Organotin Residue Determination in Poultry and Turkey Sample Survey in the United States

Robert L. Epstein,*,† Evan T. Phillippo,‡ Royce Harr,† William Koscinski,‡ and Gail Vasco‡

Science and Technology, Food Safety and Inspection Service (FSIS), U.S. Department of Agriculture, 300 12th Street S.W., Washington, D.C. 20250, and Eastern Laboratory, Food Safety and Inspection Service, College Station Road, Athens, Georgia 30604

A method was developed to determine the presence of dibutyltin dilaurate, commonly known as butynorate, a coccidiostat and anthelmintic used in poultry. The method was used to support a 2-year statistically based random survey from January 1988 through January 1990 to determine the incidence of dibutyltin residues in young turkeys in the United States. Described are the performance characteristics of the high-pressure liquid chromatography method using postcolumn morin fluorescent derivative. The 2-year survey program was based on samples collected in 25 states having sufficient turkey production, and the subsequent findings showed an 8% positive rate with 90% of the positives from 5 states.

Butynorate is an organotin drug approved for veterinary use in the United States to treat and control hexamitiasis, an endemic and life-threatening disease in turkeys. It is also used in combination with piperazine and phenothiazine as an anthelmintic in chicken (*Feed Additive Compendium*, 1982). Some organotin compounds are suspect carcinogens (Horton, 1977).

Dibutyltin diacetate was placed on the suspect carcinogen list, as a result of feeding studies in rats (U.S. FDA, 1979). Butynorate may also be a suspect carcinogen, since dibutyltin diacetate and butynorate have similar metabolism forming the diol and the glucuronide metabolite.

Unpublished withdrawal data indicate the presence of butynorate residues in turkey liver for approximately 28 days with depletion in muscle tissue in less than 7 days (Frahm, 1973). Butynorate does not have an established tolerance for residues in poultry tissues, because the drug's approval predates 1958. On December 18, 1986, a feeding regimen of butynorate at 0.375% for prevention of hexamitiasis and coccidiosis in turkeys was published (*Fed. Regist.*, 1986), changing the withdrawal time from 7 to 28 days.

The FSIS–USDA conducted a survey from January 1988 through January 1990 to determine possible butynorate residues in young turkeys. The survey was based on sampling 1 in 500 000 turkeys slaughtered in the United States on a statistically random basis. On the basis of the number of samples collected, at least a 1% incidence of butynorate residues in turkeys can be determined with a 95% confidence. The minimum reporting concentration was 0.2 mg/kg, using liver as the tissue of highest residue concentration. Most, 90%, of the positive butynorate turkey liver samples were limited to five states. There were no positive findings of butynorate in turkey muscle tissue.

METHODOLOGY DEVELOPMENT

The original method for determining organotin compounds in tissue (Corbin, 1973) was based on hydrolysis of the glucuronide conjugate and any ester residues to the diol form with spectrophotometric quantitation of a pyrocatechol violet–tin complex for organotin. This method was used by the sponsor to generate withdrawal data and was also used by FSIS for a limited 2-month–60-sample turkey liver survey in 1982. The method was found to be unsuitable, because of a 2 sample/day throughput and its nonspecificity for dibutyltin.

This 1982 survey also used atomic absorption (AA) to achieve greater productivity and verify that background concentration of tin in turkey liver is less than 0.02 mg/kg. The sample preparation and extraction of tissue for AA analysis of tin is an ashing technique with conversion to the hydride followed by (AA) analysis at 224.3 nm in a hydrogen–argon flame. Details of the procedure are depicted in Figure 1. In the 1982 survey all samples were analyzed by both the AA and spectrophotometric techniques without any significant findings of tin.

In planning the more extensive 1988–1990 survey, AA was to be used for rapid screening of liver samples for tin. However, a method was still needed to specifically determine the dibutyltin component. Several published methods were reviewed for their utility in determining residues of dibutyltin in tissue. The first method (Sasaki, 1988) was based on gel permeation chromatography followed by gas chromatography (GC) of the Grignard reagent generated methyl derivative. The second method, also a GC method (Tsuda, 1986), was based on hydride generation. Neither method achieved suitable recoveries of dibutyltin based on fortifications of tissue with dibutyltin dilaurate (DBTDL).

