

# **Report for 2002OR10B: Environmental Analysis and Impact Assessment of Endocrine Disrupters in the Willamette River: A Web-Based Information System**

- Conference Proceedings:
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**Report Follows:**

**Title:****Environmental Analysis and Impact Assessment of Endocrine Disrupters in the Willamette River: A Web-Based Information System****Introduction:**

Endocrine disruption (ED) or endocrine disrupting chemicals (EDCs) has been the focus of an increasing number of scientific investigations in recent years. An environmental endocrine disrupter is defined as an external compound that interferes with or mimics natural hormones in the body that are responsible for the maintenance, reproduction, development, and/or behavior of an organism (Folmar, 1993; Fry and Toone, 1981; Goodbred et al., 1996; Jobling et al., 1996; Schmitt et al., 1990; Smith et al., 1997; U.S. Environmental Protection Agency, 1997). Hypotheses about which chemicals may be endocrine disrupters, about the mechanisms through which they operate, and about which animals may be affected have been discussed in numerous publications (Aboul-Kassim and Simoneit, 2001; Bevans et al., 1996; Colborn and Clement, 1992; Henny et al., 1996; Facemire et al., 1995; Guillette et al., 1994; Davis and Bortone, 1992; EPA, 1997); however, few regional or national studies related to assessment and water quality have been conducted to test these hypotheses.

The U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA) program recently found evidence of endocrine disruption in common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) collected from waterways that contain synthetic organic compounds. At least 45 synthetic chemicals from several chemical groups have been identified as potentially having endocrine disrupting effects (Aboul-Kassim and Simoneit, 2001). These chemicals include the following groups: (a) Herbicides: atrazine, 2,4-D; (b) Fungicides: vinclozolin; (c) Insecticides: DDTs, carbaryl; (d) Nematocides: aldicarb; and (e) Industrial Chemicals: phenols, PCBs, phthalates. Some of these chemicals have the potential to cause reproductive impairment in aquatic organisms (Colborn and Clement, 1992; Henny et al., 1996; Facemire et al., 1995). Alteration in blood concentrations of sex steroid hormones and vitellogenin may be associated with reproductive impairment and other critical reproductive factors (Guillette et al., 1994; Davis and Bortone, 1992; EPA, 1997). Evidence indicates concentrations of sex steroid hormones (estrogen and testosterone) and vitellogenin (egg protein produced by females) were different in fish from contaminated and reference sites.

The current pollution problem in the Willamette River is the result of various anthropogenic activities and the introduction of several synthetic chemicals. An initial investigation carried out by the Department of Civil, Construction and Environmental Engineering at Oregon State University has indicated that the river is contaminated by organic pollutants. Some of these can cause endocrine disruption.

**Problem Statement:**

The Willamette River of Oregon (Figure 1) is the 10<sup>th</sup> largest river in the United States. In the 1930s, the Willamette was so polluted that fish were dying and the water was no longer safe for human use. Decades of local effort resulted in significant improvements to water quality and, by the 1970s, the Willamette became a model for what concerned citizens can accomplish in environmental restoration.

Recently, another critical milestone in the history of the Willamette River has approached. A study performed in 1997 by the US-EPA and the Oregon-DEQ found that the aquatic environment of the river is contaminated with organic pollutants that causes several fish species to die or have deformation.

It is believed that, as in past successful efforts to improve the Willamette, the best approach would be an organized research plan with full synergistic involvement and participation by all interested private sectors, academic institutions and state parties. It is believed that the Department of Civil, Construction and Environmental Engineering at Oregon Sate University is currently playing a major role in the current state problem by developing a web-based information system of EDCs characteristic to the Willamette River.

### **Research Objectives and Approach:**

The Willamette aquatic environment is contaminated with organic pollutants. According to the current state's water pollution, there is a real need to achieve an efficient and cost-effective investigation that will be helpful to the State of Oregon, DEQ and EPA for their assessment and management programs and feasibility study.

The present project aims at building a web-based information system (WBIS) to investigate EDCs in the Willamette river aquatic environment and their potential effects on fish, and to provide background information on contaminant levels at different locations of the study sites. The main objectives of the present proposal are to help:

- Characterize the potential impact of organic contaminants based on the organic chemical composition of both water and sediment samples;
- Estimate the potential ecological effect of organic contaminants present in the Willamette river environment;
- Assign the potential impact to a certain fraction(s) or a group of individual contaminants; and Model the ultimate fate and transport of the characterized endocrine disrupting contaminant(s) in the study area.

The proposed WBIS consists mainly of three fundamental approaches (i.e., modules): Characterization and Source Partitioning (CSP), CHEModynamics (CHEM) and Environmental Impact Assessment (EIA) modules. These modules can be explained as follows:

- The "CSP" module is based on characterization and identification of the EDCs of both water and sediments from the Willamette River. The main pollution sources and transport of these EDCs can be further assessed based on some known pollutant locations in the river, hot spots, chemical indices, and linear programming, artificial intelligence and Monte Carlo Simulation models.
- The "CHEM" module is based on both physical and chemical properties of the characterized EDCs, partitioning data, and multimedia modeling software. This allows determining the fate and transport of these EDCs.
- The "EIA" module is based on information that will be collected from the literature about the relationship between chemical species and endocrine disrupters.

The proposed WBIS aids the elucidation of the impact, fate, and transport of EDCs present in the aquatic environment of the Willamette River.

