UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 2, 2003

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RAB2/HED (7509C)

Date: July 16/03
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DP Barcode: D278386

Petition: 1F06313

Citation: 45405110 Tilting, N. (2001) Residues in Milk and Edible Tissues Following Oral Administration of BAS 510 F to Lactating Dairy Cattle: Final Report: Lab Project Number: 42401: 2000/1017228. Unpublished study prepared by CEM Analytical Services Limited. 178 p.

Sponsor: BASF Corporation

Background

The information contained herein was compiled by Dynamac Corporation (20440 Century Boulevard, Suite 100, Germantown MD 20874), contractor, under the supervision of RAB2/HED. This DER has undergone secondary review by RAB2, and reflects current HED and Office of Pesticide Programs (OPP) policies. This DER was also peer-reviewed by PMRA.

Executive Summary

BASF Corporation has submitted a dairy cattle feeding study with BAS 510 F. Lactating dairy cattle were dosed with BAS 510 F for 29-30 days at feeding levels equivalent to 1.8, 5.9, and 20.2 ppm in the diet; one cow from the 20.2 ppm dosing group was not sacrificed until 7 days after dosing ceased to demonstrate residue depletion. The feeding levels were intended to

(1)

represent 1x, 3x, and 10x the maximum theoretical dietary burden (MTDB) of BAS 510 F to ruminants, which the petitioner calculated to be 1.8 ppm, based on a theoretical diet consisting of carrot culls, almond hulls, and potato waste, and maximum residue levels based on preliminary field trial results.

Residues of BAS 510 F in milk samples collected over the course of the dosing period were <0.01 ppm for the 1x group, <0.01-0.013 ppm for the 3x group and <0.01-0.09 ppm for the 10x group; residues of M510F01 were below the LOQ (<0.01 ppm) in all milk samples from all three dosing groups. Milk samples from day 21 of the study were separated into skim milk and cream. Residues of BAS 510 F and M510F01 were each below the LOQ (<0.01 ppm) or at the LOQ (0.011 ppm BAS 510 F in one 10x-dose sample) in skim milk samples from all dosing groups. Quantifiable residues of BAS 510 F were observed in cream from the 1x, 3x, and 10x groups at 0.023-0.045 ppm, 0.10-0.12 ppm, and 0.24-0.37 ppm, respectively. Residues of M510F01 were below the LOQ (<0.01 ppm) in all cream samples. Based on the residues observed in milk samples from the 10x group, residues appeared to peak on day 18 of the study and then plateau at a lower level for the remainder of the dosing period.

In tissue samples from the 1x, 3x, and 10x dosing groups, respective residues of BAS 510 F were: (i) <0.025 ppm, <0.025 ppm, and <0.025-0.033 ppm in muscle samples; (ii) <0.025-0.053 ppm, 0.057-0.099 ppm, and 0.21-0.27 ppm in fat samples; (iii) <0.025 ppm, <0.025 ppm, and 0.06-0.08 ppm in liver samples; and (iv) <0.025 ppm, <0.025 ppm, and <0.025-0.044 ppm in kidney samples. Residues of M510F01 were below the LOQ (<0.025 ppm) in muscle and fat samples from all three dosing groups and in liver and kidney samples from the 1x dosing group. Quantifiable residues of M510F01 were observed in liver and kidney from the 3x and 10x groups; residues were 0.026-0.039 ppm and 0.09-0.12 ppm, respectively, in liver samples and 0.038-0.063 ppm and 0.14-0.29 ppm, respectively, in kidney samples. Combined residues of BAS 510 F and M510F01 were 0.17-0.18 ppm in liver and <0.17- <0.32 ppm in kidney from the 10x dose group.

Residues of BAS 510 F and M510F01 were each below the LOQ (<0.01 ppm) in milk samples collected 3 and 7 days after dosing ceased and were each below the LOQ (<0.025 ppm) in samples of muscle, fat, liver, and kidney from a cow sacrificed 7 days after dosing ceased.

