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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
~~WASHINGTON, D.C. 20460~~
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Beltsville, Maryland 20705

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

April 19, 1983

MEMORANDUM

TO: Martin F. Kovacs, Jr. Ph.D
Chemist, Residue Chemistry Branch
Hazard Evaluation Division

THRU: Warren R. Bontoyan, Section Head
Chemical Operations Branch

SUBJECT: PP#2F2728 Results of Method Trial for Iprodione and Its Metabolites in Liver and Milk

The Chemical Operations Branch was requested by the Residue Chemistry Branch, Hazard Evaluation Division to conduct a method trial on the fungicide Iprodione (3(3,5-dichlorophenyl) -N- (1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide) and two of its metabolites in beef liver and milk. Iprodione is a product of Rhone-Poulenc Chemical Company. The analytical methods entitled "Analytical Method for Determination of Iprodione and Its Nonhydroxylated Metabolites in Bovine Milk" (2/10/82; ADC Project #623-A); Analytical Method for Determination of Iprodione and Its Nonhydroxylated Metabolites in Bovine Tissues" (2/10/82;ADC Project#623-B); and Determination of Hydroxylated Iprodione (RP-26019) Metabolites in Cow Milk By Electron-Capture Gas Chromatography" (12/8); Rhone-Poulenc Method no. 159) were followed for this method trial. The initial request to fortify milk at the 0.005 ppm level and liver at .025 ppm, was unworkable as the controls showed interferences at these levels. Milk samples were fortified at .01 and .02 ppm with Iprodione and the nonhydroxylated metabolite. Beef liver was run at .05 and .1 ppm fortifications of the same compounds. The hydroxylated metabolite was run on milk only, at fortification levels of .01 and .02 ppm.

Method summary

The hydroxylated metabolite is extracted from the milk using acetone. The samples are cleaned-up using an aluminum

sulfate precipitation and a hexane/acetonitrile partition. Any conjugated metabolites present are released using a mild acid hydrolysis. This is followed by methylation using diazomethane. All the hydroxylated metabolites are then converted to a common moiety, 4-methoxy-3,5-dichloro aniline, by means of basic hydrolysis. The aniline is then derivatized using HFBA to form the heptafluorobutyrate. The final derivative is cleaned up by Florisil Column Chromatography and the final determination is made by gas chromatography using electron-capture detection and a DEGS column.

The Iprodione and nonhydroxylated metabolites are extracted from the liver samples with acetone. The samples are cleaned-up using aluminum sulfate precipitation and a hexane/acetonitrile partition. The Iprodione and nonhydroxylated metabolites are then connected to a common moiety, 3,5-dichloroaniline by means of basic hydrolysis. The aniline is then derivatized to the heptafluorobutyrate, cleaned-up on a florisil column, and determined by gas chromatography on a carbowax column, using an electron-capture detection.

Comments

1. The method appears to be satisfactory and the recoveries were generally good at the levels reported. However, I was unable to meet the initial request of a .005 ppm fortification level in milk due to interferences. The methods provided by the manufacturer do not mention any fortification levels lower than 0.01 ppm, and the tolerance level is .02 ppm. There were no problems with the samples fortified at the 0.01 ppm level.

2. A 6-foot column was used instead of a 4-foot column in the hydroxylated metabolite method. The substitution was made only because of availability and either length of column should work.

3. For nonhydroxylated metabolites and the parent compound, the run temperature for the carbofuran column had to be lowered from the 200° - 215° C specified in the method to 185° C in order to separate an impurity causing high control values.

4. It might be suggested that another solvent be substituted for benzene in the HFBA derivatization step as a safety factor. Benzene is suspected to be a strong carcinogen.

Mark W. Law

Mark W. Law
Chemist
Analytical Chemistry
Laboratories Section

Results

All recoveries corrected for controls

Tissue	Standard	Fortification level (ppm)	ppm recovered	Percent recovery
Milk	Iprodione	.01	.0092	92
		.01	.0119	119
		.02	.0221	110.5
		.02	.0217	108.5
Milk	RP 32490	.01	.0106	106
		.01	.0113	112
		.02	.0260	103
		.02	.0220	110.1
Milk	RP 36114	.01	.00746	74.6
		.01	(A)	
		.02	.0132	66.0
		.02	.0131	65.7
Liver	Iprodione	.05	.0382	76.4
		.05	.0430	86.0
		.1	.092	92.0
		.1	.095	95.0
Liver	RP 32490	.05	.0456	91.2
		.05	.0427	85.4
		.1	.0961	96.1
		.1	.0945	94.5

(A) This sample discarded after partial loss.