GENERAL PAPERS

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Symposia Papers Presented Before the Division of Environmental Chemistry
American Chemical Society
San Diego, CA March 13-17, 2005

QUALITATIVE ANALYSIS OF ENDOCRINE DISRUPTING CHEMICALS (EDCs) IN WATER BODIES OF SOUTHEASTERN PARTS OF TEXAS USING RETENTION TIME LOCKING TECHNIQUE (RTL) ON GC/MS

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Abstract

A large number of compounds are suspected to have endocrine disrupting effects in humans and wildlife. Presently, there is very little information about the distribution of endocrine disrupting chemicals (EDCs) in the environment. This is partially due to the fact that laboratory analysis of large groups of compounds such as EDCs is very cumbersome and costly. In this study water and sediment samples collected from various locations of southeastern Texas were extracted using EPA protocols and tested for 597 pesticides and other suspected EDCs using Agilent Technologies Retention Time Locking (RTL) method on GC-MS. The main advantage of this method is in the identification of a large number of compounds without running any standards, except for the one compound needed for "locking" retention times. Using this RTL method a total of 6 pesticides, 13 industrial chemicals and several phthalate esters were found in the study area. Pesticides were distributed mainly in different creeks located in Nueces and Kleberg counties. Industrial chemicals were found mainly in Three Rivers area and along the Nueces River. Conn Brown Harbor in Aransas County showed high phthalate contamination as well as pesticides used for protecting ship hulls. Both pesticides and industrial chemicals were detected in the lower Rio Grande Valley area. The RTL technique used in this study proved to be a fast and cost effective tool for qualitative analysis of a large number of EDCs in this considerably large area.

Introduction

The endocrine system, also referred to as the hormonal system, is a complex system to regulate a wide range of biological processes. Hormones are biochemicals, produced by endocrine glands that travel through the bloodstream and cause responses in other parts of the body. They are active at very low concentrations (ng/mL to pg/mL, *i.e*., ppb or ppt) and bind specifically to target receptor sites on cell surfaces or within cell nucleus (Sesay and Cullen, 2001). Many studies have demonstrated the potential threat of endocrine disrupting chemicals to humans and wildlife (Akingbemi *et al*., 2000; Colborn *et al*., 1996; Gray, 1992; Howsdeshell *et al.*, 1999; Peterson *et al.*, 1998). Most of the potential or known endocrine disrupting chemicals (EDCs) are pesticides, plasticizers (phthalates), polychlorinated biphenyls (PCBs), dioxins, detergents and other commercial chemicals. Phthalates are ubiquitous pollutants of global concern because of their widespread occurrence, toxicity and endocrine disrupting properties. Among the various classes of known or suspected EDCs, pesticides are of particular concern because of their direct application to the land and because of the possibility of contamination of our food sources. There is concern that certain pesticide chemicals and other substances may modify the normal functioning of human and wildlife endocrine or hormone systems and cause developmental, behavioral and reproductive problems. Most pesticides of environmental concern fall into categories of organochlorines, organophosphates, carbamates and synthetic pyrithoirds.

Organochlorine pesticides were introduced in 1940's and rapidly became the pesticides of choice worldwide. However, their use has been severely restricted since the 1980's due to their resistance to degradation and lipophilic nature. These properties make organochlorine pesticides very persistent in the environment (Harris *et al.* 2001).

In Texas, surface water quality is tested through a program called Surface Water Quality Monitoring Program (SWQM) which is conducted by the Texas Commission on Environmental Quality (TCEQ) regional and central offices and is closely coordinated with the Clean Rivers Program (CRP) to enhance the spatial coverage of monitoring sites. During 2003, TCEQ, CRP and United States Geological Survey (USGS) monitored 1,739 fixed monitoring sites. However, most sites only tested for physical properties such as dissolved oxygen, pH, water temperature, specific conductance, nutrient levels and dissolved minerals as well as fecal coliform (bacteria). Monitoring of toxic substances in water, sediment, fish tissue, toxicity testing of ambient water and sediment and biological sampling (benthic macroinvertibrates and fish community elevations and habitat assessments) are additional coverages that are included at about only 100 sites due to much higher costs associated with laboratory analytical determinations of toxic substances and time required for sampling and analysis (TCEQ, Sept. 2002). The organics tested by TCEQ at selected sites includes only 45 pesticides and 63 semi volatile organic substances (TCEQ, 2002). Thus, this study was performed to gather baseline information to see if further testing is needed.