Residues of M510F53 (marker compound for bound/conjugated residues) were <0.01 ppm in milk samples from all three dosing groups and were <0.05 ppm in liver from the 1x and 3x dosing groups; quantifiable residues were observed in liver from the 10x group at 0.08-0.09 ppm. We note that the levels of M510F53 observed in this study are significantly different from the levels of M510F53 observed in the goat metabolism study (see DER for MRID 45405025). In the goat metabolism study, residues of M510F53 were 1.13 ppm in liver following dosing for 5 days at ~32 ppm.

Samples of milk, skim milk, cream, muscle, fat, liver, and kidney were analyzed for residues of BAS 510 F and M510F01 (including glucuronide conjugate M510F02) using an adequate LC/MS/MS method (Method 471/0; see DER for MRID 45405106). Samples of milk and liver

were analyzed for bound/conjugated residues using a GC/MS method (Method 476/0); radiovalidation data are required for this method before it can be determined to be adequate for data collection purposes (see DER for MRID 45405105).

Samples of animal commodities were stored frozen (-18 C) for 25-104 days (0.8-3.4 months) prior to analysis for residues of BAS 510 F and M510F01 by method 471/0. Samples of milk and liver were stored frozen for 76-106 and 90-91 days, respectively, prior to analysis using method 476/0. The available storage stability data (see DER for MRID 45405108) demonstrate that residues of BAS 510 F and M510F01 are stable for up to 5.5 months in cow milk, liver, and muscle, and are adequate to support the storage intervals and conditions of milk, skim milk, muscle, liver, fat and kidney samples from this study.

The submitted cattle feeding study is deemed acceptable, provided it is determined by radiovalidation data that method 476/0 is adequate for data collection purposes (see DER for MRID 45405105).

Both Agencies will calculate the MTDB of BAS 510 F on cattle and will rely on the results of the current study to establish animal (cattle, goat, hogs, horses, sheep) commodity tolerances/MRLs if needed.

GLP Compliance

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The petitioner stated that the study was conducted in accordance with the GLP regulations established in Germany (Appendix 1 to §19a Section 1, Chemikaliengesetz of 25-July-1994; Official Bulletin/Federal Republic of Germany I 1994, p. 1703) instead of U.S. EPA GLP regulations.

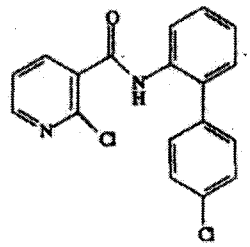
1. Materials and Methods

1.1. Test Substance

Active Ingredient

Common Name:	Nicobifen (ISO, proposed)
IUPAC Name:	2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
CAS Name:	3-Pyridinecarboxamide, 2-chloro-N-(4'chloro[1,1'-biphenyl]-2-yl)-
CAS Number:	188425-85-6
Company Name:	BAS 510 F
Other Synonyms:	BASF Registry Number 300355

Chemical Structure:



1.2. Trial Animals and Dosing

Breed:	Holstein crossbred
Diet and Water:	Total mixed ration (grass and maize silage, ground wheat, sugar beet molasses, rapeseed meal, and minerals) and water were provided *ad libitum*. Feed consumption was measured daily.
Acclimation period:	6 days prior to treatment
Predosing:	None
Type of Dosing:	Oral, in feed
Dosing Vehicle:	BAS 510 F was diluted with ethanol and mixed with sugar beet molasses. The cattle were fed the dosed sugar beet molasses twice daily. If the entire portion was not consumed, it was mixed with the total mixed ration feed and subsequently consumed.
Groups/Dosage Rate:	3 dosing groups (1x, 3x, and 10x) and one control group

Number/Group: 3 cows per dosing group, except for the 10x group which had 5 cows; one cow was removed from the 10x group on day 18 due to mastitis.

Dosage Rates: Average dose rates (over the dosing group) based on average feed intake during the dosing period were:
1x: 1.8 ppm
3x: 5.9 ppm
10x: 20.2 ppm

Duration of Dosing: Animals in the 1x and 10x dose groups were dosed for 29 consecutive days. Animals in the 3x dose group were dosed for 30 consecutive days.

