this processing study, only limited processing factors were obtained, for a discussion on processing factors for the proposed residue definition, please refer to section 2.7.6 of the Volume 1.

Bixlozone

Residues of bixlozone in oilseed rape seed (RAC) were <0.01 and 0.027 mg/kg. Residues of bixlozone were <0.01 mg/kg in raw oil, <0.01 and 0.044 mg/kg in crude oil, <0.01 and 0.038 mg/kg in press cake and <0.01 and 0.039 mg/kg in refined oil.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for bixlozone are shown in Table 7-110.

2,4-dichlorobenzoic acid

Residues of 2,4-dichlorobenzoic acid in oilseed rape seed (RAC) were <0.01 mg/kg. Residues of 2,4-dichlorobenzoic acid were <0.01mg/kg in raw oil, crude oil and refined oil and <0.01 and 0.015 mg/kg in press cake.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.
The results for 2,4-dichlorobenzoic acid are shown in Table 7-111.

5'-hydroxy-bixlozone

Residues of 5'-hydroxy-bixlozone in oilseed rape seeds (RAC) were <0.01 mg/kg. Residues of 5'-hydroxy-bixlozone in all processed fractions were <0.01 mg/kg.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.
The results for 5'-hydroxy-bixlozone are shown in Table 7-112.

2,2-dimethyl-3-hydroxy propionic acid

Residues of 2,2-dimethyl-3-hydroxy propionic acid in oilseed rape seeds (RAC) were <0.05 mg/kg. Residues of 2,2-dimethyl-3-hydroxy propionic acid in all processed fractions were <0.05 mg/kg.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.
The results for 2,2-dimethyl-3-hydroxy propionic acid are shown in Table 7-113.

Table 7-110 bixlozone results from processing trials on oilseed rape

Product	**bixlozone residue (mg/kg)**	
	Trial S16-05489-01 NEU	**Trial S16-05489-02 SEU**
Seeds (RAC)	<0.01	0.027

Raw oil	<0.01	nd
Crude oil	<0.01	0.044
Press cake	<0.01	0.038
Refined Oil	<0.01	0.039

nd = not determined

Table 7-111 2,4-dichlorobenzoic acid results from processing trials on oilseed rape

Product	**2,4-dichlorobenzoic acid residue (mg/kg)**	
	Trial S16-05489-01 NEU	**Trial S16-05489-02 SEU**
Seeds (RAC)	<0.01	<0.01
Raw oil	<0.01	nd
Crude oil	<0.01	<0.01
Press cake	<0.01	0.015
Refined Oil	<0.01	<0.01

nd = not determined.

Table 7-112 5'-hydroxy-bixlozone results from processing trials on oilseed rape

Product	**5'-hydroxy-bixlozone residue (mg/kg)**	
	Trial S16-05489-01 NEU	**Trial S16-05489-02 SEU**
Seeds (RAC)	<0.01	<0.01
Raw oil	<0.01	nd
Crude oil	<0.01	<0.01
Press cake	<0.01	<0.01
Refined Oil	<0.01	<0.01

nd = not determined

Table 7-113 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on oilseed rape

Product	**2,2-dimethyl-3-hydroxy propionic acid residue (mg/kg)**	
	Trial S16-05489-01 NEU	**Trial S16-05489-02 SEU**
Seeds (RAC)	<0.05	<0.05
Raw oil	<0.05	nd
Crude oil	<0.05	<0.05
Press cake	<0.05	<0.05
Refined Oil	<0.05	<0.05

nd = not determined

Conclusions

The submitted processing studies attempted to assess the impact of processing on the magnitude of residue levels of bixlozone, and other analytes (2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic

acid) over processing of oilseed rape and relevant fractions. For all analytes tested, <LOQ residues were observed in the RAC in at least one of the two analysed trials, and there was a high frequency of <LOQ residues across the analytes (RAC and processed fractions)

HSE Remarks

The applicant aimed to increase chances of getting positive residues by carrying out the application with a shortened PHI (30±3 days). However, there was a high frequency of <LOQ results observed throughout the trials.

The applicant provided acceptable method validation work for the analytes in some processed fractions including flowers, plant without pods, pods, seeds, oil and aqueous waste. Press cake results (for animal feed) were not fully supported by the method validation work available (only as procedural recoveries) as only one individual recovery was carried out at each fortification level. These procedural recoveries were acceptable and do not indicate a concern with the performance of the analytical method in the press cake.

The submitted trials used field trials to generate incurred residues for the processing study, which was then assessed by following a scheme of practice simulating industrial practice at a laboratory scale. Full details of each of the simulated processes were provided.

Although two processing trials are available for oilseed rape, the derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results). For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

Samples (of RAC and processed fractions) were stored in the freezer for up to around 470 days prior to extraction and analysis. A range of freezer storage stability data are available for these analytes, including freezer storage stability data on oilseeds that show stability of the analytes for a period of up to 2 years. As none of the freezer storage stability data for these analytes (across range of commodities tested) show indications of instability, it is considered that the availably storage stability, including that for oilseed, are sufficient to support the frozen periods of storage in the oilseed fractions considered and stored here (up to 470 days, 1.3 years).

B.7.5.3.4. *Maize*

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.5.2-04, Semrau, J., 2018
Title:	Determination of residue of bixlozone after one application of F9600-4 SC in maize and in processed fractions of maize at 2 sites in Northern and Southern Europe 2016
Report No.:	S16-05486
Guidelines:	OECD Guidance Document on Overview of Residue Chemistry Studies, OECD Test Guideline 509: Crop Field Trials, OECD Guideline 508: Magnitude of the pesticide residues in processed commodities, EU Guidance document SANCO/3029/99 rev. 4 for generating and reporting methods of analysis in support of pre-registration data regulations.
GLP	yes

Materials and methods

A processing study on maize (varieties: *ES Ewrojet*, *Kamil* maize), was conducted outdoors at a location in Germany (S16-05486-01 - N EU zone) and a location in Italy (S16-05486-02 - S EU zone) during the 2016/2017 growing season. However, only the SEU trial (S16-05486-02) was taken forward for processing. Normal agricultural procedures were followed, and no unusual weather events were recorded. Trial S16-05486-02 comprised of three plots, one untreated control (plot 1) and two treated plots (plot 2 and 3). Treated processing samples were taken from plot 3 only. In treated plot 2, a single foliar application was made of F9600-4 SC (an SC formulation containing 396 g/L bixlozone) was made at BBCH 12, at a target rate of 0.758 L product/ha (equivalent to 307 g a.s./ha) at a spray volume of 400 L/ha. In treated plot 3, a single application of F9600-4 SC was made, at a rate of 0.758 L product/ha (equivalent to 307 g a.s./ha), at 30 days before harvest using a spray volume of 400 L/ha. Samples for residue analysis of the crop were taken from plot 2 and the untreated plot (plot 1) at growth stage BBCH 89 (normal commercial harvest). Samples for processing were taken from the untreated control and from plot 3 at BBCH 89 (normal commercial harvest). Samples from trial S16-05486-01 were not taken forward for processing and are not considered further in this section.

The application to plot 2 was made broadly in accordance with the proposed GAP for use on maize (which is a single application at 375 g a.s./ha on maize). Therefore, the results from this trial can be considered supportive of the use on primary crops. These results can be considered supportive information when considering the proposed use on maize as a primary crop. A summary of these results is presented in section 7.3.3.

The same active substance had not been applied to the trial sites in previous seasons or as maintenance products during the trials. Additionally, the similar active substance clomazone was also not applied either in the previous three seasons or during the trials.

Residues of bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were not found (<LOQ, <0.01 mg/kg) in any of the untreated control samples. Positive residues of 2,2-dimethyl-3-hydroxy-propionic acid were found in some of the untreated control samples for some limited processed fractions as listed in Table 7-122 (denoted as 'UTC' with the corresponding treated results). All other untreated samples of other matrices (whole plant, straw and grain) from the trials did not contain residues of 2,2-dimethyl-3-hydroxy-propionic acid above the LOQ (<0.2 mg/kg). It is difficult to fully understand or explain the reasoning for residues in the untreated controls. Further discussion on the occurrence of residues, especially 2,2-dimethyl-3-hydroxy propionic acid, in untreated control samples can be found in section 2.7.3 and 2.7.6 of Volume 1. In this processing trial, treated and untreated plots were situated ≥ 10 m apart.

Samples were stored under frozen (< -18°C) conditions prior to extraction. Samples of the RAC were stored frozen for up to 195 days. Samples of the processing fractions were stored frozen for up to 216 days. No storage stability data was available for the processed fractions of cereals. Please refer to discussion of the storage stability in Vol 1 (section 2.7.1). Samples of the extracts were stored (between 1 °C and 10 °C) for up to 5 days between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

The processing of fresh maize grain samples started with a cleaning step, impurities (debris) were removed, and the grain was sorted by size, and the grain with a size of >2.5mm was kept. The sample 'cleaned grains' was taken. This grain was then processed, simulating industrial practice at a laboratory scale, into bran, flour, starch, protein, meal and oil. The processing was carried out in the following stages:

Milling of maize grain into flour:

The grain was milled using a "Bühler Mahlautomat" mill type into eight fractions. Two of the fractions are bran fractions ('coarse bran' and 'fine bran') and the remaining six are the 'flour fractions'. 'Fine bran', 'coarse bran' and 'flour' were sampled. The fin bran was purified into 'fine bran purify', 'middling' and 'topping'. The samples 'fine bran purified and 'middlings' were taken.

Starch processing:

Water and flour (water: flour = 1:2.5) were added to a kneading machine and kneaded for 10 minutes. A 3% aqueous table salt solution was added in several steps (rough ratio of dough: salt solution = 0.17-0.19). The dough was kneaded throughout each step. After each step the starch/salt solution was drained. The processed finished when no more starch dissolved in the water/ salt solution (shown by the solution staying clear). The sample 'wet protein' was taken. The starch/ salt solution was separated by decantation and the sample 'wet starch' was taken.

The fractions of wet starch and wet protein were dried using a drying oven at 70 °C until the moisture content was <15%. The samples 'dried starch' and 'dried protein' were taken.

Maize oil:

First the grains were ground using a grinding malt. 2.0 kg of a table salt solution (300g salt per kg of tap water) were added per 1.0 kg of ground seeds of maize and mixed for 20 minutes and the germs were separated. The germs were dried in an oven at 60 °C for 17-21 hours until the moisture content was 1.4-2.4%. The conditioned maize seeds were pressed in an oil extrusion press and the samples 'meal (press cake)' and 'raw oil' were taken.

The raw oil was centrifuged until clear and the supernatant was recovered. The sample 'crude oil' was taken. 0.4 mL of a citric acid solution (625 g/L) was added per 100 g of crude oil and the mix was dried in an oven at 95 °C for 35 minutes. Following this 1.8 mL of a soda solution (118 g/L) was added per 100 g of degummed crude oil and was left in the oven at 95 °C for a further 50 minutes. The neutralised oil was cooled and centrifuged to remove the soap waste. The oil was cleaned with demineralised water in the oven at 95 °C for 20 minutes. The water was removed with a settle funnel and this was repeated twice. The sample 'aqueous waste' was taken. The oil was stored at 7 °C for 20-22 hours and then put into an oven at 200 °C for 75 minutes. The sample 'refined oil' was taken.

Details of how each processed fraction was produced are given in Figure 7-24 - Figure 7-27. The mass of each processed fraction is given in Table 7-114.

Figure 7-24 Cleaning of maize grains

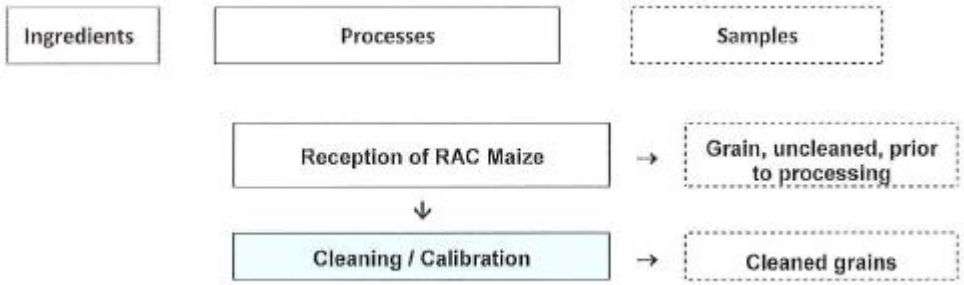


Figure 7-25 Processing of maize grains into flour

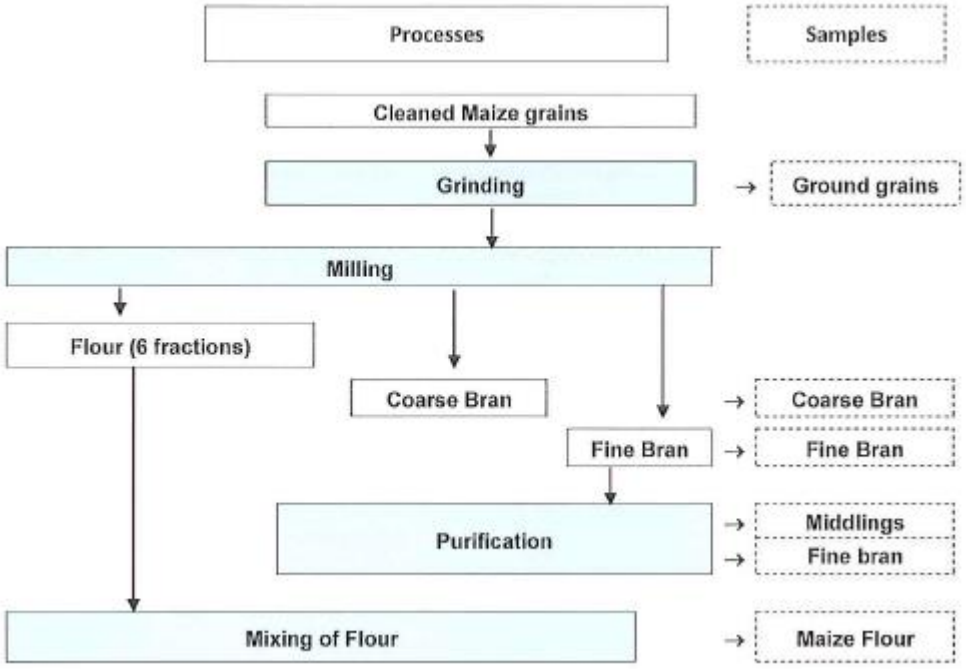


Figure 7-26 Processing of flour into starch and meal

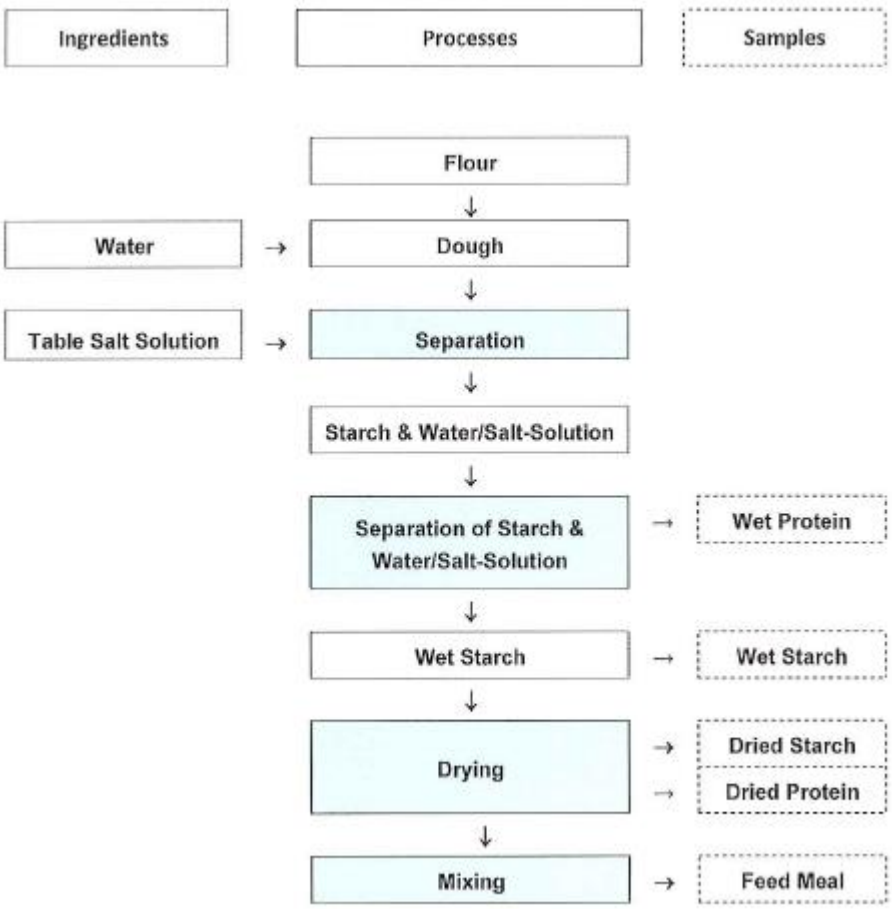


Figure 7-27 Processing of maize grains into refined oil

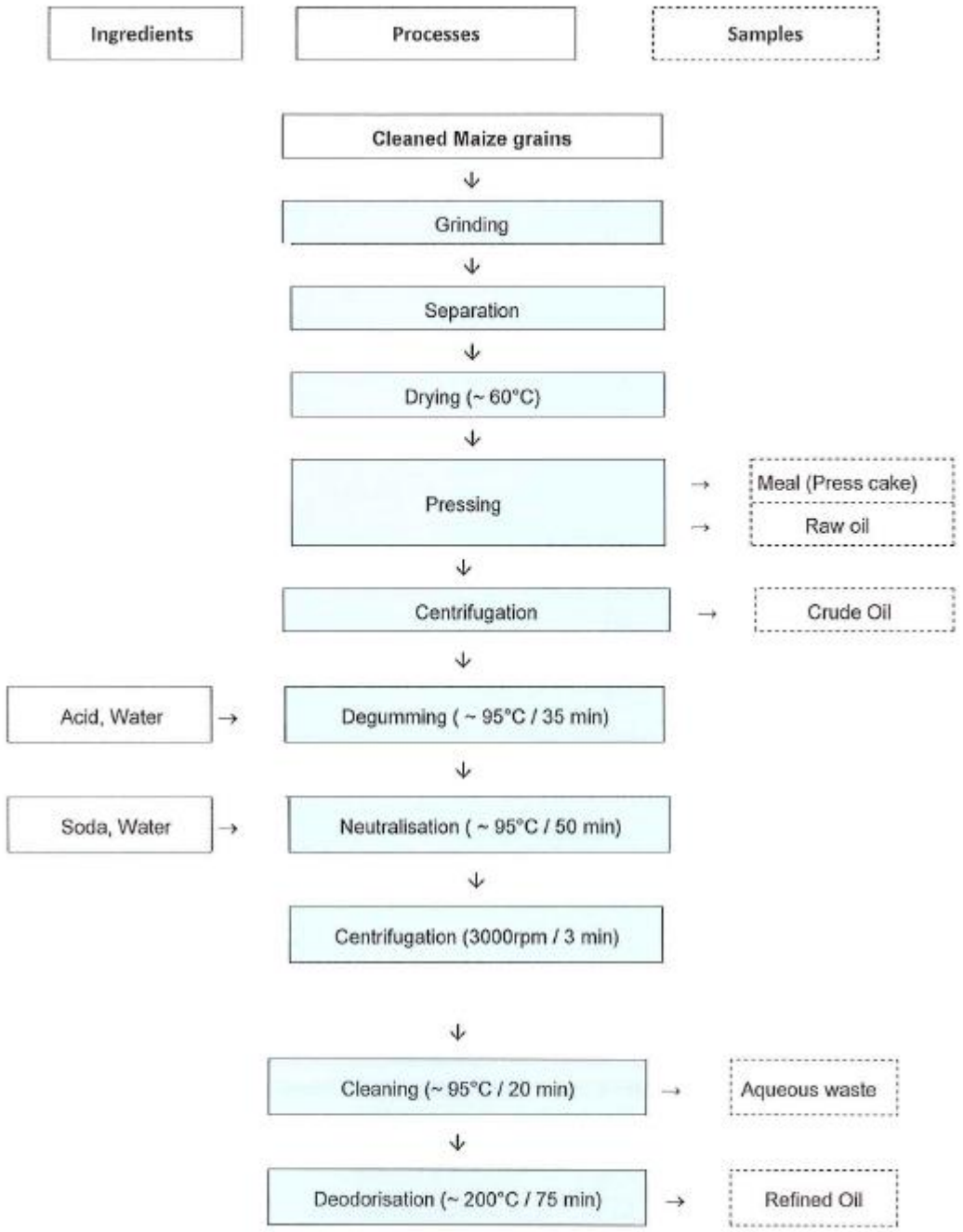


Table 7-114 Weight of processed fractions for each trial

Processed fraction	**Sample weight (kg)**	
	L16-05486-02-002A (untreated control)	**L16-05486-02-006A (treated)**
Grain	36.7	28.7
Cleaned grains	33.3	26.4
Flour	2.2	1.8
Coarse bran	2.8	1.3

Processed fraction	**Sample weight (kg)**	
	L16-05486-02-002A (untreated control)	**L16-05486-02-006A (treated)**
Fine bran	7.7	5.3
Middlings	3.6	2.4
Toppings	0.67	0.63
Purified fine bran	2.7	1.7
Wet protein	2.3	1.46
Wet starch	1.59	1.88
Dried protein	0.70	0.32
Dried starch	0.40	0.47
Feed meal	0.40	0.20
Raw oil	0.21	0.13
Crude oil	0.10	0.06
Refined oil	0.04	0.03

The level of residues in the RAC and each processed fraction was determined for bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid (method number CAM-0180/001 and CAM-0180-version 2). The LOQ for bixlozone, 2,4-dichlorobenzoic acid and 5'-hydroxy-bixlozone is 0.01 mg/kg. In some processing trials, for 2,2-dimethyl-3-hydroxy-propionic acid, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples (see Volume 1, section 2.7.4). In this case, an LOQ of 0.2 mg/kg was also supported by the validation data presented within the residue field trials study report (acceptable precision, accuracy, linearity and specificity data).