The last method evaluated for the determination of dibutyltin was based on modifications of a high-pressure liquid chromatography (HPLC) postcolumn fluorescence method using a morin (pentahydroxyflavone) derivative (Yu and Arakawa, 1983). The major modification in the method substituted a silica Sep-Pak in place of the Minipore filter. This method produced satisfactory data for the determination of dibutyltin in tissue and was used by FSIS to verify the presence of the drug in AA positive samples.

EXPERIMENTAL PROCEDURES

Apparatus. The high-pressure liquid chromatography (HPLC) instrumentation for postcolumn analysis consisted of a Waters (Millipore Corp., Milford, MA) Model 840 system with fluroescence detector 420, equipped with Varian (Sunnyvale, CA) pump 2010. The chromatographic columns from Alltech Associates (Deerfield, IL) were Adsorbosphere 287203 (150 × 4.6 mm, 5 μm), the connecting tee U-429 was purchased from Rainin Inc. (Woburn, MA), and the pulse dampener was constructed in-house from a 10-m length of 0.01-in. stainless steel tubing. The

† FSIS, Washington, DC.
‡ FSIS, Athens, GA.

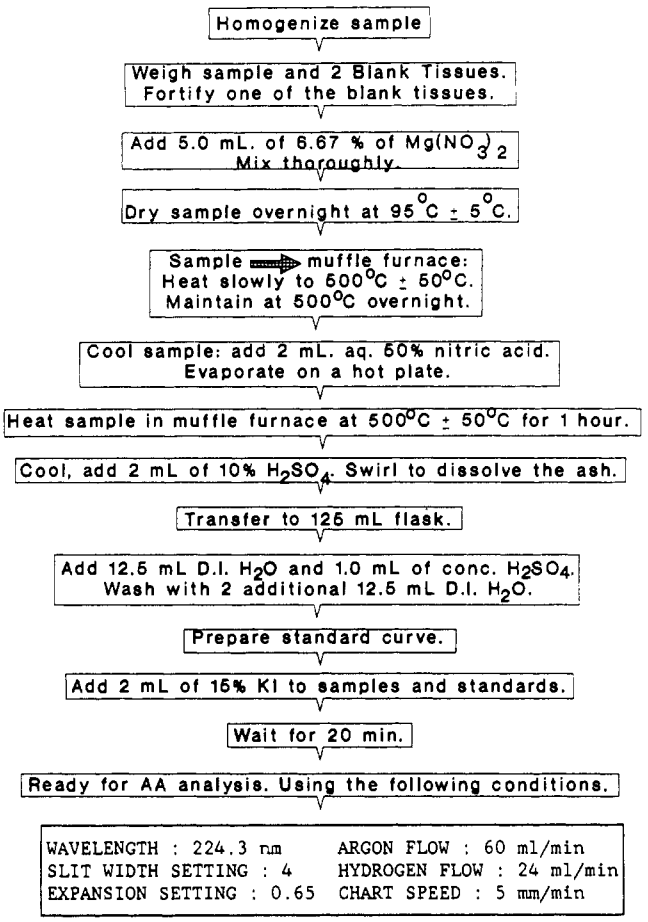


Figure 1. Atomic absorption analytical procedure scheme for the determination of elemental tin in poultry tissue.

Table I. Instrumentation Conditions for Dibutyltin Analysis in Poultry Tissue

chromatography	
instrument	HPLC, Waters Model 840
HPLC pump	Varian Model 1020
analytical column	adsorbosphere CN column, 150 mm × 4.6 mm, 3–5-μm particle size, Alltech Associates Inc., P/N 28703
guard column	CN, 3–5-μm particle size
mobile phase	hexane/ethyl acetate (95:5) containing 3% acetic acid
column flow rate	2.5 mL/min
detection	
detector	fluorescence detector, Waters Model 420
derivatizing reagent	0.005% morin in ethanol
flow rate	1.3 mL/min
detector setting	excitation, 420 nm emission, 495 nm
retention time	3.15 ± 0.10 min

specifications for operation of the HPLC postcolumn fluorescence analysis system are found in Table I. Other equipment used for preparation of the tissue samples included a Eberbach Shaker Model 6010 (Scientific Instruments, Ann Arbor, MI), silica Sep-Pak cartridges 51900 from Waters, a rotary vacuum evaporator (Buchler Instruments, Saddle Brook, NJ), and a Sorvall (E. I. du Pont, Newton, CT) Model T6008 centrifuge.

