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Analysis of toxic wastes in tissues from aquatic species Applications of matrix solid-phase dispersion

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Abstract

One of the difficult tasks in studying toxic wastes in the environment is the process involved in their extraction from biological matrices. This review addresses the regulatory aspects which mandate subsequent analyses in aquatic species and the studies which have addressed these problems from a variety of analytical perspectives. In this regard, the tissue extraction process known as matrix solid-phase dispersion is also reviewed and data are presented indicating that it may provide a generic process for the extraction and subsequent analysis of pesticides, polynuclear aromatic hydrocarbons and polychlorinated biphenyls.

Keywords: Matrix solid-phase dispersion; Extraction methods; Reviews; Environmental analysis; Polychlorinated biphenyls; Polynuclear aromatic hydrocarbons; Pesticides; Organochlorine compounds

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1. Introduction

One of the more significant and difficult tasks associated with studying the effects of chemical pollutants and toxic wastes in the environment is their analysis in biological matrices (muscle, fat, liver, etc.). This process is further complicated when such analyses must be applied to a variety of species, particularly aquatic species (fish, mussels, oysters, crayfish, frogs, turtles, etc.). To this we add the confounding need to assay for a wide range of chemically, divergent pollutant classes, such as herbicides, pesticides, polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), in the same or multiple samples. Existing 'official' methods, many of which have been promulgated as regulations, accomplish these needs but possess particular disadvantages when applied to such analyses, disadvantages that can be described in terms of time, cost, scale, their own inherent environmental impacts, and technical deficiencies that arise from the failure to apply newer analytical techniques and technologies.

To understand the significance of the impact of these compounds on aquatic environments, it is appropriate to examine the environmental and analytical perspectives that have led to the selection of these chemicals for investigation and to the necessity to provide regulatory methodology. The following review addresses the issues of aquatic environmental contamination by these compounds as measured in the aquatic species that they contaminate. It will also review some of the regulatory considerations which have led to the development of much of the analytical methodology used for these purposes.

This report also reviews the methodology that has been applied to the analysis of these pollutants in aquatic species and environments. In this regard, our laboratory has been investigating the use of an extraction methodology called matrix solid-phase dispersion (MSPD) [1], in combination with various chromatographic techniques, to analyze different classes of environmentally significant compounds in biological samples. These studies have been performed with the goal of improving existing screening tests and/or analytical methods utilized to investigate and monitor environmental contamination by addressing needed modifications to the techniques that are typically used to prepare biological samples for instrumental analysis. These MSPD methods are also reviewed and research is described that represents an effort to demonstrate the feasibility of improving environmental analytical methods by employing a generic MSPD technique.

It is anticipated that any improvements in methodology, especially those developing a more rapid, generic and environmentally friendly approach to such analyses, could contribute to research conducted for the purpose of protecting human health and the environment, making it easier to acquire chemical residue data from complex matrices. Indeed, the development of analytical methods in the field of environmental science and toxicology serve to provide valuable 'tools' from which the quality and quantity of research in these disciplines can be addressed and improved.

1.1. Ambient contamination of the environment

The contamination of the environment by several major classes of pollutants has been documented in all corners of the globe. Contamination has been reported in ground waters, surface waters, soils and sediments. Since 1975, over 70 different pesticides have been detected in ground waters in the USA [2]. Residues of organochlorine pesticides have also been found to be present in drinking water supplies [3–5]. The unrestricted agricultural use of chlorinated pes-

ticides and disposal of PCBs in the past have led to the detection of residues (ng to $\mu g\ g^{-1}$ or ml levels) in waterways, marine sediments and soil [6–18].

PAHs originating from highly populated urban areas have also found their way into aquatic sediments (ng to $\mu g\ g^{-1}$ levels). Anthropogenic sources have been reported to be 45% from urban run-off, 20% from sewage effluents, 20% from atmospheric deposition and 15% from other sources [19]. Other contamination from anthropogenic sources has also been reported in sediments from the South Carolina coast [20] to the Gulf of Mexico [8].

1.2. Fish and marine animals

The widespread contamination of various species of animals from anthropogenic chemicals such as chlorinated pesticides, PCBs, and PAHs has also been well documented. A summary of some of the studies documenting environmental contamination by chlorinated pesticides, PCBs, and PAHs, is given in Table 1 [21–54]. The information is categorized according to species, country, environmental contaminant, and reference. Although this review does not address all of the research conducted in this field, it does indicate the interest this subject has generated throughout the world and underscores the necessity for rapid, generic and environmentally friendly methods for the analysis of such toxic wastes.

The interest in monitoring environmental contamination has been relatively high over the past two decades. Governmental agencies and academic institutions have conducted numerous studies to document residues in various aquatic species in an effort to determine cause and effect linkages between the presence of these compounds and the observation of detrimental effects and to monitor the effective management of these substances. The US National Academy of Science ranked chemically contaminated seafood fourth in importance of potential health hazards [55]. Concerns such as these have ultimately manifested as seafood inspection programs [56].

In the USA, the National Pesticide Monitoring Program documented the residues of organochlorine chemicals in freshwater fish from 1980 to 1981 [21]. Other work in the North American continent has found residue levels of chlorinated pesticides and PCBs in salt and freshwater fish [14,22–25]. Cause and effect associations of reproductive problems of snapping turtle egg development in regions of relatively high PCB contamination have also been implicated [39]. Earlier studies of snapping turtles indicated that heavy fat bodies of the female did not necessarily prevent the incorporation of PCBs into the eggs [40].

Contamination of seafood based foodstuffs with organochlorine pesticides, PCBs, and/or PAHs have been documented in countries all over the world including Viet Nam [26], the Persian Gulf region [27,28], Egypt [29–31], Kenya [32,33], India [34], Spain [35], Italy [36], Chile [37] and Finland [38].

1.3. Aquatic species as biomonitors/environmental sentinels

The aforementioned studies have addressed the exposures to these environmental contaminants occurring in native aquatic species. In this manner, the native species have unwittingly offered themselves as sentinels to such events. Thus, the practice of analyzing biological matrices for environmental contaminants has been used extensively as a general environmental monitoring strategy for aquatic habitats. In some cases the levels of environmental contaminants in water are too low to detect or quantify by conventional analytical methods, requiring significant efforts at concentrating residues. This effort is further complicated if the contamination originated from a point source into a moving body of water. As a result, the use of biomonitors or environmental sentinels has been proposed as a method to determine the status of contamination by chemicals [57]. This idea takes advantage of the bioaccumulating ability of some organisms for lipophilic organic chemicals. The use of mussels (Mytilus species, for example) has been used int he past in the US Environmental Protection Agency (EPA) 'Mussel Watch' program [58]. Some of the rationale for the sentinel organism approach in which bivalves, such as mussels and oysters, are utilized are listed here [57]; (a) bivalves are distributed over a wide geographic area, (b) bivalves are sedentary (immobile), (c) they possess good bioconcentration capabilities (concentration factors are 10^2 to 10^5 over their habitat), (d) they provide for the assessment of