The method is fully validated in maize straw, grain and whole plant fractions as well as a range of other commodity groups (including high acid, high oil and high water). Full details of the sample preparation and validation data for these methods are given in DAR CA B5 Section B.5.1.2.5. Validation in the processed fractions was carried out within the processing study reports by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the recoveries. Control material was taken from the control plots in the trials. Three (3) fortifications of untreated control samples at the level of LOQ (0.01 mg/kg) and three (3) fortifications at the level of tenfold LOQ mg/kg) were performed, representing a reduced validation data set. The recovery data for the processed fractions studied in this way (flour, starch and oil) are considered adequately addressed even though a lower number of validations per fortification level were carried out as the results are acceptable (recovery range, mean recovery and %RSDs). Some matrices were only studied (in terms of method validations) in a more limited way, with only one recovery per fortification level: bran, middlings, protein and aqueous waste. The limited procedural recovery data does not indicate a concern with the performance of the analytical method in these matrices. Details of the procedural recoveries are given in Table 7-115 to Table 7-118. The mean recoveries were all within the acceptable range (70-110%), with the exception of the recovery of 2,4-dichlorobenzoic acid in starch at 111%, as this is just outside the range and recoveries at all other fortification levels are acceptable this is considered acceptable.

Table 7-115 Procedural recovery data for bixlozone in maize commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	274 → 159 *m/z* quantification	0.01	100; 98; 106	101	4.1 (3)
		0.10	105; 100; 114	106	6.7 (3)
	274 → 123 *m/z* confirmation	0.01	91; 102; 108	100	8.6 (3)
		0.10	107; 102; 118	109	7.5 (3)
Flour	274 → 123 *m/z* quantification	0.01	103; 100; 93	99	5.2 (3)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
		0.10	98; 99; 104	100	3.2 (3)
	274 → 125 *m/z* confirmation	0.01	113; 93; 95	100	11.0 (3)
		0.10	96; 98; 103	99	3.6 (3)
Starch	274 → 159 *m/z* quantification	0.01	95; 97; 104	99	4.8 (3)
		0.10	94; 92; 95	94	1.6 (3)
	274 → 123 *m/z* confirmation	0.01	92; 93; 97	94	2.8 (3)
		0.10	96; 90; 95	94	3.4 (3)
Oil	274 → 159 *m/z* quantification	0.01	60; 81; 83	75	17.0 (3)
		0.10	76; 82; 83	80	4.7 (3)
	274 → 123 *m/z* confirmation	0.01	61; 74; 86	74	17.0 (3)
		0.10	76; 85; 82	81	5.7 (3)
Bran	274 → 159 *m/z* quantification	0.01	109	-	-
		0.10	94	-	-
Middlings	274 → 159 *m/z* quantification	0.01	108	-	-
		0.10	99	-	-
Protein	274 → 159 *m/z* quantification	0.01	109	-	-
		0.10	96	-	-
Aqueous waste	274 → 159 *m/z* quantification	0.01	108	-	-
		0.10	93	-	-

Table 7-116 Procedural recovery data for 2,4-dichlorobenzoic acid in maize commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	189 → 145 *m/z* quantification	0.01	113; 97; 100	103	8.2 (3)
		0.10	110; 107; 107	108	1.6 (3)
	189 → 145 *m/z* confirmation*	0.01	96; 92; 98	95	3.2 (3)
		0.10	109; 106; 103	106	2.8 (3)
Flour	189 → 145 *m/z* quantification	0.01	94; 93; 106	98	7.4 (3)
		0.10	110; 103; 110	108	3.8 (3)
	189 → 147 *m/z* confirmation	0.01	94; 101; 96	97	3.7 (3)
		0.10	91; 87; 91	90	2.6 (3)
Starch	189 → 145 *m/z* quantification	0.01	99; 108; 115	107	8.5 (3)
		0.10	120; 106; 107	111	7.0 (3)
	189 → 147 *m/z* confirmation	0.01	99; 112; 100	104	7.0 (3)
		0.10	109; 111; 108	109	1.4 (3)
Oil	189 → 145 *m/z* quantification	0.01	95; 95; 107	99	7.0 (3)
		0.10	96; 98; 99	98	1.6 (3)
	189 → 147 *m/z*	0.01	85; 72; 93	83	13.0 (3)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
	confirmation	0.10	95; 102; 97	98	3.7 (3)
Bran	189 → 145 *m/z* quantification	0.01	105	-	-
		0.10	108	-	-
Middlings	189 → 145 *m/z* quantification	0.01	107	-	-
		0.10	110	-	-
Protein	189 → 145 *m/z* quantification	0.01	102	-	-
		0.10	110	-	-
Aqueous waste	189 → 145 *m/z* quantification	0.01	94	-	-
		0.10	102	-	-

*Confirmation transition was determined by repeating in another sequence with a different column. In general, additional confirmatory analysis is not required when primary methods are shown to be specific to the analyte of interest. The confirmatory data have been presented for completeness only.

Table 7-117 Procedural recovery data for 5'-hydroxy-bixlozone in maize commodities (method number CAM-0180)

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	290 → 175 *m/z* quantification	0.01	81; 90; 86	86	5.3 (3)
		0.10	79; 79; 76	78	2.2 (3)
	290 → 175 *m/z* confirmation*	0.01	78; 76; 80	78	2.6 (3)
		0.10	72; 74; 75	74	2.1 (3)
Flour	290 → 175 *m/z* quantification	0.01	81; 84; 73	79	7.2 (3)
		0.10	71; 73; 66	70	5.2 (3)
	290 → 111 *m/z* confirmation	0.01	85; 75; 92	84	10.0 (3)
		0.10	73; 73; 65	70	7.0 (3)
Starch	290 → 175 *m/z* quantification	0.01	100; 106; 113	106	6.1 (3)
		0.10	102; 84; 87	91	11.0 (3)
	290 → 111 *m/z* confirmation	0.01	84; 90; 100	91	8.8 (3)
		0.10	103; 85; 85	91	11.0 (3)
Oil	290 → 175 *m/z* quantification	0.01	81; 108; 115	101	18.0 (3)
		0.10	103; 110; 103	105	3.8 (3)
	290 → 111 *m/z* confirmation	0.01	75; 103; 109	96	19.0 (3)
		0.10	101; 110; 102	104	4.7 (3)
Bran	290 → 175 *m/z* quantification	0.01	105	-	-
		0.10	83	-	-
Middlings	290 → 175 *m/z* quantification	0.01	99	-	-
		0.10	92	-	-
Protein	290 → 175 *m/z* quantification	0.01	89	-	-
		0.10	79	-	-

Sample material	Mass transition	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Aqueous waste	290 → 175 *m/z* quantification	0.01 0.10	108 95	- -	- -

*Confirmation transition was determined by repeating in another sequence with a different column. In general, additional confirmatory analysis is not required when primary methods are shown to be specific to the analyte of interest. The confirmatory data have been presented for completeness only.

Table 7-118 Procedural recovery data for 2,2-dimethyl-3-hydroxy propionic acid in maize commodities (method number CAM-0180)

Sample material	Mass transition*	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
Grain	117 → 87 *m/z* quantification	0.2 2.0	76; 95; 79 98; 89; 91	83 93	12.0 (3) 5.1 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	84; 96; 90 113; 104; 88	90 102	6.7 (3) 12.0 (3)
Flour	117 → 87 *m/z* quantification	0.2 2.0	86; 83; 80 86; 83; 80	83 83	3.6 (3) 3.6 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	88; 88; 71 81; 75; 71	82 76	12.0 (3) 6.7 (3)
Starch	117 → 87 *m/z* quantification	0.2 2.0	86; 91; 94 96; 80; 89	90 88	4.5 (3) 9.1 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	82; 92; 89 98; 86; 92	88 92	5.9 (3) 6.5 (3)
Oil	117 → 87 *m/z* quantification	0.2 2.0	100; 100; 97 92; 107; 101	99 100	1.7 (3) 7.5 (3)
	117 → 87 *m/z* confirmation	0.2 2.0	85; 85; 84 83; 89; 87	85 86	0.7 (3) 3.5 (3)
Bran	117 → 87 *m/z* quantification	0.2 2.0	80 86	- -	- -
Middlings	117 → 87 *m/z* quantification	0.2 2.0	110 94	- -	- -
Protein	117 → 87 *m/z* quantification	0.2 2.0	79 91	- -	- -
Aqueous waste	117 → 87 *m/z* quantification	0.2 2.0	97 97	- -	- -

**Confirmation transition was determined by repeating in another sequence with a different column. In general, additional confirmatory analysis is not required when primary methods are shown to be specific to the analyte of interest. The confirmatory data have been presented for completeness only.*

Results and discussion

The effect of processing on residues of bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid from maize grain (sampled at BBCH 89) to processed fractions was determined in one trial. The hydrolysis study on bixlozone indicated that the metabolites 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and

2,2-dimethyl-3-hydroxy propionic acid were not formed from parent bixlozone on processing (see Section B.7.5.1); however, as they could be present in the RAC, a consideration of their levels in processed commodities was conducted within the scope of this processing study. In accordance with OECD 508, the application conditions used in the trails should aim to allow for quantifiable residues to allow the study of processing and impact of processing on residues to be observed. This is why the applicant designed the trial (plot 3) with a shortened harvest interval to increase the chances of observing positive residues. Although an acceptable strategy, the residues in this study (and across processed fractions) were sometimes low and this was not ideal for the setting of robust processing factors. Where positive residues were not observed (especially in RAC prior to processing) it was not possible to derive a reliable processing factor. Therefore, from this processing study, only limited processing factors were obtained, for a discussion on processing factors for the proposed residue definition, please refer to section 2.7.6 of the Volume 1.

Bixlozone

Residues of bixlozone in maize grain (RAC) were <0.01 mg/kg. Residues of bixlozone in all processed fractions were <0.01 mg/kg.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for bixlozone are shown in Table 7-119.

2,4-dichlorobenzoic acid

Residues of 2,4-dichlorobenzoic acid in maize grain (RAC) were <0.01 mg/kg. Residues of 2,4-dichlorobenzoic acid in all processed fractions were <0.01 mg/kg.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 2,4-dichlorobenzoic acid are shown in Table 7-120.

5'-hydroxy-bixlozone

Residues of 5'-hydroxy-bixlozone in maize grain (RAC) were <0.01 mg/kg. Residues of 5'-hydroxy-bixlozone in all processed fractions were <0.01 mg/kg.

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 5'-hydroxy-bixlozone are shown in Table 7-121.

2,2-dimethyl-3-hydroxy propionic acid

Residues of 2,2-dimethyl-3-hydroxy propionic acid in maize grain (RAC) were 0.27 mg/kg. Residues of 2,2-dimethyl-3-hydroxy propionic acid were <0.2mg/kg in flour, wet starch, wet protein, dried starch, dried protein, feed meal, raw oil, crude oil and refined oil, 0.63 mg/kg in coarse bran, 0.26 mg/kg in fine bran and fine bran purified, 0.28 mg/kg in middlings and 0.44 mg/kg in meal (presscake).

The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results) For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

The results for 2,2-dimethyl-3-hydroxy propionic acid are shown in Table 7-122.

Table 7-119 Bixlozone results from processing trials on maize

Product	Trial L16-05486-02-006A
	Bixlozone residue (mg/kg)
Grain (RAC)	<0.01
Coarse bran	<0.01
Fine bran	<0.01
Fine bran purified	<0.01
Middlings	<0.01
Flour	<0.01
Wet starch	<0.01
Wet protein	<0.01
Dried starch	<0.01
Dried protein	<0.01
Feed meal	<0.01
Raw oil	<0.01
Meal (presscake)	<0.01
Crude oil	<0.01
Refined oil	<0.01

Table 7-120 2,4-dichlorobenzoic acid results from processing trials on maize

Product	Trial L16-05486-02-006A
	2,4-dichlorobenzoic acid residue (mg/kg)
Grain (RAC)	<0.01
Coarse bran	<0.01
Fine bran	<0.01
Fine bran purified	<0.01
Middlings	<0.01
Flour	<0.01
Wet starch	<0.01
Wet protein	<0.01
Dried starch	<0.01
Dried protein	<0.01
Feed meal	<0.01
Raw oil	<0.01
Meal (presscake)	<0.01
Crude oil	<0.01
Refined oil	<0.01

Table 7-121 5'-hydroxy-bixlozone results from processing trials on maize

Product	Trial L16-05486-02-006A
	5'-hydroxy-bixlozone residue (mg/kg)
Grain (RAC)	<0.01
Coarse bran	<0.01
Fine bran	<0.01
Fine bran purified	<0.01
Middlings	<0.01
Flour	<0.01
Wet starch	<0.01
Wet protein	<0.01
Dried starch	<0.01
Dried protein	<0.01
Feed meal	<0.01
Raw oil	<0.01
Meal (presscake)	<0.01
Crude oil	<0.01
Refined oil	<0.01

Table 7-122 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on maize

Product	Trial L16-05486-02-006A
	2,2-dimethyl-3-hydroxy propionic acid residue (mg/kg)
Grain (RAC)	0.27
Coarse bran	0.63 [UTC: 0.76]
Fine bran	0.26
Fine bran purified	0.26 [UTC: 0.24]
Middlings	0.28
Flour	<0.20
Wet starch	<0.20
Wet protein	<0.20
Dried starch	<0.20
Dried protein	<0.20
Feed meal	<0.20
Raw oil	<0.20
Meal (presscake)	0.44 [UTC: 0.82]
Crude oil	<0.20
Refined oil	<0.20

Conclusions

The submitted processing study attempted to assess the impact of processing on the magnitude of residue levels of bixlozone, and other analytes (2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid) over processing of maize and relevant fractions. For all analytes tested, only one trial was taken forward for processing and a high frequency of <LOQ residues were observed. From the limited data available a concentration of residues was observed for 2,2-dimethyl-3-hydroxy propionic acid in coarse bran, middlings and meal (presscake).

HSE Remarks

The applicant aimed to increase chances of getting positive residues by carrying out the application with a shortened PHI (28 days). However, there was a high frequency of <LOQ results observed throughout the trial.

The applicant provided acceptable method validation work for the analytes in some processed fractions including flour, starch and oil.

The submitted trials used field trials to generate incurred residues for the processing study, which was then assessed by following a scheme of practice simulating industrial practice at a laboratory scale. Full details of each of the simulated processes were provided.

Only one processing trial has been conducted. The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results). For a full discussion of the setting of processing factors for the currently intended uses please refer to section 2.7.6 of the Volume 1.

Samples (of RAC and processed fractions) were stored in the freezer for up to around 200 days prior to extraction and analysis. Although a range of freezer storage stability data are available for these analytes, specific freezer storage stability data have not been provided on these analytes for grain. This is discussed further in Vol 1, section 2.7.1.

B.7.6. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS

B.7.6.1. Metabolism in rotational crops

As the representative uses being considered are crops which may be grown in rotation, a consideration of the residues in rotational crops is necessary.

All mg/kg expression of residues in all the write up of the rotational crop metabolism study are as mg parent equivalents/kg (mg parent eq./kg).

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.6.1-01, Desai, M., 2019
Title:	Uptake and metabolism of [^{14}C]F9600 in confined rotational crops
Report No.:	14019-RPT04253 (Report amendment date: December 23, 2019)
Guidelines:	OECD Guideline for the Testing of Chemicals, 502 Metabolism in Rotational Crops, January 2007. U.S EPA OPPTS Guideline 860.1300 U.S. Nature of the Residue – Plants, Livestock (August 1996). OPPTS 860.1850 Confined Accumulation in Rotational Crops
GLP	yes

Materials and methods

Materials

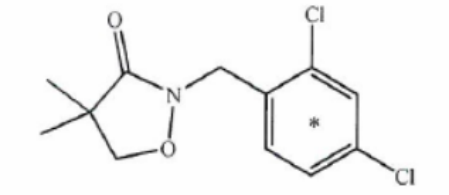
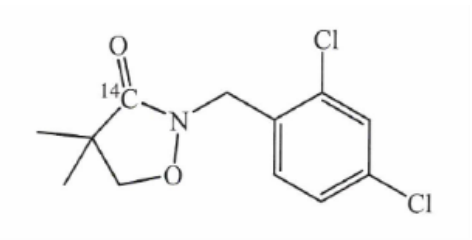
1. Test Material

Test Material:	[Phenyl -U-^{14}C]bixlozone
Lot/batch No.:	CFQ42017
Radiochemical Purity:	99.6% (nominal) 99.6% (actual)
Specific Activity:	(bulk) 56 mCi/mmol; 7.50 MBq/mg (diluted, nominal) 20 mCi/mmol; 2.68 MBq/mg (diluted, certified): 21.57 mCi/mmol; 2.89 MBq/mg

Test Material

Test Material:	[Carbonyl -C5-^{14}C]bixlozone
Lot/batch No.:	CFQ42018
Radiochemical Purity:	99.9% (nominal) 99.5% (actual)
Specific Activity:	(bulk) 59 mCi/mmol; 7.91 MBq/mg (diluted, nominal) 20 mCi/mmol; 2.68 MBq/mg (diluted, certified): 20.49 mCi/mmol; 2.75 MBq/mg
	(bulk) 59 mCi/mmol (diluted) 20 mCi/mmol (nominal); 20.5 mCi/mmol (certified)

Figure 7-28 Structures of radiolabelled bixlozone

 [Phenyl -U-^{14}C]bixlozone **(***denotes ^{14}C position) "Phenyl label"	 [Carbonyl -C5-^{14}C]bixlozone "Carbonyl label"

Methods

Test system

A 'confined' metabolism study on the rotational crops lettuce (variety Salad Bowl), radish (variety Crimson Giant) and wheat (variety Certified WB Patron) grown outdoors in Fresno, California, USA was carried out in 2014-2015. All selected crops were planted in fifteen wooden boxes (each 3 ft x 5 ft) with sandy loam soil (soil column depth ~50 cm, pH in 1:1 soil: water ratio = 7.0, Percentage sand = 67%). Each box was lined with heavy gauge plastic. No crops had been grown, or pesticides applied to the test system for 3 years before the trial. No radiolabelled material had ever been applied to the planting area prior to the trial. Weather data were reported for the trials and no exceptional events were noted.

Applications

One spray application of test substance in EC formulation 'blank' was applied to bare soil in the confined boxes. Six plots (boxes) were treated with phenyl-labelled bixlozone, three at a targeted rate of 100 g a.s./ha and three at a targeted rate of 300 g a.s./ha. A further six plots were treated with carbonyl-labelled bixlozone, three at a targeted rate of 100 g a.s./ha and three at a targeted rate of 300 g a.s./ha. A further three plots were used as controls, one for each crop. The control plots were located more than 60 m from the treated plots to limit the risk of contamination. Plastic sheeting approximately 7-foot was erected all around the plot to block the wind during each application. All plastic barriers were removed after each application.

Actual application rates ranged from 269-288 g a.s./ha for the high dose rate (representing a little underdosing, 0.9 to 0.96 (underdosing rate), as summarised in Table 7-123 (which also compared the application rates to the anticipated N rates). When taking account of the highest estimated soil exposures arising from the intended uses (i.e. taking account of soil plateau contribution following year to year use, as well as the maximum seasonal application rate) the actual applications were underdosed (around 0.9N for wheat and barley GAPs, around 0.6N for oilseed rape, and around 0.5N for the maize GAP).

The test substance was applied to the bare soil of 4 plots (one high rate and one low rate for each label) for each replanting interval time that was intended, at plant back intervals of (intended) 30 days (DAT, days after treatment), 120 DAT and 310 DAT before planting. Soil was ploughed approximately three inches prior to planting. Lettuce, radish and wheat were planted into each treated plot. Each plot had three planting sectors, so that each crop took up about a third of each plot space. In the 30 and 120 DAT plots for lettuce, phytotoxicity effects were observed and additional seeds were planted in these areas 33 days after the initial planting. As such for lettuce the 30 and 120 DATs were not adhered to, see the actual plant back intervals recorded in the table below. Data presented through the study report is from the 300 g a.s./ha rate applications, except 30 DAT immature lettuce from the phenyl-label. The 100 g a.s./ha rate application was used (slightly overdosed application rate was 104 g a.s./ha) due to phytotoxicity observed in the higher rate plot. A summary of the study design is given in Table 7-124.

Table 7-123 Summary of treatment rates of [^{14}C]bixlozone

Label	Target treatment rate (g a.s./ha)	Intended plant back interval (DAT)*	Actual plant back interval (DAT)*	% of target	rate (g a.s./ha)	Ranges N rate (wrt maximum seasonal dose) &	Ranges N rate (wrt maximum seasonal dose + soil plateau contribution^) &
Phenyl	300	30 120 310	30 (radish, wheat) 63 (lettuce) 120 (radish, wheat) 153 (lettuce) 310 (lettuce, radish, wheat)	89.7 92.9 93.6	269 279 281	1.35-1.44 (W/B) 0.90-0.96 (O) 0.72-0.77 (M)	0.86-0.92 (W/B) 0.57-0.62 (O) 0.46-0.49 (M)
Carbonyl	300	30 120 310	30 (radish, wheat) 63 (lettuce) 120 (radish, wheat) 153 (lettuce) 310 (lettuce, radish, wheat)	92.8 93.9 96.1	278 282 288		
Phenyl	100 (immature lettuce) £	30	30 (lettuce)	104.3	104	0.52 (W/B) 0.35 (O) 0.28 (M)	0.33 (W/B) 0.22 (O) 0.18 (M)

*DAT -Days after treatment
£ - The 30DAT for immature lettuce (phenyl) were the only results reported here for the low dose treatment (circa 100 g a.s./ha). These results were used as phytotoxicity had been observed in the higher application rate treatment (circa 300 g a.s./ha).
& W/B is wrt wheat and barley intended GAP of 1 application of 200 g a.s./ha
& O is wrt wheat and barley intended GAP of 1 application of 300 g a.s./ha
& M is wrt wheat and barley intended GAP of 1 application of 375 g a.s./ha
^ see Vol 1 section 2.7.7 where the N rate accounting for both the maximum seasonal application rate and the contribution from the soil plateau contribution is explained.