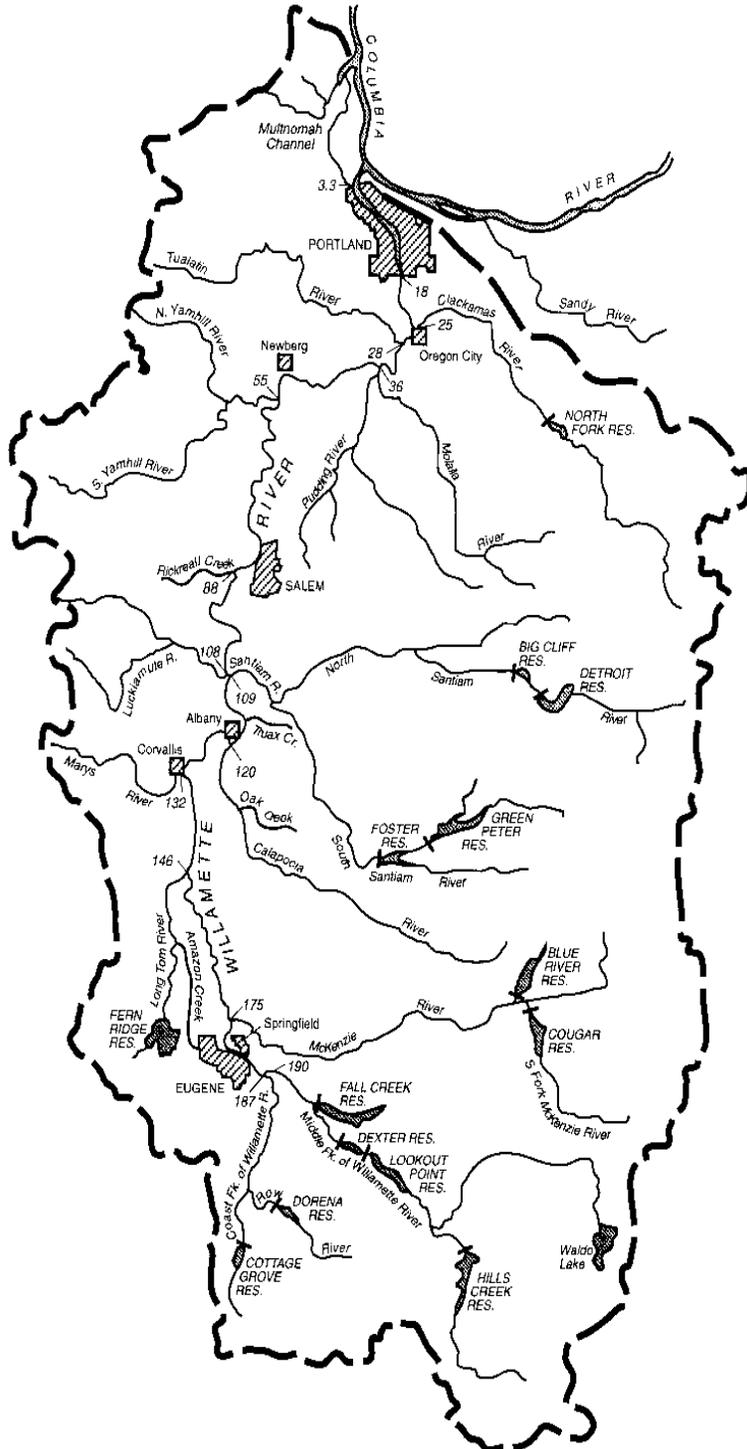


Figure 1. The Willamette River, Oregon

## **Methods, Procedures, and Facilities:**

The present WBIS project uses the state of knowledge and information in the fields of environmental organic chemistry, toxicology, computer science/programming and environmental engineering. This requires the use of state of the art instruments and latest modeling techniques. The following is a summary:

### **Sampling**

Water samples were collected by Niskin bottles from various locations in the Willamette River (Figure 1), which include Portland, Salem, Albany, Corvallis and Eugene stations.

### **Characterization and Identification of EDCs**

The following sections summarize the approach used to characterize and identify the molecular compositions of EDCs in the aquatic environment of the Willamette River, as follows:

#### Extraction and separation

An extraction protocol originally developed and revised by Aboul-Kassim (1994; 1998) and Aboul-Kassim and Simoneit (2001) was modified and verified for the qualitative and quantitative analyses of different organic classes found in the aquatic environment of the Willamette River. In brief, water samples were liquid/liquid extracted with methylene chloride-methanol (2:1). All the extracts (EOM) was concentrated to 2 ml and hydrolyzed overnight with 35 ml of 6% KOH/methanol. The corresponding neutral and acidic fractions were successively recovered with *n*-hexane (4x30 ml), the latter after acidification (pH 2) with 6*N* HCl. The acidic fractions, previously reduced to 0.5 ml, were esterified overnight with 15 ml of 10% BF<sub>3</sub>/methanol. The BF<sub>3</sub>/methanol complex was destroyed with 15 ml of water, and the methyl esters were recovered by extraction with 4x30 ml of *n*-hexane. The neutrals were fractionated by long column chromatography. The following fractions were collected:

- a) 45 ml of *n*-hexane (aliphatic hydrocarbons, F1),
- b) 25 ml of 10% methylene chloride in *n*-hexane (monoaromatic hydrocarbons "MAHs", F2),
- c) 40 ml of 20% methylene chloride in *n*-hexane (polycyclic aromatic hydrocarbons "PAHs", F3),
- d) 25 ml of 50% methylene chloride in *n*-hexane (esters and ketones, F4),
- e) 25 ml of methylene chloride (ketones and aldehydes, F5), and
- f) 50 ml of 10% methanol in methylene chloride (alcohols, F6).

