

Appendix IV

Analytical Methods

Introduction

The Food Safety and Inspection Service (FSIS) requires analytical methods for detecting, quantifying, and identifying residues that may be present in meat, poultry, and processed egg products. These methods can be used by the Agency for monitoring and surveillance activities to determine whether a product is adulterated and for human risk assessment evaluations. The Agency uses available methodology to take appropriate regulatory action against adulterated products, consistent with the reliability of the analytical data. This section describes the types of methods used by FSIS to conduct analyses.

KEY TO ABBREVIATIONS

APCI -- Atmospheric Pressure Chemical Ionization

Confirm -- Confirmatory Method

Determ. -- Determinative Method

ECD -- Electron Capture Detector

ELISA -- Enzyme-Linked Immunosorbent Assay

GC -- Gas Chromatography

GPC -- Gel Permeation Chromatography

HPLC -- High Performance Liquid Chromatography

Method Detection Limit -- The lowest amount of individual residue or sample component that can be reliably observed or found in the sample matrix by the current appropriate analytical methodology.

Minimum Reportable Level – The lowest level at which the analytical result is reported.

MS -- Mass Spectrometry

NA -- Not Applicable

ppb -- Parts per billion

ppm -- Parts per million

SIM -- Selected-Ion Monitoring Mode

TBD -- To Be Determined

Table AIV
Analytical Methods
2004 National Residue Program

Compound Class	Compound	Analytical Method			Minimum Proficiency Level ^a		
		Screen	Determinative (quantitative)	Confirmatory (identification)	Screen	Determinative (quantitative)	Confirmatory (identification)
Antibiotics	Carbadox		GC-ECD	TBD		15 ppb	TBD
	Chloramphenicol		GC	GC-MS		0.30 ppb	0.30 ppb
	Florfenicol		HPLC	GC-MS		1.9 ppm (L)	1.9 ppm (L)
Antibiotics : beta-Lactams	Penicillin	7-Plate Bioassay	Bioassay			0.01 ppm	
Antibiotics : Tetracyclines	Chlortetracycline	7-Plate Bioassay	Bioassay	HPLC (chemistry)	0.5 ppm	0.08 ppm	
	Oxytetracycline						
	Tetracycline						
Antibiotics: Macrolides	Clindamycin	7-Plate Bioassay		MS			0.1 ppm
	Erythromycin		Bioassay			0.05 ppm	0.1 ppm
	Lincomycin						0.1 ppm
	Pirlimycin						0.1 ppm
	Tilmicosin		HPLC- Ion Pairing		300 ppb (M) 600 ppb (L,K)		600 ppb
	Tylosin		Bioassay		0.2 ppm		0.1 ppm
Antibiotics: Aminoglycosides	Amikacin	7-Plate Bioassay		MS			1.0 ppm (L,K), 0.4 ppm (M)
	Apramycin						0.4 ppm (K) 0.1 ppm (L,M)
	Dihydrostreptomycin		Bioassay				0.4 ppm (L,K,M)
	Gentamicin		Bioassay		0.15 ppm		0.1 ppm (K,M)
	Hygromycin						1.0 ppm (L,K) 0.4 ppm (M)
	Kanamycin						4.0 ppm(L), 2.0 ppm (K), 0.4 ppm (M)
	Neomycin		Bioassay		0.25 ppm		0.1ppm (K,M)
	Spectinomycin				10.0 ppm		1.0 ppm (L) 0.4 ppm (K) 0.25 ppm (M)
	Streptomycin		Bioassay		0.1 ppm		0.4 ppm (L,K,M)
	Tobramycin						1.0 ppm (L) 0.1 ppm (K,M)

Table AIV – *continued*
Analytical Methods
2004 National Residue Program

Compound Class	Compound	Analytical Method			Minimum Proficiency Level ^a		
		Screen	Determinative (quantitative)	Confirmatory (identification)	Screen	Determinative (quantitative)	Confirmatory (identification)
Arsenicals	Arsenicals		AA	AA		0.2 ppm	0.2 ppm
Avermectins	Ivermectin, Doramectin, Moxidectin		HPLC	APCI/LC/MS		7.5 ppb	25 ppb
beta -Agonists	Cimaterol	ELISA			6 ppb		
	Clenbuterol	ELISA		LC-MS-MS	3 ppb		TBD
	Ractopamine		HPLC	LC/MS		1 ppb	1 ppb
	Salbutamol	ELISA			3 ppb		
Hormones, synthetic	DES		GC-MS	GC-MS		0.5 ppb	1.0 ppb
	Zeranol		GC-MS	GC-MS		0.5 ppb	1.0 ppb
Nonsteroidal Anti-inflammatory Drugs (NSAIDs)	Phenylbutazone	ELISA		LC-MS-MS	50 ppb		50 ppb
	Flunixin	ELISA	HPLC	LC/MS	50 ppb	31.3 ppb	125 ppb
Steroids	Melengesterol Acetate		GC	LC/MS		5 ppb	12.5 ppb
Sulfonamides	Sulfapyridine	TLC	GC-MS			0.05 ppm	0.05 ppm
	Sulfadiazine						
	Sulfathiazole						
	Sulfamerazine						
	Sulfamethazine						
	Sulfachloropyridazine						
	Sulfamethoxypyridazine						
	Sulfaquinoxaline						
	Sulfadimethoxine						
	Sulfathoxypyridazine						
	Sulfaphenazole						