Reagents. All solvents were distilled in glass (Burdick & Jackson Laboratories, Inc., Muskegon, MI). Hydrochloric and acetic acids and the NaCl to prepare the 0.85% saline were of reagent grade (Baker, Phillipsburg, NJ). The morin and ethanol to prepare the 0.005% ethanolic solution were purchased from Sigma Chemical Co. (St. Louis, MO) and Thomas Scientific (Swedesboro, NJ), respectively.

Standards. DBTDL, catalog no. D12470, was purchased from Pfaltz and Bauer (Waterbury, CT). The standard stock solution of 1000-μg/mL in hexane was prepared by weighing 25 mg of DBTDL into a 25-mL volumetric flask and diluting to volume. This solution is stable at 39 °F for 1 month. A standard intermediate solution of 10 μg/mL is prepared by diluting the standard stock solution 1:100 with hexane. The intermediate standard solution prepare solutions for standard curves at concentrations of 1.25, 2.5, and 5.0 μg/mL of DBTDL in hexane. The DBTDL standard for fortifying tissues is prepared form the standard stock solution at 25 μg/mL, a 1:40 dilution with hexane, and is stable at 39 °F for 2 weeks. Add 100 μl of this solution to 5 g of tissue to produce a 0.5 mg/kg fortification. Reanalysis of the working standard solutions after 90 days showed they were stable, producing the same analytical response by HPLC.

Sample Preparation. Partially thaw frozen liver tissue in a refrigerator. Cut the sample into small cubes and blend in a food processor or another suitable blender until the consistency of a puree is obtained. Place in a plastic bag and keep frozen at −20 °C until ready for analysis.

Extraction and Isolation. Weigh 5.0 g of thawed tissue into a 50-mL polypropylene centrifuge tube. Add 10 mL of saline solution and shake vigorously for 10 s. Slowly add 8 mL of HCl, shake for 10 s, and allow to stand for 5 min. Add 3 g of NaCl and 20 mL of ethyl acetate. Shake to mix well and place on a mechanical shaker at high speed for 10 min. Centrifuge at 3000 rpm for 5 min and transfer the supernatant to a 100-mL round-bottom flask by using a disposable pipet. Repeat the extraction procedure and combine extracts.

Evaporate the solution by using a rotary evaporator under reduced pressure at 30–35 °C to 0.5–1.0 mL. Transfer the solution to a 15-mL centrifuge tube by using 9 mL of hexane and mix for 10 s. Centrifuge for 10 min at 0 to −10 °C for 10 min at 1500 rpm. Transfer the hexane layer through a Sep-Pak (prewashed with 3 mL of hexane) into a 10-mL centrifuge tube. Use a 10-mL syringe to push the sample with gentle pressure through the Sep-Pak. Wash the Sep-Pak with 5 mL of hexane aliquots until 15 mL of eluate is collected. Evaporate to a final volume of 1 mL under N_2 at 35 °C for HPLC determination.

HPLC Analysis Considerations. The flow rate of the mobile phase through the guard and analytical columns is 2.5 mL/min, and the morin flow rate is 1.3 mL/min. The injected sample passes through the columns and is mixed with morin inside the tee just before it reaches the detector. The reaction of dibutyltin with morin is instantaneous. Detector settings are 420 nm for excitation and 495 nm for emission. A pulse dampener was attached to the pump for the flow of the morin solution. The extraction procedure applicable to turkey/chicken liver and muscle is summarized in Figure 2, and the requirements for HPLC fluorescence analysis are specified in Table I.

Calculations. Calculate the milligrams per kilogram as DBTDL in the sample as

$$\text{mg/kg DBTDL} = (CV)/W$$

where V equals final volume (usually 1 mL), W is the sample weight in grams, and C is the concentration of the fortified sample determined from the standard curve.