Table 1
Aquatic environmental contamination studies

Species	Location	Environmental contaminant	Ref.
>60 Fresh water fish species	USA	Chlorinated pesticides, PCBs	[21]
Herring (*Clupea harenqus*)	Canada	PCBs	[22]
Winterflounder (*Pseudopleuronectes americanus*)	USA	PCBs	[23]
Lake trout (*Salvelinus namaycush*)	USA	Chlorinated pesticides	[24]
Striped bass (*Morone saxatilis*)	USA	Chlorinated pesticides	[14]
Burbot (*Lota lota*)	Canada	Chlorinated pesticides, PCBs	[25]
8 Species fish, 3 species shellfish	Viet Nam	Chlorinated pesticides, PCBs	[26]
24 Edible marine organisms	Kuwait	Chlorinated pesticides, PCBs	[27]
14 Fish species	Arabian Gulf/Iraq	PAHs	[28]
Catfish (*Clarias lazero* and *Tilapia nilotica*)	Egypt	Chlorinated pesticides	[29]
5 Fish species	Egypt	Chlorinated pesticides, PCBs	[30]
34 Fish species	Egypt	Chlorinated pesticides	[31]
6 Fish species	Kenya	Chlorinated pesticides	[32]
5 Fish species	Kenya	Chlorinated pesticides	[33]
4 Fish species	India	Chlorinated pesticides	[34]
Trout (*Salmo trutta fario*)	Spain	Chlorinated pesticides	[35]
6 Fish species	Italy	Chlorinated pesticides	[36]
11 Edible fish and shellfish	Chile	Chlorinated pesticides	[37]
Blue mussels (*Mytilus edulis*) and 3 fish species	Finland	PAHs	[38]
Snapping turtle (*Chelydra s. serpentina*)	Canada	Chlorinated pesticides, PCBs	[39]
Snapping turtle (*Chelydra s. sepentina*)	USA	PCBs	[40]

Table 1. (*continued*)

Species	Location	Environmental contaminant	Ref.
3 Fish species, squid (*Loligo forbesi*), queen scallop (*Chlamys opercularis*)	UK	Chlorinated pesticides, PCBs	[41]
Mussels (*Mytilus galloprovincialis*)	Northwest Mediterranean	Chlorinated pesticides, PCBs	[42]
Mussels and oysters	USA	Chlorinated pesticides, PCBs PAHs	[42]
Clams	USA	Chlorinated pesticides, PCBs	[43]
Oysters	USA	Chlorinated pesticides, PCBs PAHs	[8]
Blue crab (*Callinectes sapidus*)	USA	PCBs	[44]
Shrimp (*Panaeus setiferus* and *Panaeus aztecus*)	USA	Chlorinated pesticides	[45]
Various aquatic species	Japan	Chlorinated pesticides, PCBs	[46]
Lean fish (*Barbus xanthopterus*) and indian shad (*Tenualosa ilisha*)	Iraq	Chlorinated pesticides	[47]
Tench fish (*Tinca tinca*) and rudd fish (*Scardinius erthropthalmus*)	France	Chlorinated pesticides	[48]
Various aquatic species	Hong Kong	Chlorinated pesticides	[49]
Mullet	Australia	Chlorinated pesticides	[50]
Crayfish (*Procambarus clarkii*)	USA	Chlorinated pesticides	[51]
7 Fish species, shrimp (*Panaeus setiferus, Panaeus aztecus*), blue crab (*Callenectus sapidus*)	USA	PCBs	[6]
Crayfish (*Procambarus clarkii* and *Procambarus acutus acutus*)	USA	Chlorinated pesticides	[52]
Crayfish (*Procambuaus clarkii*)	USA	Chlorinated pesticides	[53]
Crayfish (*Procambarus clarkii*), 3 fish species, eel (*Anguilla anguilla*), frog (*Rana perezi*)	Spain	Chlorinated pesticides	[54]

biological activity of chemicals, (e) bivalve xenobiotic metabolic activity is extremely low compared to fish or crustacea, (f) they have stable local populations allowing multiple sampling over different time periods, (g) they are hardy, surviving in polluted environments, (h) they can be transplanted to other areas for investigative purposes, and, (i) bivalves are valuable as a commercial seafood such

that environmental contamination becomes an issue of public health.

Although it is generally recognized that the use of bivalves as environmental sentinels is a practical approach to pollutant analysis, it is also recognized that the interpretation of chemical concentrations in bivalves must be made with an understanding of their limitations [59]. These limitations should include but not be limited to the seasonal reproductive and lipid cycles that effect the uptake and storage of chemicals, the uptake rates, and the depuration rates. Nonetheless, mussels have been studied as biomonitors in Mobile Bay [59] and in fresh water rivers [60,61]. Other investigations have also used species other than mussels to monitor environmental contamination in water [62,63]. Catfish (*Ictalurus punctatus*, for example) are also a logical model species to investigate new analytical sample preparation techniques since they are filter feeders, bottom dwellers and an important food source [64].

1.4. Regulatory aspects

A number of legislative events enacted since the late 1960s have initiated the development and standardization of analytical methods for environmental contaminants in general. An overview of early environmental legislation has been presented by Foster [65]. The first modern major legislative act approved in 1969 by the USA Congress was the National Environmental Policy Act (NEPA) and the Environmental Quality Improvement Act (EQIA) of 1970. Shortly thereafter, Congress created the EPA from which to coordinate government action and focus authority for protecting the environment. As a result, the EPA implemented four major pieces of environmental legislation. Amendments to the Federal Insecticide, Fungicide, and Rodenticide Act (FDFRA) of 1947 and the Federal Water Pollution Control Act of 1948 (FWPCA) in 1972 placed them under the control of the EPA. Another piece of legislation in 1972 was the enactment of the Marine Protection Research and Sanctuaries Act (MPRSA) implemented to expand the USA protection of the open ocean to the extent of their authority. In 1976, the Toxic Substances Control Act (TSCA) gave the EPA regulatory control over commercial chemical use. Other significant enactments of environmental legislation since then have been Resource Conservation and Recovery Act (RCRA) and Superfund (CERCLA and SARA).

1.5. Residue monitoring

Ultimately, three federal agencies participate in the responsibilities of regulating pesticides and related compounds for the United States. The EPA (see above), the US Department of Agriculture (USDA), and the Food and Drug Administration (FDA). The EPA has the responsibility of approving the use of pesticides as well as establishing residue tolerances in foods [66]. The USDA enforces these tolerances in meat and poultry and the FDA is in charge of enforcing residue tolerances for any other food products shipped in interstate commerce [67] although the FDA does play a role in aiding the EPA in establishing residue goals [68].

Currently, the FDA conducts an extensive residue monitoring program each year involving the analysis of over 20 000 samples using multiple residue methods. An overview of this program and the results since 1978 has been described [69–73]. Data collection and dissemination programs have been implemented by the FDA and are described by [74,75]. Seafood and seafood products, although not required to pass unified, continual federal inspections by the FDA, remain a concern with the public because of the specter of environmental contaminants [56]. In 1965, the US Bureau of Commercial Fisheries began a program, the National Pesticide Monitoring Program, that lasted till 1972, to monitor chlorinated pesticide residues in shellfish populations off the coast of 15 states [76]. The results demonstrated that DDT was virtually ubiquitous. This program was continued to 1976 and indicated that pesticide concentrations were declining [77]. In 1977, the EPA conducted a one time sampling event of bivalves from the estuaries previously monitored under the National Pesticide Monitoring Program which demonstrated that the presence of pesticides had continued to decline dramatically [78].

The Food Safety and Inspection Service (FSIS) of the USDA is responsible for monitoring the residues of chemicals that might be present in meat and poultry products. This responsibility is manifested into the National Residue Program (NRP). Described

by [79,80], the NRP enables the sampling and testing of meat and meat products for residues from pesticides, environmental contaminants and animal drugs.

The National Oceanic and Atmospheric Administration (NOAA) created the National Status and Trends (NS&T) Program to assess the effects of human activity on the quality of coastal and estuarine habitats throughout the USA. This program was designed to monitor chemical contamination and the biological effects of chemical contamination. Two efforts were begun in 1984 and 1986 to monitor the tissues of benthic fish (The Benthic Surveillance Program) and bivalve molluscs and sediments (The Mussel Watch Program), respectively. Results and some of the early trends from these programs are reviewed by [81,82].

A problem arising from the existence of so many monitoring and data gathering programs is the lack of integration of the enormous amounts of information that is generated. As a result, policy and decision makers seldom have easy access to this information from which to prioritize research or determine to what extent policy goals are being attained. From this concern, the EPA has initiated an integrated program for monitoring ecological status and trends known as the Environmental Monitoring and Assessment Program (EMAP) [83]. The problem of integrating various sources of environmental monitoring and residue data are even greater when one considers this issue globally.