The last fraction and an aliquot of the total extract were derivitized prior to gas chromatographic-mass spectrometric (GC-MS) analysis for further qualitative molecular examination by silylation with bis(trimethylsilyl)trifluoroacetamide. A recovery experiment for the long column chromatography was carried out using several deuterated standards.

Various PCB congeners and pesticide fractions were also be analyzed according to the methods described by Aboul-Kassim and Simoneit (2001). Measurement of the PCB congeners and pesticides required a clean up of the lipid matrix, liquid-liquid partitioning procedure followed by isolation of the PCBs and pesticides using a normal-phase LC procedures. The normal-phase LC procedures separated the analytes into two fractions, one containing the PCBs and the lower polarity chlorinated pesticides (HCB, 2,4'-DDE, and 4,4'-DDE) and the second fraction

containing the more polar chlorinated pesticides. The separation of PCBs and pesticides reduced the possible co-elution of many of the pesticides with PCB congeners of interest. These two fractions were analyzed by gas chromatography-electron capture detector (GC-ECD). Other trace organic pollutants (e.g., phthalate esters) were determined and characterized according to Aboul-Kassim and Simoneit (2001).

#### Instrumental analyses

All samples were analyzed by gas chromatography (GC), GC-ECD, GC-MS. The GC-MS analyses of the samples were performed using a Hewlett-Packard 5973 MSD quadrupole mass spectrometer operated in the electron impact mode at 70eV and coupled to an HP Model 6890 gas chromatograph. The GC is equipped with a 30 m x 0.25 mm i.d capillary column coated with DB-5 (J & W Scientific, film thickness 0.25  $\mu\text{m}$ ) and operated using helium as carrier gas.

#### Identification and quantification

Compound identification was based on comparison with the GC retention times and/or mass fragmentation patterns of standard reference materials and with the help of the Library Molecular marker identification was tabulated as follows:

- (a) *Positive*, when the sample mass spectrum, authentic standard compound mass spectrum, and their retention times agreed well;
- (b) *Probable*, same as above except no standards were available, but the sample mass spectrum agreed very well with the standard library;
- (c) *Possible*, same as above except that the sample spectrum contained information from other compounds but with minor overlap; and
- (d) *Tentative*, when spectrum contained additional information from possibly several compounds with overlap.

Identification and response factors of several EDCs were determined using a suite of standard compounds. Quantification was based on the application of perdeuterated compounds for each respective EDC fraction as internal standards.

#### Organic carbon analysis

Organic carbon analyses will be carried out for all the Willamette river samples using a Carlo Erba NA-1500 CNS analyzer. The concentrations of all EDCs will be calculated relative to the total organic carbon (TOC) content of the samples.

#### Characterization and Source Partitioning (CSP) Module

EDCs data and other chemical indices of the compositions of the aquatic environment of the Willamette river samples were examined statistically in order to determine any significant environmental variations, and to construct a source-partitioning model specific for the Willamette. All statistical analyses and modeling approaches were performed using extended Q-mode factor analysis, linear programming and artificial intelligence-neural network programs and verified using Monte Carlo Simulation.

#### CHEModynamics (CHEM) Module

Fate and transport of various organic pollutants have been studied in different environmental media (Aboul-Kassim, 1998; Aboul-Kassim and Simoneit 2001). Chemodynamic behavior of EDCs characteristic to the Willamette was investigated based on both physical and chemical

properties of the characterized EDCs, partitioning data, and multimedia modeling software. This allowed determining the fate and transport of these EDCs.

### **Environmental Impact Assessment (EIA) Module**

The "EIA" module is based on information that was gathered from the literature about the relationship between chemical species and endocrine disruption. Various databases have been searched and summarized which helped build the proposed WBIS.

### **Facilities**

The analyses were conducted in the Environmental Engineering Laboratory at Oregon State University. Adequate chemical laboratory space and the following instrument facilities, for organic chemical identification and characterization as well as environmental ecotoxicological analyses, were present under the direction of or accessible to the principal investigator. This included: (a) a temperature programmable gas chromatograph coupled to a Chemstation data system (Hewlett-Packard 5890) for high molecular weight compound analyses, (b) one Hewlett-Packard 5973 MSD quadrupole GC-mass spectrometer coupled to a Chemstation data system, (c) elemental (CHN) analysis, (d) temperature controlled environmental chambers, (e) sample collection and coring equipment, and (f) multimedia modeling software.