Table 7-124 Study design: rotational crops

	Lettuce	**Radish**	**Wheat**
Intended application rate [g a.s./ha]	300 (or 100)	300	300
plant back interval (PBI) in days after treatment (DAT)	63[2)] (C/P), 153[2)] (C/P), 310 (C/P) [3)]	30 (C/P), 120 (C/P), 310 (C/P) [3)]	30 (C/P), 120 (C/P), 310 (C/P) [3)]
sampled matrices	Immature: lettuce (BBCH 43) Mature: lettuce (BBCH 49)	Mature: roots, tops (BBCH 49)	Immature: forage (BBCH 39), hay (BBCH 75)

	Lettuce		Radish		Wheat	
					Mature: grain, straw (BBCH 89)	
sampling [DAP] 1)	immature lettuce	66	roots	53	forage	45
					hay	91
	mature lettuce	111	tops	53	grain	102
					straw	102

1) DAP days after planting – crop samples harvested at these timing for each PBI.
2) due to phytotoxicity additional seeds were planted into the same treated plot for lettuce samples 30 days after initial planting and therefore the crops represent a longer PBI. As the initial seedlings did not germinate it is unlikely that radioactivity would have been lost to the initial crop.
3) (C/P) relates to carbonyl and phenyl labels.

Sampling

Lettuce

Samples of immature lettuce were collected at approximately 50% of mature size, 66 days after planting (BBCH 43). Approximately 20% of the crop in each plot was sampled. Immature lettuce samples were not collected from the high rate (30 DAT plot for the phenyl label) due to an insufficient crop caused by phytotoxicity. Leaves were cut one inch above the soil line using a grape knife. Damaged leaves were not collected. Samples of mature lettuce were taken in the same way as immature lettuces at 111 days after planting (BBCH 49).

Radish

Samples of radish roots and tops were collected 53 days after planting (BBCH 49). The tops were cut off the roots using pruning shears and roots were pulled from soil, soil was removed by gently shaking and then roots were washed with water.

Wheat

Samples of immature wheat forage were taken 45 days after planting (BBCH 45). Samples of hay were taken 91 days after planting (BBCH 75). Approximately 15 percent of the crop from each plot was taken for the sample at initial sampling point as wheat forage sample and another 15 percent of the crop from each plot was taken at hay stage and the remaining crop was harvested at maturity. Forage samples were cut above the ground with a grape knife. Hay samples were collected similarly by cutting at two inches above soil line and then dried on racks. Samples of grain and straw were collected 102 days after planting (both BBCH 89). Wheat grain and straw were collected from the plots by removing heads from the plants, as per typical harvest practices. Grain was separated from the seed heads by use of threshing blocks. Seed and resulting chaff fell into collecting trays. Grain was separated from the chaff by using moving air to blow the lighter chaff, allowing the grain to drop into a separate collection.

Samples were then stored in a freezer at ≤-20°C until shipment. In the laboratory, all samples were stored in a freezer at *ca*. -20°C, whether as frozen samples, homogenised powder prior to extraction or as frozen extracts. Initial sample analysis and some metabolite profiling along with LC-MS characterization/identification was performed within ca. 6 months from RAC harvest (ca. 9 months for wheat grain 30 and 120 DAT samples). The latest analyses of samples was conducted after a much longer period. For further details see section on 'Storage Stability' below. Please also refer to the introductory text on '*Sample storage periods' at the start of section B7.2*.

Analysis

TRR

Combustion analysis for the determination of the TRR in plant samples was performed using finely homogenized/ground crops (immature and mature lettuce, radish tops and roots and wheat forage, hay, straw, and grain). Triplicate aliquots of each representative plant sample were used for combustion. The TRR was determined using biological sample oxidizer and measured evolved ^{14}Carbon dioxide ($^{14}CO_2$) by LSC.

Extraction

The 310 DAT samples for immature lettuce in both labels, and the carbonyl-labelled 310 DAT mature lettuce sample were not subjected to extraction due to low TRR.

The following extraction procedure was carried out for all crops.

All other samples were first extracted by blending with acetonitrile/water (80:20). The mixture was centrifuged and filtered under vacuum to separate solids. The remaining solids were then re-blended with a solution of acetonitrile/water (80:20) and the process repeated twice more. (For wheat samples, the extraction procedure was then repeated two more times with methanol: water (50:50)). For all samples, extracts were combined, assigned Ext-1 fraction, and assayed by LSC. Ext-1 fraction was concentrated and analysed by HPLC.

Where relevant, see below paragraph, a fraction of Ext-1 was also concentrated to dryness and reconstituted in aqueous solution (AQ-1), which was subjected to acid hydrolysis (1N HCl) under reflux. The mixture was cooled, neutralised and concentrated under nitrogen evaporation then reconstituted in acetonitrile: water (25:75) before assay by HPLC/LC-MS/MS analysis.

The report was not clear, however the applicant clarified (in response to an HSE letter) that acid hydrolysis was performed only on the sample extracts that triggered the criterion (10% TRR and 0.01 mg/kg) for identification. Therefore, samples with TRR levels not meeting this trigger were not included in the results tables and are listed below for clarity.

Lettuce:

-Phenyl label: Immature 310 DAT samples and mature 310 DAT samples

-Carbonyl label: Immature 310 DAT samples and mature 310 DAT samples

Radish:

-Carbonyl label: Leaf/Tops 310 DAT samples and Roots 310 DAT samples

Wheat:

-Phenyl label: Grain 310 DAT samples

-Carbonyl label: Grain 30, 120 and 310 DAT samples

Wheat PES

The remaining post-extraction solid samples (PES-1) from the initial solvent extraction were air-dried and combusted, followed by LSC. Subsamples of PES-1 were subjected to further processing by enzyme hydrolysis. Samples were individually suspended in 100mM sodium acetate buffer (pH ~5.0). Cellulase solution was prepared in 100 mM sodium acetate buffer. PES-1 fractions were then mixed with the cellulase solution and incubated for ~24 hours. After incubation the mixture was centrifuged. The supernatant was transferred to a centrifuge tube and aliquots were taken for LSC. The procedure was repeated on PES-1 subsamples to demonstrate sequential enzyme hydrolysis for amylase, pectinase, and protease enzymes. The solid residues (PES-2) were transferred into a container for further hydrolysis.

The PES-2 samples were subjected to mild acid and mild base hydrolysis. The entire PES-2 fraction from each sample was suspended in 1 N HCl (twice). The mixture was stirred for ca. 24 hours and then centrifuged. A single aliquot of the aqueous fraction was taken for LSC. The solid fraction after acid hydrolysis was individually suspended in 1 N NaOH (twice) and the mixture was vortex-mixed and stirred at ambient temperature for ca. 24 hours. The sample was then centrifuged, neutralized and the volume measured, a single aliquot of the aqueous fraction was taken for LSC. This was repeated for the solid fraction after base hydrolysis.

Samples were then subjected to more extreme acid and base treatments (sequentially) 6N.

The solid fraction after base hydrolysis from each sample was suspended in 6 N HCl (twice). The mixture was stirred for ca. 24 hours and then centrifuged. A single aliquot of the aqueous fraction was taken for LSC. The solid fraction after acid hydrolysis was individually suspended in 6 N NaOH (twice). The mixture was stirred for ca. 24 hours and then centrifuged. A single aliquot of the aqueous fraction was taken for LSC.

The radioactivity in the final PES was analysed.

Radish PES

For the phenyl-labelled 310 DAT root sample and the carbonyl-labelled 120 DAT root sample, the remaining post-extraction solid samples (PES-1) from the initial solvent extraction were air-dried and combusted, followed by LSC.

Subsamples of PES-1 were subjected to further processing by enzyme hydrolysis (cellulase followed by alpha-amylase only (alpha amylase was only conducted for the phenyl labelled samples))- see methods details below for lettuce.

The radioactivity in the final PES was analysed.

Lettuce PES

The carbonyl-labelled 120 DAT mature lettuce sample was not subjected to further analysis of the PES. In all remaining lettuce samples post-extraction solid samples (PES-1) from the initial solvent extraction were air-dried and combusted, followed by LSC. Subsamples of PES-1 were subjected to further processing by enzyme hydrolysis. Samples were individually suspended in 100mM sodium acetate buffer (pH ~5.0). Cellulase solution was prepared in 100 mM sodium acetate buffer. PES-1A fractions were then mixed with the cellulase solution and incubated for ~24 hours. After incubation the mixture was centrifuged. The solid residues (PES-1B) were transferred into a container for further enzyme hydrolysis. Samples were individually suspended in 100mM potassium phosphate buffer (pH ~7.2) and alpha amylase enzyme. PES-2 fractions were then incubated for ~24 hours. After incubation the mixture was centrifuged. The supernatant was transferred to a centrifuge tube and aliquots were taken for LSC. For the phenyl-labelled 310 DAT mature lettuce sample, further hydrolysis was carried out (on PES-1C). Samples were individually suspended in 100mM sodium acetate buffer (pH ~7.2) and pectinase enzyme. PES fractions were then incubated for ~24 hours. After incubation the mixture was centrifuged. The supernatant was transferred to a centrifuge tube and aliquots were taken for LSC.

The radioactivity in the final PES was analysed.

Identification

An initial run of assay of metabolites by HPLC (using reference standards) was made in order to scope out the potential metabolites present and to confirm their analytical separation prior to the further identification work using the analysis of the actual samples outlined below. The reference standards used in this initial scoping work were as follows: bixlozone, dimethyl malonic acid, 2,2-dimethyl-3-hydroxypropionic acid, 4,4-dimethyl 3-isoxazolidinone, 2,4-dichlorobenzoic acid, bixlozone dimethyl malonamide, bixlozone-hydroxy-isobutyramide, 3'-hydroxy-bixlozone, 'F9600 ring open acid', 2,4-dichlorobenzyl alcohol, 4-hydroxymethyl-bixlozone, 5-hydroxy-bixlozone, 5'-hydroxy-bixlozone, 6'-hydroxy-bixlozone, 5-keto-bixlozone.

Components of the residue were identified by LC-MS. A list of the standards stated in the metabolism study report (tabular 'Summary of F9600 reference standards assayed by LC/MS') is as follows: bixlozone (F9600), dimethyl malonic acid (M132/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-hydroxy isobutyramide/ bixlozone (F9600)-Dimethyl-Isobutyramide (M261/1), bixlozone dimethyl malonamide (M289/2), 5'-hydroxy-bixlozone (M289/3) and 4-hydroxymethyl-bixlozone (M289/4).

Selected extracts and isolated materials were analysed by LC/RAM/ESI-MS[9] and MS/MS. Components of the residue were analysed by LC/MS. The proposed structures were supported by HCD-MS[10]. All metabolites were further confirmed by comparison of the LC retention time, high-resolution full scan MS, and/or MS/MS data with those of available authentic standards.

Metabolites confirmed by LC-MS/MS stated in the metabolism study report ('Summary of metabolites of F9600 identified by LC-MS/MS') is as follows: dimethyl malonic acid (M132/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-hydroxy isobutyramide/ bixlozone (F9600)-Dimethyl-Isobutyramide (M261/1), bixlozone dimethyl malonamide (M289/2), 5'-hydroxy-bixlozone (M289/3), 4-hydroxymethyl-bixlozone (M289/4). These were metabolites identified/confirmed by LC/MS/MS and these correspond to both major metabolites with matching reference standards (overlap with the LC/MS list) as well as those characterized based on accurate mass and fragmentation pattern (supportive information on this identification data was provided in the study).

It was noted that Conjugates (M451/2 and M467/1 [glucoside conjugates of bixlozone, bixlozone (F9600)-[O, glucoside] and Dihydroxy -bixlozone (F9600) conjugate, respectively]) were tentatively characterised/identified by LC-ESI FTMS and comparison of the protonated molecular ion and retention time to that of the unconjugated equivalents.

A reference standard for 3'-hydroxy-bixlozone (M289/6) was also provided in the report, and this was proposed as was proposed as tentatively identified in 'set 2' by HPLC analysis.

[9] Liquid Chromatography/Radioactivity monitor/Electrospray Ionization with mass spectroscopy detection and tandem mass spectroscopy detection

[10] Higher-energy collisional dissociation with Mass Spectroscopy detection

Storage stability

As stated above, samples were not all analysed within a period of 6 months since sampling. Samples were stored frozen whether as collected field samples, homogenised samples (powder) or as extracts. The following indicates the timelines over which samples were stored:

Frozen storage prior to 'initial analyses'. Depending on matrix, samples within this period were stored as frozen extracts for periods of 1 to 4 months, or 9 months in the case of some wheat grain samples. Processing of samples was typically done after a period of frozen storage of samples stored for 2 weeks. The periods of frozen storage between harvest of the RAC and the 'initial analyses' are given in Table 7-125.

Table 7-125 Time of frozen storage of samples in rotational crop metabolism study (initial analysis)

Sample	Time of frozen storage of samples (either as frozen harvest samples, homogenised powder, or extracts) between RAC harvest and 'initial analysis'.
30 DAT wheat grain (phenyl)	9 months
120 DAT wheat grain (phenyl)	9 months
30 DAT wheat forage (phenyl)	3 months
120 DAT mature lettuce (phenyl)	4.5 months
30 DAT mature lettuce (carbonyl)	4.5 months
120 DAT mature lettuce (carbonyl)	4.5 months
Other rotational crop matrices for which metabolite identification is reported (for an overview, please see metabolism summary tables in Vol 1)	Within 6 months

Frozen storage up to the time in which analysis on the matrices were completed in this study are given in Table 7-126.

Table 7-126 Time of frozen storage of samples in rotational crop metabolism study complete analysis)

Sample	Time of frozen storage of samples between RAC harvest and complete analysis
30 DAT wheat forage (phenyl)	2.4 years
120 DAT mature lettuce (phenyl)	2.2 years
30 DAT mature lettuce (carbonyl)	2.2 years
120 DAT mature lettuce (carbonyl)	2.2 years
Other rotational crop matrices for which metabolite identification is reported.	Not specified (applicant did not confirm in their letter response to HSE).

Storage stability investigations

No storage stability comparisons were made within this rotational crop metabolism study (e.g. chromatogram comparisons for extracts analysed initially and later on in the study).

Results and discussion

Total radioactive residue

Lettuce

30 DAT

The TRR in mature samples were up to 0.018 mg/kg. In the carbonyl, the immature lettuce sample contained more TRR (0.045 mg/kg), than the corresponding mature lettuces (0.017 mg/kg). The immature lettuce phenyl sample was unusual as it was the low dose sample, so had a low residue of 0.01 mg/kg (TRR).

120 DAT

For both labels, the TRR was higher in the immature samples than the corresponding mature samples. In the phenyl-label mature and immature samples contained 0.026 and 0.034 mg/kg respectively. In the carbonyl-label a similar pattern was observed with 0.042 and 0.082 mg/kg TRR in the mature and immature samples respectively. The TRR in the 120 DAT samples were notably higher than the corresponding 30 DAT sample in all cases.

310 DAT

In both labels TRR in the 310 DAT samples were <0.01 mg/kg. A similar pattern was observed in both labels and for both immature and mature samples with TRR levels ranging from 0.004-0.008 mg/kg.

Radish

30 DAT

The TRR for both labels was highest in the leaf/ top samples, with 0.090 mg/kg and 0.208 mg/kg for the phenyl and carbonyl labels respectively. The TRR in the root samples was lower than that observed in the tops with 0.049 mg/kg and 0.055 mg/kg for the phenyl and carbonyl label respectively.

120 DAT

In radish root samples for both labels, the 120 DAT samples contained less TRR than the 120 DAT tops samples and the corresponding 30 DAT root samples. The TRR for roots was 0.021 and 0.034 mg/kg for the phenyl- and carbonyl-labelled samples respectively. In the carbonyl label the level of TRR observed in the radish tops was significantly lower than in the 30 DAT sample (0.057 mg/kg), however, in the phenyl-labelled sample the level in radish tops is slightly higher than that in the 30 DAT sample (0.107 mg/kg)

310 DAT

In the 310 DAT samples, the TRR was lower than the corresponding 120 and 30 DAT samples for roots and tops in both labels. In the carbonyl-labelled samples the TRR in both roots and tops was 0.005 mg/kg. In the phenyl-labelled samples higher TRRs of 0.017 mg/kg were observed in the tops and 0.012 mg/kg in the roots.

Wheat

30 DAT

In the phenyl labelled wheat samples, the highest TRR was observed in hay (0.590 mg/kg), followed by straw (0.227 mg/kg), forage (0.207 mg/kg) and lowest in grain (0.037 mg/kg). A similar distribution was seen with the Carbonyl-label, however in this case the TRR in straw was similar to that in hay (TRR highest in straw: 0.359 mg/kg, lower in hay at 0.345 mg/kg, forage at 0.163 mg/kg, and grain at 0.019 mg/kg). The TRR in grain was slightly higher with the phenyl-label compared with the carbonyl-label.

120 DAT

In all samples, less TRR was observed at 120 DAT in comparison with the corresponding 30 DAT sample. In both labels a similar distribution of TRR was observed as in the 30 DAT samples. In the phenyl-label, the highest TRR was observed in hay (0.325 mg/kg), followed by straw (0.230 mg/kg), forage (0.130 mg/kg) and lowest in grain (0.033 mg/kg). In the carbonyl-label, the highest TRR was observed in straw (0.339 mg/kg), followed by hay (0.200 mg/kg), forage (0.084 mg/kg) and lowest in grain (0.016 mg/kg).

310 DAT

In all samples, significantly less TRR was observed at 310 DAT in comparison with the corresponding 120 and 30 DAT samples. In the phenyl-label, the highest TRR was observed in hay (0.086 mg/kg), followed by straw (0.032 mg/kg), forage (0.028 mg/kg) and lowest in grain (0.009 mg/kg). In the carbonyl-label, the highest TRR was observed in straw (0.107 mg/kg), followed by hay (0.082 mg/kg), forage (0.031 mg/kg) and lowest in grain (0.007 mg/kg).

No total radioactive residues were found in plants grown in the control test material (≤ 0.001 mg/kg).

A summary of the TRRs in the treated plants is presented in Table 7-127.

Table 7-127 Total radioactive residue after foliar spray application of bixlozone following application at around 300 g a.s./ha.

Crop/ Matrix		**TRR measured by combustion** *[mg parent eq./kg]*		
		30 DAT[1)]	120 DAT[1)]	310 DAT
Phenyl-label				
Lettuce[1)]	Immature	2)- see below the table	**0.034**	*0.005*
	mature	**0.018**	**0.026**	**0.008**
Radish	leaf/ tops	0.090	0.107	0.017
	roots	0.049	0.021	**0.012**
Wheat	forage	**0.207**	**0.130**	**0.028**
	hay	**0.590**	**0.325**	**0.086**
	straw	**0.227**	**0.230**	**0.032**
	grain	**0.037**	**0.033**	0.009
Carbonyl-label				
Lettuce[1)]	Immature	**0.045**	**0.082**	*0.005*
	mature	**0.017**	**0.042**	*0.004*
Radish	leaf/ tops	0.208	0.057	0.005
	roots	0.055	**0.034**	0.005
Wheat	forage	**0.163**	**0.084**	**0.031**
	hay	**0.345**	**0.200**	**0.082**
	straw	**0.359**	**0.339**	**0.107**
	grain	**0.019**	**0.016**	0.007

1) Due to phytotoxicity effects additional seeds were planted 33 days after the initial planting for lettuce in the 30 and 120 DAT plots, and samples of lettuce therefore represent 63 and 153 DAT respectively for these samples rather than 30DAT and 120DAT respectively.
2) The TRR for the application of phenyl label bixlozone at around 300 g as/ha was not analysed (phytotoxicity affected the samples). Therefore the low dose sample was analysed (for the ca 100 g as/ha application). This was found to contain **0.010** mg/kg (TRR).

Residues typed in italic were not taken forward for extraction. Samples typed in bold were taken forward for further enzyme and/or acid/base analysis of the PES. For all others, organic extraction only was carried out.

Extractability

The extractabilities of ^{14}C residues from lettuce (immature and mature), radish tops, radish roots, wheat forage, hay, straw and grain are summarized in Table 7-128 - Table 7-131.

The samples had been initially extracted with a combination of acetonitrile/water and methanol/water solvent mixtures from all commodities. The remaining radioactivity after this initial extraction was termed PES-1.

See section on '*Extraction'* under methods above for details of samples that were/were not taken forward to extraction (and onward analytical work including acid hydrolysis work on the initially extracted residues), due to lower levels of radioactivity.

High initial extractability of ^{14}C residue was seen in both immature and mature lettuces (>81% TRR for total extract for both labels at all plant back intervals), radish roots and tops (>89% TRR for total extract for both labels at all plant back intervals) and in wheat forage and hay (>74% TRR for total extract for both labels at all plant back intervals). The initial solvent extractability from wheat straw was between 67-86% TRR with the lowest extraction in the 310 DAT samples for both labels. The initial solvent extractability of wheat grain samples was lower, *ca* 52-55 and *ca* 45-47% of TRR, for the phenyl-label and the carbonyl-label respectively for both the 30 and 120 DAT samples. The 310 DAT wheat grain samples for both the labels were not subject to further extraction based on the level of TRR (<0.01 mg/kg).