1.3. Sample Collection Procedures

Cows were milked twice daily, and samples were refrigerated (~4 C). The morning and evening milk collections were pooled, and aliquots were removed and stored frozen (-18 C). On day 21 of the study, milk samples were separated into cream and skim milk. Milk, skim milk, and cream samples were shipped in insulated containers to CEM Analytical Services (Berkshire, England), where they were stored frozen (-18 C). Animals from the 1x and 10x groups were sacrificed on day 29 of dosing, and animals from the 3x group were sacrificed on day 30 of dosing. One cow in the 10x group was not sacrificed until 7 days following the last dose, to measure residue depletion. After sacrifice, which occurred in the afternoon following the last morning dose (or 7 days following the last morning dose), cattle carcasses were transferred to CEM Analytical Services where samples of muscle, fat, liver, and kidney were collected. Tissue samples were homogenized and frozen. Samples were shipped frozen (-18 C) from CEM Analytical Services to the analytical facility (BASF, Limburgerhof, Germany). The storage conditions at the analytical facility are detailed in Table 1.3.1.

Table 1.3.1. Summary of Storage Conditions

Matrix	Commodity or Extract	Storage Temperature (°C)	Duration (days)	
			Method 471/0	Method 476/0
Milk	RAC	-18	25-53	76-106
Cream	RAC	-18	85-86	Not analyzed
Skim milk	RAC	-18	78-79	Not analyzed
Muscle	RAC	-18	82-84	Not analyzed
Liver	RAC	-18	100-103	90-91
Kidney	RAC	-18	100-104	Not analyzed
Fat	RAC	-18	78-83	Not analyzed

Samples were analyzed within 1-12 days (method 471/0) or 2-17 days (method 476/0) of extraction.

1.4. Analytical Methods

Samples of cattle matrices were analyzed for residues of BAS 510 F and its hydroxy metabolite M510F01 (including glucuronide conjugate M510F02) using LC/MS/MS method 471/0. Residues were extracted with methanol, subjected to enzyme hydrolysis with β-glucuronidase and arylsulfatase (to convert M510F02 to M510F01), and cleaned up by liquid/liquid partitioning and silica gel solid phase extraction prior to LC/MS/MS analysis; refer to the DER for MRID 45405106 for a full description of the method. Residues of M510F01 were calculated as parent equivalents. The LOQs were 0.01 ppm for milk and 0.025 ppm for tissues.

Selected samples of milk and liver were also analyzed using GC/MS method 476/0. This method is a common moiety method in which non-extractable or conjugated residues are released, cleaved, and derivatized by microwave hydrolysis using acetic acid, and determined as metabolite M510F53. Refer to the DER for MRID 45405105 for a full description of the method. The LOQs were 0.01 ppm for milk and 0.05 ppm for liver. This information on non-extractable or conjugated residues was generated for risk assessment. Concurrent recoveries from different matrices are presented below (Table 2.1).

2. Results

Table 2.1. Summary of Concurrent Analytical Method Validation.

Commodity Matrix	Fortification Level (ppm)	Recoveries (%)	Mean Recovery ± SD
Method 471/0 - BAS 510 F			
Milk	0.01	73.46, 76.25, 77.09, 77.81, 77.83, 79.64, 81.36, 82.19, 82.52, 83.04, 83.43, 84.68, 87.29, 87.42, 89.38, 89.84, 100.94, 101.64	85.4 ± 7.6
	0.1	75.57, 77.46, 78.48, 79.03, 80.57, 82.59, 82.68, 83.84, 86.28, 88.59, 88.84, 89.33, 89.89, 91.17, 91.23, 94.02, 95.05, 105.76	
Skim milk	0.01	73.61, 81.67	81.5 ± 5.6
	0.1	83.94, 86.64	
Cream	0.01	87.51, 90.13	89.8 ± 2.4
	0.1	88.70, 93.01	
Muscle	0.025	89.73, 91.89	90.0 ± 4.5
	0.25	83.96, 94.58	
Liver	0.025	82.44, 84.04	84.4 ± 6.7
	0.25	77.64, 93.67	

