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Short Communication

Use of high-performance liquid chromatography for the estimation of polychlorinated biphenyls and *p,p*-DDE residues in marine mammals

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Abstract

We present a screening technique for the detection of polychlorinated biphenyls (PCBs) and *p,p*-DDE residue levels in marine mammal blubber using high-performance liquid chromatography (HPLC). This method modifies the standard extraction and clean-up methods for organochlorines for use with HPLC and uses a method of chemical derivatization to separate and semi-quantify the two organochlorines with HPLC. © 1998 Elsevier Science B.V.

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1. Introduction

Organochlorines are pollutants of the marine environment. Although banned in many countries, use of these substances continues [1,2]. Because of increased volatility in warmer regions where these compounds are used, and long range transport in the atmosphere, these pollutants have attained worldwide distribution, particularly in the world's oceans [3–8]. Being highly persistent, many organochlorines accumulate in organisms that have relatively poor metabolic and excretory capabilities [9,10], leading to biomagnification along the food chain to top level predators, such as fish-eating birds and many marine mammals [11–13]. This accumulation is most often detected in blubber or fat tissue, due to the high lipid solubility of organochlorines [14–17].

Polychlorinated biphenyls (PCBs) and *p,p*-DDE, a major metabolite of the insecticide DDT, have been found in high concentrations in the blubber of marine mammals [18–23]. These substances are highly persistent, and *p,p*-DDE, along with certain PCB congeners, have been linked to many chronic problems, ranging from neoplasms to dysfunctions in the reproductive and immune systems [24–27].

The presence of PCBs and *p,p*-DDE is usually determined using gas chromatography (GC) or thin-layer chromatography (TLC) [28,29]. In the past, high-performance liquid chromatography (HPLC) had been little used because of poor ultraviolet (UV) absorbance properties of the organochlorines [30,31]. However, it has been demonstrated that PCBs and *p,p*-DDE can absorb UV well enough at shorter wavelengths, enabling HPLC to be used for the detection and semiquantification of these two compounds [32,33]. There are many laboratories that do not have the expensive GC–MS equipment needed for more sophisticated analysis of contaminants, and

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with PCBs and *p,p*-DDE occurring in varying amounts in marine mammals [20], there is a need for a reliable PCB and DDE screen. This short communication describes a technique for the extraction of PCBs and *p,p*-DDE from marine mammal blubber, and the use of chemical derivatization with chromium trioxide to separate and semiquantitate PCBs and *p,p*-DDE with HPLC, in order to screen for the presence of these contaminants in blubber samples. The extraction and clean-up procedure presented below is based on the multiresidue method recommended by the U.S. Food and Drug Administration (FDA) [34], with modifications incorporated for dealing with marine mammal blubber and for use with HPLC.

2. Experimental

2.1. Sample acquisition and storage

Blubber samples were taken from dead stranded marine mammals during standard stranding workups, wrapped in aluminum foil and frozen. Samples came from both freshly dead specimens and from slightly decomposed specimens.

2.2. Extraction of organochlorines

The samples were thawed, cut into cubes, and ground in a mortar with acid-washed sand, reagent grade hexane and anhydrous sodium sulfate (J.T. Baker, Phillipsburg, NJ, USA). The hexane containing the extracted organochlorines was decanted off, and this procedure was repeated four additional times. The decanted supernatant was run through a sodium sulfate column and concentrated to a volume of less than 15 ml in a Kuderna-Danish evaporator (Kontes Glassware, NJ, USA).

2.3. Clean-up of extract

The concentrated extract was cleaned by partitioning between hexane and acetonitrile (J.T. Baker). The cleaned extract was again run through a sodium sulfate column and concentrated to a volume of less than 5 ml, as above. Final clean-up was accomplished by passing the concentrated extract through a column of activated Florisil (J.T. Baker) with a mobile phase of 150 ml of hexane at a flow-rate of 5 ml min^{-1}. The eluate was then concentrated to a volume of less than 3 ml.