Outside the USA, there are over 50 international organizations that are involved with pesticides and other pollutants. However, there are legitimate concerns pertaining to the ability of developing nations to possess the resources necessary to evaluate toxicity from contaminated food or perform residue monitoring. As a result, the United Nations has several organizations to address the problems of conflicting agencies and countries with limited resources. A review of these efforts and the problems that exist has been written by [84].

Even with the existence of substantial legislation and monitoring programs enacted to protect the consumer from harmful chemical residues in food, the public remains concerned over the issue. According to a review conducted by [85] on dietary pesticide risk assessment, a recent consumer attitude survey, 80% of USA shoppers surveyed stated that pesticide residues in foods were a major concern. The subject of pesticides and food safety has been reviewed by [86]. Residue concerns in seafoods are discussed by [55]. Some of the more recent FDA action levels are given in Table 2 from information compiled by [55].

1.6. Residue analysis

The analyses of residues (whether drugs, pesticides or other environmental contaminants) in biological matrices were initially developed and performed on the basis of individual analytes [87]. It was not until the advent of chromatographic techniques (paper chromatography, thin-layer chromatography, gas chromatography and high-performance liquid chromatography) that multi-residue analyses were routinely employed. The classical method developed by [88] for the analysis of chlorinated pesticide residues in foods using paper chromatography was pivotal in terms of the evolution of multi-residue analyses. According to [87], the Mills method, which utilized solvent partitioning techniques for compound isolation and columns (e.g., Florisil) for fractionation and sample clean-up, became the foundation of the FDA monitoring program for the analytical techniques utilized for multiple residue analyses.

Further refinement of the Mills method was published in 1963 using gas chromatography and a halogen-specific detector [89] and became known as the MOG method [87]. Subsequent collaborative

Table 2
FDA action levels for some environmental contaminants in fish and shellfish

Contaminant	FDA action level (μg/g)
Polychlorinated biphenyls (PCBs)	2.0
Polynuclear aromatic hydrocarbons (PAHs)	No action level
Dichlorodiphenyltrichloroethane (DDT)	5.0
Dichlorodiphenyldichloroethane (DDE)	5.0
Diphenylethanedichlorophenylethane (TDE)	5.0
Chlordane	0.3
Heptachlor	0.3
Heptachlor epoxide	0.3
Dieldrin	0.3
Toxaphene	5.0

studies following the Mills method eventually led to the publication of multi-residue methods in the AOAC (Association of Official Analytical Chemists) Official Methods of Analysis (1990) and are the basis of most multi-residue methods that are utilized today. A more thorough review of the evolution of these early multi-residue methods has been reported [90].

Analytical methods utilized for the determination of residues or environmental contaminants in biological matrices can typically be divided into two separate procedures. The first part involves the preparation and subsequent clean-up (if required) of the sample prior to analysis. The second part of the method is known as the analytical finish and usually describes procedures that are associated with the isolation and detection of the prepared sample extract by an instrument. Insufficient sample clean-up of biological matrices precludes reliable results from the analytical instrument (gas chromatograph or high-performance liquid chromatograph) used because of the deterioration of these systems due to accumulation and interference by matrix contaminants [91].

Modern multi-residue analyses primarily involve the use of a variety of sample preparation and processing techniques followed by analysis on chromatographic devices coupled with specific and non-specific detectors. The review of multi-residue methods addressed here is organized in a similar manner; a description of the sample preparation/clean-up studies related to the analysis of polyaromatic hydrocarbons, chlorinated pesticides, and PCBs. References are organized according to the analyte(s), matrix, sample preparation technique, sample clean-up, and analytical finish (Table 3; [92–161]).

1.7. Sample preparation

Although the analysis of tissue residues for pesticides and other environmental contaminants by chromatographic methods has rapidly evolved, the sample preparation techniques initially developed decades ago are, for the most part, still being used today. Typically, extraction procedures involve the homogenization of the sample (whole animal, edible portions, specific organs, etc.) with an organic solvent used to extract the compounds of interest. The homogenate is then subjected to additional clean-up procedures to isolate the target compounds from other coextracted materials (e.g., lipids). The majority of the sample preparation methods utilize these basic techniques, with variations in the solvent or solvents used, number of partitioning steps, and various adsorption columns (e.g., Florisil, silica, gel permeation chromatography, etc.) used for sample clean-up. These techniques have been reviewed in detail [90,91,162,163]. However, there are other sample extraction techniques that vary from the typical. These are supercritical fluid extraction (SFE) and MSPD.

SFE involves the use of heated, pressurized carbon dioxide (CO_2), which becomes a fluid under supercritical conditions and can be used to extract lipophilic compounds from complex matrices. Once extracted, the compounds of interest are isolated and concentrated when the carbon dioxide is removed. Some of the problems associated with this technique are related to the costs of the special apparatus required, the need to exclude and remove water from the sample and the need for additional sample clean-up prior to final chromatographic analysis; generally, lipophilic analytes are readily extracted with lipophilic matrix components. A primary advantage is that it provides an alternative to using organic solvents [91], although the CO_2 solvent stream is often modified in its polarity by inclusion of small amounts of organic solvents such as methanol. A second advantage is that modern instrumentation provides the ability to perform automated SFE, enhancing the throughput of such analyses.

MSPD, a tissue disruption/extraction method developed in 1989 [1], involves the blending of octadecylsilyl (ODS)-derivatized silica (C_{18}) or other chemically modified solid supports with the tissue sample in a mortar and pestle. The MSPD process incorporates the classical methods of the use of abrasives to disrupt sample architecture and the use of a 'solvent' or detergent to disrupt cellular membranes and components, with the 'solvent' now being bound to the abrasive solid support. In this manner the tissue can be completely disrupted and distributed over the surface of the solid support so as to maximize the interactions of the solid support and bonded liquid phase chemistry and interactions with

Table 3
Multi-residue analytical methods and studies

Analyte(s)	Matrix	Sample extraction	Sample clean-up	Analytical finish	Ref.
PAHs	Coal ash	Heated solvent	None	GC–MS	[92]
PAHs	River sediment, dust	SFE N_2O, SFE CO_2, SFE ethane	None	GC–MS (SIM)	[93]
PAHs	Soil	Homogenization, Soxhlet	None	RP-HPLC–UV	[94]
PAHs	Standards	NA	NA	GC–UV–fluor	[95]
PAHs	Particulates, dust, tea, river water	Soxhlet, ultrasonic, vacuum sublimation,	Solvent partitioning, acid-base re-extraction, C_{18}, XAD 2, GPC, silica gel, alumina column	HPTLC–fluor	[96]
PAHs	Fish muscle	Soxhlet	GPC	GC–FID, GC–MS	[97]
PAHs	Particulates, river sediment	Solvent extraction	Silica gel, alumina columns, GPC	GC–FID, GC–FPD, GC–MS	[98]
PAHs	Coal tar	NP-HPLC	None	GC–FID, GC–MS, RP-HPLC–fluor	[99]
PAHs	Fish bile	None	None	3-column RP-HPLC–fluor	[100]
PAHs	Standards	None	None	Low-temperature phosphorimetry	[101]
BaP metabolites	Rat microsomes, human mononuclear leukocytes	Ultra-high centrifugation	Precipatation, centrifugation	RP-HPLC–fluor	[102]
BaP metabolites	Human hair	Precipitation and centrifugation, solvent extraction	None	RP-HPLC–fluor	[103]
PAHs	Lobster digestive gland	Solvent extraction	Solvent partitioning	RP-HPLC–UV, GC–MS	[104]
Chlorinated pesticides	Milk, butter	Solvent extraction	Florisil column	Paper chromatography	[105]
Chlorinated pesticides	Milk, butter animal fat, animal tissue	Solvent extraction	Solvent partitioning, Florisil, MgO–Celite columns, saponification	GC–ECD, GC–ELCD, TLC	[106]
Chlorinated pesticides	Seal blubber, fish liver and muscle, vegetable oil, shellfish tissues	Soxhlet extraction	Alumina, silica columns	GC–ECD	[107]
Chlorinated pesticides, PCBs	Fish tissues	Solvent	GPC, Florisil column	GC–ECD, GC–MS	[108]

