Determination of Pentachlorophenol in Milk and Blood of Dairy Cattle

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Rapid, similar analytical methods are presented for the determination of pentachlorophenol (PCP) residues in milk and blood from dairy cattle. The method for milk employs a hexane/2-propanol extraction, base partition and wash, reacidification, and extraction with hexane; for blood, PCP is extracted from acidified solution with hexane. The PCP is then determined, underivatized, by gas–liquid chromatography on a 2% SP1000/1% H_3PO_4 column with electron-capture detection. The identity of PCP is confirmed by treatment with diazomethane and gas chromatography of the pentachloroanisole derivative. Recoveries were >90% for milk and >87% for blood over a range of fortification levels. The limit of quantitation is 4 ppb for milk and 10 ppb for blood. The limit of detection for both substrates is 1.0 ppb. Details of method optimization and its application to the analysis of milk containing bioincurred residues of PCP are discussed.

In late 1975, when cattle in Michigan became ill, conditions led authorities to suspect pentachlorophenol (PCP) as the causative agent (*Chem. Eng. News*, 1977; *Chem. Mark. Rep.*, 1977). To investigate this problem, the Bureaus of Food and Veterinary Medicine of the Food and Drug Administration (FDA) jointly conducted a feeding study with commercial PCP in dairy cattle (Firestone et al., 1979). Methodology was developed to determine PCP in whole milk and whole blood samples, and the method is reported here.

Numerous methods have been developed which reflect a wide variety of analytical approaches for the determination of PCP in many sample types. Methods have been reported for the determination of PCP in urine (Barthel et al., 1969; Rivers, 1972), tissue (Barthel et al., 1969; Gee et al., 1974; Bevenue and Beckman, 1967), clothing (Barthel et al., 1969), soil (Stark, 1969; Yip, 1970), water (Stark, 1969; Rudling, 1970; Chau and Coburn, 1974), fish (Stark, 1969; Yip, 1970), fats and waxes (Higginbotham et al., 1970), blood (Barthel et al., 1969; Rivers, 1972; Bevenue et al., 1968), and milk (Erney, 1978). PCP in most instances was determined as a derivative by using gas–liquid chromatography (GLC). These reports provided a background for the development of methodology for the determination of PCP in whole milk and whole blood consistent with the specific requirements of simplicity, similarity, and speed needed for their application in a cow feeding study.

MATERIALS AND METHODS

Reagents. Hexane, 2-propanol, benzene, and acetone were distilled in glass (Burdick and Jackson Laboratories, Muskegon, MI). Diazomethane was prepared from Diazald (Cat. No. D2800-0, Aldrich Chemical Co., Inc., Milwaukee, WI), using procedure 1 given in the Aldrich bulletin for diazomethane preparation. Reference standards were supplied as primary standards by the Environmental Protection Agency, Pesticide Reference Standards Section, Beltsville, MD. Concentrated stock solutions of PCP were prepared in benzene; dilutions for GLC and for recovery experiments were prepared in hexane.

All extraction and cleanup procedures were performed in Teflon-lined screw-cap test tubes. With these tubes, shaking, heating, and centrifuging were easily accomplished. Transfers were kept to a minimum, reducing possible losses, and the speed and efficiency of the analyses were increased.

Gas–Liquid Chromatography. GLC was carried out on a Packard 7000 Series gas chromatograph equipped with a titanium tritide electron-capture detector. Column temperature was 175 °C and detector temperature was 200 °C. The electrometer setting was usually 1×10^{-9} amp at a detector voltage of 50 V. Under these conditions, 2.0 ng of PCP gave a 50% full-scale response. PCP levels were determined as the underivatized phenol by comparison with a standard. The nitrogen carrier gas flow rate was 120 mL/min. Oxygen and water-free ultrahigh-purity nitrogen were used to prevent H_3PO_4-coated column deterioration. A Hewlett-Packard 1-mV full-scale recorder with a chart speed of 0.5 in./min was used.