Table 2.1. Summary of Concurrent Analytical Method Validation.			
Commodity Matrix	Fortification Level (ppm)	Recoveries (%)	Mean Recovery ± SD
Kidney	0.025	81.33, 97.59	85.8 ± 7.9
	0.25	81.23, 82.87	
Fat	0.025	86.84, 88.37	89.6 ± 3.0
	0.25	89.42, 93.92	
Method 471/0 - M510F01			
Milk	0.01	69.34, 72.77, 73.67, 74.67, 76.17, 79.87, 80.91, 80.97, 81.34, 86.38, 86.89, 87.10, 88.85, 89.22, 90.80, 91.16, 93.83, 99.72	85.7 ± 7.3
	0.1	76.04, 80.64, 81.97, 82.22, 84.03, 85.95, 87.10, 87.89, 88.71, 88.77, 88.82, 88.92, 89.15, 90.76, 91.51, 91.67, 92.75, 102.93	
Skim milk	0.01	80.76, 83.33	85.5 ± 4.4
	0.1	87.17, 90.70	
Cream	0.01	71.72, 91.04	88.3 ± 11.5
	0.1	92.23, 98.15	
Muscle	0.025	87.96, 93.11	90.8 ± 4.9
	0.25	85.77, 96.49	
Liver	0.025	87.64, 89.70	85.4 ± 5.5
	0.25	77.25, 86.86	
Kidney	0.025	88.38, 88.43	86.7 ± 2.1
	0.25	84.08, 85.94	
Fat	0.025	73.59, 80.25	76.6 ± 2.8
	0.25	75.96, 76.42	
Method 476/0 - M510F53			
Milk	0.01	93.2, 107.5, 108.8	102.1 ± 7.4
	0.1	95.6, 97.6, 109.9	
Liver	0.05	80.8, 97.5	93.4 ± 10.8
	0.5	89.2, 106.0	

Table 2.2. Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix/ Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues (ppm)			
			BAS 510 F[1]	M510F01[1]	Total BAS 510 F +M510F01	M510F53[2]
Milk/Day 3	1.8	Not applicable (N/A)	—[3]	—	—	<0.01
			—	—	—	—
			—	—	—	—
Milk/Day 6	1.8	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 9	1.8	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	<0.01
			<0.01	<0.01	<0.02	—
Milk/Day 12	1.8	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 18	1.8	N/A	—	—	—	—
			—	—	—	—
			—	—	—	<0.01
Milk/Day 21	1.8	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Skim milk/ Day 21	1.8	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Cream/Day 21	1.8	N/A	0.023	<0.01	<0.033	—
			0.045	<0.01	<0.055	—
			0.025	<0.01	<0.035	—
Milk/Day 28	1.8	N/A	<0.01	<0.01	<0.02	<0.01
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 1	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 3	5.9	N/A	<0.01	<0.01	<0.02	<0.01
			<0.01	<0.01	<0.02	—
			0.012	<0.01	<0.022	—
Milk/Day 6	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—

Table 2.2. Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix/ Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues (ppm)			
			BAS 510 F[1]	M510F01[1]	Total BAS 510 F +M510F01	M510F53[2]
Milk/Day 9	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	<0.01
			<0.01	<0.01	<0.02	—
Milk/Day 12	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 15	5.9	N/A	<0.01	<0.01	<0.02	—
			0.011	<0.01	<0.021	—
			<0.01	<0.01	<0.02	—
Milk/Day 18	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			0.013	<0.01	<0.023	<0.01
Milk/Day 21	5.9	N/A	0.010	<0.01	<0.020	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Skim milk/ Day 21	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Cream/Day 21	5.9	N/A	0.113	<0.01	<0.123	—
			0.115	<0.01	<0.125	—
			0.100	<0.01	<0.110	—
Milk/Day 24	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 28	5.9	N/A	<0.01	<0.01	<0.02	<0.01
			0.010	<0.01	<0.020	—
			<0.01	<0.01	<0.02	—
Milk/Day 29	5.9	N/A	<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
Milk/Day 1	20.2	N/A	0.011	<0.01	<0.021	—
			0.013	<0.01	<0.023	—
			<0.01	<0.01	<0.02	—
			0.011	<0.01	<0.021	—
			<0.01	<0.01	<0.02	—

