DETERMINING THE PRESENCE AND POTENTIAL ESTROGENIC AND ANDROGENIC ACTIVITY OF CHEMICALS SEQUESTERED BY POCIS IN THE GOLDEN GATE NATIONAL PARK

By

D.A. Alvarez
and
S.D. Perkins

USGS/Columbia Environmental Research Center
4200 New Haven Road
Columbia, MO 65201

Prepared For:

Roger Hothem
U.S. Geological Survey
Western Ecological Research Center
Dixon Field Station
6924 Tremont Road
Dixon, CA 95620

and

Steven Goodbred
U.S. Geological Survey
Modoc Hall, CSUS
3020 State University Drive East
Sacramento, CA 95819-6027

Final Report

April 26, 2006

Prepared by:

_____

David Alvarez

Research Chemist

Reviewed by:

_____

Walter Cranor

Research Chemist

Reviewed by:

_____

Catherine Richter

Research Molecular Biologist

Reviewed by:

_____

Paul R. Heine

CERC Quality Assurance Officer

Approved by:

_____

Carl Orazio, Chief

Environmental Chemistry Branch

Approved by:

_____

Michael Mac, Center Director

Columbia Environmental Research Center

Table of Contents

Executive Summary.....4

Introduction.....5

Experimental.....6

Results and Discussion.....7

Summary.....10

Acknowledgements.....10

Literature Cited.....11

Tables.....13

Figures.....17

EXECUTIVE SUMMARY

The polar organic chemical integrative sampler (POCIS) was used in combination with the yeast estrogen screen (YES) at sites within the Crissy Marsh area of Golden Gate National Park to determine the potential of waterborne chemical(s) to induce an estrogenic effect. The YES is an effective first-tier screening tool to assess the estrogenic potential of chemicals and/or chemical mixtures. A yeast screen for androgens (YAS) and chemical analysis for unknowns were also performed on the POCIS samples. Data from the YES provides evidence that at each site, there is a chemical and/or chemicals present in the aqueous (dissolved) phase that is capable of producing an estrogenic response. These responses were greater than associated POCIS blanks and negative controls. Calculated estradiol equivalent values (EEQs) from the YES are listed in Summary Table 1. The rigorous isolation and identification of these chemicals was beyond the scope of this work. Two chemical classes known to produce a positive response in the YES (i.e., estrogen receptor agonists), fatty acid esters and phthalates, were identified in POCIS extracts from site CFO4-B1. This site had among the highest EEQ values for the study. None of the samples tested by the YAS produced a response greater than the 99% confidence limits for the negative controls.

Summary Table 1. Estradiol Equivalent (EEQ) Determinations from the Yeast Estrogen Screen (YES).

Site	EEQ[a] (ng E2/POCIS)		EEQ[a] (ng E2/POCIS)
Pharmaceutical POCIS[b]		*Pesticide POCIS*[c]	
CFO4 B1	19	CFO4 B1	24
CFO4 I1	7.0	CFO4 I1	6.5
CFO4 E1	39	CFO4 E1	7.2
CFO4 M1	7.3	CFO4 M1	6.8
CFO4 W1	13	CFO4 W1	11
CFO4 W2	1.4	CFO4 W2	4.6
Trip Blank	19	Trip Blank	1.0
Extraction Blank	1.8	Extraction Blank	1.0

[a] EEQ: EEQ (ng E2/POCIS) = EC_{50} E2 (ng E2/mL) / EC_{50} test (POCIS/mL) where POCIS/mL = % POCIS x 1 mL sample.
[b] Pharmaceutical POCIS: POCIS configuration tailored for sampling of pharmaceutically-related compounds.
[c] Pesticide POCIS: POCIS configuration tailored for sampling pesticides and many wastewater-related compounds.