RESULTS AND DISCUSSION

Table II is a statistical compilation of the performance characteristics of the DBTDL method over the 0.25–1.00 mg/kg range showing recoveries calculated as DBTDL exceeding 80% and a coefficient of variation (VC) of less than 13%. Results of sample studies consisting of 14 unknowns clearly show satisfactory method performance. The two-analyst study in turkey liver (Table III) over the range 0.25–1.00 mg/kg showed a mean recovery of at least 85% and a CV of less than 13%. Similarly, the single-analyst study of 14 unknowns extending the method into turkey muscle and chicken liver (Table IV) produced equivalent data. The limit of quantitation in tissue was estimated at 0.2 mg/kg on the basis of a 10 times electronic signal to noise ratio. The mean of 21 analytical sample

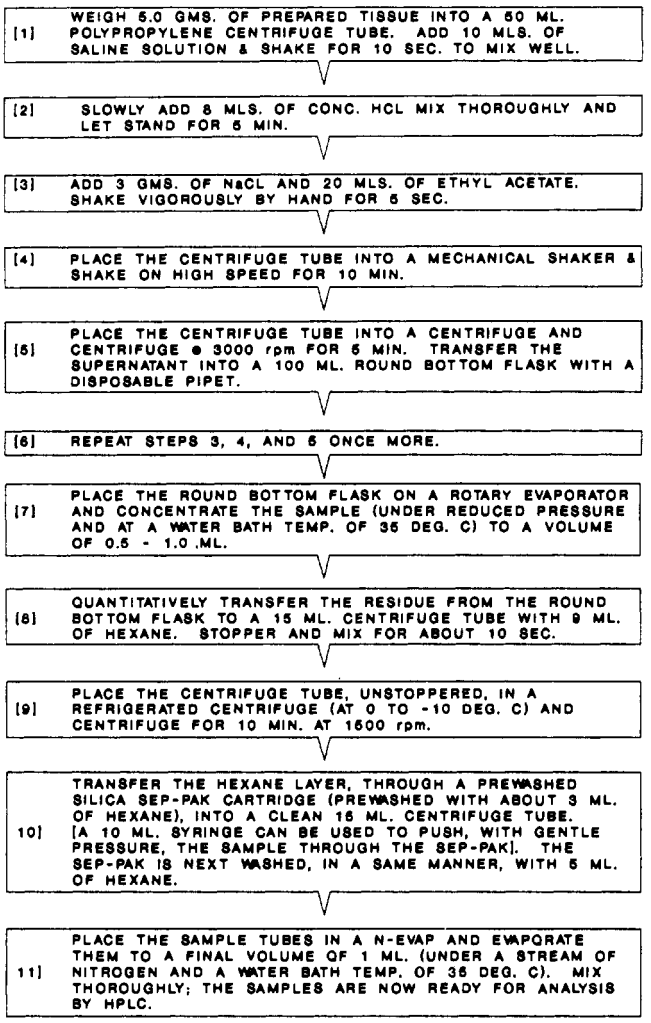


Figure 2. HPLC analytical procedure scheme for the determination of dibutyltin dilaurate (DBTDL) in poultry tissue.

Table II. Statistical Evaluation of DBTDL by HPLC Known Fortification Studies

		concn,[a] mg/kg			
species/tissues	%	0.25	0.50	1.0	combined
turkey liver[b]	recovery	94.7	88.1	83.5	88.8
$N = 6$	SD	6.4	5.5	2.0	6.9
	CV	6.8	6.2	2.4	7.8
turkey muscle	recovery	99.3	82.7	85.3	89.1
$N = 3$	SD	11.0	6.1	9.1	11.0
	CV	11.1	7.4	10.6	12.3
chicken liver	recovery	92.7	100.0	96.0	96.2
$N = 3$	SD	7.6	3.5	3.0	5.5
	CV	8.2	3.5	3.1	5.7

[a] Data represents an average from three sets of data on different days uncorrected for recovery. [b] Combined data from two analysts. SD, standard deviation; CV, coefficient of variation.

sets from the 2-year survey produced a mean recovery of 94% at 0.5 mg/kg DBTDL in turkey liver with a CV of 14%.

Figures 3–7 are respectively a representative example of a DBTDL standard at 500 ng/mL, a turkey liver blank tissue chromatogram, a fortification chromatogram at 500 μg/kg of DBTDL in turkey liver, a fortification chromatogram at 250 μg/kg in poultry liver—the limit of reliable quantitation—and an incurred turkey tissue quantitated at 500 μg/kg (the incurred tissue was also fortified with a surrogate analyte, dioctyltin dilaurate, for process control). The slightly elevated baseline at the DBTDL retention time in blank turkey liver did not bias the linear regression curve of fortified tissue. The slightly higher recoveries at the lower concentrations from Table II did not affect the utility of the method.