The GLC column used for the determination of underivatized PCP consisted of a 0.61 m × 4 mm i.d. glass column containing 2% SP1000/1% H_3PO_4 on 80–100 mesh Chromosorb W AW. The column packing was prepared as follows. With gentle warming on a steam bath, 0.40 g of SP1000 (Supelco, Inc.), which had been powdered with mortar and pestle, was dissolved in 200 mL of chloroform. To this solution was added 20 g of 80–100 mesh Chromosorb W AW. The chloroform was removed by slow rotary evaporation using a warm (ca. 50 °C) water bath. To the semidry packing was then added 0.02 g of 85.6% phosphoric acid dissolved in 200 mL of acetone. The acetone was removed by slow rotary evaporation, and when the packing appeared to be dry, it was transferred to a HI-EFF Fluidizer (Applied Science). Any packing remaining on the sides of the flask was also collected. The Fluidizer was heated (column packing ca. 65 °C) with a low flow of nitrogen for 1 h. The packing was allowed to cool to room temperature in the Fluidizer under a flow of nitrogen and then gently sieved to insure uniform 80–100-mesh coated particle size. The glass column was first rinsed with a 1% H_3PO_4 in acetone solution and then with acetone and allowed to air-dry before packing. The packing was added to the column under vacuum. (The outlet was plugged with a small piece of glass wool which had been previously soaked in a 1% H_3PO_4 in acetone solution and air-dried.) The column was then conditioned overnight at 200 °C with a low flow (ca. 30 mL/min) of nitrogen. The samples containing the methyl ether derivative of PCP (pentachloroanisole, PCA) were chromatographed on a 10% OV-101 column prepared and used as described in the "Pesticide Analytical Manual" (1972).

Sample Collection and Preparation. Whole milk samples were collected by milking into 1-qt Mason jars,

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Table I. Conditions for Acid Treatment and Extraction of Whole Milk Samples Containing Bioincurred and Added PCP

condition	bioincurred		pH of solution	fortified[b]	
	found,[a] ppm ± CV, %	no. of samples		recov,[a] % ± CV, %	no. of fortif
ES[c] only	1.50 ± 2.3	3	6.5	60 ± 9.2	3
ES + 0.05 M H_2SO_4[d]	1.87 ± 2.0	3	5.0	94 ± 1.6	3
ES + concd H_2SO_4[d]	4.77 ± 1.0	3	<1.0	92 ± 4.3	5
ES + concd H_2SO_4,[d,e] 100 °C/h	4.70 ± 1.6	3	<1.0	100 ± 4.9	3

[a] Mean ± coefficient of variation. [b] Control milk prior to dosing fortified at the 0.01-ppm level. [c] Extraction solvent, hexane/2-propanol (8:2), three 10-mL extractions. [d] Five millimeters of acid added. [e] Initial 10-mL extraction, heated.

which were then subsampled into 50-g portions and stored at 0 °C (Firestone et al., 1979). Before 10-g analytical portions were weighed, a 50-g sample was removed from the freezer, allowed to thaw in a warm water bath, and blended at medium speed 2 min.

Whole blood samples were collected in vacuum ampules (50 mL) containing 1 mL of anticoagulant solution (7.5% EDTA) and stored at 5 °C until analyzed (Firestone et al., 1979).

Analysis of Whole Milk. Five milliliters of concentrated sulfuric acid was slowly added to a 25 × 150 mm screw-cap test tube containing 10 g of whole milk. The tube was sealed with a Teflon-lined screw cap, and the contents were mixed by carefully swirling and allowed to stand at room temperature until the resulting heat dissipated (ca. 10–15 min). Then 10 mL of hexane/2-propanol (8:2, v/v) solution was added to the reaction mixture. The tube was sealed and gently mixed, and the cap then loosened to relieve any pressure buildup. The tube cap was tightened again, briefly shaken at 10-min intervals for 30 min, and then centrifuged at 2000–2500 rpm for 3 min. The upper hexane layer was removed by Pasteur pipet to a second test tube. The aqueous layer was extracted twice more by shaking for 2 min with 10-mL portions of hexane/2-propanol, centrifuging, and removing the hexane layer after each extraction as before. The combined extracts (24 mL) were shaken 2 min with 5 mL of 1 M KOH. The upper hexane layer was removed and discarded. The aqueous layer was washed with an additional 10 mL of hexane, and the hexane wash was discarded. To the aqueous layer was then added 5 mL of 6 M sulfuric acid. The acidified solution was extracted three times by shaking 2 min with 6-, 2-, and 2-mL portions of hexane. After each extraction, the tube was centrifuged 1 min and the upper hexane layer was removed. The extracts were combined in a volumetric flask for GLC. For whole milk samples containing residue levels greater than 0.05 ppm, the solution volume was adjusted (0.50 mL or more) to give an appropriate GLC response.