Table 2.2. Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix/ Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues (ppm)			
			BAS 510 F[1]	M510F01[1]	Total BAS 510 F +M510F01	M510F53[2]
Milk/Day 3	20.2	N/A	0.017	<0.01	<0.027	<0.01
			0.031	<0.01	<0.041	<0.01
			0.029	<0.01	<0.039	<0.01
			0.013	<0.01	<0.023	<0.01
			0.037	<0.01	<0.047	<0.01
Milk/Day 6	20.2	N/A	<0.01	<0.01	<0.02	—
			0.015	<0.01	<0.025	—
			0.012	<0.01	<0.022	—
			<0.01	<0.01	<0.02	—
			0.021	<0.01	<0.031	—
Milk/Day 9	20.2	N/A	0.020	<0.01	<0.030	—
			0.035	<0.01	<0.045	—
			0.026	<0.01	<0.036	—
			<0.01	<0.01	<0.02	—
			0.035	<0.01	<0.045	—
Milk/Day 12	20.2	N/A	0.015	<0.01	<0.025	<0.01
			0.022	<0.01	<0.032	<0.01
			0.022	<0.01	<0.032	<0.01
			0.013	<0.01	<0.023	<0.01
			0.022	<0.01	<0.032	<0.01
Milk/Day 15	20.2	N/A	0.024	<0.01	<0.034	—
			0.032	<0.01	<0.042	—
			0.041	<0.01	<0.051	—
			0.016	<0.01	<0.026	—
			0.032	<0.01	<0.042	—
Milk/Day 18	20.2	N/A	0.086	<0.01	<0.096	<0.01
			0.045	<0.01	<0.055	<0.01
			0.011	<0.01	<0.021	<0.01
			0.026	<0.01	<0.036	<0.01
			0.045	<0.01	<0.055	<0.01
Milk/Day 21[4]	20.2	N/A	0.028	<0.01	<0.038	—
			0.033	<0.01	<0.043	—
			0.021	<0.01	<0.031	—
			0.030	<0.01	<0.040	—
Skim milk/ Day 21[4]	20.2	N/A	0.011	<0.01	<0.021	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—
			<0.01	<0.01	<0.02	—

Table 2.2. Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix/ Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues (ppm)			
			BAS 510 F[3]	M510F01[1]	Total BAS 510 F +M510F01	M510F53[2]
Cream/Day 21[4]	20.2	N/A	0.37	<0.01	<0.38	—
			0.37	<0.01	<0.38	—
			0.24	<0.01	<0.25	—
			0.34	<0.01	<0.35	—
Milk/Day 24[4]	20.2	N/A	0.025	<0.01	<0.035	—
			0.036	<0.01	<0.046	—
			0.016	<0.01	<0.026	—
			0.030	<0.01	<0.040	—
Milk/Day 28[4]	20.2	N/A	0.029	<0.01	<0.039	<0.01
			0.033	<0.01	<0.043	<0.01
			0.018	<0.01	<0.028	<0.01
			0.036	<0.01	<0.046	<0.01
Milk/Day 32[5]	20.2	N/A	<0.01	<0.01	<0.02	—
Milk/Day 36[5]	20.2	N/A	<0.01	<0.01	<0.02	—
Muscle/Sacrifice	1.8	0	<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
Muscle/Sacrifice	5.9	0	<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
Muscle/Sacrifice	20.2	0	<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
			0.033	<0.025	<0.058	—
Muscle/Sacrifice	20.2	7	<0.025	<0.025	<0.05	—
Fat/Sacrifice	1.8	0	0.053	<0.025	<0.078	—
			<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
Fat/Sacrifice	5.9	0	0.099	<0.025	<0.12	—
			0.084	<0.025	<0.11	—
			0.057	<0.025	<0.082	—
Fat/Sacrifice	20.2	0	0.21	<0.025	<0.24	—
			0.27	<0.025	<0.29	—
			0.25	<0.025	<0.28	—
Fat/Sacrifice	20.2	7	<0.025	<0.025	<0.05	—
Liver/Sacrifice	1.8	0	<0.025	<0.025	<0.05	<0.05
			<0.025	<0.025	<0.05	<0.05
			<0.025	<0.025	<0.05	<0.05