Table 7-128 Extractability of radioactive residue from lettuce commodities. Results in italics are the only results from the low dose treatment (at around 100 g as/ha). (mg/kg = mg parent eq./kg)

Commodity	**Immature lettuce**				**Mature lettuce**					
DAT	**63**		**153**		**63**		**153**		**310**	
Residue Levels	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Phenyl-label										
TRR	100	*0.010*	100	0.034	100	0.018	100	0.026	100	0.008
Extracted residue	83.3	*0.008*	83.8	0.028	89.1	0.016	81.9	0.021	88.2	0.007
Non-extracted PES-1	16.7	*0.002*	16.2	0.006	10.9	0.002	18.1	0.005	11.8	0.001
Enzyme hydrolysis of PES-1										
PES-1A Cellulase	6.3	*0.001*	4.3	0.002	1.5	<0.001	5.6	0.002	1.0	<0.001
PES-1B α - amylase	2.6	*<0.001*	3.0	0.001	3.2	0.001	2.4	0.001	1.2	<0.001
PES-1C Pectinase	-	-	-	-	-	-	-	-	0.7	<0.001
Non-extracted PES (final)	7.8	*<0.001*	8.9	0.003	6.2	0.002	10.1	0.002	8.9	<0.001
Carbonyl-label										
TRR		*0.045*		0.082		0.017		0.042		0.004
Extracted residue	88.4	*0.040*	87.4	0.072	83.7	0.014	90.8	0.038	-	-
Non-extracted PES-1	11.6	*0.005*	12.6	0.010	16.3	0.003	9.2	0.004	-	-
Enzyme hydrolysis of PES-1										
PES-1A Cellulase	1.3	*0.001*	2.5	0.002	3.4	0.001	-	-	-	-
PES-1B α - amylase	0.9	*<0.001*	0.2	<0.001	3.3	0.001	-	-	-	-
Non-extracted PES (final)	9.4	*0.004*	9.9	0.008	0.1	0.002	-	-	-	-

Table 7-129 Extractability of radioactive residue from radish commodities. (mg/kg = mg parent eq./kg)

Commodity	**Radish tops**						**Radish roots**					
DAT	**30**		**120**		**310**		**30**		**120**		**310**	
Phenyl-label												
Residue Levels	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR		0.090		0.107		0.017		0.049		0.021		0.012
Extracted residue	93.6	0.084	93.2	0.100	92.5	0.016	90.3	0.044	92.9	0.020	86.9	0.010
Non-extracted PES-1	6.4	0.006	6.8	0.007	7.5	0.001	9.7	0.005	7.1	0.001	13.1	0.002
Enzyme hydrolysis of PES-1												
Cellulase	-	-	-	-	-	-	-	-	-	-	3.3	<0.001
Alpha amylase	-	-	-	-	-	-	-	-	-	-	3.5	<0.001
Non-extracted PES (final)	-	-	-	-	-	-	-	-	-	-	6.3	0.001
Carbonyl-label												
TRR		0.208		0.057		0.005		0.055		0.034		0.005
Extracted residue	94.9	0.197	91.4	0.052	-	-	90.0	0.050	88.7	0.030	-	-
Non-extracted PES-1	5.1	0.011	8.6	0.005	-	-	10.0	0.006	11.3	0.004	-	-
Enzyme hydrolysis of PES-1												
Cellulase	-	-	-	-	-	-	-	-	2.3	0.001	-	-
Non-extracted PES (final)	-	-	-	-	-	-	-	-	9.0	0.003	-	-

Table 7-130 Extractability of radioactive residue from wheat forage and hay commodities. (mg/kg = mg parent eq./kg)

Commodity	**Wheat forage**						**Wheat hay**					
DAT	**30**		**120**		**310**		**30**		**120**		**310**	
Phenyl-label												
Residue Levels	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR		0.207		0.130		0.028		0.590		0.325		0.086
Extracted residue	89.6	0.185	84.9	0.110	86.8	0.024	79.8	0.471	78.5	0.256	76.9	0.066
Non-extracted PES-1	10.4	0.022	15.1	0.020	13.2	0.004	20.2	0.119	21.5	0.070	23.1	0.020
Sequential enzyme hydrolysis of PES-1												
Cellulase	1.0	0.002	3.0	0.004	1.8	0.001	2.1	0.013	1.4	0.004	2.6	0.002
Alpha amylase	0.6	0.001	2.7	0.004	1.7	<0.001	1.5	0.009	1.7	0.006	1.7	0.001
Pectinase	-	-	-	-	-	-	0.6	0.004	0.8	0.002	0.08	0.001
Protease	-	-	-	-	-	-	2.1	0.012	1.4	0.004	1.7	0.002
Sequential acid and base hydrolysis of PES-2												
Acid (1N)	-	-	-	-	-	-	1.0	0.006	1.0	0.003	1.1	0.001
Base (1N)	-	-	-	-	-	-	4.5	0.026	7.5	0.025	7.9	0.007
Acid (6N)	-	-	-	-	-	-	0.9	0.005	-	-	-	-
Base (6N)	-	-	-	-	-	-	-	-	-	-	-	-
Non-extracted PES (final)	8.7	0.019	9.4	0.012	9.7	0.003	7.5	0.044	7.7	0.026	7.3	0.006
Carbonyl-label												
TRR		0.163		0.084		0.031		0.345		0.200		0.082
Extracted residue	87.7	0.143	86.8	0.073	87.9	0.027	74.6	0.257	76.7	0.153	84.4	0.066
Non-extracted PES-1	12.3	0.020	13.2	0.011	12.1	0.004	25.4	0.088	23.3	0.047	15.6	0.013
Sequential enzyme hydrolysis of PES-1												
Cellulase	1.9	0.003	2.3	0.002	2.1	0.001	2.4	0.008	2.3	0.005	2.4	0.002
Alpha amylase	1.2	0.002	2.7	0.002	-	-	1.9	0.007	1.5	0.003	1.3	0.001
Pectinase	-	-	-	-	-	-	1.1	0.004	0.9	0.002	1.0	0.001
Protease	-	-	-	-	-	-	1.4	0.005	2.0	0.004	1.2	0.001
Sequential acid and base hydrolysis of PES-2												
Acid (1N)	-	-	-	-	-	-	0.9	0.003	1.4	0.003	0.7	0.001
Base (1N)	-	-	-	-	-	-	5.2	0.018	3.4	0.007	3.8	0.003
Acid (6N)	-	-	-	-	-	-	0.8	0.003	0.6	0.001	-	-
Base (6N)	-	-	-	-	-	-	4.4	0.015	4.1	0.008	-	-
Non-extracted PES (final)	9.2	0.015	8.2	0.007	10.0	0.003	7.3	0.025	7.1	0.014	5.2	0.004

Table 7-131 Extractability of radioactive residue from wheat straw and grain commodities. (mg/kg = mg parent eq./kg)

Commodity	**Wheat straw**						**Wheat grain**					
DAT	**30**		**120**		**310**		**30**		**120**		**310**	
Phenyl-label												
Residue Levels	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR		0.227		0.230		0.032		0.037		0.033		0.009
Extracted residue	79.4	0.180	75.0	0.173	67.6	0.021	55.5	0.021	52.5	0.017	-	-
Non-extracted PES-1	20.5	0.047	25.0	0.058	32.4	0.010	44.5	0.016	47.5	0.016	-	-
Sequential enzyme hydrolysis of PES-1												
Cellulase	1.8	0.004	2.3	0.005	3.9	0.001	4.0	0.001	4.3	0.001	-	-
Alpha amylase	1.7	0.004	1.4	0.003	3.0	0.001	4.0	0.001	4.9	0.002	-	-
Pectinase	0.5	0.001	0.8	0.002	1.7	0.001	2.7	0.001	2.4	0.001	-	-
Protease	1.0	0.002	1.2	0.003	1.9	0.001	4.9	0.002	5.3	0.002	-	-
Sequential acid and base hydrolysis of PES-2												
Acid (1N)	0.4	0.001	0.5	0.001	1.4	<0.001	2.3	0.001	2.5	0.001	-	-
Base (1N)	4.1	0.010	5.9	0.014	8.8	0.003	6.9	0.003	9.1	0.003	-	-
Acid (6N)	0.8	0.002	0.8	0.002	1.0	<0.001	1.4	0.001	1.1	<0.001	-	-
Base (6N)	4.9	0.011	4.8	0.011	-	-	6.4	0.002	15.8	0.005	-	-
Non-extracted PES (final)	5.3	0.012	7.3	0.017	10.7	0.003	11.9	0.004	2.1	0.001	-	-
Carbonyl-label												
TRR		0.359		0.339		0.107		0.019		0.016		0.007
Extracted residue	85.8	0.308	86.5	0.293	77.8	0.083	45.9	0.009	47.4	0.008	-	-
Non-extracted PES-1	14.2	0.051	13.5	0.046	22.2	0.024	54.1	0.010	52.6	0.008	-	-
Sequential enzyme hydrolysis of PES-1												
Cellulase	1.3	0.005	1.3	0.004	7.9	0.009	4.5	0.001	1.2	<0.001	-	-
Alpha amylase	1.3	0.005	1.2	0.004	4.1	0.004	7.9	0.001	9.9	0.002	-	-
Pectinase	0.5	0.002	0.4	0.001	-	-	3.0	0.001	2.0	<0.001	-	-
Protease	0.6	0.002	0.6	0.002	-	-	7.9	0.002	1.2	<0.001	-	-
Sequential acid and base hydrolysis of PES-2												
Acid (1N)	0.4	0.001	0.4	0.001	-	-	3.5	0.001	0.8	<0.001	-	-
Base (1N)	4.2	0.015	3.1	0.010	-	-	5.9	0.001	3.3	0.001	-	-
Acid (6N)	-	-	-	-	-	-	0.8	<0.001	0.8	<0.001	-	-
Base (6N)	-	-	-	-	-	-	6.8	0.001	6.1	0.001	-	-
Non-extracted PES (final)	5.9	0.021	6.5	0.022	10.2	0.011	13.8	0.003	27.3	0.002	-	-

Approximately 10-18% TRR (0.004-0.005 mg/kg) remained as unextracted residues from lettuce samples and all lettuce PES-1 samples were subjected to further processing, including enzyme hydrolysis of the PES-1, except immature lettuce samples for the 310 DAT PBI which did not require further consideration (due to a TRR of <0.01 mg/kg). In radish samples less than 10% TRR (0.001 to 0.003 mg/kg) remained as unextracted residues in the majority of samples. In two samples the PES-1 contained more than 10% TRR, with 13.1% TRR (0.002 mg/kg) in the 310 DAT, phenyl label root sample and 11.3% TRR (0.004 mg/kg) in the 120 DAT carbonyl labelled root sample. These samples were subjected to further enzyme hydrolysis of the PES-1. Wheat forage samples contained approximately 10-15% TRR (0.004-0.022 mg/kg) as unextracted residues in both labels at all plant back interval and subsequently the PES-1 was subjected to further enzyme hydrolysis. In wheat hay samples higher levels of radioactivity remained in the PES-1 for both labels at all plant back intervals (15.6% TRR, 0.013 mg/kg – 25.4% TRR, 0.088 mg/kg). Hay PES-1 samples were subjected to further processing including enzyme hydrolysis and acid/ base hydrolysis. In wheat straw samples, higher levels of radioactivity were detected in the phenyl labelled samples in terms of %TRR (<32.4% TRR, 0.010 mg/kg), however the absolute values of radioactivity were comparable in both labels (0.010-0.058 mg/kg). All wheat straw PES-1 samples were subjected to enzyme hydrolysis and acid/ base hydrolysis. In wheat grain samples, lower levels of extraction were observed. In the 310 DAT grain samples for both labels the absolute values of radioactivity were low (<0.005 mg/kg) and therefore despite high levels of %TRR (<69.8%) no further processing of the PES-1 was carried out. In the 30 and 120 DAT samples for both labels, higher levels of radioactivity were observed (44.5-54.1% TRR, 0.008-0.016 mg/kg) and subsequent enzyme and acid/ base hydrolysis of the PES-1 was carried out.

In all crops, extractability was broadly comparable for both labels.

Characterisation and Identification

Characterisation of the PES-1 following initial solvent extraction (where undertaken):

Lettuce: In all lettuce samples the PES-1 represented around up to 19%TRR. Cellulase hydrolysis and alpha amylase hydrolysis each extracted ca. 1-6% TRR. The final PES contained up to 10%TRR.

Radish: In the carbonyl-labelled root samples (120 DAT), the PES-1 represented 11.3% TRR (0.004 mg/kg). Cellulase hydrolysis extracted a further 2.3% TRR (0.001 mg/kg) and the final PES was 9% TRR (0.003 mg/kg). In the phenyl-labelled root samples (310 DAT), the PES-1 represented 13.1% TRR (0.002 mg/kg). Sequential cellulase and alpha amylase extracted a further 3.3% TRR (<0.001 mg/kg) and 3.5% TRR (<0.001 mg/kg) respectively. The final PES was 6.3% TRR (0.001 mg/kg).

Wheat (forage):

The PES-1 after organic extraction of the forage samples represented ≈10-15% TRR in both labels at all replant interval timings. Alpha amylase and cellulase hydrolysis each extracted around 2 or 3 %TRR, and the amount remaining in the final PES was 8-10%TRR.

Wheat (hay):

The PES-1 after organic extraction of the hay samples represented ≈16-25% TRR in both labels at all replant interval timings. In all of the sequential treatments to release base (1N) hydrolysis (and also base 6N hydrolysis) extracted the most significant levels of TRR. 1N (base) hydrolysis released 3-8%TRR and 6N (base) hydrolysis released up to a further 5%TRR. Lower levels (<3%TRR, typically representing low mg/kg amounts of radioactivity) were released by cellulase, alpha amylase, pectinase, protease, acid (1N) and acid (6N) hydrolysis, when these treatments were attempted. The final PES remaining represented 5 to 8%TRR.

Wheat (straw):

The PE1 after organic extraction of the 30 DAT straw samples represented ≈13-25% TRR 30 DAT and 120 DAT samples). Higher amounts (up to 32%TRR) were observed in the 310 DAT samples. Similarly to hay the base treatments (1N and also 6N) were the most effective treatment for releasing radioactivity as part of the sequential steps. This suggest some similarity in nature of these unextracted residues across hay and straw. Cellulase, alpha amylase, pectinase, protease, acid (1N) and acid (6N) hydrolysis individually extracted much lesser amounts of radioactivity, but step wise release of radioactivity still occurred. The amount remaining in the final PES was 5-10%TRR.

Wheat (grain):

The PES-1 after organic extraction of the straw samples represented ≈45-55% TRR. Both the 30 DAT and 120 DAT PES-1 were investigated further using the sequential enzyme (various) and base and acid hydrolysis steps. Whilst some appreciable (%TRR) amounts were released by some of the enzyme treatments (e.g. alpha amylase up to

10%TRR), once again base treatment (1N or 6N) was the most effective method of releasing the residues 3%TRR to 16%TRR. The amount remaining in the final PES was up to 27%TRR, representing only 0.002 mg/kg.

Metabolite identification:

The applicant provided metabolite distributional data on the acid hydrolysed fractions, obtained when the initial solvent extracts had been treated with acid. Please refer to the methods section which clarifies the samples that were/were not taken through this process.

Data on the organic extracts was available as the integration of the peaks in the organic extract was also presented in the GLP amendment study report but not prepared into a metabolite distribution table. HSE agrees that the metabolite distribution data presented in the study report from acid hydrolysis of the organic extracts presents a detailed assessment of metabolites in rotational crops.

An overview over the components of the extracted residue is given below in Table 7-132 - Table 7-135. Metabolites found at >10% TRR are discussed in detail for each matrix below. Structures of the metabolites are outlined in the appendices to Volume 1.

Lettuce

30 DAT

Immature Lettuce

For lettuce the '30 DAT' equates to 63 DAT due to the replanting 33 days after initial seeds were planted. It is also noted that for the phenyl labelled, 30 DAT immature lettuce sample the 100 g a.s./ha application was analysed due to phytotoxicity in the 300 g a.s./ha application sample. In the phenyl-labelled 30 DAT immature lettuce sample the total TRR by combustion was 0.01 mg/kg. For immature lettuce, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 27.1% TRR (0.003 mg/kg) as M289/2 and 16.6% TRR (0.002 mg/kg) was postulated as the conjugate M467/1. A further three metabolites were identified at low levels (maximum of 2.8% TRR, <0.001 mg/kg). Parent bixlozone was identified at a total level of 2.3% TRR (<0.001 mg/kg). A total of 30.5% TRR (0.011 mg/kg) material was assigned as unknowns, including 11 regions, with the highest individual unknown component level at 7.6% TRR (0.001 mg/kg). It is noted that 10 of the unknown regions were detected at <0.001 mg/kg and therefore when summed the total unknowns of 0.011 mg/kg is not an accurate representation.

For immature lettuce, in the carbonyl-label the acid hydrolysis of the organic extracts enabled 26.9% TRR (0.012 mg/kg) to be identified as metabolite M132/1 and 17.5% TRR (0.008 mg/kg) as metabolite M289/2. Parent bixlozone was detected at low levels (2.9% TRR, 0.002 mg/kg). A peak at 7.9% TRR (0.008 mg/kg) was postulated as a conjugate M467/1. A total of 28.4% TRR (0.012 mg/kg) was attributed to unknown metabolites, containing 5 regions at a maximum level of 9.0% TRR (0.004 mg/kg).

Mature Lettuce

In the mature lettuce samples for the phenyl label, the acid hydrolysis of the organic extracts enabled 30.7% TRR (0.006 mg/kg) to be identified as metabolite M289/2 and 11.4% TRR (0.002 mg/kg) was postulated as the conjugate M467/1. A further two metabolites were present at a maximum level of 3.2% TRR (0.001 mg/kg). A total of 47.1% TRR (0.007 mg/kg) was assigned as unknown material containing a total of 13 individual regions at a maximum individual 'unknown' level of 9.1% TRR (0.002 mg/kg). As in the immature lettuce samples the actual concentration in mg/kg of the unknown regions is not representative of the %TRR due to a number being present <0.001 mg/kg. The distribution of metabolites in the phenyl label were broadly comparable between immature and mature lettuce samples.

For mature lettuce, in the carbonyl-label the acid hydrolysis of the organic extracts enabled 39.8% TRR (0.007 mg/kg) to be identified as metabolite M132/1 and 11.8% TRR (0.002 mg/kg) as metabolite M289/2. A peak at 7.8% TRR (0.001 mg/kg) was postulated to be the conjugate M467/1. No parent bixlozone was detected. A total of 21.4% TRR (0.004 mg/kg) was attributed to unknown metabolites, containing 4 regions at a maximum individual 'unknown' level of 8.1% TRR (0.001 mg/kg). No further characterisation was carried out. The metabolic pattern differs a bit between immature and mature lettuce samples, with the conjugate M467/1 detected in the mature samples and not in the immature samples whereas the metabolite M289/2, present in the immature samples, was not identified in the mature samples.

120 DAT

Immature Lettuce

For lettuce the 120 DAT equates to 153 DAT due to the replanting 33 days after initial seeds were planted. For immature lettuce, in the phenyl-label, the acid hydrolysis of the organic extracts enabled 45.6% TRR (0.015 mg/kg) to be identified as metabolite M289/2 and 6.8% TRR (0.002 mg/kg) as the metabolite M289/4. A total of 31.5% TRR (0.010 mg/kg) was assigned as 'unknowns', including 5 regions with a maximum individual unknown level of 9.9% (0.001 mg/kg).

For immature lettuce, in the carbonyl-label the acid hydrolysis of the organic extracts enabled 46.3% TRR (0.038 mg/kg) to be identified as metabolite M132/1 and 9.0% TRR (0.007 mg/kg) as M289/2. A peak at 25% TRR (0.002 mg/kg) was postulated to be the conjugate M467/1. A total of 28.4% TRR (0.012 mg/kg) was attributed to unknown metabolites, containing 8 regions with a maximum individual unknown level of 8.3% TRR (0.007 mg/kg).

Mature Lettuce

In the mature lettuce samples for the phenyl label, the acid hydrolysis of the organic extracts enabled identification 31.0% TRR (0.008 mg/kg) as the metabolite M289/2 and 22.7% TRR (0.002 mg/kg) was postulated as the conjugate M467/1. A further two metabolites were present at a maximum level of 4.9% TRR (0.001 mg/kg). A total of 21.0% TRR (0.005 mg/kg) was assigned as unknown regions containing a total of four individual regions at a maximum individual 'unknown' level of 7.6% TRR (0.002 mg/kg). The distribution of metabolites in the phenyl label were comparable between immature and mature lettuce samples.

For mature lettuce, in the carbonyl-label the acid hydrolysis of the organic extracts enabled identification 46.7% TRR (0.020 mg/kg) as the metabolite M132/1 and 16.0% TRR (0.007 mg/kg) as metabolite M289/2. A peak at 10.0% TRR (0.004 mg/kg) was postulated as the conjugate M467/1. Parent bixlozone was detected at 2.2% TRR (0.001 mg/kg). One unknown region was detected at 6.5% TRR (0.003 mg/kg).

Radish

30 DAT

Radish Leaves

In the phenyl-labelled 30 DAT radish tops sample, the acid hydrolysis of the organic extracts enabled identification of 24.4% TRR (0.022 mg/kg) as parent bixlozone. The metabolite M261/1 was identified at 19.1% TRR (0.017 mg/kg). A further 3 metabolites were identified at low levels (maximum of 6.4% TRR, 0.006 mg/kg). A total of 34.6% TRR (0.032 mg/kg) was assigned as unknowns, including 4 regions at a maximum individual unknown level of 13.2% TRR (0.012 mg/kg).

For radish tops, in the carbonyl-label the acid hydrolysis of the organic extracts enabled identification of parent bixlozone at a level of 25.9% TRR (0.054 mg/kg). M261/1 was detected at a level of 26.6% TRR (0.055 mg/kg). Three other metabolites were detected at a maximum level of 6.5% TRR (0.013 mg/kg). A total of 33.1% TRR (0.068 mg/kg) was attributed to unknown metabolites, containing 2 regions at a maximum individual unknown level of 19.9% TRR (0.041 mg/kg).

Radish Roots

In the radish root samples for the phenyl label, the acid hydrolysis of the organic extracts enabled identification of parent bixlozone at a level of 75.7% TRR (0.037 mg/kg). One unidentified region at a level of 14.6% TRR (0.007 mg/kg) was detected.

In the radish root samples for the carbonyl label, the acid hydrolysis of the organic extracts enabled identification of parent bixlozone at a level of 40.3% TRR (0.022 mg/kg). Dimethyl-malonic acid (M132/1) was found at 14.2% TRR (0.008 mg/kg). A further 3 metabolites were present at a maximum level of 4.5% TRR (0.003 mg/kg). A total of 23.3% TRR (0.013 mg/kg) was assigned as unknown material containing a total of 3 individual regions at a maximum individual unknown level of 10.4% TRR (0.006 mg/kg).

120 DAT

Radish Leaves

For radish tops/leaves, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 37.3% TRR (0.040 mg/kg) as the metabolite M261/1. 11.8% TRR (0.013 mg/kg) of M289/2, 10.7% TRR (0.012 mg/kg) and

10.5% TRR (0.011 mg/kg) of M190/1 were also identified. A total of 23.0% TRR (0.025 mg/kg) was assigned as unknown material, including 3 regions at a maximum individual unknown level of 10.6% TRR (0.011 mg/kg).