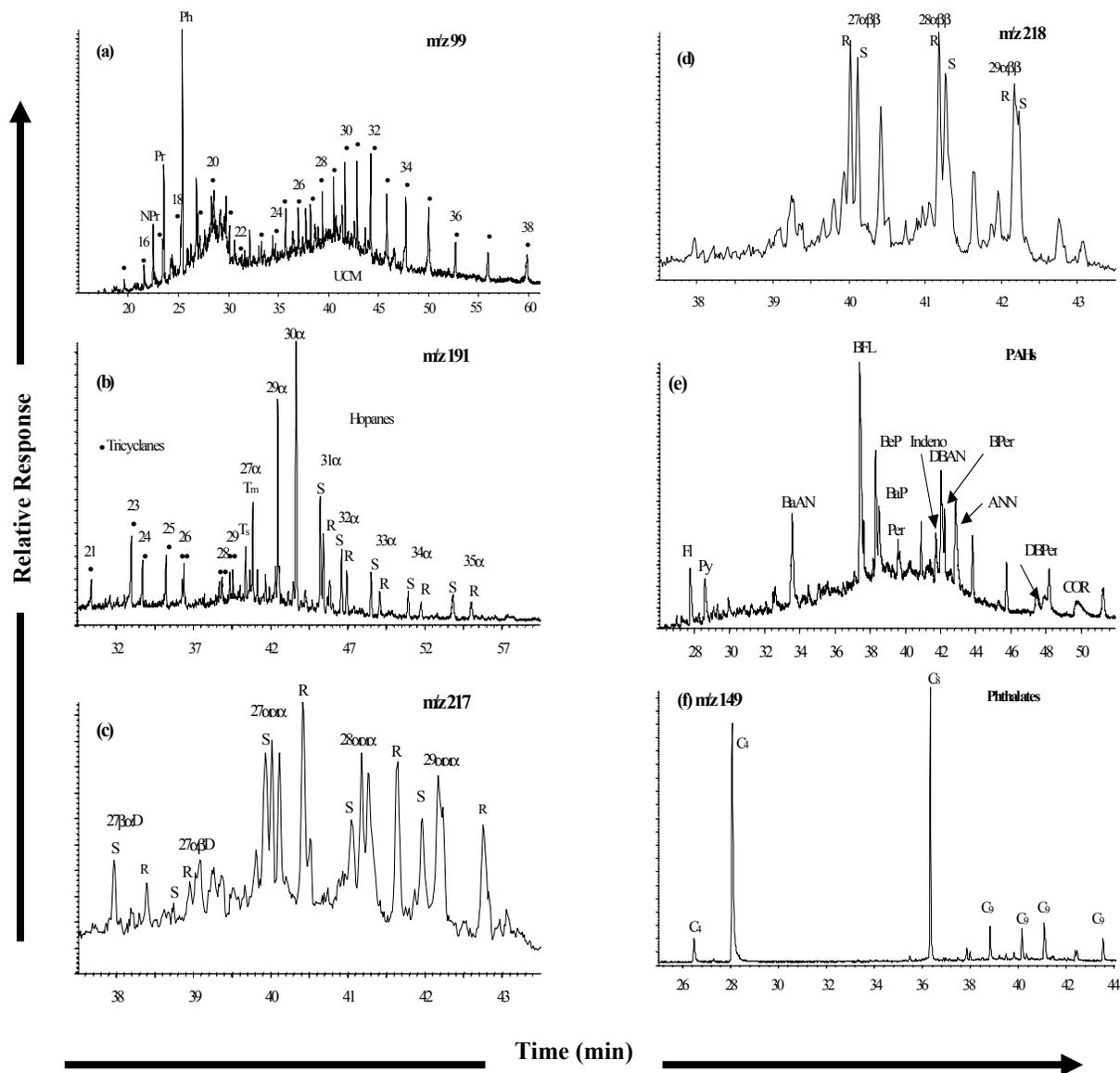
### **Principle Findings and Significance:**

The current pollution in the Willamette River is the result of anthropogenic activities. The present investigation indicated the presence of several chemicals of complex organic mixtures (COMs) or possible organic wastewater contaminants (OWCs). Regardless of sampling locations, the group of COMs/OWCs of anthropogenic origin includes aliphatic hydrocarbons (*n*-alkanes, hopanes, steranes, UCM), polycyclic aromatic hydrocarbons (PAHs) and phthalates. Typical characteristic examples of gas chromatography-mass spectrometry (GC-MS) fingerprinting of these compounds are shown in Figure 2. Complete information about the contaminant chemical name, Composition and molecular weight are shown in Table 1. Chemical structures of both aliphatic and aromatic hydrocarbons are shown in Figures 3 and 4, respectively.

Results of this study will be shared with environmental, assessment and management teams from the: (a) City of Portland - Bureau of Environmental Services; (b) Port of Portland - Environmental Program; (c) Oregon-Department of Environmental Quality; (d) USGS – Portland Office; (e) EPA – Region X - Portland Office; and (f) concerned local citizens in the Willamette area who live and share the river will be also informed.

Based on the principal findings of the current investigation:

- a comprehensive research project was submitted to the United States Geologic Survey in March 2003. The project is entitled: "OCCURRENCE AND JOINT MULTICOMPONENT TOXIC/GENOTOXIC INTERACTION MODELING OF PHARMACEUTICALS, HORMONES AND OTHER ENDOCRINE DISRUPTING CHEMICALS IN THE WILLAMETTE RIVER", with Kenneth J. Williamson, and Bernd R.T. Simoneit (Co-Investigators), \$399,537 for two years
- a collaboration or an interaction with the Port of Portland and City of Portland research teams has been established.