1. INTRODUCTION

During 1998 through 2000, the Golden Gate National Recreation Area, in cooperation with the Golden Gate National Parks Conservancy, restored Crissy Marsh (a 7.3-ha tidal marsh) along the San Francisco Bay front in the Presidio of San Francisco. In addition to the restored tidal marsh, the site includes dunes and dune swale habitat, a re-created historic airfield, and landscaped areas to accommodate recreational use. The Presidio Trust is designing a wastewater treatment plant scheduled to become operational in the summer of 2007, which will provide tertiary-treated recycled wastewater primarily for irrigation of Crissy Airfield, a landscaped area adjacent to the marsh. About 6 ha of the Crissy Airfield has an underdrain system that discharges through a single pipe into the marsh. Planned water treatment processes are not designed to remove and do not monitor certain emergent contaminants including pharmaceuticals and the active ingredients in personal care products (PPCPs). Rainfall and over-irrigation could flush these residual contaminants into the underdrain system and eventually into the marsh where they could individually or collectively cause harm to the tidal marsh ecosystem. Many of these contaminants, including steroid hormones, have been documented to cause endocrine disruption in fish and other wildlife (Desbrow et al., 1998; Purdom et al., 1994). Since hormones, pharmaceuticals and personal care products are neither measured by sewage treatment plants nor regulated by state or federal agencies, data regarding their presence and abundance are rare. No data exist for these compounds for the Crissy Marsh or its vicinity.

USGS scientists from the region around San Francisco are collaborating with the USGS Columbia Environmental Research Center (CERC) to generate baseline data on the presence and effects of select anthropogenic contaminants prior to the operation of the new wastewater treatment plant. An integrative sampler, the polar organic chemical integrative sampler (POCIS), was used to sample the complex mixtures of chemicals potentially present in designated areas of the marsh. Sequestered chemical residues were tested by the yeast estrogen screen (YES) and yeast androgen screen (YAS) to determine the chemical(s) potential to produce an estrogenic and/or androgenic response. The extracts also were screened by chemical analysis to look for natural and synthetic hormones and other chemicals present.

The YES assay uses recombinant yeast cells which are transfected with the human estrogen receptor. The recombinant yeast cells also contain expression plasmids carrying a reporter gene (*lac-z*) situated downstream from a promoter sequence which incorporates an estrogen response element (ERE). Following the binding of a suitable agonist, the yeast cells undergo a cascade of events which results in the release of β-galactosidase into the growth media. The β-galactosidase interacts with a chromogenic substrate, chlorophenol-red-β-D-galactopyranoside (CPRG), in the growth media producing a color change (yellow to red) which can be measured spectrophotometrically. This color change is a measure of the estrogenic potential of chemicals in the sample. The YAS assay is similar to the YES with the exception of the yeast cells are transfected with the human androgen receptor and an androgen response element (ARE)-*lac-z* reporter. Thus,

the YES and YAS assays specifically measure receptor binding and transcriptional activation of the estrogen and androgen receptor, respectively.

The POCIS are designed to mimic key aspects of the bioconcentration process and an organism's exposure to hydrophilic organic contaminants. The POCIS consists of a solid phase sorbent or mixture of sorbents contained between two sheets of a microporous polyethersulfone membrane. Sampling of compounds with low to moderate octanol-water partition coefficients (K_{ow}s) is integrative (i.e., extracted residues are constantly accumulated without significant losses back into the environment) and analyte concentrations can be reported as time-weighted average values. Two configurations of the POCIS, differing by the type of sorbents used, are currently available. The "pesticide" POCIS is the original design optimized for the sampling of many pesticides, hormones, wastewater-related compounds, and most water-soluble organic chemicals. The "pharmaceutical" POCIS was designed to allow for the recovery of certain classes of chemicals which contain multiple functional groups (i.e., pharmaceuticals). Overlap exists in the types of chemicals sampled by each configuration. Detailed description of these two sampler types has been described by Alvarez et al. (2004). The combination of the POCIS and the YES assay has successfully been used to measure the estrogenic potential of chemical mixtures in past studies (Petty et al., 2004; Vermeirssen et al., 2005).