Table III. Unknown Sample Studies by HPLC

	DBTDL in turkey liver,[a] mg/kg					
	analyst 1			analyst 2		
sample[b]	added	found	% recovery	added	found	% recovery
1	0.75	0.56	75	0.25	0.24	96
2	0	0	NA	1.00	0.90	90
3	1.0	0.78	78	0.38	0.30	79
4	0	0	NA	0	0	NA
5	0.50	0.39	78	0.50	0.53	106
6	0.75	0.56	75	0	0	NA
7	0.25	0.23	92	0.375	0.37	99
8	0.38	0.40	105	0.75	0.60	80
9	0.50	0.45	90	1.00	0.73	73
10	0.25	0.24	96	0.25	0.22	88
11	0	0	NA	0.50	0.39	78
12	0.38	0.33	87	0	0	NA
13	0	0	NA	0.75	0.71	95
14	1.0	0.80	80	0	0	NA
x			85			88
SD			8.0			11
CV			9.4			12.5

[a] All values uncorrected for recovery. [b] Samples 1–7 and 8–14 were analyzed on different days. NA, not applicable; SD, standard deviation; x, mean recovery; CV, % coefficient of variation; HPLC, high-pressure liquid chromatography; DBTDL, dibutyltin dilaurate.

Table IV. Unknown Sample Studies by HPLC

	DBTDL in poultry,[a] mg/kg					
	turkey muscle			chicken liver		
sample[b]	added	found	% recovery	added	found	% recovery
1	0.25	0.19	76	0.25	0.23	92
2	1.0	0.77	77	1.0	1.02	102
3	0.38	0.27	72	0.38	0.38	100
4	0	0	NA	0	0	NA
5	0.50	0.45	90	0.50	0.51	102
6	0	0	NA	0	0	NA
7	0.38	0.38	100	0.38	0.38	100
8	0.75	0.67	89	0.75	0.75	100
9	1.0	0.83	83	1.0	1.1	110
10	0.25	0.22	88	0.25	0.34	136[c]
11	0.50	0.37	74	0.50	0.44	88
12	0	0	NA	0	0	NA
13	0.66	0.75	88	0.75	0.69	92
14	0	0	NA	0	0	NA
x			84			98
SD			9			6
CV			10.7			6.1

[a] All values uncorrected for recovery. [b] Samples 1–7 and 8–14 were analyzed on different days. [c] Outlier. NA, not applicable; SD, standard deviation; x, mean recovery; CV, % coefficient of variation; HPLC, high-pressure liquid chromatography; DBTDL, dibutyltin dilaurate.

SURVEY STUDY

From January 1988 through January 1990, 1031 turkey samples were collected from 25 states and analyzed for butynorate. Twenty-five states did not slaughter sufficient numbers of samples to be included in the survey. The sampling rate was about 1 sample per 500 000 young turkeys slaughtered.

Samples approximately 1 lb in weight, were collected by Agency inspectors on designated dates, frozen at −20 °C, and then shipped to the laboratory. Upon receipt at

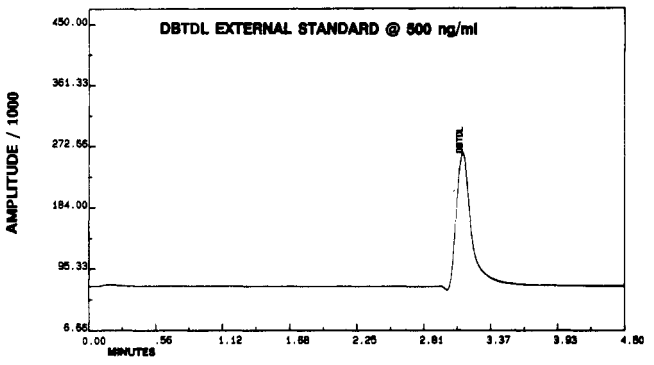
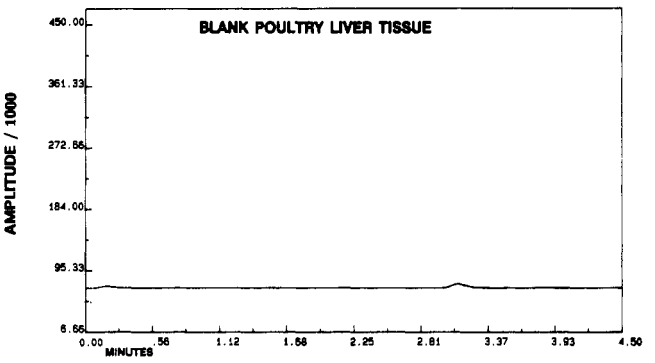


Figure 3.