2.4. HPLC determination of organochlorines

The procedure used in our laboratory for the detection of PCBs and *p,p*-DDE involved first injecting a 25-μl sample of the cleaned eluate into an isocratic HPLC system with a variable wavelength UV detector (Beckman-Altex, Berkeley, CA, USA) to establish that PCBs and/or *p,p*-DDE were present. (Other organochlorines do not absorb UV well enough to be detected at the concentrations encountered in marine mammal blubber). A prepacked LiChrosorb SI 60 5-μm adsorption column, 250 mm×4 mm I.D. (Merck, Darmstadt, Germany) was used with a mobile phase of 95:5 (v/v) hexane–chloroform (HPLC grade, J.T. Baker) at a flow-rate of 1.4 ml min^{-1} and detection was at 205 nm. If significant PCB/DDE concentrations were detected, the eluate was subjected to chromium trioxide (Mallinckrodt, St. Louis, MO, USA) oxidation to convert the *p,p*-DDE present to *p,p*-DCBP (dichlorobenzophenone). One of the major problems in the detection and quantification of *p,p*-DDE and PCBs is that the two compounds have similar retention times in GC, TLC and HPLC systems [22,35]. A variety of methods have been devised to overcome this problem, including column chromatography and chemical derivatization [36–38]. The oxidation of *p,p*-DDE to *p,p*-DCBP, a process that does not affect the PCBs, has been shown to allow the separation and semiquantitative determination of PCB and *p,p*-DDE residues by GC or TLC, as *p,p*-DCBP has a different retention time from *p,p*-DDE [39–41]. This separation procedure also worked with adsorption HPLC (Fig. 1). A 25-μl sample of this oxidized eluate was then injected into the HPLC system to determine the levels of PCBs and *p,p*-DDE present in the sample.

2.5. Quantification

Estimation of the concentration was done by comparing the average peak area of PCBs and *p,p*-DDE with peak areas for known concentrations of standards of Aroclor 1254 and *p,p*-DCBP. Following

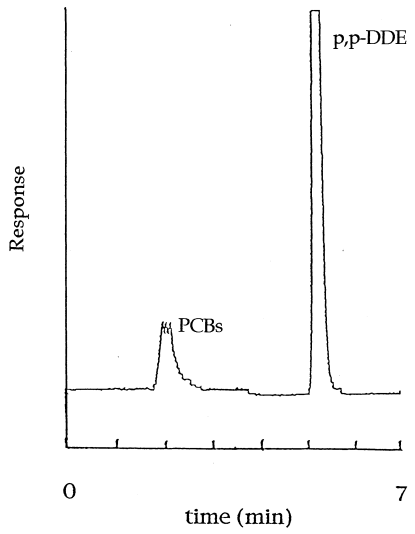

Fig. 1. HPLC chromatogram of a cleaned and oxidized solution of Aroclor 1254 and *p,p*-DDE (both at 100 μg g^{-1}), showing the separation achieved with the conversion of *p,p*-DDE to *p,p*-DCBP.

the suggestion of Bakalyar and Henry [42], peak areas were used rather than peak heights, as the flow-rate could be precisely controlled in this system, whereas the mobile phase composition could not. PCBs and *p,p*-DCBP could be determined in a concentration range of 1 μg g^{-1}–1 mg g^{-1}, with no loss in linearity (Fig. 2).

2.6. Control and recovery

As a control, an extract was analyzed from beef that had been specially raised to minimize exposure to PCBs and DDT. There were no detectable residues in this extract. Recovery experiments were performed by spiking samples for which residue levels had already been determined with known concentrations of Aroclor 1254 and *p,p*-DDE. In addition, standard solutions of both Aroclor 1254 and *p,p*-DDE were run through the entire experimental protocol to determine recovery. Recovery of Aroclor 1254 averaged 82.5±11.7%, while that of *p,p*-DDE averaged 85.6±9.3%. A validation of the method and recovery was performed using a sample of whale blubber that had been analyzed by the U.S. National Institute of Standards and Technology (NIST). Recoveries from this sample, SRM-1945, were 81.6%, for *p,p*-DDE, and 82.5%, for PCBs.