To determine residue levels of PCP in whole milk at less than 0.05 ppm, a supplemental cleanup was required. The final solution volume for samples containing PCP residue levels less than 0.05 ppm was concentrated on a steam bath to 0.5 mL. To this was added 0.5 mL of concentrated sulfuric acid and the mixture was carefully swirled. After separation of the layers, PCP was determined by injection of an aliquot from the hexane layer. A 0.01-ppm residue level could be quantitated by injecting 5 μL (100 mg of sample equivalent injected). The limit of quantitation (10% full-scale recorder deflection) for 100 mg of equivalent of whole milk injected was 4 ppb. The limit of detection (twice the base line noise) was ca. 1.0 ppb.

Analysis of Whole Blood. Five milliliters of 6 M sulfuric acid and 6 mL of hexane were added to a 20 × 150 mm screw-cap test tube containing 2.05–2.10 g (2.0 mL) of whole blood. The tube was sealed with a Teflon-lined screw cap, briefly shaken, and placed in a boiling water bath where it was heated 45 min with shaking at 15-min intervals. (Samples were heated in a fume hood. Periodic shaking was accomplished with the tube wrapped in a cloth towel. No safety problems arose.) After cooling to room temperature, the tube was centrifuged at 2000–2500 rpm. The upper hexane layer was removed by Pasteur pipet to a 10-mL volumetric flask and the aqueous hydrolysate was extracted twice more by shaking for 2 min each with 2-mL portions of hexane. Without adding more hexane, the mixture was vigorously shaken once more and centrifuged, and the last traces of hexane were collected. The combined extracts (9.5–10 mL) were diluted or concentrated and analyzed as described for milk. The limit of quantitation for whole blood (20 mg of sample equivalent injected) was 10 ppb; the limit of detection was ca. 1.0 ppb.

RESULTS AND DISCUSSION

Extraction. In order to accommodate the large number of samples involved in the FDA dairy cow feeding study, closely related methods were developed which could be utilized for the analysis of both whole milk and whole blood. Fast, accurate, and reliable methods, utilizing similar reagents and glassware, were needed. In devising these methods, advantage was taken of the stability of PCP in acid and its rapid conversion under alkaline conditions to an aqueous soluble salt.

An 8:2 mixture of hexane/2-propanol was used as the solvent for the milk extraction. The presence of 2-propanol proved effective in reducing emulsions. Strongly acidic conditions favored the extraction of PCP. Sample fortification revealed that the initial extraction step of the method for whole milk removed 93% of the PCP added. The second extraction increased the recovery to 98%. The third extraction was included in the procedure since three extractions can be carried out quickly and a third extraction provides assurance of complete recovery. Complete separation of the aqueous and organic phases did not occur after the first extraction with a few of the milk samples. Adding a few drops of 2-propanol, carefully swirling, and centrifuging resolved the problem. Alternatively, removing as much of the incompletely separated upper layer as possible and continuing the extraction with the second 10 mL of solvent gave complete separation for the second extraction.

To elucidate factors influencing the total extraction of PCP from milk containing bioincurred residues using this methodology, samples from the cow feeding study were extracted after they were subjected to different treatments with acid and heat. As shown in Table I, the highest residue level found resulted from treatment of whole milk with 5 mL of concentrated sulfuric acid. These conditions produced a solution temperature of approximately 80 °C, resulting from the heat of mixing. Heating the acidified milk-extraction solvent mixture for 1 h at 100 °C did not affect the recovery, but resulted in a more complex chromatogram (Figure 1A,C).