(Continued on p. 296)

Table 3. (*continued*)

Analyte(s)	Matrix	Sample extraction	Sample clean-up	Analytical finish	Ref.
Chlorinated pesticides	Dairy products, vegetables	Solvent extraction	Florisil column	GC–ECD	[109]
Chlorinated pesticides, PCBs	Fish muscle	Solvent extraction	H_2SO_4	GC–ECD	[110]
Chlorinated pesticides	Corn oil standards	None	GPC	GC–ECD	[111]
Chlorinated pesticides	Spiked vegetables	Solvent extraction	Florisil column	GC–ECD	[112]
Chlorinated pesticides, PCBs	Fish tissue	Solvent extraction	Florisil column	RP-TLC, GC–ECD	[113]
Chlorinated pesticides, PCBs	Chicken fat, fish tissue	Solvent extraction	Florisil column	GC–ECD, GC–ELCD	[114]
Chlorinated pesticides	Vegetables, fruits	Solvent extraction	Solvent partitioning, Florisil column	GC–ECD	[115]
Chlorinated pesticides	Animal fat	Saponification	Alumina column, H_2SO_4	GC–ECD	[116]
Chlorinated pesticides, PCBs	Seawater	Solvent extraction	Alumina column	GC–ECD	[117]
Chlorinated pesticides	Milk	Solvent extraction	Florisil column	GC–ECD	[118]
Chlorinated pesticides	Butterfat, corn oil	None	GPC	GC–ECD, GC–NPD	[119]
Chlorinated pesticides	Vegetable oil, butterfat	Dry Florisil column, solvent	Solvent partitioning, Florisil colum extraction	GC–ECD	[120]
Chlorinated pesticides, PCBs	Fish tissue	Solvent extraction	Florisil column	GC–ECD	[121]
Chlorinated pesticides	Soil	Solvent extraction study	None	None	[122]
PCBs	Fish tissue	Solvent extraction	Florisil, alumina, silica gel, saponification, H_2SO_4 wash, solvent partitioning, TLC	GC–ECD	[123]
Chlorinated pesticides	Seawater	Solvent extraction	Alumina column, H_2SO_4 wash, ETOH–KOH	GC–ECD	[124]
Chlorinated pesticides	Beef fat	Sweep co-distillation	Florisil column	GC–ECD	[125]

Table 3. (*continued*)

Analyte(s)	Matrix	Sample extraction	Sample clean-up	Analytical finish	Ref.
Chlorinated pesticides	Fish tissue	Solvent extraction	Alumina/silicic acid column	GC–ECD	[126]
Chlorinated pesticides, PCBs	Vegetable and animal fat	Solvent extraction	GPC, tandem capillary columns	GC–ECD	[127]
Chlorinated pesticides	Animal feed	Cyclic steam distillation	Solvent extraction	GC–ECD	[128]
Chlorinated pesticides	Adipose tissue, soil, water, oil sludge	Solvent extracton, C_{18} concentration	Alumina/silicic acid column, combustion	Colorimetric determination	[129]
PCBs	Standards, fish tissue	Referenced	Referenced	GC–ECD, COMSTAR (qualitative and quantitative algorithm)	[130]
PCBs	Air	Florisil/solvent desorption	Perchlorination	GC–ECD, GC–MS	[131]
PCBs	Standards	None	None	GC–ELCD, GC–MS, response factor calibration	[132]
PCBs	Standards	None	Tandem capillary columns	GC–ECD	[133]
Chlorinated pesticides, PCBs	Adipose tissue	Solvent extraction	Solvent partitioning, H_2SO_4 wash, Florisil column, alumina column, GPC, silica gel, forced volatilization,	GC–ECD	[134]
Chlorinated pesticides, PCBs	Human milk	Liphophilic gel,	Solvent partitioning, alumina column, silica gel column	GC–ECD, GC–MS	[135]
Chlorinated pesticides, PCBs	Human serum	Solvent extraction	Florisil column, H_2SO_4 wash, silica gel column	GC–ECD	[136]
Chlorinated pesticides, PCBs	Spiked fish oil, standards	Not available	Florisil column	GC–ECD	[137]
PCBs	Mussel and fish tissue	Solvent extraction	H_2SO_4 wash, alumina	GC–ECD, GC–MS, GC–NICI-MS	[138]
PCBs	Fish muscle	Solvent extraction	GPC, silica gel column, Florisil column	GC–ECD	[139]
PCBs	Cereal, fruit, vegetables, meat, eggs	Solvent extraction and partitioning	H_2SO_4 wash, silica gel column	GC–ECD	[140]

(*Continued on p. 298*)

Table 3. (*continued*)

Analyte(s)	Matrix	Sample extraction	Sample clean-up	Analytical finish	Ref.
Chlorinated pesticides	Fish tissue, bird tissue, eggs	Solvent extraction	Alumina/silicic acid column	GC–ECD	[141]
Chlorinated pesticides, PCBs	Mussel and penguin tissue, sediment, particulates	Solvent extraction	Alumina column, silica gel column, GPC, NP HPLC	GC–ECD	[142]
Chlorinated pesticides, PCBs	Fish tissues	Solvent extraction	GPC, Florisil column, silica gel column	GC–ECD, GC–MS, DIP-MS-SIM	[143]
PCBs	Fish muscle	Solvent extraction	Florisil column	GC–ECD	[144]
Chlorinated pesticides	Sediment, vegetation, coal	Solvent extraction	Florisil column	GC–ECD	[145]
Chlorinated pesticides	Animal and vegetable fat, vegetable oil	Solvent extraction and partitioning	Preparative RP HPLC	GC–ECD	[146]
Chlorinated pesticides	Apple	Solvent extraction	None	GC–ECD	[147]
PCBs	Sediment	Soxhlet extraction	Florisil column, silica gel column, H_2SO_4 wash, Mercury	GC–ECD	[148]
Chlorinated pesticides	Formulated products	Solvent extraction	None	GC–FID	[149]
Chlorinated pesticides, PCBs	Fish muscle, crab muscle	Solvent extraction	C_{18} column, Florisil column	GC–ECD	[150]
Chlorinated pesticides	Animal fat	Solvent extraction	GPC	GC–ECD	[151]
Chlorinated pesticides	Vegetables, vegetable fat	SFE	GPC, Florisil column	GC–ECD	[152]
Chlorinated pesticides	Fish tissue, chicken tissue, vegetables, vegetable fat	Solvent extraction	Size-exclusion chromatography	GC–ECD	[153]
Chlorinated pesticides, PCBs	Fruits, vegetables	Solvent extraction	None	GC–MS	[154]
Chlorinated pesticides	Standards	None	None	GC–FID–ECD	[155]
Chlorinated pesticides	Vegetable and meat based foods	Solvent extraction	GPC, Florisil column	Simultaneous GC-ECD and GC–ELCD	[156]

Table 3. (*continued*)

Analyte(s)	Matrix	Sample extraction	Sample clean-up	Analytical finish	Ref.
Chlorinated pesticides	Animal tissue and fat	SFE	None	EIA	[157]
Chlorinated pesticides	Chicken fat, lard	SFE	Alumina column, silica column	GC–ECD	[158]
Chlorinated pesticides	Produce	Solvent extraction	None	GC–ECD, GC–MS-SIM	[159]
Chlorinated pesticides	Vegetables, fruits	Solvent extraction	None	GC–AED	[160]
Chlorinated pesticides	Human tissues	Solvent extraction	H_2SO_4 wash, solvent partitioning, alumina	GC–ECD, GC–NICI–MS column, Florisil column, silica gel/carbon column	[161]