2. EXPERIMENTAL

2.1. Receipt of POCIS extracts

Sixteen extracts, each in an amber glass ampoule, were received from Environmental Sampling Technologies (EST Labs, St. Joseph, MO) per instructions from the co-investigators for testing by the YES assay and chemical analysis at CERC. These extracts are from both the "pesticide" and "pharmaceutical" POCIS configurations. All extracts were in methanol as the carrier solvent. Site identifications, POCIS type, and number of POCIS per sample are listed in Table 1.

2.2. Sample Processing and Analysis

The POCIS samples were processed by members of the Environmental Chemistry Branch of CERC according to established protocols (Alvarez et al., 2004; Alvarez et al., 2005; Rastall et al., 2004; Routledge and Sumpter, 1996) and other methods developed as needed. Testing methods are summarized in the following text. Extracts from the passive samplers were tested for the estrogenic and/or androgenic potential of sequestered contaminants using the YES and YAS assays. The extracts were also screened on a gas chromatograph coupled with a mass selective detector (GC-MSD) to determine the presence of select natural and synthetic hormones and any other unknown contaminants present.

> *YES and YAS screening of POCIS extracts:* Methods for YES and YAS are identical with the exception of the use of the appropriate yeast culture. Figure 1 depicts the preparation of aliquots of the sample, positive and negative controls on

a test plate. The percentage of sample added to each sample well is listed in Tables 1 and 2. Yeast cultures are removed from storage and grown for 24 hours at 30 ºC to determine their viability prior to inoculating the test plates. Sample extracts are solvent exchanged into ethanol to prevent damage to the test plate. The test plates are prepared by adding a positive control in the first row and alternating negative controls (200 µL ethanol) and test sample (100 µL extract diluted with 100 µL ethanol in triplicate) in the following rows. All samples and controls are then serially diluted across the test plate. The liquid in each well is allowed to evaporate prior to adding 200 µL of assay medium containing ≈ 4 x 10^7 recombinant yeast cells and CPRG. The plates are gently agitated, sealed, and incubated at 30 ºC for up to 72 hours. Each day, the plates are inspected for the conversion of CPRG in the positive controls to determine the speed of plate development. After 24 to 48 hours, the plates are allowed to finish developing at room temperature. YAS plates generally will develop faster that YES plates. The plates were read using a Labsystems Multiskan MS type 352 with the Genesis II software (Labsystems, Finland) measuring the absorbance at 540 and 620 nm.

Chemical Analysis of the POCIS extracts: The instrument used was an Agilent 6890 GC equipped with an Agilent 7683 autosampler (Agilent Technologies, Inc., Wilmington, DE) with a HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) capillary column. One µL of each sample extract (1 mL volume) was injected using the "cool-on-column" technique with helium as the carrier gas. The following temperature program was used to separate chemicals present in the sample: injection at 50 °C, held for 2 min, then 25 °C/min to 130 °C, held for 1 min, followed by 6 °C/min to 310 °C and held at 310 °C for 5 min. Analytes were detected with a 5973 MSD (Agilent Technologies, Inc., Palo Alto, CA) in full scan mode. Detector zone temperatures were set at 310 °C for the MSD transfer line, 150 °C at the quadrupole, and 230 °C at the source. Selected hormones and unknown chemicals were identified by matching retention times and mass spectra to genuine standards and to a NIST Mass Spectral Library (1998).

2.3. Special Safety Requirements

All of the experiments were conducted in accordance with the CERC Safety Plan. In general, the plan calls for appropriate protective wear, use of chemical and biological safety hoods as needed, proper records and handling of used materials, training of individuals that will be handling toxic substances, appropriate equipment and plans to handle accidents, and accident reporting requirements for the employee to notify the supervisor and safety officer in the case of an event. Proper sterilization techniques were used in all tests using the yeast cultures.