In order to evaluate the ability of the extraction step of

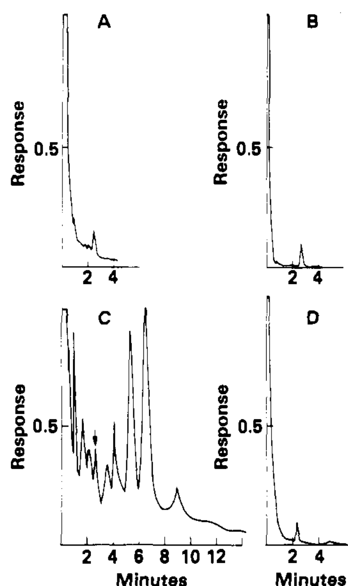

Figure 1. Gas–liquid chromatograms of whole milk, illustrating the determination of PCP at levels lower than 0.05 ppm: (A) before supplemental cleanup, (B) after supplemental cleanup, (C) added heat (100 °C for 1 h applied to first 10-mL extraction solvent mixture before supplemental cleanup), (D) heated sample after supplemental cleanup. Each chromatogram represents 100 mg of sample equivalent injected, at 2–3 ppb of PCP.

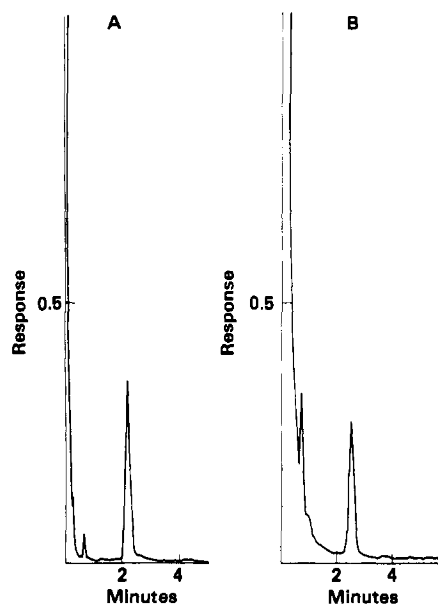

Figure 2. Typical gas–liquid chromatograms of (A) whole milk without supplemental cleanup at levels above 0.05 ppm, 10 mg of sample equivalent injected (0.08 ppm); (B) blood, 10.5 mg of sample equivalent injected (0.022 ppm).

the method to recover free PCP from whole milk, a set of experiments was carried out with milk fortified with PCP reference standard. Different conditions of heat and acid were used in the extraction. As shown in Table I, recoveries from milk samples that were not treated with acid averaged 60%. Addition of acid had a marked effect on the fortification experiments, resulting in recoveries greater than 90%.

Although the precise factors which control the release of PCP residues from milk for analysis under these conditions have not been described, some general observations can be made. In the absence of mineral acid, a strong interaction between PCP and the milk matrix is evident. The ionization of PCP probably plays a role, considering the native pH of milk (ca. 6.5) and pK_a of PCP (4.7) (Cessna and Grover, 1978). As the phenolate anion, the free PCP residue cannot be extracted efficiently from milk into hexane. The ionization can be overcome by adjusting the pH.

The addition of acid, which both shifts the equilibrium and effectively competes with the interactive sites, appears to be the key to the recovery of PCP added directly to whole milk. Comparison, however, with the amount of bioincurred residue extracted, using 0.05 M sulfuric acid and concentrated sulfuric acid, indicates a more precise dependence on acid strength for the release and recovery of bioincurred PCP residue from milk. In addition to being present in free form, PCP can be bio-incorporated into milk and exist in conjugated forms, e.g., as its glucuronide or sulfate. The increase (>60%) in the amount of incurred residue determined when the sample is treated with concentrated sulfuric acid may reflect the hydrolysis of these bioincurred forms of PCP.

Numerous bioincurred compounds, including phenols, existing as glucuronide and sulfate conjugates, have been identified in cows' milk (Brewington et al., 1973, 1974). If the hydrolysis of these conjugates is the source of the difference observed in Table I, then such forms account for at least 60% of the total PCP found. Such an interpretation is in agreement with Brewington et al. (1973, 1974), who suggest that, of the compounds released from cows' milk, a substantial percentage may be present in the form of conjugates. Furthermore, Akitake and Kobayashi (1975) and Tashiro et al. (1970) have shown that treatment of the glucuronide and sulfate conjugates of PCP with acid and heat releases PCP in free form.

The whole blood samples also required acidic conditions for quantitative recovery of PCP. Without the addition of acid, the recoveries from fortified blood were low and variable (18–40%). Treatment of duplicate fortified samples with acid (no heat), followed by extraction with hexane, gave PCP recoveries of 87 and 88%. Heating an acidified sample 30–45 min gave quantitative (95–100%) recovery. This need for both acidic conditions and heat for extraction of PCP from blood is consistent with previous results (Bevenue et al., 1968).