Abbreviations: AED, atomic emission detection; DIP, direct insertion probe; ECD, electron-capture detection; EIA, enzyme immunoassay; ELCD, electrolytic conductivity detection; FID, flame ionization detection; FLUOR, fluorescence; FPD, flame photometric detection; GC, gas chromatography; GPC, gel permeation chromatography; HPLC, high-erformance liquid chromatography; MS, mass spectrometry; NA, not applicable; NICI, negative ion chemical ionization; NP, normal-phase; NPD, nitrogen–phosphorus detection; RP, reversed-phase; SFE, supercritical fluid extraction; SIM, selected ion monitoring; TLC, thin-layer chromatography; UV, ultraviolet detection.

the individual cellular components and, thus, their subsequent interactions with one another. The contents are transferred to a syringe-barrel column and the compounds of interest are eluted, ready, in many cases, for direct analysis. This technique has been found to be useful for the extraction of a variety of drugs and other materials in a wide variety of solid sample matrices, including compounds that are known to comprise toxic wastes. In some cases co-columns of material, such as alumina, silica, Florisil or additional C_{18} or some other absorption or solid-phase extraction (SPE) supports have been placed at the bottom of the syringe-barrel column prior to addition of the MSPD blend or have been used post elution as a second column, in order to further assist the extraction process and remove co-eluting interferences. This can also be accomplished by literally stacking small columns or extraction disc materials in a sequence. The collected elution solvent(s) may then be processed for appropriate analysis, such as evaporation of an aliquot for addition to an immunoassay or for liquid chromatographic or gas chromatographic analysis. Applications of this method for the specific classes of compounds that comprise toxic wastes are discussed in more detail below.

1.8. Matrix solid-phase dispersion analysis of chlorinated pesticides and related agricultural compounds

Our laboratory has reported several methods using MSPD for the extraction and analysis of chlorinated pesticides in catfish muscle [164], oysters [165], crayfish hepatopancreas [166] and bovine fat [167]. The MSPD methods for the isolation of these compounds from these varied matrices are essentially identical. For example, the MSPD isolation of 9 chlorinated pesticides from beef fat or catfish muscle employed 2 g of C_{18} and a 2 g Florisil co-column, added to the syringe blend prior to addition of the MSPD blend, to remove lipids from the sample. Similarly, the same method was applied to the extraction and analysis of 14 chlorinated pesticides from crayfish hepatopancreas, a particularly difficult matrix. Slight modification of this procedure, altering the elution solvent to acetonitrile–methanol (9:1, v/v) provided methodology for the extraction and

analysis of 14 chlorinated pesticides in oysters. In all cases the extract was analyzed directly by GC with electron-capture detection (ECD), giving excellent recoveries, limits of detection and consistent data following repeated injection.

We have also examined the application of these methods to the analysis of incurred chlorinated pesticide residues and conducted side-by-side comparisons of the method and results with a more classical method applied by the State laboratory [168]. Like the MSPD method, the classical method used a ratio of tissue (in grams) to volume of solvent (in ml) of 1:21. However, the larger sample size (50 g vs. 0.5 g) of the official method required far more solvent and produced much more solvent waste (1070 ml vs. 10 ml) while producing nearly equivalent analytical results. The 'official' methods used by various regulatory agencies are, in general, more environmentally hazardous in this regard and may actually contribute more pollution than they eventually detect or ameliorate. This is generally true of most classical and/or official methods, whether for pesticides or for PAHs or PCBs. Further, it was estimated that for a 30-sample batch the official method would require 4–6 weeks for completion and consume and pollute with over 30 l of solvent whereas the MSPD approach would take approximately 3 days to complete and would expend only 300 ml of solvent. In general, comparable MSPD methodologies for drugs and their metabolites have been found to reduce solvent use by 95% and sample preparation time by 90% when compared to classical approaches.

In further applications of this technique, Ling and Huang [169] have recently reported a MSPD method for the isolation of 16 chlorinated pesticides as well as PCBs from fish tissues utilizing a C_{18} MSPD blend over a co-column of acidic silica. Excellent recoveries and detectability were reported and were found to compare favorably with results obtained by classical methods. Schenck and Wagner [170] have reported a similar approach to the analysis of organochlorine and organophosphorous pesticides in milk. Five compounds from each class were isolated from milk blended with C_{18}, eluting with acetonitrile, followed by a Florisil post column clean-up step. This approach provided comparable results to a more classical method in side-by-side comparison. MSPD has also been applied to the analysis of carbofuran in corn [171] and the analysis of amitraz and pirimicarb in fruits and vegetables [172].

1.9. Matrix solid-phase dispersion extraction and analysis of benzo[a]pyrene and polychlorinated biphenyls in catfish muscle tissue

Given the applications of MSPD for the compounds examined as described above, we were curious as to whether or not a generic process for the isolation of pesticides, PAHs and PCBs could be developed, so as to potentially isolate and analyze all three classes of compounds from the same sample, perhaps in a single elution. Based on the data obtained in our laboratory for the isolation of chlorinated pesticides in a variety of species using an acetonitrile elution we sought to apply this method to the analysis of PAH and PCB residues. As part of an ongoing study of benzo[*a*]pyrene (BaP) metabolism, we examined the extraction of this compound from fortified fish muscle tissues. We also applied the same procedure to the extraction and analysis of fish muscle tissues fortified with Arocor 1254. The results of these examinations are presented below.

2. Materials and methods

2.1. Reagents and expendable materials

Solvents were obtained from commercial sources and were of the highest purity available. These materials were used without further purification. Water for HPLC analyses was triple-distilled and further treated with a Modulab Polisher 1 water purification system (Continental Water Systems, San Antonio, TX, USA). BaP was obtained from Sigma, St. Louis, MO, USA, and [$^2H_{12}$]chrysene, used as an internal standard or surrogate spike, was supplied by the EPA Quality Assurance Materials Bank. The MSPD bulk C_{18} packing used was octadecylsilyl derivatized silica, 40 μm particle size, 18% carbon load, endcapped (obtained from Analytichem International, Harbor City, CA, USA). The C_{18} was cleaned by sequentially washing in a 50-ml column containing bulk C_{18} (22 g) with 2 column volumes

each of hexane, dichloromethane (DCM) and methanol by vacuum aspiration until dry.

Stock BaP and internal standard solutions were prepared to a concentration of 100 $\mu g\,ml^{-1}$ by dissolving pure standard in acetonitrile and diluting with acetonitrile to the appropriate $\mu g\,ml^{-1}$ level. [$^2H_{12}$]Chrysene internal standard stock solution was originally obtained from the EPA at a concentration of 1000 $\mu g\,ml^{-1}$. The stock solutions were subsequently admixed and then serially diluted with acetonitrile to make 0.10, 0.25, 0.50, 1.00 and 2.00 $\mu g\,ml^{-1}$ BaP standard solutions containing 0.50 $\mu g\,ml^{-1}$ internal standard [$^2H_{12}$]chrysene in each mixture.

The polychlorinated biphenyls standard (Aroclor 1254) was obtained from the Quality Assurance Materials Branch, EPA (The EPA Quality Assurance Materials Bank, 2 Triangle Drive, RTP, NC, USA). The preparation of standard stock PCB (Aroclor 1254) solution, 100 $\mu g\,ml^{-1}$, was performed by transferring 0.2 ml of the EPA standard solution (5000 $\mu g\,ml^{-1}$) into a 10.0-ml volumetric flask and filling to volume with hexane. The stock solution of Aroclor 1254 was transferred to 1.0 ml glass vials and stored at 4°C until used. The working standard solution of Aroclor 1254 (10 $\mu g\,ml^{-1}$) was prepared for daily use by diluting 1 ml of the Aroclor 1254 standard stock solution into a 10-ml volumetric flask and filling to volume with hexane.