Table 2.2. Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix/ Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues (ppm)			
			BAS 510 F[1]	M510F01[1]	Total BAS 510 F +M510F01	M510F53[2]
Liver/Sacrifice	5.9	0	<0.025	0.030	<0.055	<0.05
			<0.025	0.026	<0.051	<0.05
			<0.025	0.039	<0.064	<0.05
Liver/Sacrifice	20.2	0	0.060	0.12	0.18	0.08
			0.080	0.091	0.17	0.09
			0.070	0.11	0.18	0.09
Liver/Sacrifice	20.2	7	<0.025	<0.025	<0.05	<0.05
Kidney/Sacrifice	1.8	0	<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
			<0.025	<0.025	<0.05	—
Kidney/Sacrifice	5.9	0	<0.025	0.046	<0.071	—
			<0.025	0.038	<0.063	—
			<0.025	0.063	<0.09	—
Kidney/Sacrifice	20.2	0	<0.025	0.29	<0.32	—
			0.044	0.18	0.22	—
			<0.025	0.14	<0.17	—
Kidney/Sacrifice	20.2	7	<0.025	<0.025	<0.05	—

[1] Residues of BAS 510 F and M510F01 determined using method 471/0; residues of M510F01 reported as parent equivalents.
[2] Nonextractable residues determined as M510F53 using method 476/0.
[3] Not analyzed.
[4] One cow was removed from the study due to mastitis.
[5] Dosing ceased on Day 29.

Table 2.3. Summary of Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Analyte	Residue Levels (ppm)			
				Maximum	Highest Average	Mean[1]	Std. Dev.
Milk	1.8	Not applicable (N/A)	BAS 510 F	<0.01	N/A	<0.01	0
			M510F01	<0.01	N/A	<0.01	0
			M510F53	<0.01	N/A	<0.01	0
Skim milk	1.8	N/A	BAS 510 F	<0.01	N/A	<0.01	0
			M510F01	<0.01	N/A	<0.01	0
Cream	1.8	N/A	BAS 510 F	0.045	N/A	0.031	0.012
			M510F01	<0.01	N/A	<0.01	0

Table 2.3. Summary of Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Analyte	Residue Levels (ppm)			
				Maximum	Highest Average	Mean[1]	Std. Dev.
Milk	5.9	N/A	BAS 510 F	0.013	0.011 (Day 18)	0.010	0.0006
			M510F01	<0.01	N/A	<0.01	0
			M510F53	<0.01	N/A	<0.01	0
Skim milk	5.9	N/A	BAS 510 F	<0.01	N/A	<0.01	0
			M510F01	<0.01	N/A	<0.01	0
Cream	5.9	N/A	BAS 510 F	0.12	N/A	0.11	0.009
			M510F01	<0.01	N/A	<0.01	0
Milk	20.2	N/A	BAS 510 F	0.086	0.043 (Day 18)	0.025	0.014
			M510F01	<0.01	N/A	<0.01	0
			M510F53	<0.01	N/A	<0.01	0
Skim milk	20.2	N/A	BAS 510 F	0.011	N/A	0.010	0.0004
			M510F01	<0.01	N/A	<0.01	0
Cream	20.2	N/A	BAS 510 F	0.37	N/A	0.33	0.06
			M510F01	<0.01	N/A	<0.01	0
Muscle	1.8	0	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
Muscle	5.9	0	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
Muscle	20.2	0	BAS 510 F	0.033	N/A	0.028	0.004
			M510F01	<0.025	N/A	<0.025	0
Muscle	20.2	7	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
Fat	1.8	0	BAS 510 F	0.053	N/A	0.034	0.016
			M510F01	<0.025	N/A	<0.025	0
Fat	5.9	0	BAS 510 F	0.10	N/A	0.08	0.021
			M510F01	<0.025	N/A	<0.025	0
Fat	20.2	0	BAS 510 F	0.27	N/A	0.24	0.030
			M510F01	<0.025	N/A	<0.025	0
Fat	20.2	7	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
Kidney	1.8	0	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0