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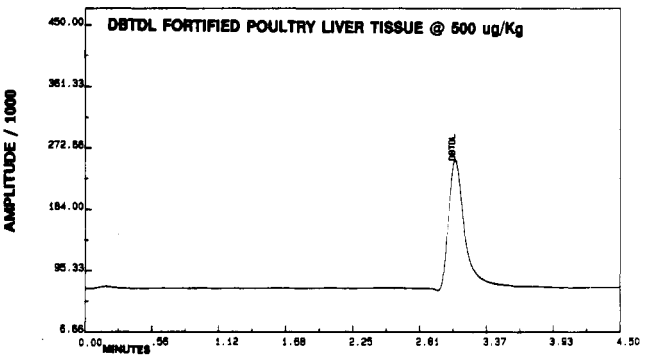

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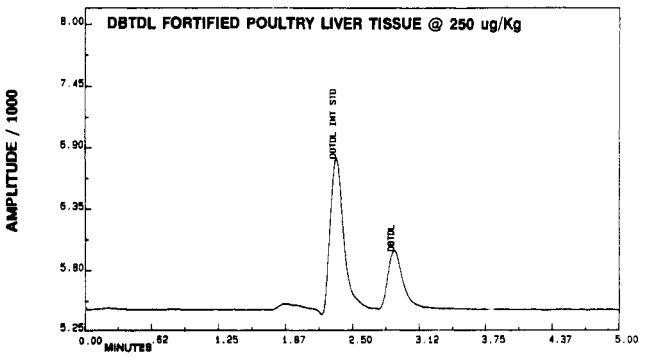

Figure 6.

the laboratory the sample was thawed and the entire sample homogenized and blended in a food processor. The homogenized sample was frozen at −20 °C until ready for analysis. Separate tissue aliquots were used for all replicate analyses.

All liver tissues were initially analyzed by AA. Samples in which the total tin by AA exceeded 0.1 mg/kg (equivalent to 0.5 mg/kg DBTDL) were subjected to further analysis by the HPLC method. All HPLC positives equal to or greater than 0.20 mg/kg were reanalyzed and

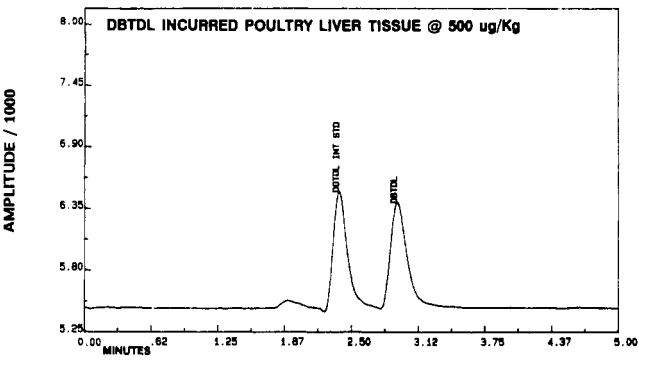

Figure 7.

Table V. Distribution of Butynorate Positives by State

	turkey liver tissue, mg/kg			
state[a]	no. of samples	positives samples	% positives	range of positives
AR	61	15	24.6	0.2–5
CA	135	0	0	
CO	12	0	0	
GA	2	0	0	
IL	3	0	0	
IN	52	0	0	
IA	57	0	0	
MA	1	0	0	
MI	19	2	10.5	0.2–0.3
MN	162	15	9.3	0.2–1
MO	71	22	31.0	0.2–6
NE	12	0	0	
NY	2	0	0	
NC	187	3	1.6	0.5–3
OH	3	1	33.3	2
OK	2	1	50.0	2
OR	10	0	0	
PA	25	1	4.0	1
SC	22	0	0	
SD	9	0	0	
TX	29	11	37.9	0.2–2
UT	17	0	0	
VA	106	0	0	
WV	2	0	0	
WI	23	22	47.8	0.2–2
total	1031	82	8.0	0.2–6

[a] Samples were collected in 25 states. There were insufficient numbers of turkeys slaughtered in the remaining 25 states to meet the 1/500 000 sampling rate.

the individual values calculated to two significant figures. The two values were also subjected to the 95% confidence interval test (ASTM, 1979), and if the criteria for 95% confidence were not met, the samples were reanalyzed in duplicate and the new mean was reported. All mean values were reported to one significant figure.