Table AIV – *continued*
Analytical Methods
2004 National Residue Program

Compound Class	Compound	Analytical Method			Minimum Proficiency Level ^a		
		Screen	Determinative (quantitative)	Confirmatory (identification)	Screen	Determinative (quantitative)	Confirmatory (identification)
Sulfonamides	Sulfatroxazole		TLC	GC-MS		0.05 ppm	0.05 ppm
	Sulfisoxazole						
	Sulfadoxine						
CHCs/COPs/PCBs	Aldrin		GPC with GC-EC	GC-MS		0.10 ppm	
	<i>alpha</i> -BHC					0.10 ppm	0.01 ppm
	Captan				0.04 ppm		
	Carbophenothion				0.06 ppm		
	Chlorfenvinphos					0.06 ppm	
	Chlorpyrifos					0.10 ppm	
	<i>cis</i> -chlordane					0.30 ppm	
	Coumaphos-O					0.20 ppm	
	Coumaphos-S					0.20 ppm	
	Dieldrin					0.10 ppm	0.01 ppm
	Endosulfan I				0.01 ppm		
	Endosulfan II					0.06 ppm	
	Endrin					0.10 ppm	0.03 ppm
	HCB					0.10 ppm	0.01 ppm
	Heptachlor epoxide					0.10 ppm	0.10 ppm
	Heptacholr					0.10 ppm	0.01 ppm
	Kepone				0.06 ppm		
	Lindane					0.10 ppm	0.01 ppm
	Linuron				0.50 ppm		
	Methoxychlor					0.50 ppm	0.15 ppm
	Mirex					0.10 ppm	

Table AIV – *continued*
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		Screen	Determinative (quantitative)	Confirmatory (identification)	Screen	Determinative (quantitative)	Confirmatory (identification)
CHCs/COPs/PCBs (continued)	Nonchlor		GPC with GC-EC	GC-MS		0.15 ppm	
	o,p'-DDT				0.15 ppm		
	Oxychlordane					0.06 ppm	0.1 ppm
	p,p'-DDE					0.10 ppm	0.02 ppm
	p,p'-DDT					0.15 ppm	0.04 ppm
	p,p'-TDE					0.15 ppm	0.04 ppm
	PCB 1260					0.50 ppm	
	PCB 1254					0.50 ppm	
	Phosalone				0.02 ppm		
	Ronnel					0.06 ppm	
	Stirofos					0.06 ppm	
	Toxaphene					1.00 ppm	
	<i>trans</i> -chlordane					0.30 ppm	

a Minimum Proficiency Level: The lowest amount of individual residue or sample component that FSIS requires its laboratories to reliably detect, quantify, or confirm. This is usually the lowest amount for which the method used by FSIS laboratories has been validated.

Key:

L = Liver

K = Kidney

M = Muscle

AA = Atomic Absorption Spectroscopy

CHCs = Chlorinated hydrocarbons

COPs = Chlorinated organophosphates

PCBs = Polychlorinated biphenyls

GC = Gas Chromatography

MS = Mass Spectroscopy

GPC = Gel Permeation Chromatography

TLC = Thin Layer Chromatography

Table AIV – *continued*
Analytical Methods
2004 National Residue Program

ECD = Electron Capture Detection

ELISA = Enzyme Linked Immunosorbent Assay

ppm = parts per million

ppb = parts per billion

APCI = Atmospheric Pressure Chemical Ionization

HPLC = High Performance Liquid Chromatography

TBD = To be determined

Appendix V

Statistical Table

Table V, *Statistical Table*, indicates the number of samples required to ensure detection of a violation that affects a given percentage of the sampled population.

Table A V
Statistical Table
2004 FSIS National Residue Program

Percentage Violative in Sampled Population	Probability of Detection (Percent)			
	90	95	99	99.9
	Samples Required			
10	22	29	44	66
5	45	59	90	135
1	230	299	459	688
0.5	460	598	919	1,379
0.1	2,302	2,995	4,603	6,905
0.05	4,605	5,990	9,209	13,813