3. RESULTS AND DISCUSSION

3.1. Quality Control

Quality control measures used in this project included positive and negative controls in the YES/YAS determinations as well as Trip and Extraction blanks in the POCIS sampling. Positive and negative controls are included with each test plate to account for any variability in the preparation of the plate (i.e., serial dilution, handling, contamination, etc.) and at the basic level, to ensure that the test is working. POCIS extraction blanks account for any bias introduced during the extraction of sequestered chemicals from the POCIS matrix. POCIS trip blanks provide a measure of any potential bias introduced due to processing of the samples in the laboratory in addition to any potential contamination of the samplers during transport, storage, and during the act of sampler deployment and retrieval.

3.2. Acceptance or Rejection Criteria for Results

Method limits of detection and of quantification are estimated from low-level standards as determined by the signal-to-noise ratio of the response from either the bioindicator assay or instrumental analysis. For the positive identification and quantification of each analyte, the following criteria were established:

a. The estrogenic or androgenic response must be greater than the 99% confidence limits as determined from the negative controls.
b. Analyte identification by GC-MSD will be considered tentative with a greater than 80% spectral match to a NIST mass spectral library.
c. Quantitation and detection limits for the instrumental analysis will be in accordance with CERC's Environmental Chemistry Branch guidelines.

3.3. YES and YAS analysis

For the YES, each sample exhibited the typical sigmodial dose-response curve associated with receptor-mediated responses. All gave either maximal (Type I) or sub-maximal (Type II) responses capable of determining EC_{50} concentrations. Photographs of representative test plates are shown in Figure 2 and dose-response curves are shown in Figure 3.

Estradiol equivalent factors (EEQ) for the samples were calculated by measuring the EC_{50} for the 17β-estradiol (E2) positive control and determining the percent of sample required to give an equivalent response (same adjusted absorbance indicating the equivalent amount of conversion of CPRG). The adjusted absorbance, compensating for turbidity caused by the growing yeast, is calculated as

$$Abs_{\text{corrected}} = Abs_{540} - (Abs_{620} - Abs_{\text{MNC620}}) \quad (1)$$

where Abs_{540} and Abs_{620} are the measured absorbances at 540 and 620 nm, respectively, and Abs_{MNC620} is the mean negative control absorbance at 620 nm. Since plate to plate variability can occur during the development, EEQs are calculated using the positive control from that plate instead of an average value.

$$EEQ = EEQ\ (ng\ E2/POCIS) = EC_{50}E2\ (ng\ E2/mL)\ /\ EC_{50}test\ (POCIS/mL) \qquad (2)$$

The $EC_{50}E2$ values ranged from 17 ng/L to 100 ng/L with an average of 36 ng/L. Serial dilutions of the POCIS extracts were run in triplicate with variations ranging from 0.14% to 23%, however, most replicates had less the 1% variation.

All sites had EEQ values significantly above baseline and above any response related to the POCIS matrix (co-extractable materials from the membrane and/or sorbent) indicating the presence of one or more chemicals capable to producing an estrogenic response (Table 3). Sites CFO4-B1 and CFO4-E1 had the highest EEQ values for both POCIS configurations. Samples from CFO4-M1 showed the typical sigmodial curve at higher dilutions, however, a decrease in absorbance at the lower dilutions (higher sample concentrations) is indicative of toxicity or other non-toxic inhibitory activity of the yeast due to chemicals present in the sample. The site replicates, CFO4-W1 and CFO4-W2, were not as reproducible as expected. The pharmaceutical and pesticide POCIS from within the same deployment canister (CFO4-W1 vs. CFO4-W2) gave similar EEQ values. It is unknown why there is a 2.5 to 10-fold difference in the CFO4-W1 and CFO4-W2 values. It may be possible that there was some differences in how the samplers were deployed (i.e., differences in flow conditions, samplers covered by sediment, etc.) or that there was some procedural differences during processing. There is not enough information at this stage to make any definitive conclusions.

In general, EEQ values were similar for both POCIS configurations within the same site which is in agreement with data presented by Vermeirssen et al. (2005). This would be expected as chemicals are sampled equally by both configurations. Differences arise in the lower (or not at all) recovery of some chemicals from the pesticide configuration. Although it has a higher predicted capacity, the triphasic sorbent admixture used in the pesticide POCIS can bind nearly irreversibly to chemicals with multiple polar functional groups as seen in many pharmaceuticals.