For radish tops/leaves, in the carbonyl-label the acid hydrolysis of the organic extracts enabled identification of parent bixlozone at a level of 17.1% TRR (0.001 mg/kg). 29.7% TRR (0.017 mg/kg) was found as M132/1 and 12.0% TRR (0.007 mg/kg) as M261/1. Three other metabolites were present at a maximum level of 8.5% TRR (0.005 mg/kg). A total of 16.7% TRR (0.011 mg/kg) was attributed to unknown material, containing 3 regions at a maximum individual unknown level of 13.6% TRR (0.008 mg/kg).

Radish Roots

In the radish root samples for the phenyl label, the acid hydrolysis of the organic extracts enabled identification of 33.2% TRR (0.007 mg/kg) as parent bixlozone. The metabolites M190/1 and M289/2 were identified at a level of 22.5% TRR (0.005 mg/kg) and 14.3% TRR (0.003 mg/kg) respectively. A total of 22.8% TRR (0.005 mg/kg) was assigned as unknown material containing a total of 3 individual regions at a maximum individual unknown level of 12.3% TRR (0.003 mg/kg).

For radish root samples, in the carbonyl-label the acid hydrolysis of the organic extracts enabled identification of parent bixlozone at a level of 35.6% TRR (0.012 mg/kg). The only metabolite found was M132/1 at a level of 20.3% TRR (0.017 mg/kg). A total of 32.8% TRR (0.012 mg/kg) was attributed to unknown metabolites, containing 3 regions at a maximum individual unknown level of 13.5% TRR (0.005 mg/kg).

310 DAT

Radish Leaves

For radish tops/leaves, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 19.3% TRR (0.003 mg/kg) as parent bixlozone. The metabolite M261/1 was identified at 23.5% TRR (0.004 mg/kg) and three other metabolites were identified at a maximum level of 7.4% TRR (0.001 mg/kg). A total of 35.4% TRR (0.006 mg/kg) was assigned as unknown material, including 4 regions at a maximum individual unknown level of 11.5% TRR (0.002 mg/kg).

Radish Roots

In the radish root samples for the phenyl label, the acid hydrolysis of the organic extracts enabled identification of 27.1% TRR (0.003 mg/kg) as parent bixlozone. The metabolites M190/1 and M289/2 were identified at 23.8% TRR (0.003 mg/kg) and 4.9% TRR (0.001 mg/kg) respectively. A total of 25.7% TRR (0.005 mg/kg) was assigned as unknown material containing a total of 5 individual regions at a maximum individual unknown level of 9.0% TRR (0.003 mg/kg).

Wheat

30 DAT

Forage

For wheat forage, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 34.5% TRR (0.072 mg/kg) as the metabolite M289/3 and 14.2% TRR (0.029 mg/kg) as M190/1. A total of 38.5% TRR (0.081 mg/kg) of material was assigned as unknowns, including 7 regions at a maximum individual unknown level of 7.1% TRR (0.015 mg/kg).

In the wheat forage samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled identification of 21.9% TRR (0.036 mg/kg) as the metabolite M289/3. 18.2% TRR (0.030 mg/kg) was postulated to be M132/1 and M261/1 was identified at 5.6% TRR (0.009 mg/kg). A total of 42.1% TRR (0.069 mg/kg) was assigned as unknow material containing a total of 7 individual regions at a maximum individual unknown level of 16.1% TRR (0.026 mg/kg).

Hay

For wheat hay, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 23.5% TRR (0.139 mg/kg) as the metabolite M289/3 and two other metabolites were identified at a maximum level of 7.9% TRR (0.047 mg/kg). A total of 41.8% TRR (0.248 mg/kg) of unknowns were detected, including 11 regions at a maximum individual unknown level of 7.3% TRR (0.043 mg/kg).

In the wheat hay samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled 44.4% TRR (0.153 mg/kg) to be found as M132/1, however this characterisation contained split peaks (peaks observed at 31.9% TRR,

0.110 mg/kg at a retention time of 2.88-3.38 mins and at 12.5% TRR, 0.043 mg/kg at a retention time of 4.38-5.38 mins). . The applicant has stated that the split peaks and shifts in retention time observed in the chromatography of this sample were due the polar nature of the metabolite M132/1 and the similarity in pH between the metabolite and the mobile phase. This split in RT was only seen in hay samples and not in straw and forage. Presence of M132/1 is plausible in these rotational crop samples, although the identification is uncertain.

Two other metabolites (M261/1 and M289/3) were identified at a maximum level of 6.2% TRR (0.021 mg/kg). A total of 13.4% TRR (0.046 mg/kg) of radioactivity was assigned as unknown, with a total of 4 individual regions at a maximum individual unknown level of 7.1% TRR (0.025 mg/kg).

Straw

For wheat straw, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 31.7% TRR (0.072 mg/kg) as the metabolite M289/3 as well as 5 other metabolites at a maximum level of 8.0% TRR (0.018 mg/kg). A total of 22.9% TRR (0.051 mg/kg) of material was assigned as unknown material, including 7 regions at a maximum individual unknown level of 3.9% TRR (0.009 mg/kg).

In the wheat straw samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled identification of 36.6% TRR (0.132 mg/kg) as the metabolite M289/3. Three other metabolites were identified at a maximum level of 6.5% TRR (0.023 mg/kg). A total of 32.8% TRR (0.118 mg/kg) of material was assigned as unknown material containing a total of 5 individual regions at a maximum individual unknown level of 10.7% TRR (0.039 mg/kg).

Grain

For wheat grain, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 29.6% TRR (0.011 mg/kg) as the metabolite M190/1 and 11.5% TRR (0.004 mg/kg) as the metabolite M289/2. A total of 14.5% TRR (0.006 mg/kg) was assigned as unknown material, including 3 regions at a maximum individual unknown level of 10.0% TRR (0.004 mg/kg).

120 DAT

Forage

For wheat forage, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 33.6% TRR (0.044 mg/kg) as the metabolite M289/3 and 6.9% TRR (0.009 mg/kg) as the metabolite M261/1. A total of 44.4% TRR (0.058 mg/kg) was assigned as unknown material, including 7 regions at a maximum individual unknown level of 7.9% TRR (0.010 mg/kg).

In the wheat forage samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled identification of 33.0% TRR (0.028 mg/kg) as the metabolite M289/3. The metabolite M261/1 was identified at 9.5% TRR (0.008 mg/kg) and 4.5% TRR (0.004 mg/kg) was postulated as M132/2. A total of 39.9% TRR (0.034 mg/kg) of material was assigned as unknown material containing a total of 6 individual regions at a maximum individual unknown level of 11.1% TRR (0.009 mg/kg).

Hay

For wheat hay, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 39.1% TRR (0.128 mg/kg) as the metabolite M289/3 and two other metabolites were identified at a maximum level of 8.9% TRR (0.029 mg/kg). A total of 23.7% TRR (0.077 mg/kg) of material was assigned as unknown, including 7 regions at a maximum individual unknown level of 7.4% TRR (0.024 mg/kg).

In the wheat hay samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled 13.6% TRR (0.027 mg/kg) to be found as M132/1, however this characterisation contained split peaks (peaks observed at 5.5% TRR, 0.011 mg/kg at a retention time of 2.88-3.38 mins and at 8.1% TRR, 0.016 mg/kg at a retention time of 4.38-5.38 mins). The applicant has stated that the split peaks and shifts in retention time observed in the chromatography of this sample were due the polar nature of the metabolite M132/1 and the similarity in pH between the metabolite and the mobile phase. This split in RT was only seen in hay samples and not in straw and forage. Presence of M132/1 is plausible in these rotational crop samples, although the identification is uncertain.

25.4% TRR (0.051 mg/kg) was identified as M289/3 and M261/1 was found at a level of 6.3% TRR (0.013 mg/kg). A total of 25.0% TRR (0.050 mg/kg) was assigned as unknown material containing a total of 4 individual regions at a maximum individual unknown level of 8.1% TRR (0.016 mg/kg).

Straw

For wheat straw, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 41.4% TRR (0.095 mg/kg) as the metabolite M289/3 as well as 4 other metabolites at a maximum level of 7.3% TRR (0.017 mg/kg). A total of 12.9% TRR (0.029 mg/kg) was assigned as unknown material, including 3 regions at a maximum individual unknown level of 7.1% TRR (0.016 mg/kg).

In the wheat straw samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled identification of 33.1% TRR (0.112 mg/kg) as the metabolite M289/3. The metabolite M132/1 was characterised from a peak containing 33.7% TRR (0.114 mg/kg). Two other metabolites were identified at a maximum level of 9.7% TRR (0.033 mg/kg). A total of 7.6% TRR (0.026 mg/kg) was assigned as unknown material, including 2 regions at a maximum individual unknown level of 5.8% TRR (0.020 mg/kg).

Grain

For wheat grain, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 24.2% TRR (0.008 mg/kg) as the metabolite M190/1 and 9.4% TRR (0.003 mg/kg) as the metabolite M289/2. A total of 18.8% TRR (0.007 mg/kg) was assigned as unknown material, including 5 regions at a maximum individual level of 8.6% TRR (0.003 mg/kg).

310 DAT

Forage

For wheat forage, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 22.9% TRR (0.006 mg/kg) as the metabolite M289/2 and 18.4% TRR (0.005 mg/kg) as the metabolite M289/3. Two other metabolites were identified at a maximum level of 7.6% TRR (0.002 mg/kg). A total of 37.8% TRR (0.010 mg/kg) was assigned as unknown material, including 4 regions at a maximum individual unknown level of 25.2% TRR (0.007 mg/kg).

In the wheat forage samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled identification of 16.6% TRR (0.005 mg/kg) as the metabolite M289/3. A peak at 18.4% TRR (0.006 mg/kg) was postulated to be M132/1 and M261/1 was found at 4.2% TRR (0.001 mg/kg). A total of 48.7% TRR (0.016 mg/kg) was assigned as unknown material containing a total of 3 individual regions at a maximum individual unknown level of 29.7% TRR (0.009 mg/kg).

Hay

For wheat hay, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 42.3% TRR (0.036 mg/kg) as the metabolite M289/3 and two other metabolites were identified at a maximum level of 5.1% TRR (0.004 mg/kg). A total of 26.9% TRR (0.024 mg/kg) was assigned as unknown material, including 2 regions at a maximum individual unknown level of 19.2% TRR (0.017 mg/kg).

In the wheat hay samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled 33.0% TRR (0.027 mg/kg) to be suggested as metabolite M132/1, however as in the hay samples at both 30 and 120 DAT, this characterisation contained split peaks (see the discussions regarding this uncertain metabolite assignation above for the hay samples at 30 DAT and 120 DAT). M289/3 was identified at a level of 35.8% TRR (0.029 mg/kg) and M261/1 was identified at a level of 5.2% TRR (0.004 mg/kg). A total of 10.3% TRR (0.008 mg/kg) was assigned as unknown material containing a total of 3 individual regions at a maximum individual unknown level of 29.7% TRR (0.009 mg/kg).

Straw

For wheat straw, in the phenyl-label, the acid hydrolysis of the organic extracts enabled identification of 19.4% TRR (0.006 mg/kg) as the metabolite M289/3. The metabolite M289/2 was identified at a level of 13.9% TRR (0.004 mg/kg) and M190/1 was identified at a level of 11.6% TRR (0.004 mg/kg). M261/1 was also identified at 3.9% TRR (0.001 mg/kg). A total of 13.2% TRR (0.003 mg/kg) was assigned as unknown material, including 3 regions at a maximum individual unknown level of 4.4% TRR (0.001 mg/kg).

In the wheat straw samples for the carbonyl-label, the acid hydrolysis of the organic extracts enabled identification of 30.8% TRR (0.033 mg/kg) as the metabolite M289/3. A peak at 27.6% TRR (0.029 mg/kg) was characterised as M132/1. Two other metabolites were identified at a maximum level of 7.5% TRR (0.008 mg/kg). One unknown region was detected containing 4.1% TRR (0.004 mg/kg).

Table 7-132 Distribution of [^{14}C]bixlozone and its metabolites in immature and mature lettuce (from the acid hydrolysed fractions of the initially solvent extracted residues) following application to bare soil at around 300 g as/ha (aside from sample denoted † which provides results based on the low dose treatment (circa 100 g as/ha)). (mg/kg = mg parent eq./kg)

Metabolite	Immature Lettuce				Mature Lettuce			
	63 DAT†		153 DAT		63 DAT		153 DAT	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
	Phenyl label							
Total TRR by combustion (mg/kg)	**0.010**		**0.034**		**0.018**		**0.026**	
bixlozone	2.3	<0.001	-	-	-	-	-	-
2,4-Dichlorobenzoic Acid (M190/1)*	1.4	<0.001	-	-	1.8	<0.001	4.9	0.001
Dihydroxy -bixlozone conjugate (M467/1)	16.6	0.002	-	-	11.4	0.002	22.7	0.006
bixlozone-dimethyl-malonamide (M289/2)	27.1	0.003	45.6	0.015	30.7	0.006	31.0	0.008
bixlozone-glucoside (M451/2)	2.8	<0.001	-	-	-	-	-	-
4-hydroxyl-methyl -bixlozone (M289/4)	2.5	<0.001	6.8	0.002	3.2	0.001	3.2	0.001
Total unknown	30.5[a]	0.011[a]	31.5[b]	0.01[b]	42.2[c]	0.014[c]	21.0[d]	0.005[d]
Total 'identified'	**52.7**	**0.009**	**52.4**	**0.017**	**47.1**	**0.007**	**61.8**	**0.009**
Final non-extracted/ PES	7.8	<0.001	8.9	0.003	6.2	0.002	10.1	0.002
	Carbonyl label							
Total TRR by combustion (mg/kg)	**0.045**		**0.082**		**0.017**		**0.042**	
bixlozone	2.9	0.002	-	-	-	-	2.2	0.001
Dimethyl-malonic acid (M132/1)	26.9	0.012	46.3	0.038	39.8	0.007	46.7	0.020
Dihydroxy -bixlozone conjugate (M467/1)	7.9	0.004	2.5	0.002	7.8	0.001	10.0	0.004
bixlozone-dimethyl-malonamide (M289/2)	17.5	0.008	9.0	0.007	11.8	0.002	16.0	0.007
4-hydroxyl-methyl -bixlozone (M289/4)	-	-	-	-	-	-	3.4	0.001
Total unknown	28.4[e]	0.012[e]	29.6[f]	0.024[f]	21.4[g]	0.004[g]	6.5[h]	0.003[h]
Total 'identified'	**55.2**	**0.012**	**57.8**	**0.047**	**59.4**	**0.010**	**78.3**	**0.033**
Final non-extracted/ PES	9.4	0.004	9.9	0.008	0.1	0.002	9.2	0.004

' - ' considered but not detected (no associated radioactivity)

*Applicant states a possible shift in retention time due to high matrix or pH of mobile phase. Peaks at 13.38-13.88 min and 17.13-17.88 min (for the purposes of this table, the two peaks identified in the study report have been summed). The applicant's presentation of the data is given in the table, however HSE considers that this information (and level of residue associated

with 2,4-Dichlorobenzoic Acid (M190/1)) is uncertain, especially considering the large (around 4 mins) shift in RT. In view of the primary crop metabolism of bixlozone, and the metabolism in soil, 2,4-Dichlorobenzoic Acid is not an unexpected rotational crop metabolite.

†Due to phytotoxicity, the 100 g a.s./ha rate application was used for determination of residues in the 30 DAT (corresponding to 63 DAT) immature lettuce sample.

a - Includes 11 individual components. Max. 7.6% TRR, 0.001 mg/kg.
b - Includes 5 individual components. Max. 9.9% TRR, 0.003 mg/kg.
c - Includes 13 individual components. Max. 9.1% TRR, 0.002 mg/kg.
d - Includes 4 individual components. Max. 7.6% TRR, 0.002 mg/kg.
e - Includes 5 individual components. Max. 9.0% TRR, 0.004 mg/kg.
f - Includes 8 individual components. Max. 8.3% TRR, 0.007 mg/kg.
g - Includes 4 individual components. Max. 8.1% TRR, 0.001 mg/kg.
h - Includes 1 individual component.

Table 7-133 Distribution of [^{14}C]bixlozone and its metabolites in radish tops and roots (from the acid hydrolysed fractions of the initially solvent extracted residues) following application to bare soil at around 300 g as/ha. (mg/kg = mg parent eq./kg)

Metabolite	Radish leaves/ tops						Radish roots					
	30 DAT		120 DAT		310 DAT		30 DAT		120 DAT		310 DAT	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
	Phenyl label											
Total TRR by combustion (mg/kg)	**0.090**		**0.107**		**0.017**		**0.049**		**0.021**		**0.012**	
bixlozone	24.4	0.022	-	-	19.3	0.003	75.7	0.037	33.2	0.007	27.1	0.003
2,4-Dichlorobenzoic Acid (M190/1)	-	-	10.5	0.011	4.6	0.001	-	-	22.5	0.005	23.8	0.003
bixlozone-dimethyl-malonamide (M289/2)	6.4	0.006	11.8	0.013	2.3	<0.001	-	-	14.3	0.003	4.9	0.001
bixlozone-hydroxy-isobutyramide (M261/1)	19.1	0.017	37.3	0.040	23.5	0.004	-	-	-	-	-	-
4-hydroxy-methyl - bixlozone (M289/4)	4.9	0.004	10.7	0.012	7.4	0.001	-	-	-	-	-	-
5'-OH-bixlozone (M289/3)	4.2	0.004	-	-	-	-	-	-	-	-	-	-
Total unknown	34.6[a]	0.032[a]	23.0[b]	0.025[b]	35.4[c]	0.006[c]	14.6[d]	0.007[d]	22.8[e]	0.005[e]	25.7[f]	0.005[f]
Total 'identified'	**65.8**	**0.059**	**70.3**	**0.076**	**68.6**	**0.011**	**75.7**	**0.037**	**70.0**	**0.015**	**55.8**	**0.007**
Final non-extracted/ PES	6.4	0.006	6.8	0.007	7.5	0.001	9.7	0.005	7.1	0.001	6.3	0.001
	Carbonyl label											
Total TRR by combustion (mg/kg)	**0.208**		**0.057**		**0.005**		**0.055**		**0.034**		**0.005**	
bixlozone	25.9	0.054	17.1	0.010	-	-	40.3	0.022	35.6	0.012	-	-
Dimethyl-malonic acid (M132/1)	-	-	29.7	0.017	-	-	14.2	0.008	20.3	0.007	-	-
bixlozone-dimethyl-malonamide (M289/2)	2.9	0.006	8.5	0.005	-	-	-	-	-	-	-	-
bixlozone-hydroxy-isobutyramide (M261/1)	26.6	0.055	12.0	0.007	-	-	4.5	0.003	-	-	-	-

4-hydroxy-methyl - bixlozone (M289/4)	6.5	0.013	4.6	0.003	-	-	2.4	0.001	-	-	-	-
5'-hydroxy-bixlozone (M289/3)	3.1	0.006	2.8	0.002	-	-	2.4	0.001	-	-	-	-
Total unknown	33.1[g]	0.068[g]	16.7[h]	0.011[h]	-	-	23.3[i]	0.013[i]	32.8[j]	0.012[j]	-	-
Total 'identified'	**65.0**	**0.134**	**74.7**	**0.044**	**-**	**-**	**63.8**	**0.035**	**55.9**	**0.019**	**-**	**-**
Final non-extracted/ PES	5.1	0.011	8.6	0.005	-	-	10.0	0.006	9.0	0.003	-	-

' - ' considered but not detected (no associated radioactivity)

a - Includes 4 individual components. Max. 13.2% TRR, 0.012 mg/kg.
b - Includes 3 individual components. Max. 10.6% TRR, 0.011 mg/kg.
c - Includes 4 individual components. Max. 11.5% TRR, 0.002 mg/kg.
d - Includes 1 individual component.
e - Includes 3 individual components. Max. 12.3% TRR, 0.003 mg/kg.
f - Includes 5 individual components. Max. 9.0% TRR, 0.001 mg/kg.
g- Includes 2 individual components. Max. 19.9% TRR, 0.041 mg/kg.
h - Includes 3 individual components. Max. 13.6% TRR, 0.008 mg/kg.
i - Includes 3 individual components. Max. 10.4% TRR, 0.006 mg/kg.
j - Includes 3 individual components. Max. 13.5% TRR, 0.005 mg/kg.