Materials and Methods

The samples were collected according to the procedures outlined in Chapter 4, Section 1 of SW-846, *Sampling Considerations*. Nonvolatile and semi-volatile organic compounds were extracted and concentrated from the water samples using the protocols outlined in the EPA SW-846 Method 3510, *Separatory Funnel Liquid-Liquid Extraction.* The extracted samples were analyzed on the gas chromatograph/mass selective detector (GC/MSD) using new retention time locking (RTL) techniques. Sediment samples for this study were collected according to the procedures outlined in Chapter 4, Section 1 of SW-846, *Sampling Considerations* as well*.* Samples were extracted using EPA SW-846 Method 3540C *Soxhlet Extraction.* Sediment samples were then concentrated to 5 mL using a Kuderna-Danish apparatus for clean up using an HPLC. A GC/MSD (model 6890N GC system; model 5973 MSD) supplied by Agilent Technologies, Inc. was used for this experiment. This instrument, is equipped with a 7683 series auto sampler and the RTL–MSD pesticide library and RT database. This allows for the screening of 567 of the most common pesticides and endocrine disrupting chemicals (EDCs) known to be in use worldwide.

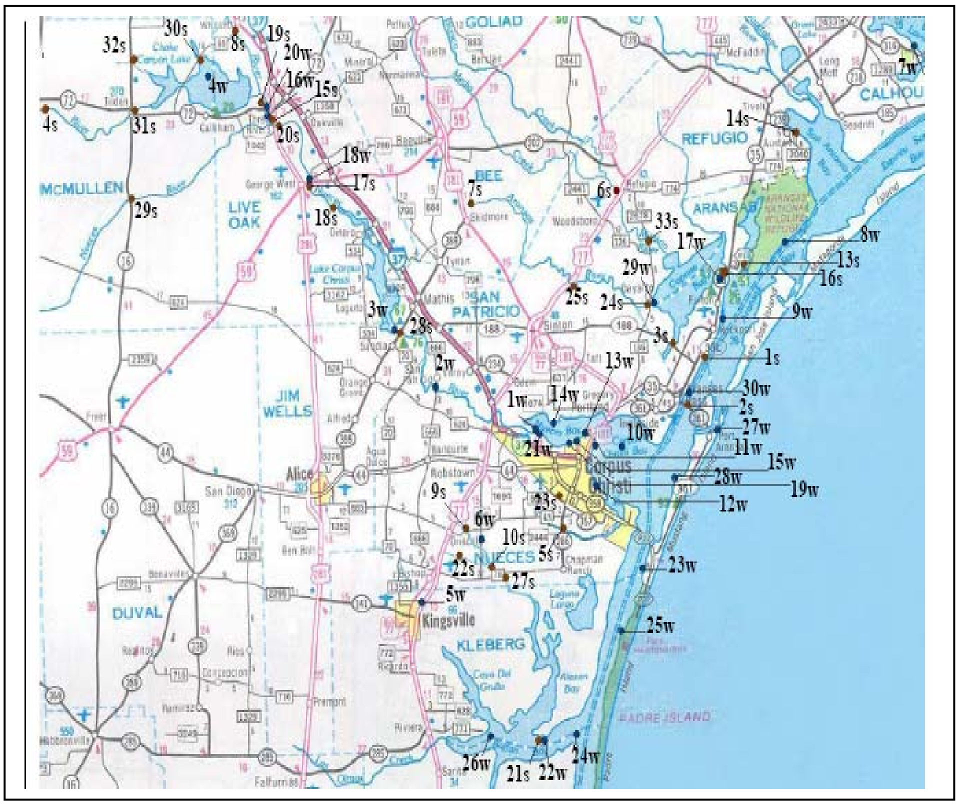

Figure 1. The water and sediment sample locations in southeastern Texas.

Results

During the period of this study, 30 water samples and 35 sediment samples were collected, extracted and analyzed. Of several compounds that appeared in samples 2,4-dichlorophenol (2,4DCP), aniline, atrazine and methoxychlor were the most important chemicals from an endocrine disrupting point of view. Atrazine and methoxychlor are two well known endocrine disruptors whose effect has been documented in multiple studies. Atrazine was found in Petronila Creek, and Methoxychlor was found in San Fernando Creek. Both areas are highly dominated by row crop agriculture. Atrazine is classified as a harmful possible human carcinogen by the U.S. EPA, while methoxychlor is considered to be a proestrogenic endocrine disruptor that also shows estrogenic activity and reproductive toxicity in mammals (Hu and Kupfer, 2002; Cupp *et al*. 2003).