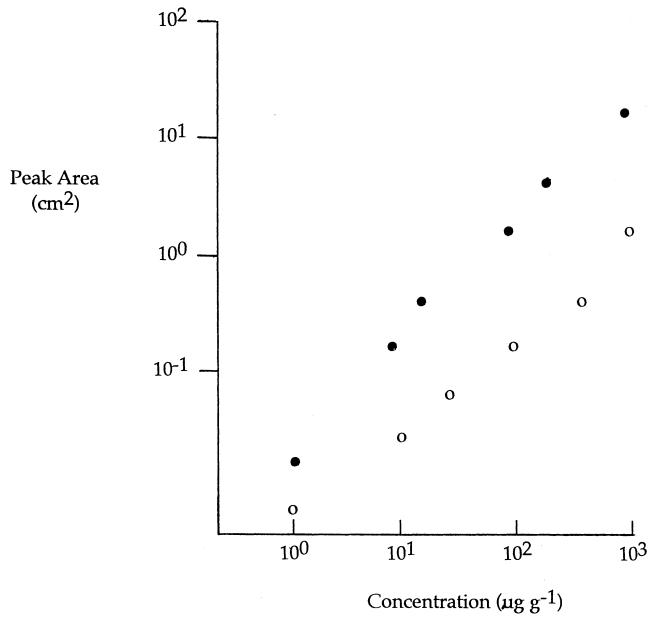

Fig. 2. Peak areas versus concentration of Aroclor 1254 (○) and *p,p*-DCBP (●), demonstrating linearity of response from 1 μg g^{-1} to 1 mg g^{-1}.

3. Results and discussion

The screening of tissues for the presence of PCBs and *p,p*-DDE with HPLC involves the use of hexane for extraction and clean-up instead of petroleum ether, as called for in the FDA procedure [34], and requires only the hexane eluate from the Florisil column for analysis. This eluate contains PCBs and *p,p*-DDE, along with *o,p*-DDE, aldrin, heptachlor, mirex, hexachlorobenzene, and some *o,p*-DDT and *trans*-nonachlor present in the sample [20,43]. UV absorption by organochlorines, other than PCBs and *p,p*-DDE, present in this hexane fraction is poor or nonexistent at the concentrations encountered in marine mammals, so PCBs and *p,p*-DDE can be determined using a UV detector without interference from any other organochlorine contaminants [30,31]. Since a UV detector is the one most often used with HPLC, screening marine mammal tissues for these two organochlorine contaminants is especially feasible. PCBs and *p,p*-DDE are the most commonly occurring organochlorines currently found in marine mammal blubber, with *p,p*-DDE representing by far the largest percentage of total DDE [44–46]. Since *p,p*-DDE is the most prevalent metabolite of DDT in marine mammals, the determination of *p,p*-DDE levels provides a good approximation of total DDT accumulation [47–53].

Generally, in reports of organochlorine residue levels in marine mammals only total PCB concentrations are given [19,20,44,54]. These levels are determined by comparing peak characteristics and retention times of the samples to those of standard Aroclor 1254 or mixtures of Aroclor 1254 and Aroclor 1260 [21,54–56], similar to the procedure used here with HPLC. However, PCB is a generic name used in referring to 209 different isomers and congeners with a varying number of chlorine atoms substituted in the biphenyl rings [3]. Using GC, these individual congeners can be separated and identified, while in adsorption HPLC, PCBs elute only as a series of peaks, with incomplete resolution between the peaks. The determination of the concentration of PCBs using HPLC is therefore that of total PCBs. Consequently, because of the fact that PCBs occur in varying amounts in marine mammals [44–51,53–56], we recommend the use of this screening technique as a means of identifying samples carrying physiologically significant loads; samples which would, therefore, be good candidates for individual congener analysis by GC. We have used this protocol as a screening technique to examine blubber samples from a total of 55 different marine mammals [57]. Values obtained ranged from 1.0–24.3 $\mu g\ g^{-1}$ (wet weight) for PCBs and 0.5–53.9 $\mu g\ g^{-1}$ (wet weight) for *p,p*-DDE. For those species examined, the values obtained were consistent with those reported in the literature, determined by GC. We found using HPLC that PCBs could be detected down to 1 $\mu g\ g^{-1}$ and *p,p*-DDE down to 0.5 $\mu g\ g^{-1}$. Since HPLC is non-destructive to the sample, this protocol can be used to obtain clean fractions of PCBs for detailed GC analysis. Sample preparation is therefore another advantage of this procedure.

4. Conclusions

HPLC can be used to screen blubber samples from marine mammals for the presence of PCBs and *p,p*-DDE. It has the potential to do so for other organisms. Although it cannot replace GC for detailed analysis of organochlorines, it provides a good semiquantitative estimate of the total concentrations of these two specific contaminants. It does so economically, and is useful in a laboratory that has a need to perform a reliable screen for these two pollutants, but has no access to the more costly gas chromatographic equipment.

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