The pharmaceutical POCIS Trip Blank had an unusually high EEQ which was not present in the Extraction Blank. It is likely that this is due to some unknown contamination of the sample and not due to the POCIS itself. No such contamination was observed in the pesticide POCIS Trip Blank. In general, the POCIS extraction and trip blanks elicited an estrogenic response only at the highest sample concentrations.

None of the samples elicited an androgenic response in the YAS. Most samples showed no significant difference from the negative controls and remained within the 99% confidence interval. Toxic effects of the samples at the lower dilutions/higher concentrations were more evident in the YAS in samples from CFO4-B1, CFO4-E1, and CFO4-M1. These toxic effects were possibly were masked in the YES by the highly estrogenic nature of the samples.

3.4. Chemical Analysis

Analysis of the POCIS extracts by GC-MSD resulted in few identifiable peaks above the background noise (Table 4). In general, the unknown scans were not significantly different than those of the extraction and trip blanks (Figure 4). A more rigorous processing and analysis scheme requiring cleanup and fractionation of sample extracts and analytical measurements targeting specific chemicals may have increased the number of chemicals detected. Such a rigorous scheme was beyond the scope of this project. The most notable chemicals identified were from Site CFO4 B1 and included assorted fatty acid esters and di-n-octyl phthalate. Fatty acid esters have been reported to induce an estrogenic response (Lebo et al., 2004) and di-n-octyl phthalate is a weak estrogen agonist (Beresford et al., 2000). This data corresponds to elevated measurements of EEQ in POCIS from that site. As previously noted, the pesticide POCIS trip blank had an unexpected increase in EEQ. A few phthalates and high molecular weight hydrocarbons were identified in the extracts which may have contributed to the EEQ. These chemicals were unique to this sample complicating source identification.

The natural hormone, 17β-estradiol, and its metabolites, estriol and estrone, along with the synthetic hormone 17α-ethynylestradiol were targeted in the unknown scans. No matches to the retention time or mass spectra of genuine standards of these chemicals were present in any sample. In addition, standard mixtures of PAHs and current-use pesticides were run to aid in peak identification. As with the hormones, no matches were found.

4. SUMMARY

Data from the YES provides evidence that at each site, there is a chemical and/or chemicals present in the aqueous (dissolved) phase that is capable to causing an estrogenic response. The rigorous isolation and identification of these chemicals was beyond the scope of this work. Two chemical classes known to produce a positive response in the YES (i.e., estrogen receptor agonists), fatty acid esters and phthalates, were identified in POCIS extracts from site CFO4-B1. This site had among the highest EEQ values for the study. None of the samples indicated any androgenic potential in the YAS as the response of the samples were not significantly greater than the negative controls. The data, expressed as estradiol equivalents, can be used as a relative scale for ranking study sites. Use of EEQs as a direct measure of effects to organisms is beyond the design of this study. Vermeirssen et al. (2005) reported excellent correlation between EEQ values estimated from POCIS extracts and those from whole water samples and bile from caged fish. However, that study saw a low correlation between EEQ values and estrogen –induced effects (vitellogenin induction) in brown trout.

5. ACKNOWLEDGEMENTS

The technical assistance from Dr. Andrew Rastall, University of Heidelberg, Germany, is greatly appreciated in the processing and interpretation of the YES and YAS results. The

use of specialized laboratory space and equipment from other CERC science branches was critical to the completion of this work and their cooperation is also acknowledged.

6. LITERATURE CITED

Alvarez, D.A., Petty, J.D., Huckins, J.N, Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., Manahan, S.E. 2004. Development of a Passive, In Situ, Integrative Sampler for Hydrophilic Organic Contaminants in Aquatic Environments. *Environ. Tox. Chem.* 23, 1640-1648.