Table 7-134 Distribution of [^{14}C]bixlozone and its metabolites in wheat forage and hay (from the acid hydrolysed fractions of the initially solvent extracted residues) following application to bare soil at around 300 g as/ha. (mg/kg = mg parent eq./kg)

Metabolite	Wheat forage						Wheat hay					
	30 DAT		120 DAT		310 DAT		30 DAT		120 DAT		310 DAT	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
	Phenyl label											
Total TRR by combustion (mg/kg)	**0.207**		**0.130**		**0.028**		**0.590**		**0.325**		**0.086**	
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
2,4-Dichlorobenzoic Acid (M190/1)	14.2	0.029	-	-	7.6	0.002	-	-	8.9	0.029	5.1	0.004
bixlozone-hydroxy-isobutyramide (M261/1)	-	-	6.9	0.009	5.6	0.002	6.4	0.038	7.0	0.023	2.6	0.002
5'-hydroxy-bixlozone (M289/3)	34.5	0.072	33.6	0.044	18.4	0.005	23.5	0.139	39.1	0.128	42.3	0.036
bixlozone-dimethyl-malonamide (M289/2)	-	-	-	-	22.9	0.006	7.9	0.047	-	-	-	-
Total unknown	38.5[a]	0.081[a]	44.4[b]	0.058[b]	37.8[c]	0.01[c]	41.8[d]	0.248[d]	23.7[e]	0.077[e]	26.9[f]	0.024[f]
Total 'identified'	**48.7**	**0.101**	**40.6**	**0.053**	**54.5**	**0.015**	**37.8**	**0.224**	**55.0**	**0.180**	**50.0**	**0.042**
Final non-extracted/ PES	8.7	0.019	9.4	0.012	9.7	0.003	7.5	0.044	7.7	0.026	7.3	0.006
	Carbonyl label											
Total TRR by combustion (mg/kg)	**0.163**		**0.084**		**0.031**		**0.345**		**0.200**		**0.082**	
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
Dimethyl-malonic acid (M132/1)*	18.2	0.030	4.5	0.004	18.4	0.006	44.4	0.153	13.6	0.027	33.0	0.027
bixlozone-hydroxy-isobutyramide (M261/1)	5.6	0.009	9.5	0.008	4.2	0.001	1.2	0.004	6.3	0.013	5.2	0.004
5'-hydroxy-bixlozone (M289/3)	21.9	0.036	33.0	0.028	16.6	0.005	6.2	0.021	25.4	0.051	35.8	0.029
Total unknown	42.1[g]	0.069[g]	39.9[h]	0.034[h]	58.7[i]	0.016[i]	13.4[j]	0.046[j]	25.0[k]	0.050[k]	10.3[l]	0.008[l]
Total 'identified'	**45.7**	**0.075**	**47.0**	**0.040**	**39.2**	**0.012**	**51.8**	**0.178**	**45.3**	**0.091**	**74.0**	**0.060**

Final non-extracted/ PES	9.2	0.015	8.2	0.007	10.0	0.003	7.3	0.025	7.1	0.014	5.2	0.004

‘ - ‘ considered but not detected (no associated radioactivity)
*M132/1 Applicant states a possible shift in retention time as well as split peaks. Peaks at 2.88-3.38 min and 4.38-5.38 min (for the purposes of this table, the two peaks identified in the study report have been summed)

a - Includes 7 individual components. Max. 7.1% TRR, 0.015 mg/kg.
b - Includes 7 individual components. Max. 7.9% TRR, 0.01 mg/kg.
c - Includes 4 individual components. Max. 25.2% TRR, 0.007 mg/kg.
d - Includes 11 individual components. Max. 7.3% TRR, 0.043 mg/kg.
e - Includes 7 individual components. Max. 7.4% TRR, 0.024 mg/kg.
f - Includes 2 individual components. Max. 19.2% TRR, 0.017 mg/kg.
g - Includes 7 individual components. Max. 16.1% TRR, 0.026 mg/kg.
h - Includes 6 individual components. Max. 11.1% TRR, 0.009 mg/kg.
i - Includes 3 individual components. Max. 29.7% TRR, 0.009 mg/kg.
j - Includes 4 individual components. Max. 7.1% TRR, 0.025 mg/kg.
k - Includes 4 individual components. Max. 8.1% TRR, 0.016 mg/kg.
l - Includes 3 individual components. Max. 4.1% TRR, 0.003 mg/kg.

Table 7-135 Distribution of [^{14}C]bixlozone and its metabolites in wheat straw and grain (from the acid hydrolysed fractions of the initially solvent extracted residues) following application to bare soil at around 300 g as/ha. (mg/kg = mg parent eq./kg)

Metabolite	Wheat straw						Wheat grain					
	30 DAT		120 DAT		310 DAT		30 DAT		120 DAT		310 DAT	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
	Phenyl label											
Total TRR by combustion (mg/kg)	**0.227**		**0.230**		**0.032**		**0.037**		**0.033**		**0.009**	
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
2,4-Dichlorobenzoic Acid (M190/1)	8.0	0.018	7.3	0.017	11.6	0.004	29.6	0.011	24.2	0.008	-	-
bixlozone-dimethyl-malonamide (M289/2)	3.3	0.008	-	-	13.9	0.004	11.5	0.004	9.4	0.003	-	-
bixlozone-hydroxy-isobutyramide (M261/1)	6.2	0.014	4.0	0.009	3.9	0.001	-	-	-	-	-	-
3'-hydroxy-bixlozone (M289/6)	3.2	0.007	6.6	0.015	-	-	-	-	-	-	-	-
4-hydroxy-methyl - bixlozone (M289/4)	4.1	0.009	2.9	0.007	-	-	-	-	-	-	-	-
5'-hydroxy-bixlozone (M289/3)	31.7	0.072	41.4	0.095	19.4	0.006	-	-	-	-	-	-
Total unknown	22.9[a]	0.051[a]	12.9[b]	0.029[b]	13.2[c]	0.003[c]	14.5[d]	0.006[d]	18.8[e]	0.007[e]	-	-
Total 'identified'	**56.5**	**0.128**	**62.2**	**0.143**	**48.8**	**0.015**	**41.1**	**0.015**	**33.6**	**0.011**	-	-
Final non-extracted/ PES	5.3	0.012	7.3	0.017	10.7	0.003	11.9	0.004	2.1	0.001	-	-
	Carbonyl label											
Total TRR by combustion (mg/kg)	**0.359**		**0.339**		**0.107**		**0.019**		**0.016**		**0.007**	
bixlozone	-	-	-	-	-	-	-	-	-	-	-	-
Dimethyl-malonic acid (M132/1)	5.4	0.019	33.7	0.114	27.6	0.029	-	-	-	-	-	-
bixlozone-hydroxy-isobutyramide (M261/1)	6.5	0.023	9.7	0.033	7.5	0.008	-	-	-	-	-	-

4-hydroxy-methyl - bixlozone (M289/4)	4.5	0.016	2.5	0.009	7.5	0.008	-	-	-	-	-	-
5'-hydroxy-bixlozone (M289/3)	36.6	0.132	33.1	0.112	30.8	0.033	-	-	-	-	-	-
Total unknown	32.8[f]	0.118[f]	7.6[g]	0.026[g]	4.1[h]	0.004[h]	-	-	-	-	-	-
Total 'identified'	**53.0**	**0.190**	**79.0**	**0.268**	**73.4**	**0.078**	-	-	-	-	-	-
Final non-extracted/ PES	5.9	0.021	6.5	0.022	10.2	0.011	-	-	-	-	-	-

' - ' considered but not detected (no associated radioactivity)

a - Includes 7 individual components. Max. 3.9% TRR, 0.009 mg/kg.
b - Includes 3 individual components. Max. 7.1% TRR, 0.016 mg/kg.
c - Includes 3 individual components. Max. 4.4% TRR, 0.001 mg/kg.
d - Includes 3 individual components. Max. 10.0% TRR, 0.004 mg/kg.
e - Includes 5 individual components. Max. 8.6% TRR, 0.003 mg/kg.
f - Includes 5 individual components. Max. 10.7% TRR, 0.039 mg/kg.
g - Includes 2 individual components. Max. 5.8% TRR, 0.020 mg/kg.
h - Includes 1 individual component.

Storage stability investigations- results

No storage stability comparisons were made within this rotational crop metabolism study (e.g. chromatogram comparisons for extracts analysed initially and later on in the study).

Translocation and proposed metabolic pathway

Different to the primary crop metabolism, parent bixlozone was found in the rotational crop metabolism, indicating the potential for uptake from the soil of bixlozone, whereas following primary crop application (to early post emergence crops), parent bixlozone was completely metabolised before harvest (section B.7.2).

The unchanged parent bixlozone was detected in rotational crop lettuce (small amounts) and rotational radish roots and tops.

Higher percentages of parent bixlozone were detected in the radish root samples compared to the tops. A higher level of metabolic breakdown is observed in the tops of the plant. The total radioactive residue uptake and translocation into the roots amounted to 0.049 mg/kg and 0.055 mg/kg for the 30 DAT phenyl and carbonyl labels respectively. In lettuce samples, parent bixlozone was detected at very low levels (0.002 mg/kg) in one sample only (immature lettuce, 30 DAT). Similarly, to the pattern observed in primary crop metabolism, the unchanged parent bixlozone was not detected in any of the rotational crop wheat commodities analysed showing that the compound is readily metabolised into various metabolite compounds. The total radioactive residue uptake and translocation into wheat grains amounted to only 0.037 mg/kg and 0.019 mg/kg for the 30 DAT phenyl and carbonyl labels respectively.

In lettuce samples, the main route of degradation was oxidative ring opening of the isoxazolidin-3-one ring in the parent compound, to form 2,4-dichlorobenzoic acid (M190/1) and dimethyl malonic acid (M132/1). A major metabolite route involved the formation of the metabolite bixlozone-dimethyl-malonamide (M289/2). Hydroxylation and subsequent conjugation of bixlozone also resulted in the dihydroxy-bixlozone conjugate (M467/1).

In radish samples, unchanged parent bixlozone was detected in all samples with the exception of the 120 DAT phenyl labelled tops sample. In the 30 DAT phenyl labelled root sample, bixlozone accounted for all of the identified radioactivity (75% TRR, 0.037 mg/kg) suggesting limited metabolism within radish roots. In tops the main route of degradation was via oxidative ring opening of the isoxazolidin-3-one ring in the parent compound, to form the metabolites, 2,4-dichlorobenzoic acid (M190/1) and dimethyl malonic acid (M132/1). Metabolite bixlozone-hydroxy-isobutyramide (M261/1) was also formed in the metabolic pathway. In the roots samples the metabolism was more limited with mainly only 2,4-dichlorobenzoic acid (M190/1) and dimethyl malonic acid (M132/1) observed.

For wheat grain, the main route of degradation in the phenyl labelled samples was oxidative ring opening of the isoxazolidin-3-one ring and cleavage of the parent compound, to form the metabolite 2,4-dichlorobenzoic acid (M190/1). Bixlozone-dimethyl-malonamide (M289/2) was again formed.

In wheat forage, hay and straw the most significant metabolic conversion of the parent compound is by hydroxylation in the 5'-position of the dichloro-phenyl ring to form the metabolite 5'-OH-bixlozone (M289/3). The metabolites formed upon oxidative ring opening of the isoxazolidin-3-one ring (M132/1 and M190/1) were also formed. This metabolic pathway is consistent with the pattern observed in primary wheat metabolism (although metabolite M132/1 was not thoroughly investigated in primary crop wheat metabolism samples, and the main plant metabolite in the primary crops, M118/1, 2,2-dimethyl-3-hydroxy propionic acid, was not thoroughly investigated in the rotational crop metabolism work it seems (seem bullet point remarks below)).

The applicant's proposed metabolic pathway is included in Figure 7-29.

Conclusion

The metabolism of bixlozone was investigated in rotational crops by applying phenyl-labelled or carbonyl-labelled bixlozone to bare soil contained in a confined cropping situation (wooden boxes). The application rate of the study was 300 g as/ha. A sample of immature lettuce suffered phytotoxicity and instead a sample of immature lettuce from a lower dose regime (100 g as/ha) was analysed instead. No further samples from the low dose regime were analysed.

The rotational crop metabolism study involved planting crops representative of the three following crop groupings: leafy vegetables (lettuce), root and tuber vegetables (radish) and small grains (wheat) at various plant back intervals (30, 120, 310 DAT). The application to bare soil was made at around 300 g a.s./ha, this is equivalent to the maximum seasonal use rate compared to the GAP on oilseed rape, and 1.5N compared to the GAP for cereals, however the rate in this trial does not cover the seasonal use rate in maize (0.8N compared to the GAP of 350 g a.s./ha). When accounting for an anticipated soil plateau concentrations after year on year use, as well as the maximum intended seasonal application, the application rate of 300 g as/ha is a little underdosed (0.9N for wheat and barley GAP), and somewhat further underdosed for the other intended uses (0.6N for oilseed rape GAP and 0.5N for the intended maize GAP), as explained at the start of this rotational crop metabolism study evaluation.

In the 30 and 120 DAT plots for lettuce, phytotoxicity effects were observed and additional seeds were planted in these areas 33 days after the initial planting. These plots are therefore representative of 63 and 153 DAT respectively.

For both labels, solvent extractability was fairly high for all samples (at least 67% TRR, with much higher levels of extractability for many of the commodity matrices) with the exception of wheat grain where extractability was ≈45-55% TRR (0.008-0.021 mg/kg).

Enzyme hydrolysis using various enzymes sequentially of the PES was carried on some of the wheat, lettuce and radish samples to varying degrees. Also acid and base treatments were used in wheat samples, and base hydrolysis was found to be the most successful method of releasing radioactivity from the PES, 1N and 6N NaOH treatments.

Despite efforts to release and identify radioactive residues, a large number of extracted residues remained as unknowns especially in commodities such as wheat hay and straw (that are not consumed directly by humans). Taken together they could represent appreciable amounts of radioactivity. The applicant has indicated the levels of maximum individual unknowns, and some of these did represent >10%TRR (please see the breakdown for 'unknowns' below the metabolite distribution tables for further details). For crop items that can be consumed directly by humans the maximum unidentified levels were very low and all were <0.01 mg/kg and for crop items that are animal feed items the maximum individual levels were generally low, and at a maximum level of 0.04 mg/kg.

Metabolism of bixlozone in rotational crops includes primarily, oxidative ring opening. Hydroxylation and conjugation were also observed. Unchanged parent bixlozone was detected in radish roots and tops, accounting for the largest proportion of TRR in most samples (~17-76% TRR). In lettuce samples the metabolite M289/2 accounted for the highest proportion of the radioactive residue in phenyl labelled samples (accounting for ~27-46% TRR) whereas in carbonyl labelled samples the highest proportion of the residue was accounted for by metabolite M132/1 (~27-47% TRR). A very low amount of unchanged parent bixlozone was found in lettuce (0.002 mg/kg). Similar to the primary crop metabolism, parent was not found in wheat. Also as per the primary crop situation, wheat forage, hay and straw, contained metabolite M289/3 which accounts for the main proportion of the radioactivity (~16-43% TRR). M132/1 was also detected in high proportions (~5-44% TRR). In wheat grain M289/3 is not detected, and the major metabolite detected was M190/1 (accounting for 25-30% TRR).

Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in three representative rotational crops. Overall, metabolism of bixlozone in rotational crops is considered reasonably well-elucidated, even though a number of low level metabolites could not be identified. HSE has the following remarks/observations based on this rotational crop metabolism study:

- There were a number of analytical challenges noted in this work. Some of the metabolite identification was uncertain. For one commodity, M132/1 (e.g. in hay) was allocated to two different peaks, which were summed to give a total amount of M132/1. The peaks had different retention times of 2.88-3.38 mins and 4.38-5.38 mins. The applicant has stated that the split peaks and shifts in retention time observed in the chromatography were due the polar nature of the metabolite M132/1 and the similarity in pH between the metabolite and the mobile phase. In working with low levels of radioactivity in lettuce, and the assignation of this component as M190/1, the applicant noted a possible shift in retention time due to high matrix or pH of mobile phase. Peaks at with different retention times of 13.38-13.88 min and 17.13-17.88 min (which represents a marked shift in RT) were summed to give a level of M190/1. This evaluation has presented the details to indicate some of the analytical difficulties and uncertainties.
- A number of metabolites have been only characterised in terms of their extractability and availability in the acid hydrolysed fraction of the initial solvent extract. The study did not present distributional data for before the acid hydrolysis of the solvent extract. It is noted that in other primary crop metabolism studies, that the acid hydrolysed fractions have 'revealed' more metabolites compared to the analysis of

the organic solvent extracts prior to the acid hydrolysis. It is suspected that treatment of the extract with acid is enabling conjugates to 'de-conjugate' and show 'new' residues (not previously seen in the unhydrolyzed extract). This rotational crop metabolism study has anyhow aimed to present detailed information on the acid hydrolysed fraction of the initial extract with the intention of identifying the candidate residues.

- In some/many crop fractions, there is a large proportion not identified (as extracted and non-assigned residues). For example, in some of the wheat hay and wheat forage samples there are roughly equal proportions extracted but unassigned radioactive residues compared to the amounts of identified (extracted) residues. Whilst in other commodities there are better proportions identified, there remains large numbers unknown. These have then been described to the extent that they are characterised as a large number of unidentified and small individual unknown peaks of radioactivity. In crop fractions that can be consumed by humans the largest 'unknown' was <0.01 mg/kg, and in fractions consumed by animals in feed items (and not humans) the largest 'unknown' was 0.04 mg/kg. Fractions may contain a mixture of polar metabolites of bixlozone (some might be low molecular weight components) and that identification of these is analytically challenging. It is possible that different fractions containing unknowns could contain the same metabolite (but found across different fractions).

- In terms of the PES (non-extracted radioactivity), a number of successive extractions and treatments (including enzyme/acid/base) seemed to release radioactivity so individual fractions (that were in the initial post extraction solids) were well characterised. In this study 1N and 6N base hydrolysis and alpha-amylase extractions were some of the most successful approaches that aided stepwise release of radioactivity.

- The metabolite 2,2-dimethyl-3-hydroxy propionic acid (M118/1) that was sought and found in primary crop metabolism and cereal crop field trials at significant levels is not reported as a residue in the rotational crop metabolism study or written up in terms of the extent to which it was investigated in the rotational crop metabolism study. A reference standard for M118/1 was used in the initial HPLC 'scoping' prior to onward analysis of the rotational crop samples. It is not reported in the metabolite distributional tables as a metabolite but the applicant does mention that it has been found in samples (only in the way of the excerpt stated below). The following excerpt from the rotational crop metabolism study (the only mention of the detection of M118/1) also indicates the challenges with trying to identify residues when they might involve a number of smaller polar molecules:

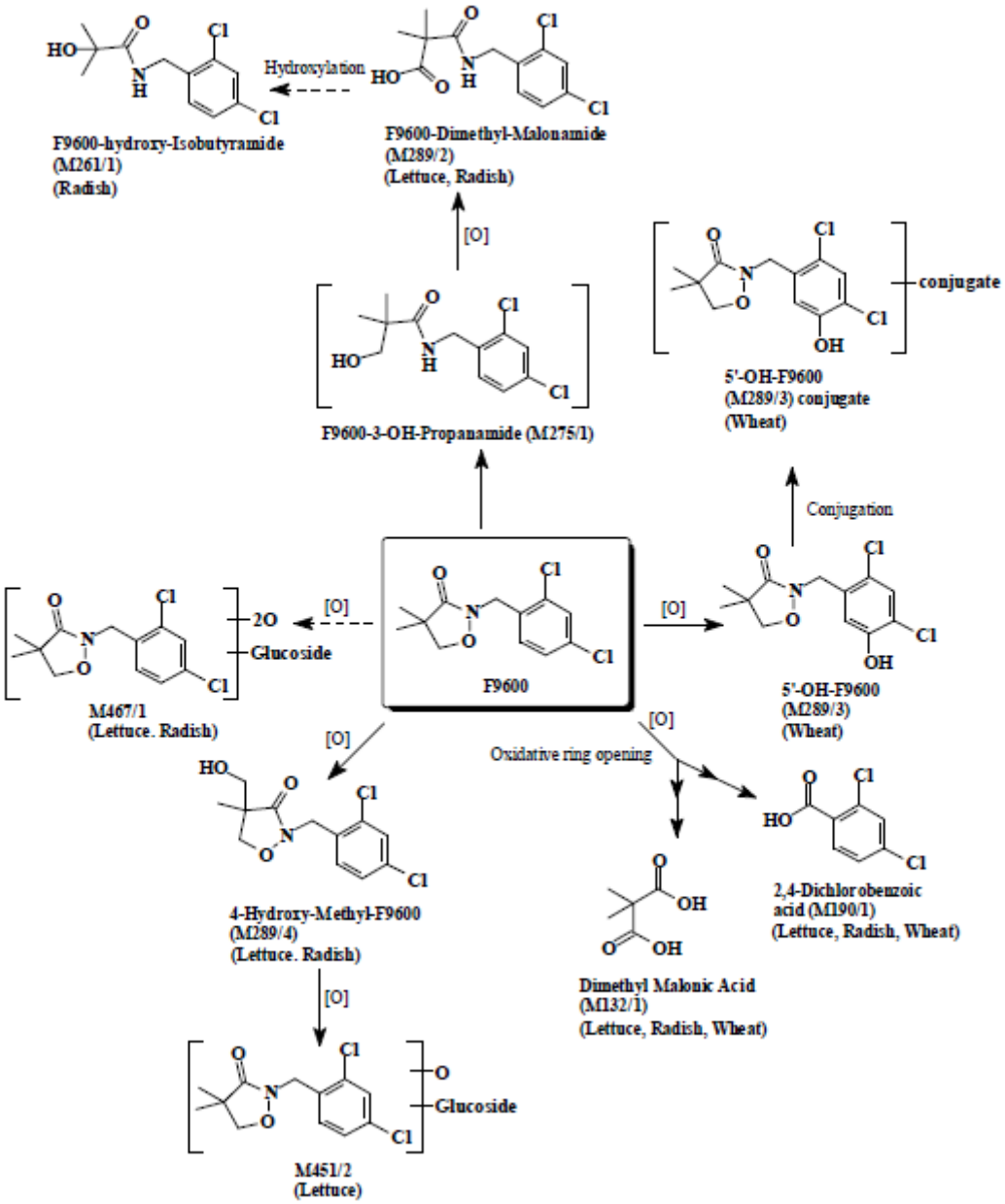
  > "2,4-dichlorobenzaldehyde appears to be oxidized to 2,4-dichlorobenzoic acid which is observed in [14C-Phenyl]F9600 label samples. Isoxazolidinone moiety may undergo oxidation, reduction and/or hydrolysis to form various pivalic acid derivatives which can oxidize and decarboxylate easily under the conditions used for hydrolysis of the conjugates. Metabolism of pivalic acid is known to produce a variety of small acids such as lactic acid and acrylic acid (Rezanka et.al., 2012). These small molecules would be expected to be polar nature. Therefore, the radioactive polar peaks seen in the chromatograms of [14C-Carbonyl]F9600 label samples are proposed to be these small molecular acids. In fact, some of these molecules such as OH-pivalic acid (2,2-dimethyl-3-hydroxypropionic acid) and dimethylmalonic acid have been identified in [14C-Carbonyl]F9600 label samples. The polar radioactive peaks that have not been labeled in the chromatograms of [14C-Carbonyl]F9600 label samples are proposed to be the compounds derived from these acids. They can readily form conjugates and also be incorporated into natural products. Since these small molecule acids, four carbon or fewer size, are devoid of any chromophores and would elute near the void volumes of the HPLC columns, they would not be amenable for spectrometric detection or structure elucidation. These small molecules are natural products (Rezanka, et al, 2012) and therefore be considered benign in nature."

- The above mentioned paper was provided by the applicant (Rezanka, et al, 2012) and describes the role of pivalic acid and related derivatives in the biosynthetic pathway of bacteria. A further discussion of the proposal that this component might be a natural residue is provided in section 2.7.4 of Vol 1 (field trials section).