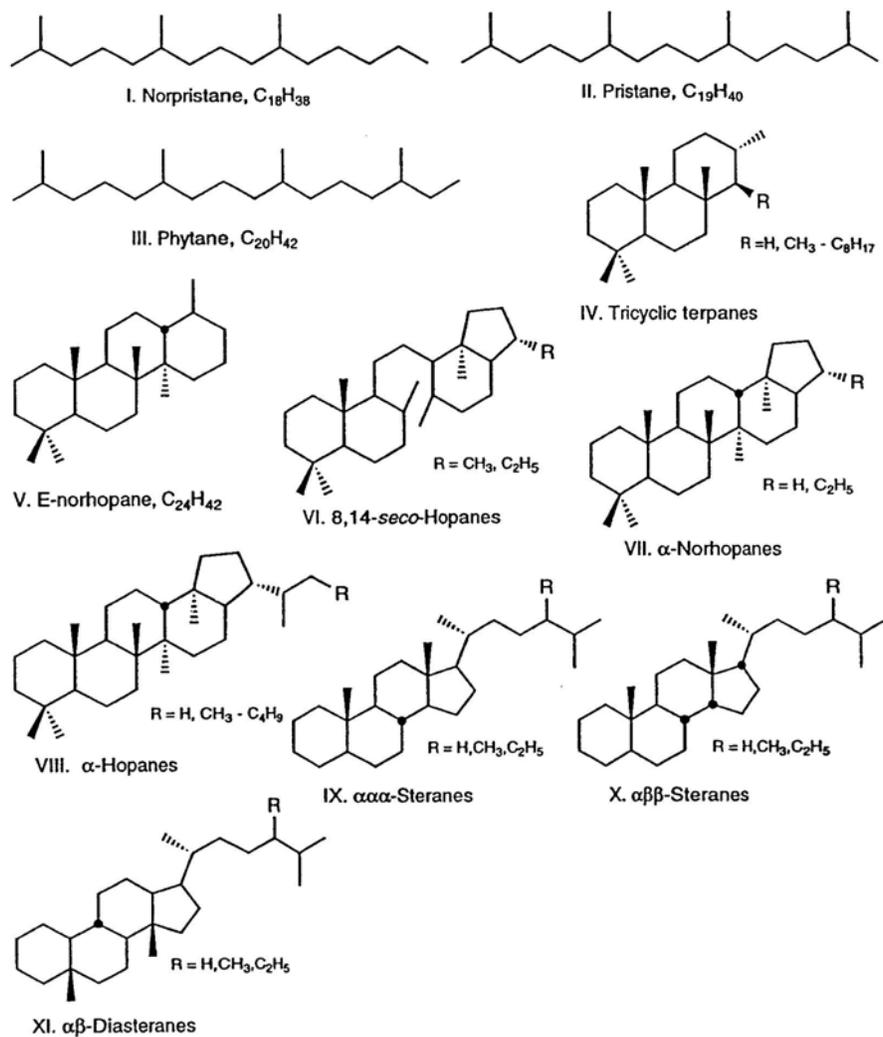
**Figure 2.** Typical GC-MS fingerprints for: (a) *n*-alkanes,  $m/z$  99 (Pr = pristane, Ph = phytane, Npr = norpristane, UCM = unresolved complex mixture, numbers over peaks indicate carbon numbers); (b) hopane series,  $m/z$  191; (c)  $\alpha\alpha\alpha$  sterane series,  $m/z$  217; (d)  $\alpha\beta\beta$  sterane series,  $m/z$  218; (e) PAH composition (Fl = fluoranthene, Py = pyrene, BaAN = benz[a]anthracene, BFL = benzo[b,k]fluoranthene, BeP = benzo[e]pyrene, BaP = benzo[a]pyrene, Per = perylene, Indeno = indeno[1,2,3-cd]pyrene, DBAN = dibenz[a,h]anthracene, BPer = benzo[ghi]perylene, ANN = anthanthrene, DBPer = dibenzo[ghi]perylene, Cor = coronene); and (f)  $C_i$ -phthalate esters,  $m/z$  149 ( $i$  = carbon number of ester groups).