Alvarez, D.A., Stackelberg, P.E., Petty, J.D., Huckins, J.N., Furlong, E.T., Zaugg, S.D., Meyer, M.T. 2005. Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream. *Chemosphere* 61, 610-622.

Beresford, N., Routledge, E.J., Harris, C.A., Sumpter, J.P. 2000. Issues Arising When Interpreting Results from an in Vitro Assay for Estrogenic Activity. *Toxicol. Appl. Pharmacol.* 162, 22-33.

Desbrow, C., Routledge, E., Brighty, G., Sumpter, J., Waldock, M. 1998. Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and in Vitro Biological Screening. *Environ. Sci. Technol.* 32, 1549-1558.

Lebo, J.A., Almeida, F.V., Cranor, W.L., Petty, J.D., Huckins, J.N., Rastall, A., Alvarez, D.A, Mogensen B.B., Johnson, B.T. 2004. Purification of Triolein for use in Semipermeable Membrane Devices (SPMDs). *Chemosphere* 54, 1217-1224.

Petty, J.D., Huckins, J.N., Alvarez, D.A., Brumbaugh, W.G., Cranor, W.L., Gale, R.W., Rastall, A.C., Jones-Lepp, T.L., Leiker, T.J., Rostad, C.E., Furlong, E.T. 2004. A Holistic Passive Integrative Sampling Approach for Assessing the Presence and Potential Impacts of Waterborne Environmental Contaminants. *Chemosphere* 54, 695-705.

Purdom, C., Hardiman, P., Bye, V., Eno, N., Tyler, C., Sumpter, J. 1994. Estrogenic Effects of Effluents from Sewage Treatment Works. *Chem. Ecol*. 8, 275-285.

Rastall, A.C., Neziri, A., Vukonvic, Z., Jung, C., Mijovic, S., Hollert, H., Nikcevic, S., Erdinger, L. 2004. The Identification of Readilhy Bioavailalble Pollutants in Lake Shkodra/Skadar Using Semipermeable Membrane Devices (SPMDs), Bioassays and Chemical Analysis. *Environ Sci & Pollut Res*. 11, 240-253.

Routledge, E.J. and Sumpter, J.P. 1996. Estrogenic Activity of Surfactants and Some of Their Degradation Products Assessed Using a Recombinant Yeast Screen. *Environ. Tox. Chem.* 15, 241-248.

Vermeirssen, E.L., Körner, O., Schönenberger, R., Suter, M., Burkhardt-Holm, P. 2005. Characterization of Environmental Estrogens in River Water Using a Three Pronged Approach: Active and Passive Water Sampling and the Analysis of Accumulated Estrogens in the Bile of Caged Fish.

Table 1. Sample Identification

EST ID[a]	Site ID	POCIS Type	Sample Compositing	mg POCIS in original sample	maximum mg POCIS in test plate	maximum % of sample in test plate
	Extraction Blank A	Pharmaceutical A	1 POCIS	200	8	4
	Extraction Blank B	Pesticide B	1 POCIS	200	8	4
04-789 A	CFO4 B1	Pharmaceutical A	2 POCIS composite	400	16	4
04-789 B	CFO4 B1	Pesticide B	1 POCIS	200	8	4
04-790 A	CFO4 I1	Pharmaceutical A	2 POCIS composite	400	28	7
04-790 B	CFO4 I1	Pesticide B	1 POCIS	200	14	7
04-791 A	CFO4 E1	Pharmaceutical A	2 POCIS composite	400	16	4
04-791 B	CFO4 E1	Pesticide B	1 POCIS	200	8	4
04-792 A	CFO4 M1	Pharmaceutical A	2 POCIS composite	400	16	4
04-792 B	CFO4 M1	Pesticide B	1 POCIS	200	8	4
04-793 A	CFO4 W1	Pharmaceutical A	2 POCIS composite	400	28	7
04-793 B	CFO4 W1	Pesticide B	1 POCIS	200	14	7
04-794 A	CFO4 W2	Pharmaceutical A	2 POCIS composite	400	28	7
04-794 B	CFO4 W2	Pesticide B	1 POCIS	200	14	7
04-795 A	Trip Blank	Pharmaceutical A	1 POCIS	200	14	7
04-795 B	Trip Blank	Pesticide B	1 POCIS	200	14	7

[a] Laboratory identification code from Environmental Sampling Technologies (EST Labs) St. Joseph, MO.