Table 2.3. Summary of Residue Data from Cattle Feeding Study with BAS 510 F.

Matrix	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Analyte	Residue Levels (ppm)			
				Maximum	Highest Average	Mean[1]	Std. Dev.
Kidney	5.9	0	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	0.063	N/A	0.049	0.012
Kidney	20.2	0	BAS 510 F	0.044	N/A	0.031	0.011
			M510F01	0.29	N/A	0.20	0.079
			Total[2]	<0.32	N/A	0.24	0.08
Kidney	20.2	7	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
Liver	1.8	0	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
			M510F53	<0.05	N/A	<0.05	0
Liver	5.9	0	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	0.039	N/A	0.032	0.006
			M510F53	<0.05	N/A	<0.05	0
Liver	20.2	0	BAS 510 F	0.080	N/A	0.070	0.010
			M510F01	0.12	N/A	0.11	0.016
			Total[2]	0.18	N/A	0.18	0.006
			M510F53	0.09	N/A	0.09	0.006
Liver	20.2	7	BAS 510 F	<0.025	N/A	<0.025	0
			M510F01	<0.025	N/A	<0.025	0
			M510F53	<0.05	N/A	<0.05	0

[1] Mean calculated using LOQ for values below the LOQ.
[2] Total BAS 510 F + M510F01; only included in this table when quantifiable residues were observed for both analytes.

The only matrix for which quantifiable residues were observed at all three dosing levels was cream. Regression analysis using both the maximum and mean residue levels demonstrated a very good correlation (r^2 >0.999), indicating a linear relationship between residue levels and dose.

Apparent residues of BAS 510 F and M510F01 were below the LOQ (0.01 ppm for milk, skim milk, and cream, and 0.025 ppm for muscle, fat, liver, and kidney) in 18 samples of milk, and 3 samples each of skim milk, cream, muscle, fat, liver, and kidney from undosed cattle.

3. Discussion

3.1. Methods

Lactating dairy cattle were dosed with BAS 510 F for 29-30 days at feeding levels equivalent to 1.8, 5.9, and 20.2 ppm in the diet; one cow from the 20.2 ppm dosing group was not sacrificed until 7 days after dosing ceased to demonstrate residue depletion. The feeding levels were intended to represent 1x, 3x, and 10x the maximum theoretical dietary burden of BAS 510 F to ruminants, which the petitioner calculated to be 1.8 ppm, based on a theoretical diet consisting of 25% carrot culls (12% dry matter, maximum residue of 0.5 ppm), 10% almond hulls (90% dry matter, maximum residue of 2.5 ppm), and 65% potato waste (15% dry matter, maximum residue of 0.1 ppm); the petitioner noted that the maximum residue levels used for determination of dietary burden were based on preliminary field trial results.

Samples of milk, skim milk, cream, muscle, fat, liver, and kidney were analyzed for residues of BAS 510 F and M510F01 (including glucuronide conjugate M510F02) using an LC/MS/MS method (Method 471/0), and samples of milk and liver were analyzed for bound/conjugated residues using a GC/MS method (Method 476/0). Concurrent method validation data included in the current submission indicate that the LC/MS/MS method is adequate for data collection purposes in animal commodities. In addition, adequate method validation data have been submitted for Method 471/0 (see DER for MRID 45405106) on milk, cream, and cow tissues. Because the microwave hydrolysis step of Method 476/0 has not been validated with fortification standards (method validation and concurrent method recovery data were generated by fortifying samples after the microwave hydrolysis step), radiovalidation data are required before Method 476/0 can be determined to be adequate for data collection purposes (see DER for MRID 45405105).