**Table 1. Contaminant Name, Chemical Composition and Molecular Weight representing the Willamette River, Oregon.**

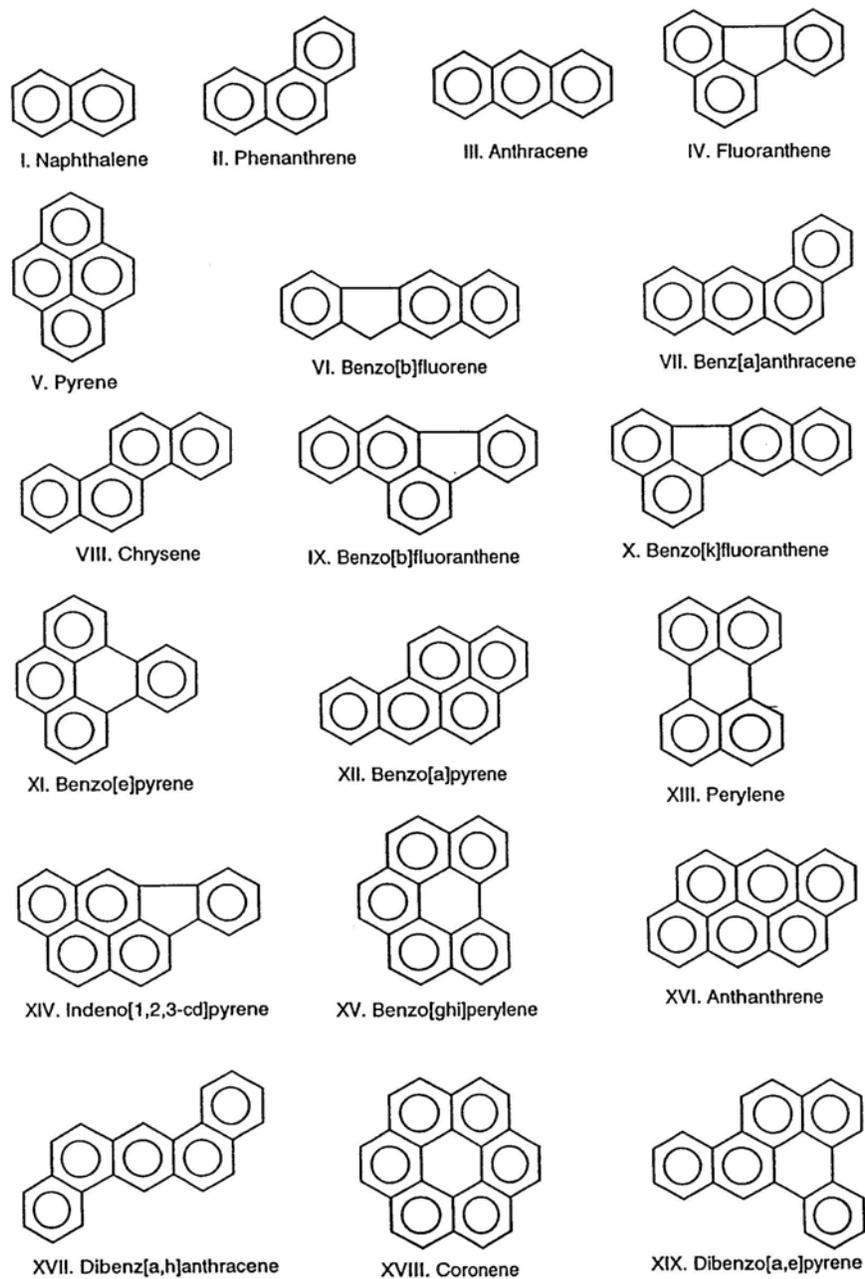
COMPOUND			CHEMICAL COMPOSITION	MW	COMPOUND			CHEMICAL COMPOSITION	MW
#	NAME				#	NAME			
<b>(I) ALIPHATIC HYDROCARBONS</b>					<b>(II) POLYCYCLIC AROMATIC HYDROCARBONS</b>				
<i>n</i> -Alkanes					Neutral PAHs				
<b>1</b>	<i>n</i> -hexadecane		C <sub>16</sub> H <sub>34</sub>	226	<b>69</b>	Quinoline “benzo[b]pyridine”		C <sub>9</sub> H <sub>7</sub> N	129
<b>2</b>	<i>n</i> -heptadecane		C <sub>17</sub> H <sub>36</sub>	240	<b>70</b>	2,3-Dimethyl quinoline		C <sub>11</sub> H <sub>11</sub> N	157
<b>3</b>	<i>n</i> -octadecane		C <sub>18</sub> H <sub>38</sub>	254	<b>71</b>	Phenanthrene		C <sub>14</sub> H <sub>10</sub>	178
<b>4</b>	<i>n</i> -nonadecane		C <sub>19</sub> H <sub>40</sub>	268	<b>72</b>	Anthracene		C <sub>14</sub> H <sub>10</sub>	178
<b>5</b>	<i>n</i> -eicosane		C <sub>20</sub> H <sub>42</sub>	282	<b>73</b>	Fluoranthene		C <sub>16</sub> H <sub>10</sub>	202
<b>6</b>	<i>n</i> -heneicosane		C <sub>21</sub> H <sub>44</sub>	296	<b>74</b>	Pyrene		C <sub>16</sub> H <sub>10</sub>	202
<b>7</b>	<i>n</i> -docosane		C <sub>22</sub> H <sub>46</sub>	310	<b>75</b>	11 H-Benzo[a]fluorene		C <sub>17</sub> H <sub>12</sub>	216
<b>8</b>	<i>n</i> -tricosane		C <sub>23</sub> H <sub>48</sub>	324	<b>76</b>	Benz[a]anthracene		C <sub>18</sub> H <sub>12</sub>	228
<b>9</b>	<i>n</i> -tetracosane		C <sub>24</sub> H <sub>50</sub>	338	<b>77</b>	Chrysene/triphenylene		C <sub>18</sub> H <sub>12</sub>	228
<b>10</b>	<i>n</i> -pentacosane		C <sub>25</sub> H <sub>52</sub>	352	<b>78</b>	Benzo[b+k]fluoranthenes		C <sub>20</sub> H <sub>12</sub>	252
<b>11</b>	<i>n</i> -hexacosane		C <sub>26</sub> H <sub>54</sub>	366	<b>79</b>	Benzo[e]pyrene		C <sub>20</sub> H <sub>12</sub>	252
<b>12</b>	<i>n</i> -heptacosane		C <sub>27</sub> H <sub>56</sub>	380	<b>80</b>	Benzo[a]pyrene		C <sub>20</sub> H <sub>12</sub>	252
<b>13</b>	<i>n</i> -octacosane		C <sub>28</sub> H <sub>58</sub>	394	<b>81</b>	Perylene		C <sub>20</sub> H <sub>12</sub>	252
<b>14</b>	<i>n</i> -nonacosane		C <sub>29</sub> H <sub>60</sub>	408	<b>82</b>	Indeno[1,2,3-cd]pyrene		C <sub>22</sub> H <sub>12</sub>	276
<b>15</b>	<i>n</i> -triacontane		C <sub>30</sub> H <sub>62</sub>	422	<b>83</b>	Dibenz[ah]anthracene		C <sub>22</sub> H <sub>14</sub>	278
<b>16</b>	<i>n</i> -hentriacontane		C <sub>31</sub> H <sub>64</sub>	436	<b>84</b>	Benzo[ghi]perylene		C <sub>22</sub> H <sub>12</sub>	276
<b>17</b>	<i>n</i> -dotriacontane		C <sub>32</sub> H <sub>66</sub>	450	<b>85</b>	Anthanthrene		C <sub>22</sub> H <sub>12</sub>	276
<b>18</b>	<i>n</i> -tritriacontane		C <sub>33</sub> H <sub>68</sub>	464	<b>86</b>	Coronene		C <sub>24</sub> H <sub>12</sub>	300
<b>19</b>	<i>n</i> -tetratriacontane		C <sub>34</sub> H <sub>70</sub>	478	<b>87</b>	Dibenzo[ae]pyrene		C <sub>24</sub> H <sub>14</sub>	302
<b>20</b>	<i>n</i> -pentatriacontane		C <sub>35</sub> H <sub>72</sub>	492	<b>Alkyl-Substituted PAHs</b>				
<b>21</b>	<i>n</i> -hexatriacontane		C <sub>36</sub> H <sub>74</sub>	506	<i>(Alkyl phenanthrene series)</i>				
<b>22</b>	<i>n</i> -heptatriacontane		C <sub>37</sub> H <sub>76</sub>	520	<b>88</b>	3-Methylphenanthrene (3MP)		C <sub>15</sub> H <sub>12</sub>	192
<b>23</b>	<i>n</i> -octatriacontane		C <sub>38</sub> H <sub>78</sub>	534	<b>90</b>	2-Methylphenanthrene (2MP)		C <sub>15</sub> H <sub>12</sub>	192
<b>24</b>	2,6,10-trimethylpentadecane (norpristane)		C <sub>18</sub> H <sub>38</sub>	254	<b>91</b>	9-Methylphenanthrene (9MP)		C <sub>15</sub> H <sub>12</sub>	192