Table 2. Percent of sample in each well across a single row of the 96-well plate.

Well number	% of POCIS sample in well	% of POCIS sample in well
1	4[a]	7[a]
2	2	3.5
3	1	1.75
4	0.5	0.875
5	0.25	4.38×10^{-1}
6	1.25×10^{-1}	2.19×10^{-1}
7	6.25×10^{-2}	1.09×10^{-1}
8	3.13×10^{-2}	5.47×10^{-2}
9	1.56×10^{-2}	2.73×10^{-2}
10	7.81×10^{-3}	1.37×10^{-2}
11	3.91×10^{-3}	6.84×10^{-3}
12	1.95×10^{-3}	3.42×10^{-3}

[a] The maximum percent of sample added to the plates was either 4% or 7% depending on the necessary dilution of the original (bulk) sample. Refer to Table 1 for a listing of the maximum percentages for each sample.

Table 3. EC_{50} and Estradiol Equivalent (EEQ) Determinations from the Yeast Estrogen Screen (YES).

Site	EC_{50} E2[a] (M E2)	EC_{50} E2 (ng/mL E2)	EC_{50} test[b] (mg POCIS)	EC_{50} test (% POCIS)	EEQ[c] (ng E2/POCIS)
Pharmaceutical POCIS					
CFO4 B1	1.4E-10	0.037	0.75	0.19	19
CFO4 I1	9.8E-11	0.027	1.5	0.38	7.0
CFO4 E1	7.1E-11	0.019	0.21	0.05	39
CFO4 M1	7.2E-11	0.020	1.1	0.27	7.3
CFO4 W1	6.4E-11	0.017	0.51	0.13	13
CFO4 W2	8.8E-11	0.024	6.6	1.7	1.4
Trip Blank	1.1E-10	0.029	0.30	0.15	19
Extraction Blank	1.2E-10	0.032	3.5	1.7	1.8
Pesticide POCIS					
CFO4 B1	1.3E-10	0.035	0.30	0.15	24
CFO4 I1	1.0E-10	0.028	0.86	0.43	6.5
CFO4 E1	3.7E-10	0.10	2.8	1.4	7.2
CFO4 M1	1.8E-10	0.050	1.5	0.73	6.8
CFO4 W1	2.4E-10	0.067	0.12	0.59	11
CFO4 W2	1.4E-10	0.038	1.7	0.83	4.6
Trip Blank	1.1E-10	0.031	6.0	3.0	1.0
Extraction Blank	1.0E-10	0.028	5.4	2.7	1.0

