Preliminary Trials of the Use of Immunoassay Screening for Chlordane in Arctic Sea Ice Cores

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ABSTRACT

Twelve ice-pack surface sediments and three ice cores taken during the 1994 AOS TransArctic Cruise were assayed for chlordane using a commercial immunoassay. The total chlordane ranged from 38 to 400 ng/g in sediments and 128 to 430 ng/L in ice. No gas chromatography/electron capture detection (GC/ECD) confirmations were performed; however, spike-recovery tests indicated that it may be possible to obtain contamination estimates for chlordane from single ice cores.

Key words: Arctic, sea ice, pesticides, immunoassays, chlordane

INTRODUCTION

The occurrence of anthropogenic, chlorinated hydrocarbons in Arctic air, sea water, snow, plankton, fish, birds and mammals has been well documented (Kawano et al. 1988, Muir et al. 1988, Norstrom et al. 1988, Iwata et al. 1993). The sum of all contaminants has been reported in the ranges of pg/m^3 in air, ng/L in sea water and snow, ng/g in plankton, fish and birds and µg/g in mammals. The extreme toxicity of some of these compounds presents a human health problem even at these low levels. Federal drinking water and food consumption recommendations are for zero intake (Sittig 1981).

The traditional method of analysis for trace-level chlorinated hydrocarbons in environmental samples is GC/ECD. This method attempts to separate each compound found in the sample, identifying and quantifying each compound by comparing its elution time and detector response to known standards. This is a difficult problem for several reasons.

The chlordane and other chlorinated hydrocarbons that have been manufactured over the decades were not pure chemicals, but often mixtures of dozens of related configurations called congeners. The PCBs (polychlorinated biphenyls) include over 200 congeners. Chlordane is relatively simple, with only a few congeners. When all the classes of pesticides, herbicides, industrial chemicals and their environmental degradation products are combined, the list of analytes totals in the hundreds. These must be detected in the presence of hundreds of naturally occurring organic chemicals that are present in overwhelmingly greater quantities.

To overcome these problems, large sample sizes must be collected, the contaminants extracted, cleaned of interfering natural substances, divided into several fractions, and then concentrated before there is any hope of producing chromatograms with identifiable peaks. Each peak must also be above the detection limit of the instrument. The required sample sizes are hundreds of liters of water or grams of sediment or tissue. Because concentrations will be low in sea ice, larger samples (i.e., tens of cores) are required. Given the difficulty and expense of obtaining cores, sampling and analyses for chlorinated hydrocarbons has rarely been done. It is therefore not known whether the sea ice is holding and transporting significant quantities of toxic chemicals or how much contamination will be released into water when the ice melts. As ice forms and metamorphoses, it may concentrate the chemicals in brine pockets, sediments or entrained plankton. As the case scenario would be that toxic contaminants are concentrated on fine sediments or in plankton. When the ice melts these could be delivered in a pulse to a localized population of higher trophic level aquatic organisms such as filter feeding fish and then to humans.

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The objective of my research was to evaluate the feasibility of using commercial immunoassays to assess chlorinated hydrocarbon contamination in the limited samples of meltwater, entrained sediments and biota that are available from single ice cores.

BACKGROUND

By using enzyme immunoassays, one can circumvent the large sample requirements and extensive cleanup procedures required by traditional chromatographic methods. Enzyme immunoassays are analytical methods based on highly selective binding reactions of antibodies with specific target analytes. Antibodies are proteins produced in response to foreign substances as part of the vertebrate immune response system. Many environmental contaminants are small molecules that cannot induce antibody production by themselves. These molecules must be covalently bound to larger carrier proteins in order to stimulate antibody production when injected into an animal. These small molecule–protein conjugates are called haptens. Through careful selection of antibodies it is possible to design immunoassays that can distinguish an analyte from a related family of compounds or a parent compound from its metabolites.

Enzyme immunoassays developed for small molecules are usually formatted as competitive enzyme-linked immunosorbent assays (ELISAs). In one common form of ELISA (Fig. 1), the target analyte is bound to an enzyme through a spacer molecule to form an enzyme–analyte conjugate. Antibodies are bound onto the surface of a solid such as the walls of a microtitre well or test tube. When a known amount of enzyme–analyte conjugate and sample containing the free target analyte are mixed with the antibodies, they compete with each other for binding sites on the antibodies. After washing away unbound conjugates and free analytes, a substrate is added which undergoes color change when oxidized by the conjugated enzyme. Quantitation is accomplished by comparing color intensity to a standard curve. The amount of enzyme conjugate retained by the antibodies is inversely proportional to the amount of target analyte in the sample. In other words, the more intense the color development, the lower the concentration of free analyte in the sample. A less intense color indicates higher concentrations of free analyte. Since many immunoassays are cross reactive with other compounds of similar structure, manufacturers generally provide a list of analytes exhibiting 50% inhibition of the antibodies on a dose-response curve. Positive results may be due to the target analyte, to cross reactive analytes or to a combination of analytes. For this reason, results of ELISA analyses are often expressed in target analyte equivalents. Such cross reactivity is an advantage when sample size is limited.