Aniline, found in the Nueces River and the Frio River, is used in the manufacture of dyes and pigments, herbicides and several other industrial processes (Sarasa *et al*., 2002) and is well known for its splenic toxicity in rats (Khan *et al*., 2003). 2,4-Dichlorophenol (2,4-DCP) appeared in Frio River sites (16w and 20w), which are both located near the Valero refinery in Three Rivers, TX. It is highly toxic and persistent in the environment (Quan *et al*., 2004) and is suspected to have endocrine disrupting effects (Takeda *et al*., 2001). After close analysis of these samples it was apparent that five suspected EDCs were found in almost all of the 30 samples. Tables 1 and 2 illustrates the name, location and sample number, as well as suspected EDCs found in each sample.

Table 1. Suspected endocrine disrupting chemicals present in water samples.

Site No.	Aniline	Phenol	p-Dichlorobenzene	2,4-Dichlorophenol	N,N-Diethyl-m-toluamide	Diethyl phthalate	Benzophenone	Atrazine	Diisobutyl phthtalate	Di-n-butylphthalate	Di-n-hexyl phthalate	Butyl benzyl phthalate	Methoxychlor	Bis (2-ethylhexyl)phthalate	Dinonyl phthalate
1w					x	x			x	x		x		x	
2w	x	x			x	x			x	x		x		x	
3w						x			x	x		x		x	
4w					x	x			x	x	x			x	x
5w					x	x			x	x	–	x	x	x	
6w			x		x	x	x	x	x	x		x		x	
7w		x			x	x	x		x	x	x	x		x	
8w					x	x			x	x		x		x	
9w		x			x	x	x		x	x	x	x		x	
10w		x			x	x			x	x				x	
11w					x	x			x	x		x		x	
12w					x	x			x	x				x	
13w					x	x			x	x		x		x	
14w		x			x	x			x	x		x		x	
15w		x			x	x			x	x				x	
16w				x	x	x			x	x		x		x	
17w		x			x	x			x	x				x	
18w	x					x			x	x		x		x	
19w					x	x			x	x		x		x	
20w	x			x	x	x			x	x		x		x	
21w					x	x			x	x		x		x	
22w		x			x	x			x	x				x	
23w					x	x			x	x		x		x	
24w					x	x			x	x	x	x		x	
25w			x		x	x			x	x		x		x	
26w					x	x			x	x		x		x	
27w		x			x	x			x	x		x		x	
28w		x			x	x			x	x		x		x	
29w		x			x	x			x	x		x		x	
30w		x			x	x				x				x	
Blank						x			x	x				x	
SB1						x									
SB2						x			x	x		x		x	