The extraction efficiency of PCP from hydrolyzed whole blood was determined in a manner similar to that for whole milk samples. A single 6-mL hexane extraction recovered 70% of the added PCP. A second extraction (2 mL) increased the recovery to 95–99%. As with the milk samples, a third extraction (2 mL) was included to allow for some possible variation in extractability. 2-Propanol was not needed in the extraction of PCP from blood since no emulsion problems existed.

Cleanup. Milk sample extracts were cleaned up by partition of the combined hexane layers into strong alkali solution. To further remove interferences, the alkaline layer was washed once with 10 mL of hexane. An additional wash with another 10 mL of hexane was used on some samples, but was found not to be necessary. The alkaline layer was then acidified and extracted with hexane for GLC. The blood samples did not require a supplemental cleanup and the hexane extract of the acid hydrolysate was directly subjected to GLC. Typical chromatograms of the determination of PCP above 0.05 ppm in whole blood and whole milk without supplemental cleanup are shown in Figure 2.

For milk samples containing residues of PCP less than 0.05 ppm, additional cleanup was required due to interferences from the sample matrix. The addition of concentrated sulfuric acid to the final hexane extract elimi-

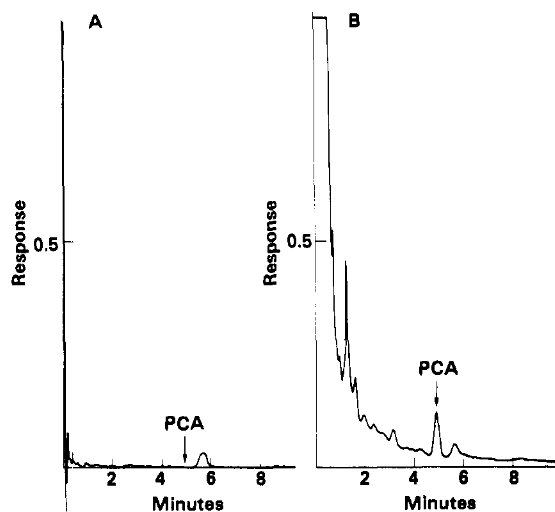

Figure 3. Gas-liquid chromatograms, illustrating derivatization of PCP to PCA; 10% OV-101 column, 175 °C. (A) Before derivatization; (B) after derivatization, 100 mg of sample equivalent injected (3 ppb of PCA).

nated the substances responsible for extraneous GLC peaks. Figure 1 shows the GLC trace before and after the additional cleanup.

Gas-Liquid Chromatography. In order to further reduce the number of method variables and at the same time increase the overall speed of sample analysis, PCP was determined as the underivatized phenol. A 0.61 m × 4 mm i.d. glass column containing 2% SP1000/1% H_3PO_4 on 80–100 mesh Chromosorb W AW was selective for PCP. This mixed liquid phase has one restriction. It must be used at less than 200 °C because at that temperature the retention time for PCP gradually decreases. As expected, the load of SP1000, along with the column temperature, determined the retention time, while the amount of phosphoric acid present did not. The phosphoric acid, however, by coating the active sites, results in a sharp peak for PCP. It is possible, though not proven, that at 200 °C the phosphoric acid slowly hydrolyzes the phthalic acid end group of the SP1000, thereby decreasing the effective load and consequently shortening the retention time. When the column was operated at 175 °C, this effect was not noticed and the column was in use for up to 6 months before there was any change in performance. At this point, retention remained constant, but the PCP peak began to show signs of broadening. This condition was quickly and effectively remedied by a few injections (four or five, 30 μL) of a solution of 1% phosphoric acid in acetone (the column was disconnected from the detector while the injections were made. After letting the column equilibrate in an oven at 175 °C for ca. 2 h, it was reconnected to the detector. The performance of the column after this treatment was identical with that before peak broadening began to occur.

The PCP response curve, under the conditions used, showed good linearity through the range of interest (0.2–5.0 ng). The limit of detection (twice the base line noise) was 1.0 ppb for both milk and blood. After an injection, the syringe was thoroughly rinsed with hexane/2-propanol (4:1) and then with hexane before the next injection. This resolved a problem, noticed in a few lower level quantitations, of a small ghost peak affecting subsequent underivatized determinations.