COMPOUND		CHEMICAL COMPOSITION	MW	COMPOUND		CHEMICAL COMPOSITION	MW
#	NAME			#	NAME		
25	2,6,10,14-tetramethylpentadecane (pristane)	C <sub>19</sub> H <sub>40</sub>	268	92	1-Methylphenanthrene (1MP)	C <sub>15</sub> H <sub>12</sub>	192
26	2,6,10,14-tetramethylhexadecane (phytane)	C <sub>20</sub> H <sub>42</sub>	282	93	Dimethylphenanthrenes	C <sub>16</sub> H <sub>14</sub>	206
27	Unresolved Complex Mixture (UCM)			94	Trimethylphenanthrenes	C <sub>17</sub> H <sub>16</sub>	220
<b>Tricyclic terpanes</b>				95	Tetramethylphenanthrenes	C <sub>18</sub> H <sub>18</sub>	234
28	C <sub>19</sub> -tricyclic	C <sub>19</sub> H <sub>34</sub>	262	<i>(Alkyl pyrene/fluoranthene series)</i>			
29	C <sub>20</sub> -tricyclic	C <sub>20</sub> H <sub>36</sub>	276	96	Methylpyrenes/fluoranthenes	C <sub>17</sub> H <sub>12</sub>	216
30	C <sub>21</sub> -tricyclic	C <sub>21</sub> H <sub>38</sub>	290	97	Dimethylpyrenes/fluoranthenes	C <sub>18</sub> H <sub>14</sub>	230
31	C <sub>23</sub> -tricyclic	C <sub>23</sub> H <sub>42</sub>	318	98	Trimethylpyrenes/fluoranthenes	C <sub>20</sub> H <sub>16</sub>	244
32	C <sub>24</sub> -tricyclic	C <sub>24</sub> H <sub>44</sub>	332	<i>(Alkyl 228 series)</i>			
33	C <sub>25</sub> -tricyclic	C <sub>25</sub> H <sub>46</sub>	346	99	Methyl-228	C <sub>19</sub> H <sub>14</sub>	242
34	C <sub>26</sub> -tricyclic (S)	C <sub>26</sub> H <sub>48</sub>	360	100	Dimethyl-228	C <sub>20</sub> H <sub>16</sub>	256
35	C <sub>26</sub> -tricyclic (R)	C <sub>26</sub> H <sub>48</sub>	360	<i>(Alkyl 252 series)</i>			
36	C <sub>28</sub> -tricyclic	C <sub>28</sub> H <sub>50</sub>	388	101	Methyl-252	C <sub>21</sub> H <sub>14</sub>	266
37	C <sub>29</sub> -tricyclic	C <sub>29</sub> H <sub>52</sub>	402	102	Dimethyl-252	C <sub>22</sub> H <sub>16</sub>	280
<b>Tetracyclic terpanes</b>				103	Trimethyl-252	C <sub>23</sub> H <sub>18</sub>	294
38	C <sub>24</sub> -tetracyclic (17,21-seco-hopane)	C <sub>24</sub> H <sub>42</sub>	330	104	Tetramethyl-252	C <sub>24</sub> H <sub>20</sub>	308
39	C <sub>28</sub> -tetracyclic (18,14-seco-hopane)	C <sub>28</sub> H <sub>50</sub>	386	<b>(III) NON-HYDROCARBONS</b>			
40	C <sub>29</sub> -tetracyclic (18,14-seco-hopane)	C <sub>29</sub> H <sub>52</sub>	400	<b>Phthalates</b>			
<b>Pentacyclic triterpanes</b>				105	Phthalic anhydride	C <sub>8</sub> H <sub>4</sub> O <sub>3</sub>	148
41	18α(H)-22,29,30-trisnorhopane (Ts)	C <sub>27</sub> H <sub>46</sub>	370	106	Dimethyl phthalate	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	194
42	17α(H)-22,29,30-trisnorhopane (Tm)	C <sub>27</sub> H <sub>46</sub>	370	107	Diethyl phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222
43	17α(H),21β(H)-29-norhopane	C <sub>29</sub> H <sub>50</sub>	398	108	Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390
44	17α(H),21β(H)-hopane	C <sub>30</sub> H <sub>52</sub>	412	<b>Phenols and Substituted Phenols</b>			
45	17α(H),21β(H)-homohopane (22S)	C <sub>31</sub> H <sub>54</sub>	426	109	Phenol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	94
46	17α(H),21β(H)-homohopane (22R)	C <sub>31</sub> H <sub>54</sub>	426	110	2,3,4,5,6-Pentachlorophenol	C <sub>6</sub> Cl <sub>5</sub> OH	266
47	17α(H),21β(H)-bishomohopane (22S)	C <sub>32</sub> H <sub>56</sub>	440	<b>Amines</b>			
48	17α(H),21β(H)-bishomohopane (22R)	C <sub>32</sub> H <sub>56</sub>	440	111	N,4-Dimethylbenzenamine	C <sub>8</sub> H <sub>11</sub> N	121