- A similar metabolite (M132/1, dimethyl malonic acid) to 2,2-dimethyl-3-hydroxy propionic acid (M118/1) was sought and found in the rotational crops metabolism study as a main metabolite. Conversely M132/1 was not so well investigated and reported in all the plant metabolism studies, but it was analysed and found in rice grain.

- The work has enabled a profiling of an overall metabolic pathway suitable for rotational crops, which shows consistency across the rotational crops studied (and with similarities to the primary crop metabolism pathways). The study has been useful for identifying marker compounds to include in the analysis of the rotational crop field trials (see Vol 1 section 2.7.3 for a discussion regarding this). The rotational crop field study covered the following main analytes found in the rotational crop metabolism which includes a good selection of marker metabolite components: bixlozone, M190/1 (2,4-dichlorobenzoic acid), M289/2 (bixlozone-dimethyl-malonamide), M261/1 (bixlozone-hydroxy-isobutyramide), M289/4 (4-hydroxymethyl-bixlozone) and M289/3 (5'-hydroxy bixlozone). The rotational crop metabolite dimethyl malonic acid (M132/1) was not included in the following field trials.

Figure 7-2930 Applicant's proposed metabolic pathway of bixlozone (F9600) in rotational crops



B.7.6.2. Magnitude of residues in rotational crops

Evaluation status:	**New data, submitted for purpose of first approval in GB**
Report:	CA 6.6.2-01, Semrau, J., 2018
Title:	Determination of residues of F9600 in rotated crops (radish, leaf lettuce, wheat) after one application of F9600-4 SC in maize at 1 site in Northern Europe and 1 site in Southern Europe 2016/2017
Report No.:	S16-01156
Guidelines:	OECD Test Guideline 509: crop field trials, OECD Test Guideline 504: Residues in rotational crops.
GLP	yes

Materials and Methods

During the growing season of 2016-2017, two outdoor field trials on three rotational crops (wheat, radish and lettuce) using the formulated product 'F9600-4 SC', an SC formulation containing 396 g/L bixlozone (the representative formulation being considered) were conducted in Germany (N EU zone), and Spain (S EU zone). Each trial consisted of five control and five treated plots at least 10 m apart. The crops and plant back intervals in each plot are shown in Table 7-136.

Table 7-136 Crops and plant back intervals

Plot	Treated/ untreated	Plant back interval (approx.)	Rotational crop
1	Untreated	30 days	Radish, Leaf lettuce
2		30 days	Wheat
3		60 days	Radish, Leaf lettuce
4		60 days	Wheat
5		220 days	Radish, leaf lettuce and wheat
6	Treated	30 days	Radish, Leaf lettuce
7		30 days	Wheat
8		60 days	Radish, Leaf lettuce
9		60 days	Wheat
10		220 days	Radish, leaf lettuce and wheat

The primary crop was maize in all plots. To all treated plots, the formulation was applied once as a foliar spray using a boom sprayer at a rate of 300 g a.s./ha (to maize at BBCH growth stage 11-13) in a spray volume of 150-400 L/ha. Using the standard FOCUS crop interception values, as suggested in the OECD guidance on residues in rotational crops, for application to maize at BBCH 10-19, 25% crop interception should be considered when considering the amount of active substance reaching the soil. In this case, the application was made to maize at BBCH 11-13, therefore an interception value of 10% is considered a more realistic estimate. BBCH 19 represents nine or more leaves unfolded and BBCH 11-13 represents one to three leaves unfolded, both in the 'leaf development stage' (principal growth stage 1) which follows 'germination stage' (principal growth stage 0), which ends at BBCH 09, 'emergence' (when coleoptile penetrates soil surface). Comparison of the application rate in this study to the proposed maximum per season rate for bixlozone in cereals and oilseeds is shown in Table 7-137.

Table 7-137 Comparison of application rate to proposed maximum seasonal application rate and soil plateau concentration

Proposed crop (GAP intended use)	Proposed maximum seasonal application rate (g a.s./ha)	Application rate required to achieve: (g a.s./ha)		Application rate in rotational field trial (g a.s/ha)	Application rate in rotational field trial considering 10% crop interception (g a.s/ha)	N rate (application rate in trial compared to maximum seasonal application rate)	N rate (application rate in trial compared to soil plateau plus maximum seasonal application rate)
		Soil plateau concentration $A_{plateau}$ - £	Soil plateau plus maximum seasonal application rate A_{total} - £				
Wheat	200	112.04	312.04	300	270	1.35	0.87
Barley	200	112.04	312.04	300	270	1.35	0.87
Maize	375	210.07	585.07	300	270	0.72	0.46
Oilseed rape	300	168.06	468.06	300	270	0.9	0.58

£ The fate and behaviour evaluation derivations of $A_{plateau}$ and A_{total} are provided in section CP.B.8.2.2

Before planting of the rotational crops with a plant back interval of 30 days, the primary crop (maize) was fully incorporated into the soil. At the planting of the crops with 60 and 220 day plant back intervals, the primary crop (maize) was cut above the soil and removed. The remaining stems were incorporating into the soil.

Crop samples were collected as outlined in Table 7-138. Control specimens were collected prior to collection of the treated specimens to avoid contamination.

Table 7-138 Sampling parameters of the rotational crop study

		Sampling event no.1	*Sampling event no.2*
Rotational crop (sampling timing, sample size)	wheat	BBCH 75-77 Hay (0.5 kg)	BBCH 89 grain (1 kg) straw (0.5 kg)
	radish	-	BBCH 49 root (2 kg, 12 pieces) top (1 kg, 12 pieces)
	lettuce	BBCH 42-43 leaf (1 kg, 12 pieces)	BBCH 49 leaf (1 kg, 12 pieces)

Weather data were reported for the trials and no exceptional events were noted. Normal agricultural practice was followed during the field stage. Samples weighing at least 1 kg were collected for mature plants, and samples weighing at least 0.5 kg were collected for immature plants, wheat grain and wheat straw, from an adequate number of places over the plot (12). The same active substance was not applied as a maintenance product during the trials or in the previous season. As explained for the primary crop field trials, clomazone was also not applied during the trials or in previous seasons (for further explanation see section B.7.3)

Samples were stored frozen at ≤-18 °C within 7 hours of harvest and maintained at this temperature until extraction. Samples of the RAC were stored frozen for up to 559 days. There are sufficient storage stability data reported under section 7.1 and summarised in Volume 1 to support this time period of storage for radish root, leaf lettuce, wheat grain and straw, for each of the analytes analysed. Samples were stored for up to 7 days under refrigerated conditions (1 – 10°C) between extraction and analysis. The stability of these extracts was confirmed by the procedural recoveries performed in parallel with the field sample analysis. Procedural recovery samples were stored under the same conditions for the same amount of time and gave acceptable results.

Samples were analysed for bixlozone, and its metabolites 2,4-dichlorobenzoic acid (M190/1), 5'-hydroxy-bixlozone (M289/3), bixlozone-dimethyl-malonamide (M289/2), bixlozone-hydroxy-isobutyramide (M261/1, also termed bixlozone-dimethyl-isobutyramide) and 4-hydroxymethyl-bixlozone (M289/4) using method number CAM-0180/001. Full details of the sample preparation and validation data are given in DAR CA B5 Section

B.5.2.1, where the method is considered fully validated in accordance with SANCO/3029/99 rev. 4. The LOQ for bixlozone, 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone, bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone is 0.01 mg/kg across the matrices of interest to this rotational crop field trial.

Details of the procedural recoveries are given in Table 7-139. Only procedural recoveries for radish leaves and roots are presented as the procedural recoveries and validation recoveries were identical within the study, with the exception of these analytical sets on radish root and leaves. A full consideration of the recoveries determined from lettuce, wheat grain and straw are presented in DAR Volume 3 B5 CA, section B.5.2.1. Mean procedural recoveries were all within the acceptable range (70-110%).

Table 7-139 Procedural recoveries for bixlozone and its metabolites in radish leaves and roots.

Sample material	Fortification level	Individual recoveries (%)	Mean recovery (%)	%RSD (n)
bixlozone, Mass Transition 274 → 159 m/z (quantification)				
Radish (leaves)	0.01	98; 86; 99; 88; 83	91	8.0 (5)
	0.10	95; 94; 89; 92; 99	94	3.9 (5)
Radish (roots)	0.01	68; 77; 82; 72; 67; 75	74	7.7 (6)
	0.10	81; 82; 80; 70; 72; 76	77	6.5 (6)
2,4-dichlorobenzoic acid, Mass Transition 189 → 145 m/z (quantification)				
Radish (leaves)	0.01	85; 78; 84; 81; 81; 94	84	6.6 (6)
	0.10	76; 76; 76; 70; 76; 91	78	9.1 (6)
Radish (roots)	0.01	92; 78; 90; 88; 86; 103	90	9.2 (6)
	0.10	99; 101; 98; 101; 90 ;93	97	4.7 (6)
5'-hydroxy-bixlozone, Mass Transition 290 → 175 m/z (quantification)				
Radish (leaves)	0.01	72; 68; 71; 76; 69; 87	74	9.5 (6)
	0.10	79; 83; 78; 85; 79; 81	81	3.4 (6)
Radish (roots)	0.01	82; 95; 102; 81; 82; 89	89	9.7 (6)
	0.10	91; 107; 109; 101; 96; 80	97	11.0 (6)
bixlozone-dimethyl-malonamide, Mass Transition 290 → 216 m/z (quantification)				
Radish (leaves)	0.01	69; 73; 76; 75; 70; 94	74	12.0 (6)
	0.10	81; 85; 85; 84; 85; 89	81	3.0 (6)
Radish (roots)	0.01	94; 95; 97; 86; 81; 81	89	8.1 (6)
	0.10	97; 105; 101; 99; 95; 82	97	8.2 (6)
bixlozone-hydroxy-isobutyramide, Mass Transition 262 → 159 m/z (quantification)				
Radish (leaves)	0.01	82; 83; 81; 88; 71; 78	81	7.1 (6)
	0.10	86; 85; 82; 84; 82; 79	83	3.0 (6)
Radish (roots)	0.01	80; 89; 85; 89; 84; 73	83	7.3 (6)
	0.10	83; 89; 87; 89; 88; 72	85	7.8 (6)
4-hydroxymethyl-bixlozone, Mass Transition 262 → 159 m/z (quantification)				
Radish (leaves)	0.01	75; 74; 76; 68; 65; 75	72	6.3 (6)
	0.10	79; 78; 75; 74; 87; 76	78	6.0 (6)
Radish (roots)	0.01	90; 93; 98; 93; 90; 97	94	3.6 (6)
	0.10	101; 108; 101; 100; 97; 81; 98; 99; 109; 106; 106	100	7.6 (11)

Results and Discussion

Field trials were conducted with the rotational crops: wheat, radish and lettuce, planted at approximately 30, 60 or 220 days after foliar spray to the primary crop maize with 300 g a.s./ha bixlozone.

Samples were taken at BBCH 49 for radish roots and leaves, BBCH 42-43 (immature) and BBCH 49 (mature) for lettuce and at BBCH 77 (hay) and BBCH 89 (straw and grain) for wheat. Residues trials data are presented in Table 7-140.

In untreated samples no residues of bixlozone and its metabolites 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone, bixlozone-dimethyl-malonamide (M289/2), bixlozone-hydroxy-isobutyramide (M261/1) and 4-hydroxymethyl-bixlozone (M289/4), exceeding the LOQ (0.01 mg/kg) were detected.

For all crop groups, cereals, root/tuber and leafy vegetables, residue levels of bixlozone and its metabolites 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone, bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone in samples from the treated plots were below the LOQ of 0.01 mg/kg at all plant back intervals, with the exception of two positive residues of bixlozone detected in the 229 day PBI samples for radish tops and immature lettuce leaves (0.013 and 0.011 mg/kg respectively).

Conclusion

Residue data was obtained for two independent field trials conducted with the rotational crops wheat, radish and lettuce, planted at approximately 30, 60 and 220 days after treatment on the primary crop maize with 300 g/ha of bixlozone. This application rate covers the maximal seasonal application rate for the intended GAPs of wheat, barley and oilseed rape, but is underdosed (0.8N) with regard to the maximum seasonal GAP rate proposed for maize. The context of the significance of residues in rotational crops is further discussed in Vol 1 also taking account of the potential for soil plateau contribution from multi-year use. Please refer to Vol 1, section 2.7.7.

Residues of the metabolites of bixlozone do not exceed the LOQ of 0.01 mg/kg for different representative succeeding crops at any plant back interval investigated. Low level residues of bixlozone were found at the 229 day PBI (radish tops 0.013 mg/kg and immature lettuce 0.011 mg/kg).

Table 7-140 Residues of bixlozone and its metabolites in rotational crops following treatment of the primary crop maize (at early leaf development stage, BBCH 11-13) at 300 g a.s./ha

Trial	Crop	Commodity	PBI	Application rate (g a.s./ha)	Growth stage at sampling (BBCH)	Residues (uncorrected, mg/kg)					
						bixlozone	2,4-dichloro benzoic acid (M190/1)	5'-hydroxy-bixlozone (M289/3)	bixlozone-Dimethyl malonamide (M289/2)	bixlozone-OH-isobutyramide (M261/1)	4-OH-methyl-bixlozone (M289/4)
S16-01156-01, Saxony, Germany	Radish	Leaves	27	310.3	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			56	305.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		0.013	<0.01	<0.01	<0.01	<0.01	<0.01
		Roots	27	310.3	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			56	305.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Lettuce	Immature leaves	27	310.3	42-43	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			56	305.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		0.011	<0.01	<0.01	<0.01	<0.01	<0.01
		Mature leaves	27	310.3	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			56	305.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Wheat	Hay	34	291.8	77 (after drying)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			60	289.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	34	291.8	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			60	289.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	34	291.8	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			60	289.8		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			229	287.7		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Trial	Crop	Commodity	PBI	Application rate (g a.s./ha)	Growth stage at sampling (BBCH)	Residues (uncorrected, mg/kg)					
						bixlozone	2,4-dichloro benzoic acid (M190/1)	5'-hydroxy-bixlozone (M289/3)	bixlozone-Dimethyl malonamide (M289/2)	bixlozone-OH-isobutyramide (M261/1)	4-OH-methyl-bixlozone (M289/4)
S16-01156-02, Aragon, Spain	Radish	Leaves	40	313.4	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			69	309.2		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Roots	40	313.4	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			69	309.2		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Lettuce	Immature leaves	40	313.4	42-43	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			69	309.2		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Mature leaves	40	313.4	49	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			69	309.2		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Wheat	Hay	35	289.1	75-77 (after drying)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			63	316.9		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Grain	35	289.1	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			63	316.9		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Straw	35	289.1	89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			63	316.9		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			230	313.1		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

HSE remark: A good range of potential metabolites (as informed by the rotational crop metabolism study) have been studied in addition to parent bixlozone. It should be noted that the primary crop metabolite 2,2-dimethyl-3-hydroxy propionic acid was not studied in these field trials (nor extensively considered in the rotational crop metabolism study, see bullet point remarks at the end of section B.7.6.1). The application rate used in the field studies is underdosed considering the maximum seasonal application rate for maize and the contribution of potential build-up of residues of bixlozone in the soil from multi-year use. See Volume 1 for a further consideration of this. Considering this underdosing and the low positive residues of bixlozone found in leafy vegetables, the potential for low residues of parent bixlozone should be considered in the risk assessment (see Volume 1). In any future rotational crop field trials, parent bixlozone appears to be the most suitable indicator analyte to explore the potential for soil residues to be taken up into crops grown in rotation. However, if significant residues of bixlozone are found, it may also be necessary to consider the potential for some metabolite residues to be also found. Please see Volume 1 for further discussion.

B.7.6.3. Conclusion on rotational crops

To investigate residues in rotational crops, a nature of the residue study and a magnitude of the residue study have been conducted in different crops representing three different crop categories, namely leafy vegetables, root and tuber vegetables and cereals.

In the nature of residues study, bixlozone, either phenyl labelled or carbonyl labelled, was applied at around 300 g a.s./ha to bare soil in a confined rotational crop setting. Rotational crops of lettuce, radish and wheat were grown in plastic lined wooden boxes containing soil into which the rotational crops were planted and different replant intervals ranging from 30 to 310 days after treatment (DAT). The work has enabled a profiling of an overall metabolic pathway suitable for rotational crops.

Based on the applicant's proposed metabolic pathway, whilst some differences are observed the main patterns of metabolism are the same as those observed in the primary crop metabolism. Oxidative ring opening was a prime metabolic route in rotational crops, leading to formation of M132/1 (dimethyl malonic acid) in rotational crops, In the rotational crop metabolism, parent bixlozone was also found, and hydroxylation and conjugation was also observed. Main rotational crop residues observed in the metabolism study also included M190/1 (2,4-dichlorobenzoic acid), M289/3 (5'-hydroxy-bixlozone), M289/2 (bixlozone-dimethyl-malonamide) and M261/1 (bixlozone (bixlozone)-dimethyl-isobutyramide, also termed bixlozone hydroxy isobutyramide).

In the magnitude of residues study, bixlozone was applied at 300 g a.s./ha to the primary crop maize. Considering 10% crop interception from this primary crop, the amount of active substance estimated to reach the soil is 270 g a.s./ha. The rotational crops were cultivated after intervals of approximately 30, 60 and 220 days, samples were taken at both mature and immature growth stages (with the exception of radish which was only samples at mature growth stages). A good range of metabolites from the rotational crop metabolism study were investigated in the field trials. The application rate, whilst covering the maximum seasonal rate for the intended uses of wheat and barley (200 g a.s./ha), does not cover the intended primary crop application rate for the oilseed rape (300 g a.s./ha) or maize GAPs (375 g a.s./ha). Please refer to Volume 1 which discusses the significance of residues in rotational crops also taking account of the potential soil plateau rate of bixlozone considering the potential for multi-year use.

Based on results obtained in the nature of the residue study conducted with two labels (Phenyl-label, carbonyl-label) and the follow up studies on magnitude of the residues, the possibly anticipated low level residue in rotational crops arising from the proposed GAP uses is parent bixlozone. Please refer to Volume 1 for a full discussion. In one field trial, no residues of parent or metabolites was found. In the other trial, low positive residues of parent bixlozone were found (up to 0.013 mg/kg in leafy crops); no residues of metabolites were found. The available rotational crop studies have not investigated a large range of rotational crops, but the positive residues found are low. In any future rotational crop field trials, parent residues are expected to be a main indicator of the possible expectation of residues in rotational crops. However, if significant residues of parent bixlozone are found, it may also be necessary to consider the potential for some metabolite residues to be also found. Please see Volume 1 for further discussion.

B.7.7. OTHER STUDIES

No additional studies have been provided.

B.7.7.1. Effect on the residue level in pollen and bee products

The applicant provided the following case:

'The intended uses/GAPs all involve early pre- and post-emergence application (BBCH 00-13) i.e. prior to flowering. Therefore residue studies in pollen and bee products are not considered necessary.'

At the date of submission (29/06/2018) there were no agreed EU guidance documents or test methods to address these data requirements. Since submission, the Technical Guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9), have been noted with an agreed implementation date of 1st January 2020. Please see Volume 1, section 2.7.8 for further discussion.

B.7.7.2. Literature Review

Overview

The applicant submitted a literature review in support of bixlozone. HSE considers that acceptable search criteria have been applied to this literature review when considering the residues and dietary exposure areas. From the available review, no issues were identified that would impact on the regulatory residues/consumer risk assessment. No studies were considered relevant in the field of residues (and dietary exposure) and therefore no reliability criteria needed to be applied. The number of studies 'returned' by the search seems relatively low, likely due to bixlozone being a new active substance.

More detailed evaluation

The applicant provided a review for bixlozone (and its metabolites, see below metabolites 1 to 14) in accordance with the EFSA Guidance (EFSA Journal 2011; 9(2):2092).

The applicant did not produce any relevant published papers for additional evaluation by HSE for incorporation into this residues chapter. Therefore no papers from this literature review cited in the below list of 'references relied upon' relating to residues.

Literature review report for bixlozone: May, 2018. Exponent Report conducted on behalf of FMC. Exponent QAID: 1508442.UK0 – 5012 FMC Tracking Number: 2018WHP-ISX4339.

Addendum to Literature review report for bixlozone. March 2020. Exponent Report conducted on behalf of FMC. Exponent QAID: 1508442.UK0 – 5415. FMC Tracking Number: 2018WHP-ISX4339-AD1

Search terms:

See the table below: the codes and IUPAC names were applied in the Dialog database searches; where available CAS numbers were used in the STN searches (Dialog search was performed for the active substance and 14 metabolites. STN search was done for active substance and 3 metabolites).

- bixlozone and other related names for the active substance (and the above named metabolites) were used.
- suitable terms relating to the assessment of residues were not used, presumably as the search on active substance and metabolites did not yield too many suggested papers or titles to assess

Active substance (bixlozone (F9600): IUPAC 2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one) and metabolites (14):

Code	IUPAC	CAS number
3-hydroxypropanamide-F9600	N-(2,4-dichlorobenzyl)-3-hydroxy-2,2-dimethylpropanamide	Not assigned
2,4-Dichlorobenzoic acid	2,4-dichlorobenzoic acid	50-84-0
5-Hydroxy-F9600	2-(2,4-dichlorobenzyl)-5-hydroxy-4,4-dimethylisoxazolidin-3-one	Not assigned
5'-Hydroxy-F9600	2-(2,4-dichloro-5-hydroxy benzyl)-4,4-dimethylisoxazolidin-3-one	Not assigned

Code	IUPAC	CAS number
6'-Hydroxy-F9600	2-(2,4-dichloro-6-hydroxy benzyl)-4,4-dimethylisoxazolidin-3-one	Not assigned
4-Hydroxy-methyl-F9600	2-(2,4-dichlorobenzyl)-4-(hydroxymethyl)-4-methylisoxazolidin-3-one	Not assigned
Dimethyl malonamide-F9600	3-((2,4-dichlorobenzyl)amino)-2,2-dimethyl-3-oxopropanoic acid	Not assigned
Dimethyl malonic acid	2,2-dimethylmalonic acid	595-46-0
F9600-isobutyramide	N-(2,4-dichlorobenzyl)isobutyramide	Not assigned
Hydroxy-Isobutyramide	N-(2,4-dichlorobenzyl)-2-hydroxy-2-methylpropanamide	Not assigned
3-hydroxypivalic acid	2,2-dimethyl-3-hydroxy propionic acid	4835-90-9
5-OH, 5'-OH Di-Hydroxy-F9600	2-(2,4-dichloro-5-hydroxy benzyl)- 5-hydroxy-4,4-dimethylisoxazolidin-3-one	Not assigned
4-carboxy-F9600	2-(2,4-dichlorobenzyl)-4-methyl-3-oxoisoxazolidine-4-carboxylic acid	Not assigned
2,4-Dichloroippuric acid	N-(2,4-dichlorobenzoyl)glycine	Not assigned

Search- methodology applied and databases used:

Time period of search: 1 January 2008 to 15 Feb 2018

The search strategy was based on a single-concept search (in both STN {STN Toxicology Database Cluster} and Dialog databases). Sources other than bibliographic databases, such as reference lists of full-text journal articles (e.g. reviews); journals' tables of contents; patents or websites of conferences or organisations, were not used. However the STN approach described and used by the applicant for the active substance and three of the metabolites is considered to be a comprehensive approach. It is used by a number of registrants in the undertaking of literature reviews.