Table 2. Suspected endocrine disrupting chemicals present in sediment samples

Site.No	Phenol	p-Dichlorobenzene	4-Methylphenol	Biphenyl	Dimethylphthalate	O-Phenylphenol	N,N-Diethyl-m-toluamide(DEET)	Diethyl phthalate	4(tert-octyl)phenol	Benzophenone	Tributyl phosphate	Tributyltin chloride	Atrazine	Diisobutyl phthalate	Di-n-butylphthalate	trans-Chlordane	cis-Chlordane	Bisphenol A	p,p'-DDE	Di-n-hexyl phthalate	Butyl benzyl phthalate	Methoxychlor	Dicyclohexyl phthalate	Bis(2-ethylhexyl)phthalate	Benzo(a)pyrene
1s	X				X	X	X	X		X		X		X							X	X		X	X
2s	X						X	X		X				X	X			X			X			X	
3s							X	X					X	X	X						X			X	
4s	X			X		X	X	X		X	X			X	X						X		X	X	X
5s							X	X					X	X	X									X	
6s							X	X						X	X						X			X	
7s							X	X						X	X									X	
8s								X						X	X					X				X	
9s	X					X	X	X		X				X	X					X		X		X	
10s							X	X		X				X	X						X	X		X	
BLNK1							X	X		X				X	X						X	X		X	
11s							X	X		X	X			X	X						X	X		X	
12s							X	X		X				X	X						X			X	
13s						X	X	X		X				X	X						X			X	
14s	X		X				X	X		X				X	X						X			X	
15s	X						X	X		X				X	X					X	X			X	
16s	X					X	X	X		X	X			X	X						X			X	
17s	X						X	X		X				X	X						X		X	X	
18s						X	X	X		X				X	X						X			X	X
19s		X				X	X	X		X				X	X						X		X	X	
BLNK2								X		X				X	X									X	
20s	X					X	X	X		X				X	X						X			X	
21s						X	X	X		X	X			X	X						X			X	
22s						X	X	X		X	X			X	X						X			X	X
23s	X					X	X	X		X	X			X	X						X			X	X
BLNK3		X						X						X										X	
24s	X					X	X	X		X	X			X	X						X			X	
25s						X	X	X		X	X			X	X						X			X	
BLNK4							X	X		X				X	X						X			X	
26s	X					X	X	X		X	X			X	X						X			X	
27s	X					X	X	X		X	X			X	X	X	X		X		X			X	X
28s		X				X	X	X		X	X			X	X						X			X	
BLNK5							X	X		X	X			X	X						X			X	
29s						X	X	X		X				X	X						X		X	X	
30s							X	X		X	X			X	X						X		X	X	
31s						X	X	X		X	X			X	X						X		X	X	
32s						X	X	X		X	X			X	X						X		X	X	
33s								X						X	X										
34s			X			X		X	X	X				X	X									X	X
35s	X					X		X		X				X	X				X		X			X	X
BLNK6						X		X		X				X	X						X			X	
SolvBlnk1								X																	
SolvBlnk2								X						X	X						X			X	

Discussion

After analysis of the thirty different water sampling sites, it was apparent that the chemical compounds diethyl phthalate, di-*n*-butyl phthalate, butyl benzyl phthalate, bis (2-ethylhexyl) phthalate, and the most common N,N,-diethyl-*m*-toluamide also known as and referred to as DEET, was present in at least twenty-six of the water samples and most of the sediment samples.

DEET is commonly used in insect repellents as an active ingredient to prevent mosquito-borne disease (Sudakin and Trevathan). DEET is also a persistent environmental contaminant that breaks down slowly in soil. A recent U.S. Geological Survey report on water contaminants listed DEET as one of the compounds most frequently found in the nation's streams. The U.S. EPA regards DEET as "slightly toxic" to birds, fish and aquatic invertebrates and possibly as an endocrine disrupting chemical in humans (EPA 2002).

The Environmental Protection Agency (EPA) has identified 1,397 sites on its National Priorities List (NPL). Diethyl phthalate has been found in at least 248 of these sites. When a chemical is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment as a chemical emission.

This is one possibility for the presence of diethyl phthalate in samples. Diethyl phthalate is manufactured for many uses. It is commonly used to make plastics more flexible. Because diethyl phthalate is not a part of the chain of chemicals (polymers) which makes up the plastics, it can be released fairly easily from these products. Because of this, contamination by samples could have occurred making this another possibility for the presence of diethyl phthalate. These plastics are found in products such as toothbrushes, automobile parts, tools, toys and food packaging. Diethyl phthalate may enter the environment in industrial wastewaters, by evaporation into the air from disposal sites, directly from consumer products, from the burning of plastic products and by leaking from landfills into soil or water including groundwater. In air, diethyl phthalate may break down into other products. It may also be deposited on the ground or in water by rain.

Di-*n*-butyl phthalate is used in the production of soft plastics, carpet backing, paints, glue, insect repellents, hairspray, nail polish and rocket fuel. Di-*n*-butyl phthalate does not dissolve easily in water, but can enter waterways by attaching to soil particles. Because di-*n*-butyl phthalate has so many uses in modern society, it has become widespread in the environment and its presence in several samples of this study was not uncommon. This is also true for butyl benzyl phthalate and bis (2-ethylhexyl) phthalate, which are very similar and have many of the same uses.

Compounds most commonly found in sediment samples include atrazine, tributyl phosphate, 4-methyl phenol, methoxychlor, benzo(a)pyrene and benzophenone. The possible sources or contamination of these possible EDCs is unknown at this time. Further studies are needed to determine seasonal variation of these chemicals in the environment and to try to determine the possible source of contamination.

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