COMPOUND		CHEMICAL COMPOSITION	MW	COMPOUND		CHEMICAL COMPOSITION	MW
#	NAME			#	NAME		
49	17 $\alpha$ (H),21 $\beta$ (H)-trishomohopane (22S)	C <sub>33</sub> H <sub>58</sub>	454	112	N,N, 3-Trimethylbezenamine	C <sub>9</sub> H <sub>13</sub> N	134
50	17 $\alpha$ (H),21 $\beta$ (H)-trishomohopane (22R)	C <sub>33</sub> H <sub>58</sub>	454	<b>Amides</b>			
51	17 $\alpha$ (H),21 $\beta$ (H)-tetrakishomohopane (22S)	C <sub>34</sub> H <sub>60</sub>	468	113	N-(2, 4-Dmethylphenyl)formamide	C <sub>9</sub> H <sub>11</sub> NO	149
52	17 $\alpha$ (H),21 $\beta$ (H)-tetrakishomohopane (22R)	C <sub>34</sub> H <sub>60</sub>	468	<b>Various Alcohols</b>			
53	17 $\alpha$ (H),21 $\beta$ (H)-pentakishomohopane (22S)	C <sub>35</sub> H <sub>62</sub>	482	114	Dicyclopentadieneol	C <sub>10</sub> H <sub>13</sub> O	149
54	17 $\alpha$ (H),21 $\beta$ (H)-pentakishomohopane (22R)	C <sub>35</sub> H <sub>62</sub>	482	115	[1,1' - Biphenyl]-2-ol	C <sub>12</sub> H <sub>10</sub> O	170
<b>Diasteranes</b>				<b>Acids</b>			
55	13 $\alpha$ ,17 $\beta$ -diacholestane (20S)	C <sub>27</sub> H <sub>48</sub>	372	116	Benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	122
56	13 $\alpha$ ,17 $\beta$ -diacholestane (20R)	C <sub>27</sub> H <sub>48</sub>	372	117	Nonanoic acid	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	158
<b>Steranes</b>				118	Decanoic acid	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172
57	5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -cholestane (20S)	C <sub>27</sub> H <sub>48</sub>	372	119	Dodecanoic Acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200
58	5 $\alpha$ ,14 $\beta$ ,17 $\beta$ -cholestane (20R)	C <sub>27</sub> H <sub>48</sub>	372	120	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228
59	5 $\alpha$ ,14 $\beta$ ,17 $\beta$ -cholestane (20S)	C <sub>27</sub> H <sub>48</sub>	372	121	Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
60	5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -cholestane (20R)	C <sub>27</sub> H <sub>48</sub>	372				
61	5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -ergostane (20S)	C <sub>28</sub> H <sub>50</sub>	386				
62	5 $\alpha$ ,14 $\beta$ ,17 $\beta$ -ergostane (20R)	C <sub>28</sub> H <sub>50</sub>	386				
63	5 $\alpha$ ,14 $\beta$ ,17 $\beta$ -ergostane (20S)	C <sub>28</sub> H <sub>50</sub>	386				
64	5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -ergostane (20R)	C <sub>28</sub> H <sub>50</sub>	386				
65	5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -sitostane (20S)	C <sub>29</sub> H <sub>52</sub>	400				
66	5 $\alpha$ ,14 $\beta$ ,17 $\beta$ -sitostane (20R)	C <sub>29</sub> H <sub>52</sub>	400				
67	5 $\alpha$ ,14 $\beta$ ,17 $\beta$ -sitostane (20S)	C <sub>29</sub> H <sub>52</sub>	400				
68	5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -sitostane (20R)	C <sub>29</sub> H <sub>52</sub>	400				



**Figure 3. Chemical Structures of Some Aliphatic Hydrocarbons**



**Figure 4. Chemical Structures of Some Polycyclic Aromatic Hydrocarbons**

## **Training and Publications:**

The present study was partially a part of the Masters' thesis for one graduate student in the Department of Civil, Construction and Environmental engineering at Oregon State University. The student has received some training about endocrine disrupting chemicals in the aquatic environment during the project.

The project approach was presented at the 5<sup>th</sup> *International Conference of the Balkan Environmental Association on "Transboundary Pollution"* during 7-10 November 2002, in Belgrade, Yugoslavia. The title of the presentation was: "ENVIRONMENTAL ANALYSIS AND IMPACT ASSESSMENT OF ENDOCRINE DISRUPTERS IN THE WILLAMETTE RIVER – PROJECT APPROACH".

In addition, the principal findings were a part of a lecture, entitled "FORENSIC ANALYSIS, CHEMODYNAMICS AND ENVIRONMENTAL IMPACT ASSESSMENT OF COMPLEX ORGANIC MIXTURES", given in the Department of Environmental Engineering at Texas A&M University-Kingsville, Texas, during January 24, 2003

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