The actual dibutyltin concentrations were 30–60% of the total tin value by AA and calculated as tin. This clearly indicates the possibility of other tin metabolites, e.g., monobutyltin and/or elemental tin by successive dealkylation.

Tin residues were not detected (no response for tin) by AA in approximately 90% of the turkey liver samples. There were 82 (8.0%) verified positive turkey liver samples analyzed by HPLC for butynorate which were equal to or in excess of 0.2 mg/kg (Table V). Five states, Arkansas, Minnesota, Missouri, Texas, and Wisconsin, accounted for 90% or 74 of the 82 butynorate positives and 33% or 346 of the total samples collected. Another five states, Michigan, North Carolina, Ohio, Oklahoma, and Penn-

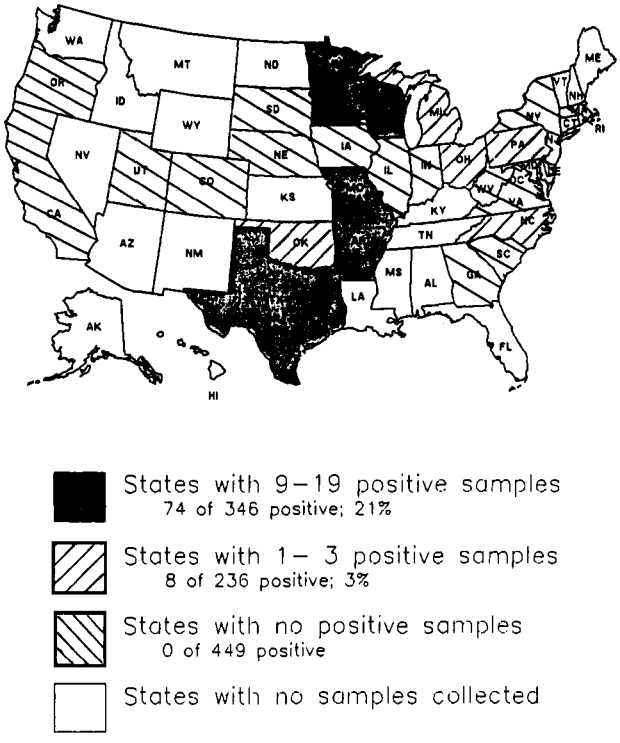

Figure 8. Distribution of butynorate in liver samples collected randomly from young turkeys from Jan 1, 1988, through Jan 16, 1990 (sampling rate approximately 1/500 000).

sylvania, accounted for the remaining 8 positive samples, representing 23% or 236 of the samples. The remaining 15 states, accounting for 43% of the samples, did not have any verified positives of dibutyltin. Of the 82 positive butynorate samples, 25 samples were equal to or greater than 1 mg/kg, 23 samples were between 0.5 and 0.9 mg/kg inclusive, and the remaining 34 samples were from 0.2 through 0.4 mg/kg. Butynorate positives in turkey liver did not exceed 6 mg/kg.

Figure 8 is a map of the United States divided into four sections illustrating the 5 states with a high significant incidence of butynorate positive samples [74 of 346 (21%)], the 5 states with a low incidence [8 of 236 (3%)], the 15 states where dibutyltin was not detected in any of the 449 samples, and the 25 states where the turkey slaughter was insufficient for sampling.

Companion turkey muscle tissue samples, which were collected from September 1988 through January 1990 and analyzed if the concentration of butynorate in liver was 0.2 mg/kg or greater, did not show any significant incidence of tin by AA. The only two positives by AA were not substantiated by HPLC.

ACKNOWLEDGMENT

We express our appreciation to Margaret Coleman of the Residue Evaluation and Planning Division, FSIS, for verification of the survey results and construction of the U.S. map.

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Received for review August 16, 1990. Accepted December 20, 1990.

Registry No. Butynorate, 77-58-7.