Syringe barrels were used as extraction columns; the 10-ml plastic syringe barrels (obtained from Becton Dickinson, Rutherford, NJ, USA), were thoroughly washed with hot soapy water, rinsed with double distilled water and acetonitrile and air dried prior to use as columns for sample extraction. A filter paper disc (Whatman No.1, 1.5 cm) was placed at the bottom of the syringe barrel to retain the column packing. All glassware was solvent rinsed.

Catfish (*Ictalurus punctatus*) muscle tissue was obtained locally from a commercial seafood supplier or was obtained from drug free catfish raised at the School of Veterinary Medicine. All tissues were frozen (−20°C) until used for this study.

2.2. High-performance liquid chromatography

Analyses of extracted samples and BaP standards were conducted utilizing a Hewlett–Packard HP 1090 HPLC system (HP 79994A Chemstation) equipped with a photodiode array detector and a programmable fluorescence detector (HP 1046A) plumbed in series. The photodiode array detector was set at 254 nm with a bandwidth of 20 nm spectrum range of 210–500 nm and a reference spectrum of 550 nm with a bandwidth of 100 nm. The fluorescence detector was set at an excitation wavelength of 260 nm and an emission wavelength of 420 nm. The solvent system was water–acetonitrile (25:75, v/v) at a flow-rate of 1.5 ml min^{-1} for 6 min. A reversed-phase octadecylsilyl (ODS) derivatized silica column (Vydac 201 TP, 5 μm, 15 cm× 4.6 mm, The Sep/a/ra/tions Group, Hesperia, CA, USA) fitted with a guard column (C_{18} cartridge; Alltech, Deerfield, IL, USA) maintained at 40°C was utilized for all determinations.

2.3. Gas chromatography

A Varian Vista 6000 gas chromatograph equipped with a DB-5 column (J and W Scientific, 25×0.25 mm I.D. 0.2-μm coating) was used for this study. The injector temperature was set at 250°C. The column temperature program was as follows: 120°C for 2 min, increase at 10°C min^{-1} to 290°C and hold for 5 min; the splitless injector was operated with a purge function activated at 0.75 min post-injection. The detector was an electron-capture detector which was operated at a temperature of 300°C. The carrier gas used was ultra high purity nitrogen at a linear flow-rate of 14 cm s^{-1}.

2.4. Preparation of sample extracts

Two (2) g of C_{18} were placed in a glass mortar and 0.5 g of fish muscle was placed onto the C_{18}. Standard BaP mixtures in acetonitrile (5 μl, 0.10, 0.25, 0.50, 1.00 and 2.00 $\mu g\,ml^{-1}$ BaP standard solutions containing 0.50 $\mu g\,ml^{-1}$ internal standard [$^2H_{12}$] chrysene in each mixture) were injected into the tissues and the fortified samples were allowed to stand for at least 5 min. This fortification level resulted in a final concentration in the tissue of 0.20, 0.50, 1.00, 2.00 and 4.00 μg of BaP and 1.0 μg of internal standard [$^2H_{12}$] chrysene per gram of tissue (wet mass). Blank control tissues were prepared similarly, except that 5 μl of acetonitrile containing

no BaP were injected into the tissue. Five replicates for each level were used resulting in 30 (n=30) fortified tissue samples.

Standard solutions of Aroclor 1254 (5 μl containing 0.10, 0.25, 0.50, 1.00 and 2.00 μg) were injected into the tissues and the fortified samples were allowed to stand for at least 5 min. This fortification level resulted in a final concentration in the tissue of 0.20, 0.50, 1.00, 2.00 and 4.00 $\mu g\, g^{-1}$ of PCB on a wet tissue mass basis. Five replicates for each level, including a blank, were used resulting in 30 (n=30) fortified tissue samples. Blank control tissues were prepared similarly, except that 5 μl of hexane containing no PCB were injected into the tissue.

The tissues were then gently blended into the C_{18} material with a glass pestle until a homogeneous mixture was observed (approximately 30 s). The resultant homogeneous C_{18}/tissue matrix was transferred into a previously prepared 10-ml syringe barrel that contained a filter disk and 2 g of Florisil which had been stored at 100°C overnight. Two additional filter paper discs were placed on the column head and the column was compressed with a syringe plunger that had the rubber end and pointed plastic portion removed. A plastic pipette tip (100 μl) was placed on the column outlet to increase the residence time of the eluting solvents on the column.

BaP and internal standard [$^2H_{12}$] chrysene or PCBs were eluted with 8 ml acetonitrile into a 10-ml conical screw-thread disposable glass centrifuge tube (Kimble, Vineland, NJ, USA). A steady flow of acetonitrile through the column was initiated by applying positive pressure (pipette bulb) to the column head and collecting the acetonitrile in the centrifuge tube. A final extract volume of 5 ml was obtained. The tube was tightly capped, and the tube contents were thoroughly vortexed. Any precipitate was removed by centrifugation and the supernatant was transferred to a clean tube. The acetonitrile extracts were subsequently evaporated to dryness under a mild stream of nitrogen in a water bath at 55°C.

The residue for the BaP samples was reconstituted with 1 ml of acetonitrile, vortexed, filtered (0.45-μm disposable syringe filter) and transferred to a glass HPLC sample vial. A portion of the extract (10 μl) was then directly analyzed by a high-performance liquid chromatograph equipped with ultraviolet (photodiode array) and fluorescence detectors.

Following evaporation to dryness under a mild stream of nitrogen in a water bath at 55°C, the residue for the PCBs was reconstituted with 1 ml of hexane, vortexed, filtered (0.45-μm disposable syringe filter) and transferred to a glass sample vial. A portion of the extract (3 μl) was then directly analyzed by GC–ECD.

2.5. Data analysis

For the BaP analyses peak area ratios for standard curves were obtained by plotting integration areas of generated peaks as a ratio to the area of the internal standard. A comparison of fortified and extracted sample peak areas to peak areas of unextracted pure standards run under identical conditions gave percentage recoveries (n=30; 30 samples, 5 replicates of each concentration). The interassay variability was calculated as follows. The mean of the peak area ratios for five replicates of each concentration (0.10, 0.25, 0.50, 1.00 and 2.00 $ng\, \mu l^{-1}$, 10 μl injection volume) was calculated. The standard deviation (S.D.) corresponding to each mean was divided by its respective mean and this resulted in the coefficient of variation for each concentration. The mean of the coefficients of variation was calculated along with its standard deviation and defined as the interassay variability, ±S.D.