Databases searched (these are regularly updated):

STN-DATABASES:
ANABSTR (Analytical abstracts)
BIOSIS (BIOSIS PREVIEWS®)
CABA (CA Abstracts)
CAplus (Chemical abstracts plus)
CAplus (Toxicology focus)
CAplus (Analytical chemistry focus)
Chemical Abstracts REGISTRY
EMBASE (Excerpta Medica)
ESBIOBASE (Elsevier Current Research in Biology and BioScience)
MEDLINE
NAPRALERT (Natural Products Alert)
RTECS (Registry of Toxic Effects of Chemical Substances)
SCISEARCH (Science Citation Index)
TOXCENTER (Toxicology Center produced by American Chemical Society CAS)

DIALOG DATABASES:
AGRICOLA
AGRIS
Aqualine
Aquatic Science & Fisheries Abstracts (ASFA)
Chemical Engineering and Biotechnology Abstracts
CSA Life Sciences Abstracts
Ecology Abstracts
ENVIROLINE®
Environment Abstracts
FSTA®
FOODLINE®: Science
GeoArchive
GEOBASE™
MEDLINE®
Meteorology & Geoastrophysical Abstracts.
Pollution Abstracts
ToxFile
Toxicology Abstracts
TOXLINE
Water Resources Abstracts

The Literature review report provided a justification for the use of each of the substituent databases. The following information was submitted in regard of each database cluster:

STN DATABASES *http://www.stn-international.de/database_list.html?&no_cache=1&cHash=*

DIALOG DATABASES

"Dialog is the premier online retrieval service with the most comprehensive content collection and most powerful search language available. Dialog is the worldwide leader in providing online-based information in science. The database holds data from more than 800 million unique records of key information, accessible via the Internet. Content areas include, but are not limited to, biomedical research, biotechnology, chemicals, environment, food and agriculture, medicine and science and technology."

A subset of the individual databases (Chemical Abstracts, Medline, SciSearch, and Agricola) are those that EFSA provided as a list of reliable databases {sent to MSs by EFSA in March 2015}. With the list of databases used by this applicant, the search covers a good range (e.g. including a global range). The STN and Dialog cluster database searches are efficient means of retrieval of papers from a large number of database searches.

The searching systems and algorithms employed by the search engines are different - the STN databases index by CAS number and items without the CAS indexing are picked up by the Dialog searches. Therefore Dialog search was performed for the active substance and 14 metabolites. STN search was done for active substance and 3 metabolites. The applicant has justified their strategy stating that the two search engines, Dialog and STN, each cover comprehensive sets of databases to capture publications relevant to human health and the environment as required according to the EFSA guidance.

Relevancy criteria:

Assessment of studies for relevance was carried out by reference to their titles and, if necessary, abstracts. Reviews of the relevance of the articles brought up in the literature search were carried out by experts in the relevant technical disciplines (e.g. residues specialist for 'Residues in or on treated products, food and feed'). The intention, if any papers were deemed relevant on the basis of abstract, was to obtain the full papers and to consider the full-text to further to determine whether the information contained in the study could impact on the endpoints and risk assessment parameters related to the active substance. In the case of this literature review no full papers needed to be assessed (papers were excluded on the basis of titles or abstracts). Although the EFSA 2011 Guidance indicates that full papers of those not excluded in the first step (rapid assessment referred to here), HSE agrees that it was possible to exclude the remaining papers on the basis of the abstracts alone, as they did not add anything to address data requirements or impact on the regulatory assessment for residues/consumer risk assessment.

The applicant provided information of their consideration of relevancy as follows:

Studies relevant to the dossier are those that inform one or more data requirement(s), including hazard identification, hazard characterisation and exposure assessment, including Residues in or on treated products, food and feed, for the active substance under assessment, its relevant metabolites, or plant protection products.

The AGES interpretation of the relevancy criteria (EFSA supporting document, 2013) also sets out aspects to do with all sub-sections of the residues work where data requirements exist (storage stability of residues, metabolism, processing etc). However for bixlozone it does not seem that any residues searches using residues key words were performed, since the search focussing on active substance and metabolites did not yield too many to search through as the initial search to narrow down yielded 37 papers only).

In the area of residues the guidance on relevancy criteria detailed used by the applicant was taken from AGES Interpretation[11] of EFSA's Guidance document:

- Studies dealing with any crop treated with the active substance are considered to be relevant and not just studies dealing with the representative crops only. A broader spectrum of relevant literature might therefore show useful information considering the MRL setting and MRL review program at EU level; 'real' residue data that do not reflect the representative uses might be considered separately 'for MRL setting'.

- Bibliographic databases may also contain useful information about minor uses. Such studies can be considered as relevant for inclusion into the dossier, although the chances of finding such studies in the literature databases are rather limited; residue trials performed on minor uses are usually conducted by growers' associations and those studies are usually not published in peer-reviewed journals.

- Genetically modified (GMO) crops are covered by the present search concept and the corresponding studies should be selected for further consideration.

- Monitoring studies are not considered data requirements for the review of the active substances. However, in some cases monitoring data can be the basis for MRL setting. Monitoring data could be included on a case-by-case basis, after careful consideration.

- For active substances that are used in stores and containers, cross-contamination may be an issue. This issue should be considered on a case-by-case basis.

Results and stepwise consideration:

[11] EFSA supporting publication 2013:EN-511

From the initial database searches 37 titles were retrieved (once duplicates were deleted).

Of these 28 records were excluded after rapid assessment of relevance (when considering title or abstract). The titles were listed in the report and HSE agrees with the exclusion of these for residues purposes. Thus, most publications were considered irrelevant after rapid assessment.

Therefore, nine were scrutinised further to consider their potential relevance (by way of referring to abstract rather than full papers). These were discounted by the applicant on this basis. This left no studies to consider the number of relevant/reliable studies according to the criteria of Klimisch *et al.* (1997). The title of these papers and abstracts were included in the literature review with a comment as to why they were not considered relevant. HSE agrees these papers to not add anything to address data requirements or impact on the regulatory assessment for residues/consumer risk assessment.

An addendum to the initial report was also submitted (following some enquiries bulleted below about the literature review):

- The search has been performed on a substance '2,4-Dichloroippuric acid'. However, the correct name of the metabolite is 2,4-dichlorohippuric acid. The applicant was requested to include the correct name of the metabolite in the literature search. The applicant addressed this in the addendum (there were no 'hits' using the revised correct name).

- The major urine metabolite carbamic acid was not included in the literature search.
  The applicant was requested to add this metabolite to the literature search. The literature review addendum covers this metabolite. The search terms employed were: Toxicity; rat; mouse; dog; rabbit; hamster; repeat dose; genotox*; mutagen*; carcinogen*; acute; irritation; chronic; toxicokinetics; reproduct*; development*; oncogen*; neurotox*; adverse; human; medical; endocrine; biotransformation; poison*; residue*; metabolism; plant; livestock; crop; goat; cow; hen; pig; bird; aquatic; fish; invertebrate; algae; sediment; bee; arthropod; earthworm; micro-organism; dietary; bioconcentration; sewage treatment; wildlife; reptil*; amphibian; soil; air; water; surface water; groundwater; degradation; aerobic; anaerobic; *sorption; mobility; hydro*; photo; biodegrad*; dissipation; accumulation; leaching; lysimeter; monitoring.
  Filters were applied to exclude document types such conference papers and patents from the search. Most of the 2502 titles (carbamic acid) retrieved were excluded following rapid assessment. Full texts of three papers (carbamic acid) were considered for potential relevance (toxicology/ecotoxicology) and were discounted as not relevant to the risk assessment on consideration of the full texts. The rationale for this was outlined in the addendum report.

- The applicant was requested to provide more information on metabolite 2,4- Dichlorobenzoic acid, CAS number 50-84-0, for which it had been indicated that it is a metabolite of the active substance spirodiclofen and that it is commercially available. The applicant responded by way of the information in the below bullet point remarks.

The FMC/Exponent literature review report concluded that "This review of the published literature for F9600 and its metabolites did **not** reveal any studies considered to significantly affect the regulatory assessment of human health, animal health or the environment.

Nevertheless, the applicant drew attention to the following bullet point remarks:

- Metabolite 2,4-Dichlorobenzoic acid, CAS number 50-84-0, is also a metabolite of the active substance spirodiclofen. Spirodiclofen is currently authorised as a plant protection product in the EU until 31/07/2020. Conclusions on the peer review of spirodiclofen assessment published in 2009 did not report any major human health, animal health or the environmental health concerns for 2,4-dichlorobenzoic acid as a major metabolite of spirodiclofen (EFSA Scientific Report (2009) 339, 1-86).
- Regarding 2,4-dichlorobenzoic acid available commercially, it is used as an intermediate in the manufacture of dyes, pharmaceuticals and other chemicals although there is currently no record of a REACH registration dossier submitted for the substance in the European Chemical Agency

(ECHA) database of registered substances. Therefore, the toxicological and ecotoxicological safety of 2,4-dicholorobenzoic acid under these uses could not be assessed.

- Metabolite 3-hydroxypivalic acid, CAS 4835-90-9, is also used as an intermediate in the manufacture of chemicals. A dossier to support the chemical under REACH was submitted in 2018 but does not appear to include any actual studies on toxicology or ecotoxicology.

[On the above point, HSE notes that the applicant has referred to some published papers (e.g. Rezanka *et al*., 2012 and Dembitsky, 2006) relating to findings of pivalic acid, see the discussion in Volume 1, section 2.7.4, regarding whether the residues of metabolite M118/1, 2,2-dimethyl-3-hydroxy propionic acid (3-hydroxypivalic acid) found in the residues trials could be of natural origin.]

HSE remarks: 2-chlorobenzyl alcohol ((2-chlorophenyl) methanol) was a main metabolite of the active substance clomazone. Clomazone is structurally similar to bixlozone and is FMC is also a main data holder for the active substance clomazone. 2,4-Dichlorobenzoic acid was included in the applicant's literature search (as it is a metabolite in plants), but the corresponding alcohol 2,4-dichlorobenzyl alcohol was not. The applicant notes that the EFSA Guidance focusses on the need for metabolites, degradation products, or transformation products of an active substance for which further assessment is required according to the data requirements and the Guidance documents applicable at the time of submitting the dossier. The applicant stated that 2,4-dichlorobenzyl alcohol was only identified as a metabolite in soil and aquatic metabolism studies, but was never found at >5%AR. Further assessment was not considered to be required which is why it was not considered to be included in the Literature search.

The applicant also referred to the Confidential section for further information, as 2,4-dichlorobenzyl alcohol is also an impurity.

HSE refers to section B.7.2 which outlines efforts seeking to identify 2,4-dichlorobenzyl alcohol in plants, and the rationale for HSE asking about this metabolite in the plant metabolism context. This metabolite was not found in the metabolism studies where its finding was investigated (canola, sugar beet, and rotational crops). The applicant also submitted a rationale for its non-finding in plants. The EU MRL Review for clomazone (EFSA, 2011) concluded that the 'alcohol' metabolite of clomazone, 2-chlorobenzyl alcohol, did not need to be included in the residue definition for clomazone on the basis of its very low toxicity.

Conclusion: HSE concludes that regarding the literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to this residues risk assessment.

B.7.8. REFERENCES RELIED ON

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	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.1/01	Rawle, N	2018	Storage Stability Study of F9600 and its Metabolites (2,4-dichlorobenzoic acid, 5-hydroxy-F9600 and 2,2-dimethyl-3-hydroxy propionic acid) in Crop Samples Stored Under Frozen Conditions CEMAS, UK, Study no: CEMS-7268 FMC Tracking no: 2015AMT-ISX2098 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.1/02	Kennedy, S	2018	Storage Stability of 5'-Hydroxy-F9600 in Crop Samples Stored Under Frozen Conditions. CEMAS, UK, Study no: CEMS-8008 FMC Tracking no: 2017RES-ISX3131 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.1/03	Fritzsch, S	2020	Storage Stability of F9600-dimethyl-malonamide, F9600-OH-isobutyramide and 4-hydroxymethyl-F9600 in Radish, Leaf Lettuce, Wheat Grain and Straw under Deep Frozen Conditions Eurofins Agroscience Services Chem GmbH, Study No. S18-04053 (FMC-1801L) 2018RES-ISA4306 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.1/01	Desai, M	2019	Nature of the Residue/ Metabolism	N	Y	Study to support new active	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			of [^{14}C]F9600 in/on Wheat Crop XenoBiotic Laboratories, Inc, (XBL) USA, Study no: XBL 14003 Report amendment 1 FMC Tracking no: 2013MET-ISX1098 GLP Unpublished			approval in GB		
KCA 6.2.1/01	McClanahan, R.	2021	Stability of Incurred 14C-F9600 Residues in Wheat. Frontage Document No. 037955-1. Frontage Laboratories, Inc., Concord, OH. FMC Tracking no: FMC-53485 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.1/02	Desai, M	2019	Nature of the Residue/ Metabolism of [^{14}C]F9600 in/on Canola Crop XenoBiotic Laboratories, Inc, (XBL) USA, Study no: XBL 14004 Report amendment 1 FMC Tracking no: 2013MET-ISX1096 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.1/02	McClanahan, R.	2021	Stability of Incurred 14C-F9600 Residues in Canola. Frontage Document No. 037967-1. Frontage Laboratories, Inc., Concord, OH. FMC Tracking no: FMC No. 53483, GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.1/03	Desai, M	2019	Nature of the Residue/ Metabolism	N	Y	Study to support new	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			of [^{14}C]F9600 in/on Sugar beet Crop XenoBiotic Laboratories, Inc, (XBL) USA, Study no: XBL 14005 Report amendment 1 FMC Tracking no: 2013MET-ISX1097 GLP Unpublished			active approval in GB		
KCA 6.2.1/03	Miner, P	2021	Stability of Incurred 14C-F9600 Residues in Sugar Beets. Frontage Document No. 037968-1. Frontage Laboratories, Inc., Concord, OH. FMC Tracking no: FMC No. 54930, GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.1/04	Desai, M	2019	Nature of the Residue/ Metabolism of [^{14}C]F9600 in/on Rice XenoBiotic Laboratories, Inc, (XBL) USA, Study no: XBL 15031 Report amendment 1 FMC Tracking no: 2015MET-ISX1892 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.1/04	Miner, P	2021	Stability of Incurred 14C- F9600 Residues in Rice. Frontage Document No. 037969-1. Frontage Laboratories, Inc., Concord, OH FMC Tracking no: FMC No. 54931, GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.2.2/01	[redacted]	2019	[^{14}C]F9600: Nature of the Residue in	Y	Y	Study to support new	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			Livestock: Metabolism of F9600 in Laying Hens [redacted] A, Study no: [redacted] 15171 Report amendment 1 FMC Tracking no: 2015MET-ISX2186 GLP Unpublished			active approval in GB		
KCA 6.2.3/01	[redacted]	2019	[^{14}C]F9600: Nature of the Residue in Livestock: Metabolism of F9600 in Lactating Goats [redacted], Study no: [redacted] 15086 Report amendment 2 FMC Tracking no: 2015MET-ISX2141 GLP Unpublished	Y	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.3.1/01	Gemrot, F	2017a	Magnitude of the Residues of F9600 in Oil Seed Rape (Raw Agricultural Commodity) after one pre emergence or post emergence application of F9600-4 SC in Northern Europe (Northern France and Germany) and Southern Europe (Italy and Spain) – 2015 and 2016 SGS AGRI MIN, France, Study No: 15SGS108 FMC Tracking no: 2015RES-ISX2146 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.3.1/02	Semrau, J	2017a	Determination of residues of F9600 after one pre-	N	Y	Study to support new active	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			emergence or post-emergence application of F9600-4 SC in Oilseed Rape at 10 sites Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-01155 FMC Tracking no: 2016RES-ISX2487 GLP Unpublished			approval in GB		
KCA 6.3.2/01	Bousquet, C	2017a	Magnitude of the Residues of F9600 in Barley (Raw Agricultural Commodity) after one pre-emergence or post-emergence application of F9600-4 SC in Northern and Southern Europe-2015 and 2016 SGS AGRI MIN, France, Study No: 15SGS110 FMC Tracking no: 2015RES-ISX2150 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.3.2/02	Chevallier, E	2017a	Magnitude of the Residues of F9600 in Wheat (Raw Agricultural Commodity) after one pre-emergence or post-emergence application of F9600-4 SC in Northern and Southern Europe-2015 and 2016 SGS AGRI MIN, France, Study No: 15SGS109 FMC Tracking no: 2015RES-ISX2149 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6.3.2/03	Semrau, J	2017b	Determination of residues of F9600 after one pre-emergence or post-emergence application of F9600-4 SC in Barley at 6 sites Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-01154 FMC Tracking no: 2016RES-ISX2486 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.3.2/04	Semrau, J	2018	Determination of residues of F9600 after one pre-emergence or post-emergence application of F9600-4 SC in Wheat at 10 sites in Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-01153 FMC Tracking no: 2016RES-ISX2485 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.3.3/01	Gemrot, F	2017b	Magnitude of the residues of F9600 in maize (Raw Agricultural Commodity) after one pre-emergence application of F9600-4 SC or F9600-28 CS in Northern Europe (Northern France and Germany) and Southern Europe (Southern France and Spain) – 2015	N	Y	Study to support new active approval in GB	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			SGS AGRI MIN, France, Study No: 15SGS072 FMC Tracking no: 2015RES-ISX1939 GLP Unpublished					
KCA 6.3.3/02	Chevallier, E	2017b	Magnitude of the residues of F9600 in maize (Raw Agricultural Commodity) after one pre-emergence application of F9600-4 SC or F9600-28 CS in Northern Europe (Northern France and Germany) and Southern Europe (Southern France and Spain) – 2015 SGS AGRI MIN, France, Study No: 15SGS073 FMC Tracking no: 2015RES-ISX1943 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.3.3/03	Semrau, J	2018	Determination of residues of F9600 after one pre-emergence or post-emergence application of F9600-4 SC in Maize at 8 sites Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-01152, FMC Tracking no: 2016RES-ISX2484 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.5.1/01	Mende, P	2017	[^{14}C]F9600: Hydrolysis under Typical Conditions (pH, Temperature and Time) of Processing	N	Y	Study to support new active approval in GB	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			Eurofins Agroscience Services GmbH, study no: S16-05475 FMC Tracking no: 2016RES-ISX2775 GLP Unpublished					
KCA 6.5.2/01	Semrau, J.	2018	Determination of residues of F9600 after one pre-emergence application of F9600-4 SC in Wheat and in processed fractions of Wheat at 2 sites in Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-05487 FMC Tracking no: 2016RES-ISX2785 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.5.2/02	Semrau, J.	2018	Determination of residues of F9600 after one pre-emergence application of F9600-4 SC in Barley and in processed fractions of Barley at 2 sites in Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-05488 FMC Tracking no: 2016RES-ISX2786 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.5.2/03	Semrau, J.	2018	Determination of residues of F9600 after one pre-emergence application of F9600-4 SC in Oilseed Rape and in processed	N	Y	Study to support new active approval in GB	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			fractions of Oilseed Rape at 4 sites in Northern and Southern Europe 2016 Eurofins Agroscience Services GmbH, study no: S16-05489 FMC Tracking no: 2016RES-ISX2787 GLP Unpublished					
KCA 6.5.2/04	Semrau, J.	2018	Determination of residues of F9600 after one application of F9600-4 SC in Maize and in processed fractions of Maize at 2 sites in Northern and Southern Europe 2017 Eurofins Agroscience Services GmbH, study no: S16-05486 FMC Tracking no: 2016RES-ISX2784 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.6.1/01	Desai, M	2019	Uptake and Metabolism of [^{14}C] F9600 in Confined Rotational Crops XenoBiotic Laboratories, Inc, (XBL) USA, Study no: XBL 14019 Report amendment 1 FMC Tracking no: 2014MET-ISX1194 GLP Unpublished	N	Y	Study to support new active approval in GB	FMC	None (new active)
KCA 6.6.2/01	Semrau, J	2018	Determination of residues of F9600 in rotated crops (radish, leaf lettuce, wheat) after one application of F9600-4 SC in maize at 1 site in Northern Europe and	N	Y	Study to support new active approval in GB	FMC	None (new active)

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	Justification if data protection is claimed	Owner	Previous evaluation
			1 site in Southern Europe 2016/2017 Eurofins Agroscience Services GmbH, study no: S16-01156 FMC Tracking no: 2016RES-ISX2558 GLP Unpublished					
	Exponent Report conducted on behalf of FMC.	2018	Literature review report for bixlozone. Exponent QAID: 1508442.UK0 – 5012 FMC Tracking Number: 2018WHP-ISX4339 Non-GLP Unpublished	N	N	Not applicable	FMC	None (new active)
	Exponent Report conducted on behalf of FMC.	2020	Addendum to Literature review report for bixlozone. Exponent QAID: 1508442.UK0 – 5415. FMC Tracking Number: 2018WHP-ISX4339-AD1 Non-GLP Unpublished	N	N	Not applicable	FMC	None (new active)