The identity of PCP in selected low level samples was confirmed by treatment with diazomethane to give the methyl ether derivative, PCA. The identity of the anisole derivative was established by GLC on a 10% OV-101 column and by mass spectral (MS) identification. A representative gas chromatogram of the PCA derivative is shown in Figure 3.

Table II. Recovery of PCP from Milk and Blood

fortif level, ppm[a]	no. of fortif	mean recov ± CV, %[b]
	Milk	
0.01	8	95.4 ± 6.0
0.05	3	98.3 ± 4.1
0.10	3	90.3 ± 0.6
1.0	3	100.7 ± 1.5
2.5	3	100.0 ± 2.0
5.0	4	101.0 ± 1.6
overall mean recov		97.3 ± 5.1
	Blood	
0.05	3	93.3 ± 2.5
0.10	3	89.0 ± 1.1
1.0	3	87.7 ± 1.7
2.5	3	92.7 ± 3.5
5.0	6	103.8 ± 3.5
15.0	9	102.1 ± 7.0
25.0	5	103.8 ± 6.9
50.0	3	99.7 ± 5.1
overall mean recov		99.8 ± 7.7

[a] Samples were fortified by direct injection of PCP standard solutions before addition of acid. [b] Coefficient of variation.

Recovery. To further establish the adequacy of the milk and blood methods used in the feeding study, the recovery of PCP through both procedures in their final forms was examined. Results are listed in Table II. The recoveries from fortification experiments ranged from 88 to 100%. Milk and blood samples collected before the start of dosing, which were used to determine background levels, were also used in the fortification recovery experiments. At the low fortifications, recovery data were calculated by subtraction of a 2–4-ppb PCP level present in the background. This low level response was identified as PCP by derivatization to PCA and then confirmed by GLC–MS. Chromatograms of the method reagent blank were free of interferences.

Samples of whole milk were fortified at a low level (0.01 ppm) and carried through the supplemental cleanup step to determine if any loss occurred. Samples (corrected for background) showed a quantitative recovery (Table II).

The accuracy of low level quantitation of the underivatized phenol, when the extract was treated with a supplemental cleanup, was confirmed by quantitation of the PCA derivative. The levels of PCP found, whether calculated either as the methyl ether derivative or as free PCP, agreed to within 6% for several samples. If samples containing low levels of PCP were not subjected to a supplemental cleanup, the comparison of quantitative results between underivatized and derivatized PCP showed that a higher level (10–15%) was being determined underivatized. This result indicates the need for a supplemental cleanup for the determination of PCP at low levels since without it coextractives in the sample matrix can contribute to the GLC response of underivatized PCP. With this as a consideration for low level determinations, this analytical approach has the utility of providing both a means of routine quantitation for PCP as the underivatized phenol at levels above 0.05 ppm, with PCP confirmation by conversion to the methyl ether derivative and requantitation as the methyl ether if necessary, and supplemental cleanup and quantitation at levels below 0.05 ppm as either the free phenol or its methyl ether. Such versatility, coupled with the accuracy for both low and high

level determinations, made these methods useful for the analysis of the large numbers of samples generated by the cow feeding study for which the methods were developed.