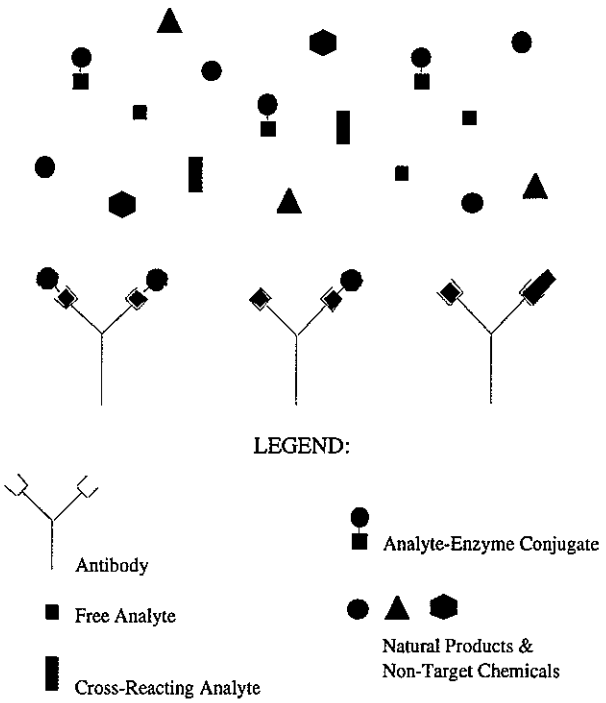

Figure 1. Competitive enzyme immunoassay format.

Whereas GC/ECD must detect 50 ng of each compound, the immunoassay need only detect 50 ng of all related compounds. Thus a sample with PCBs at a level of 5 ng/congener would not be detectable by GC/ECD but would produce a positive response by ELISA. Immunoassays for chlordane, PCBs and many other chlorinated hydrocarbons are available commercially. They have the advantage of high sensitivity (low ng range) using small sample sizes (50 µL of water or 50 mg of solid).

MATERIALS AND METHODS

Freeze-dried, surface sediments taken from twelve locations during the TransArctic Cruise were assayed for chlordane (and related compounds) and PCBs using EnviroGard immunoassay test kits (Millipore). For this test, 1.0-g samples were extracted with 2 mL of methanol. A 100-µL aliquot of the methanol extract was used for each analysis. For ice analyses, six samples were taken from three cores. Melted ice water was passed through a 0.45-µm nylon filter. The entrained sediments were weighed,

Table 1. Chlordane in surface sediments, single and triplicate analysies. Estimated chlordane is based on recovery of spike.

Sample	*Chlordane (ng/g)*	*Average*	*RSD*	*Recovery of spike (%)*	*Estimated chlordane based on recovery (ng/g)*
Single samples					
207-1	270				
215-1	44				
208-1	210				
224-1	75				
235-1	50				
Triplicate samples					
215-E3 a	105				
b	45				
c	55	68	0.47		
222-1 a	400				
b	360				
c	310	357	0.13		
223-1 a	155				
b	140				
c	190	162	0.16		
227-1 a	270				
b	130				
c	101	167	0.54		
212-2 a	150				
b	106				
c	131	129	0.17	23	561
226-1 a	38				
b	130				
c	65	78	0.61	42	186
232-1 a	47				
b	72				
c	65	61	0.29	26	235

air dried and extracted with 1.0 mL of methanol per gram of solid. Contaminants in the meltwater were extracted and concentrated using solid-phase extraction membranes (Empore-C-18, Baker). These membranes are similar in appearance and format to standard 47-mm particulate filters; however, they contain sorbent material that retains hydrophobic chemicals such as chlorinated hydrocarbons (Barceló et al. 1993). The retained chemicals are then eluted with methanol and analyzed.

RESULTS

Results for the surface sediments ranged from 38 to 400 ng/g for chlordane (Table 1). The relative standard deviations for triplicate analyses averaged 33% (13–61%). Spike-recovery experiments were performed for three subsamples by adding known amounts of standards to the previously analyzed sample/methanol mixture, then performing the assay again. The difference between the initial sample value and the sample plus spike value was compared to the spiked amount. The percentage of the spike that was recovered was then used as a correction factor for that sample. Recoveries were low (23% to 42%). Microscopic examination showed the sediments to be predominantly organic detritus.

Results for chlordane from the three ice cores are listed in Table 2. Total filterable sediment ranged from 0.040 g/300 mL to 1.44 g/700 mL. Duplicate or triplicate analyses had an average standard deviation of 13% and an average spike recovery of 105% (83–135%). The 135% recovery from sediment sample 227-4 reveals a positive interference with the chlordane test. These sediments were largely inorganic.

Table 2. Chlordane in ice and entrained sediments, single and multiple analyses. Estimated chordane is based on recovery of spike.

Sample	*ICE Chlordane (ng/L)*	*SEDIMENT Chlordane (ng/g)*	*Average*	*RSD*	*Recovery of spike (%)*	*Estimated chlordane based on recovery (ng/g)*
224 ice	233					
224 sediment		129				
232 ice	88					
232 sediment		125				
227-4 ice	100					
227-1 sediment a		80				
b		78				
c		72	77	0.05	100	77
227-4 sediment a		160				
b		105				
c		145	137	0.21	135	101
227-3 sediment a		78				
b		66	72	0.12	103	70
227-5 sediment a		50				
b		51	51	0	83	61

CONCLUSIONS

Chlordane was detected by commercial immunoassay in surface sediments and both the entrained sediment/detritus and meltwater portions of three ice cores. A modification to the manufacturer's procedure combined an initial determination with a spike-recovery assessment. The recovery-corrected estimates for chlordane are semi-quantitative and were not validated by traditional GC methods. However, it is proposed that valuable information can be obtained from single ice cores using immunoassays. The combination of traditional GC/ECD analysis of surface sediments with group-specific estimates of ice contamination could add significant information to models of global contaminant transport.

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