Samples of animal commodities were stored frozen (-18 C) for 25-104 days (0.8-3.4 months) prior to analysis for residues of BAS 510 F and M510F01. Samples of milk and liver were stored frozen for 76-106 and 90-91 days, respectively, prior to analysis using method 476/0. The available storage stability data (see DER for MRID 45405108) are adequate to support the storage intervals and conditions of milk, skim milk, muscle, liver, fat and kidney samples from this study, and demonstrate that residues of BAS 510 F and M510F01 are stable for up to 5.5 months in cow milk, liver, and muscle.

3.2. Results

Following dosing of cattle with BAS 510 F for 29-30 days at 1.8 (1x), 5.9 (3x), and 20.2 ppm (10x), residues of BAS 510 F in milk samples collected over the course of the dosing period were <0.01 ppm for the 1x group, <0.01-0.013 ppm for the 3x group and <0.01-0.09 ppm for the 10x group; residues of M510F01 were below the LOQ (<0.01 ppm) in all milk samples from all three dosing groups. Milk samples from day 21 of the study were separated into skim milk and cream. Residues of BAS 510 F and M510F01 were each below the LOQ (<0.01 ppm) or at the LOQ (0.011 ppm BAS 510 F in one 10x dose sample) in skim milk samples from all dosing groups.

Quantifiable residues of BAS 510 F were observed in cream from the 1x, 3x, and 10x groups at 0.023-0.045 ppm, 0.10-0.12 ppm, and 0.24-0.37 ppm, respectively. Residues of M510F01 were below the LOQ (<0.01 ppm) in all cream samples. Based on the residues observed in milk samples from the 10x group, residues appeared to peak on day 18 of the study and then plateau at a lower level for the remainder of the dosing period.

In tissue samples from the 1x, 3x, and 10x dosing groups, respective residues of BAS 510 F were: (i) <0.025 ppm, <0.025 ppm, and <0.025-0.033 ppm in muscle samples; (ii) <0.025-0.053 ppm, 0.057-0.10 ppm, and 0.21-0.27 ppm in fat samples; (iii) <0.025 ppm, <0.025 ppm, and 0.060-0.080 ppm in liver samples; and (iv) <0.025 ppm, <0.025 ppm, and <0.025-0.044 ppm in kidney samples. Residues of M510F01 were below the LOQ (<0.025 ppm) in muscle and fat samples from all three dosing groups and in liver and kidney samples from the 1x dosing group. Quantifiable residues of M510F01 were observed in liver and kidney from the 3x and 10x groups; residues were 0.026-0.039 ppm and 0.091-0.12 ppm, respectively, in liver samples and 0.038-0.063 ppm and 0.14-0.29 ppm, respectively, in kidney samples. Combined residues of BAS 510 F and M510F01 were 0.170-0.18 ppm in liver and <0.17-<0.32 ppm in kidney from the 10x dose group.

Residues of BAS 510 F and M510F01 were each below the LOQ (<0.01 ppm) in milk samples collected 3 and 7 days after dosing ceased and were each below the LOQ (<0.025 ppm) in samples of muscle, fat, liver, and kidney from a cow sacrificed 7 days after dosing ceased.

Residues of M510F53 (marker compound for bound/conjugated residues) were <0.01 ppm in milk samples from all three dosing groups and were <0.05 ppm in liver from the 1x and 3x dosing groups; quantifiable residues were observed in liver from the 10x group at 0.08-0.09 ppm. We note that the levels of M510F53 observed in this study are significantly different from the levels of M510F53 observed in the goat metabolism study (see DER for MRID 45405025). In the goat metabolism study, residues of M510F53 were 1.13 ppm in liver following dosing for 5 days at ~32 ppm.

4. Deficiencies

None for this study, provided it is determined by radiovalidation data that method 476/0 is adequate for data collection purposes (see DER for MRID 45405105).

5. References

None.