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LITERATURE CITED

Akitake, H., Kobayashi, K., *Bull. Jpn. Soc. Fish.* **41**, 321 (1975).
Barthel, W. F., Curley, A., Thrasher, C. L., Sedlak, V. A., Armstrong, R., *J. Assoc. Off. Anal. Chem.* **52**, 294 (1969).
Bevenue, A., Beckman, H., *Residue Rev.* **19**, 83 (1967).
Bevenue, A., Emerson, M. L., Casarett, L. J., Yauger, W. L., *J. Chromatogr.* **38**, 467 (1968).
Brewington, C. R., Parks, O. W., Schwartz, D. P., *J. Agric. Food Chem.* **21**, 30 (1973).
Brewington, C. R., Parks, O. W., Schwartz, D. P., *J. Agric. Food Chem.* **22**, 293 (1974).
Cessna, A. J., Grover, R., *J. Agric. Food Chem.* **26**, 289 (1978).
Chau, A. S. Y., Coburn, J. A., *J. Assoc. Off. Anal. Chem.* **57**, 389 (1974).
Chem. Eng. News **55**(12), 7 (1977).
Chem. Mark. Rep. **211**(12), 3 (1977).
Erney, D. R., *J. Assoc. Off. Anal. Chem.* **61**, 214 (1978).
Firestone, D., Clower, M., Borsetti, A. P., Teske, R. H., Long, P. A., *J. Agric. Food Chem.* **27**, 1171 (1979).
Gee, M. G., Land, D. G., Robinson, D., *J. Sci. Food Agric.* **25**, 829 (1974).
Higginbotham, G. R., Ress, J., Rocke, A., *J. Assoc. Off. Anal. Chem.* **53**, 673 (1970).
"Pesticide Analytical Manual", Vol. I, Food and Drug Administration, Washington, DC, 1972, Chapter 3, Sect. 301.
Rivers, J. B., *Bull. Environ. Contam. Toxicol.* **8**, 294 (1972).
Rudling, L., *Water Res.* **4**, 533 (1970).
Stark, A. J., *J. Agric. Food Chem.* **17**, 871 (1969).
Tashiro, S., Sasamoto, T., Aikawa, T., Tokunaga, S., Taniguchi, E., Eto, M., *Nippon Nogei Kagaku Hui Pao* **44**, 124 (1970).
Yip, G., *J. Assoc. Off. Anal. Chem.* **53**, 358 (1970).

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Determination of Terbutryn and Its Degradation Products in Water, Sediments, Aquatic Plants, and Fish

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A method is described for the determination of terbutryn [2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine] and several of its degradation products, N-deethylated terbutryn (DET), hydroxyterbutryn (HT), N-deethylated hydroxyterbutryn (DEHT), 2-(*tert*-butylamino)-4-(ethylamino)-*s*-triazine (EBT), and 2-(*tert*-butylamino)-4-amino-*s*-triazine (ABT), in samples from the aquatic environment. Water samples were extracted with dichloromethane, while sediment, aquatic plants, and fish tissues were extracted with aqueous acetonitrile. The hydroxytriazines were isolated by chromatography on a cation-exchange resin and cleanup on alumina and by high-pressure liquid chromatography. Recoveries of terbutryn, DET, HT, DEHT, ABT, and EBT from water (0.5–50 μg/L) ranged from 76 to 120%. Terbutryn and DET recoveries from sediment, plant material, and fish tissues (0.05–0.9 μg/g) ranged from 74 to 106%. HT and DEHT recoveries from sediment, plant material, and fish tissue (0.1–1.23 μg/g) ranged from 62 to 124%.

Terbutryn (2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine) is registered for control of broadleaf weeds in cereals in the United States (W.S.S.A., 1978), and over the past 10 years, terbutryn has been tested in several countries as an aquatic herbicide under the trade name Clarosan (Ciba-Geigy Ltd. Trademark). It is used for the control of floating and submerged aquatic plants and algae at concentrations of 100 μg/L. There are relatively few published reports on the degradation of terbutryn in the environment, but hydroxytriazines, N-deethylated triazines, and photoproducts have been reported as terbutryn decomposition products (Ciba-Geigy Ltd., 1978; Burkhard and Guth, 1976). In order to monitor the appearance of terbutryn degradation products as well as the disappearance of the parent compound in farm ponds (Muir et al., 1980), an analytical method was required that could be applied to both terbutryn and its more polar breakdown products.

Methods have been described for the analysis of terbutryn in water (Byast and Cotterhill, 1975) and in crops and soils (Ramsteiner et al., 1974; Tweedy and Kahrs, 1978). The analysis of triazine herbicide residues in fish (Hesselberg and Johnson, 1973) and fish eggs (Sirons, 1978a) has also been reported. Ramsteiner and Hormann (1979) have described a method for the determination of hydroxytriazines in plant material by use of high-pressure liquid chromatography (LC). With some modifications, these procedures appeared suitable for the analysis of terbutryn in samples from aquatic ecosystems. In the present work, procedures are described for the analysis of terbutryn in pond water, sediment, cattails (*Typha* sp.), and fish, and their application to the determination of N-deethylated terbutryn (DET), hydroxyterbutryn (HT), N-deethylated hydroxyterbutryn (DEHT), 2-(*tert*-butylamino)-4-(ethylamino)-*s*-triazine (EBT) and 2-(*tert*-bu-

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