The amount of PCB in each sample was calculated using the average of the response factors from three external standards (0.5, 1.0 and 2.0 $\mu g\, ml^{-1}$) of Aroclor 1254. The response factor for each external standard concentration was developed by dividing the sum of the area of the peaks representing PCBs against the concentration of the PCB standard. The extracted PCBs were analyzed under the same conditions as the unextracted PCB standards. The percent recovery for each fortified sample was determined by comparing the amount of PCBs determined in the sample with the amount of corresponding PCBs with which it was spiked. Interassay variability was assessed in the following manner; the percent recovery for five replicates of each concentration (0.25, 0.50, 1.0 and 2.0 $\mu g\, ml^{-1}$, 3 μl

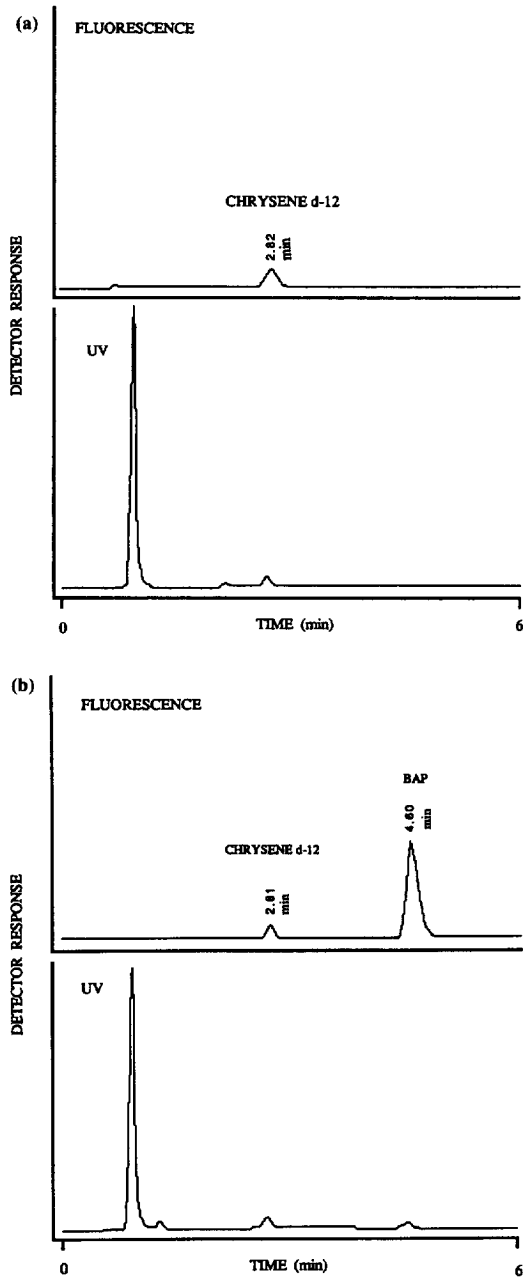

Fig. 1. (a) Representative high-performance liquid chromatograms obtained from the ultraviolet (254 nm) and fluorescence (excitation 260 nm, emission 420 nm) detector analyses (10 μl injection volume) of the acetonitrile extract of blank (control) catfish (*Ictalurus punctatus*) muscle. (b) Representative high-performance liquid chromatograms obtained from the ultraviolet and fluorescence detector analysis (10 μl injection volume) of the acetonitrile extract of BaP fortified catfish muscle.

injection volume) were averaged which resulted in a mean±S.D.). The mean of the relative standard deviations±S.D. was defined as the interassay variability.

3. Results

3.1. Benzo[a]pyrene

In the MSPD procedure developed here, the fish muscle tissue sample (0.5 g) was used to make a C_{18}/tissue matrix blend and fashioned into a column from which the BaP was eluted with acetonitrile. This resulted in an extract that had minimal interferences, as demonstrated in the HPLC chromatograms of the blank control (Fig. 1A) and the acetonitrile extracted BaP fortified (Fig. 1B) tissue. The proven limit of quantitation determined (100 ng g^{-1}) is far above the possible limits of the method, given BaPs strong fluorescence response and the fact that the data generated here utilized only 10 μl of a 1-ml reconstituted extract for analysis; injection of more sample and/or reconstitution in a smaller volume should proportionately enhance detection. The low background, while also proportionately enhanced, should still appear minimal. The average relative percentage recoveries (range of 73.5±11.0% to

Table 4
Mean percent recovery (n=5, ±S.D.) for the individual concentrations examined

Benzo[*a*]pyrene spike concentration (ng g^{-1} tissue)	Recovery (%) mean±S.D. (n=5)
200	73.5±11.0
500	98.9±4.3
1000	112.3±12.8
2000	105.6±10.9
4000	110.6±4.7
Mean recovery±S.D.	100.2±8.8
Standard curve correlation coefficient (r)	0.999
Inter-assay variability (%)	9.1±4.8

Mean percent recovery for the overall assay and the percent inter-assay variability for the range of concentrations of benzo[*a*]pyrene isolated from fortified catfish (*Ictalurus punctatus*) muscle tissue are shown.
r=Correlation coefficient from linear regression analysis.

112.3±12.8%) and interassay variability percent (9.0±4.8%) were indicative of a suitable method for the determination of BaP in fish muscle tissue (Table 4). Linear regression analyses and a corresponding correlation coefficient of 0.999 indicated the extraction technique was linear with respect to increasing concentrations for BaP.

3.2. Polychlorinated biphenyls

The acetonitrile extract of fish muscle tissue fortified with an Aroclor 1254 mix possessed minimal interferences, as illustrated by the analysis of a tissue blank (Fig. 2A) using capillary GC and ECD. Representative chromatograms of the standard and the fortified extracted samples are shown in Fig. 2B and C, respectively. Quantitation was performed by summing selected total peak areas for each of the standards and samples analyzed. Table 5 lists and summarizes the data associated with the analyses and gives the average percent recoveries for each level of fortification. The mean recovery, 71.7%, illustrates

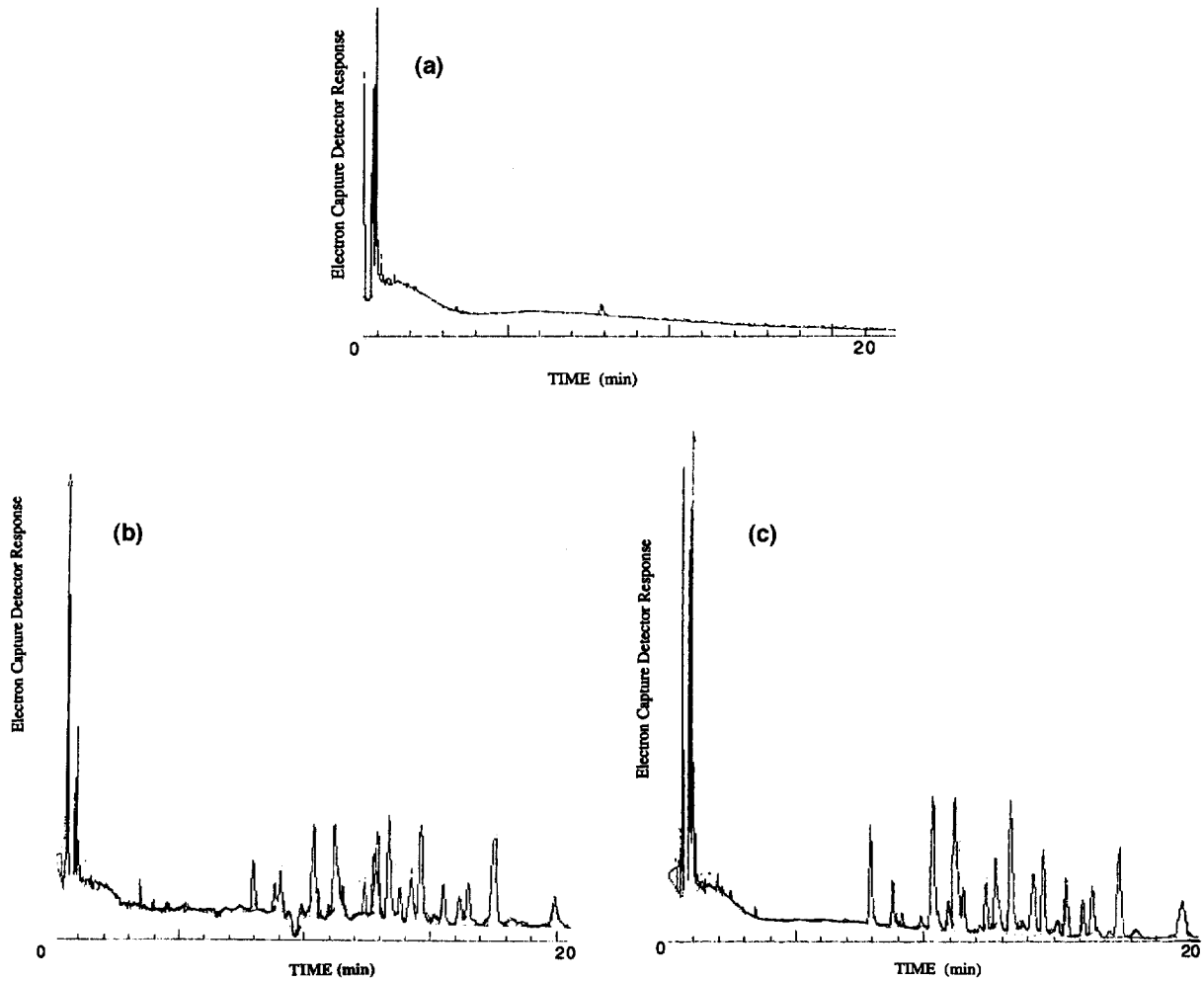

Fig. 2. (a) Representative capillary column gas chromatogram obtained from the ECD analysis of the MSPD acetonitrile extract of a catfish muscle blank control. (b) Analysis of PCB standard (Aroclor 1254) at an equivalent dilution to that obtained from tissue extracts. (c) Representative capillary column gas chromatogram obtained from the ECD analysis of the MSPD acetonitrile extract of catfish muscle tissue fortified with Aroclor 1254 standard.

Table 5
Mean percent recovery ($n=5$, ±S.D.) for the individual concentrations ($\mu g\ g^{-1}$) of PCBs (Aroclor 1254) examined as spikes in catfish muscle tissue[a]

PCB conc. ($\mu g\ g^{-1}$)	Mean recovery ($n=5$)±S.D. (%)
0.5	76.0±7.5
1	77.1±9.5
2	74.5±2.4
4	59.1±3.3
Mean recovery (±mean S.D.)	71.7±5.7

[a] The mean recovery for the range of concentrations and the mean for the S.D. are shown.

the feasibility of this approach for conducting PCB analyses in fish tissues. Although a minimum concentration of 0.5 $\mu g\ g^{-1}$ of total PCBs was examined here, it should be feasible to enhance the sensitivity of the method by reconstitution of the sample in a smaller volume of solvent (presently 1 ml) prior to GC–ECD analysis.

It should be noted that both classes of compounds were extracted by the same technique; PAHs and PCBs were isolated from fish muscle tissues using the MSPD approach (C_{18} and a Florisil co-column) with acetonitrile alone as the eluate. Thus, both classes of compounds could, theoretically, be assayed from the same sample, applying different detectors for the appropriate class. The methods defined here for the PCBs and PAHs are also quite similar, and for some identical, to the MSPD methods that have been described for the analysis of chlorinated pesticides and other agriculture pesticide-related compounds [164–168]. While not demonstrated here, in can be inferred that the use of MSPD may provide a generic procedure for the analysis of all three major classes of compounds, pesticides, PAHs and PCBs, that are most often considered as toxic wastes that impact aquatic environments and species.

4. Discussion

4.1. Matrix solid-phase dispersion analysis of benzo[a]pyrene and polynuclear aromatic hydrocarbons

The results provided here for BaP are from fortified samples, such as would be required and obtained for the preparation of standard curves or for conducting recovery studies for the qualitative/quantitative analysis of BAP residues in biological samples or for exposure studies. We have made further application of this method in the analysis of incurred residue samples with catfish dosed by intraperitoneal injection of BaP. Various tissues were subjected to the sample preparation described above; muscle, liver, bile, head kidney, etc. The same method was applicable to all tissues examined. BaP was readily identified in most of the tissues as were compounds putatively identified as hydroxy-BAP metabolites [173].

While BaP is one of the best recognized mutagen/carcinogen related PAHs, there is also great interest in being able to screen for and quantitate a range of such PAHs in aquatic species. Thus, we have also conducted experiments using a commercially available standard mix containing 16 common PAH compounds spiked into fish muscle tissue. The results [173] indicate that the same method presented here is also applicable for other compounds of this class and are assayable by both GC and LC procedures with only a few co-extracting interferences being evident. Crouch has also applied this methodology to the analysis of alkylated naphthalenes from a variety of aquatic species exposed to such compounds from a large gasoline spill [174].

4.2. Matrix solid-phase dispersion analysis of polychlorinated biphenyls

Ling et al. [175] were the first to report the application of MSPD to the extraction and analysis of PCBs in fish. Their approach used the standard MSPD technique (2 g C_{18} to 0.5 g of tissue) and combined the isolation procedure with an acidic alumina co-column for simultaneous clean-up of the sample. The elution solvent was 8 ml of hexane, which was subsequently reduced in volume to 1 ml and the extract assayed by GC–ECD. This approach was later extended to include PCBs and 16 chlorinated pesticides as described above [169], thus demonstrating the ability to use MSPD to isolate these two major classes of pollutants in a single sample using a single elution protocol. The differ-

ence between these methods and that reported here is in the use of a more polar elution solvent, acetonitrile, the use of a Florisil co-column and the extension of the approach to address chlorinated pesticides, PCBs and PAHs. We have observed that the MSPD columns can provide a separation of lipids and other non-polar materials from these target analytes if the elution solvent chosen has the correct solvent strength in relation to the matrix and column support. Thus, selecting a more polar solvent can provide adequate interactions of target analytes with the solvent while allowing the C_{18} and the major lipid components of the tissue matrix/C_{18} blend to assist in the retention of otherwise co-extracted lipid components. Nonetheless, co-columns that retain lipids without adversely effecting the recovery of the target analytes assures cleaner samples and prevents degradation of the chromatographic system as a function of the numbers of samples analyzed. In this case, both methods appear to provide adequate extraction and approaches to sample preparation that far exceed the otherwise complicated and laborious standard methods currently in use for such analyses. The methods also require no initial capital investment in instrumentation and are fully portable to the field. Such methods, preparing extracts for screening by immunoassay or other rapid techniques, could greatly speed the process and reduce the costs of toxic waste analysis. That there is a potential to conduct screening and analysis of all three major classes of pollutants from a single extract makes application of this approach a worthwhile pursuit for further research.

5. Conclusions

The ability to detect and assess the impacts of toxic wastes on aquatic environments and the species that populate the surrounding ecosystems is dependent on the ability to perform analyses of the water, sediments, soils and tissues of the affected animals. While there has been significant progress in the recent past in the ability to extract and analyze pollutants in water, mainly due to new solid phase extraction-disc technologies and advances in instrumentation sensitivity, there has yet to be such progress in performing analyses in sediment muds and in tissues from aquatic species. Automated gel permeation and SFE instrumentation have been employed to a degree but require an initial large investment in equipment and are limited in their throughput. Existing official methods involving sample disruption, solvent–solvent extractions, back-extractions and column chromatographic clean-up steps, are even more limited in their response times and expend large volumes of solvent and personnel hours. The solvent use is of particular concern since many of the methods require the use of liters of solvent even for a single sample, solvent that is often evaporated into the atmosphere, polluting thousands of liters of air. Discarded solvent must be handled as hazardous waste, adding large volumes of solvent residues to already burdened disposal sites and greatly adding to the overall costs of solvent disposal, not to mention the initial costs of solvent purchase and the unknown costs of personnel exposure. For these reasons it is anticipated that future directions for the analyses of toxic wastes and pollutants in general will proceed through the development of simple, generic and environmentally friendly methods that require little or no solvent and that are capable of providing data in rapid response to inquiries concerning contamination and waste remediation. The approach provided by MSPD and the methods described and reviewed here appear to meet these requirements. In combination with the development of immunoassay or related screening techniques that can be carried into the field to identify sites and species at risk, these methods and approaches are capable of meeting the goals of providing rapid, generic and environmentally friendly analyses of a wide range of toxic wastes and related pollutants.

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