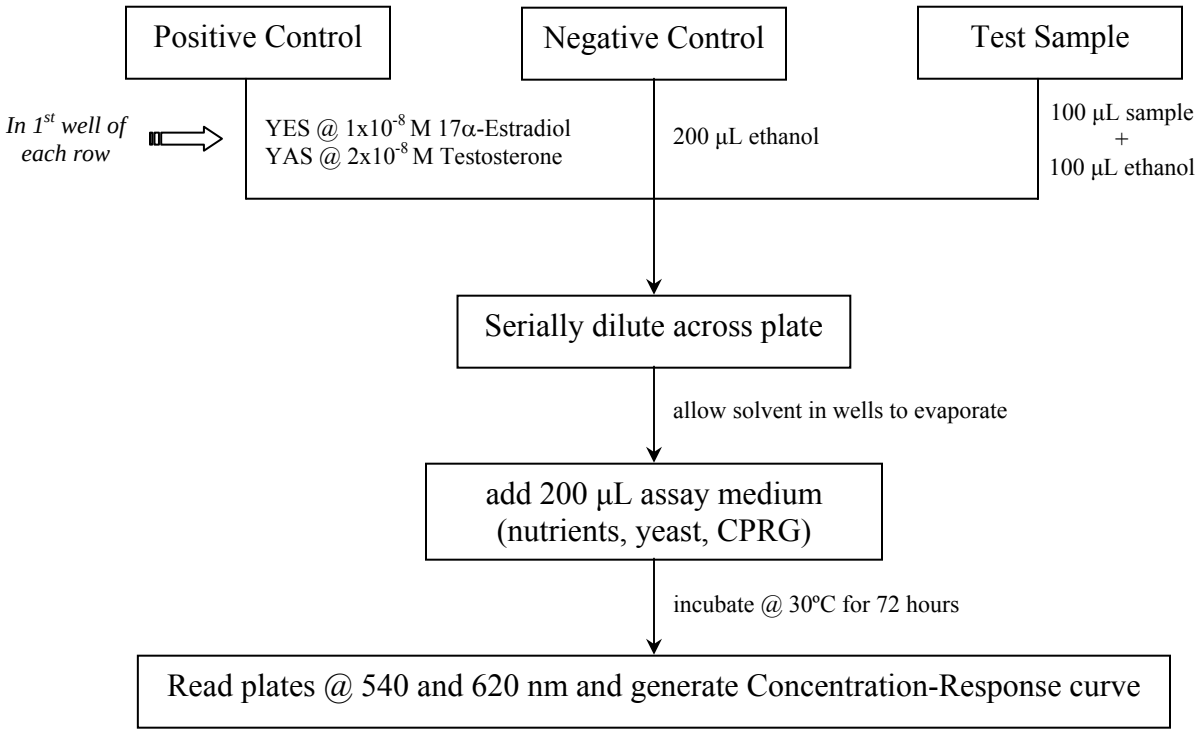
[a] EC_{50} E2: effective median concentration of 17β-estradiol producing 50% of the maximum response for the expression of *lac-Z* reporter gene of the yeast assay.
[b] EC_{50} test: concentration of sample needed to give a response equivalent to the EC_{50} E2.
[c] EEQ: EEQ (ng E2/POCIS) = EC_{50} E2 (ng E2/mL) / EC_{50} test (POCIS/mL) where POCIS/mL = % POCIS x 1 mL sample.

Table 4. Chemical Screen by GC-MSD of POCIS Extracts for Unknowns

EST ID[a]	Site ID	Identified Chemical(s)	Comments
	Extraction Blank A	None Identified [b]	
	Extraction Blank B	None Identified	
04-789 A	CFO4 B1	di-n-octyl phthalate misc. fatty acid esters	weak estrogenic response [c] estrogenic response reported [d]
04-789 B	CFO4 B1	None Identified	
04-790 A	CFO4 I1	None Identified	
04-790 B	CFO4 I1	None Identified	
04-791 A	CFO4 E1	None Identified	
04-791 B	CFO4 E1	None Identified	
04-792 A	CFO4 M1	None Identified	
04-792 B	CFO4 M1	None Identified	
04-793 A	CFO4 W1	None Identified	
04-793 B	CFO4 W1	None Identified	
04-794 A	CFO4 W2	None Identified	
04-794 B	CFO4 W2	None Identified	
04-795 A	Trip Blank	None Identified	
04-795 B	Trip Blank	misc. hydrocarbons di-ethylhexyl phthalate	

[a] Laboratory identification code from Environmental Sampling Technologies (EST Labs).
[b] No peaks were present in the TICs above background noise which could be identified according to established criteria. Peaks identified as silicone septa bleed and solvent artifacts were present in all field deployed and QC samples.
[c] Beresford, N., et al. Toxicol. Appl. Pharmacol. 162 (2000) 22-33.
[d] Lebo, J.A., et al. Chemosphere 54 (2004) 1217-1224.

Figure 1. Sample processing schematic and plate layout for the YES and YAS.



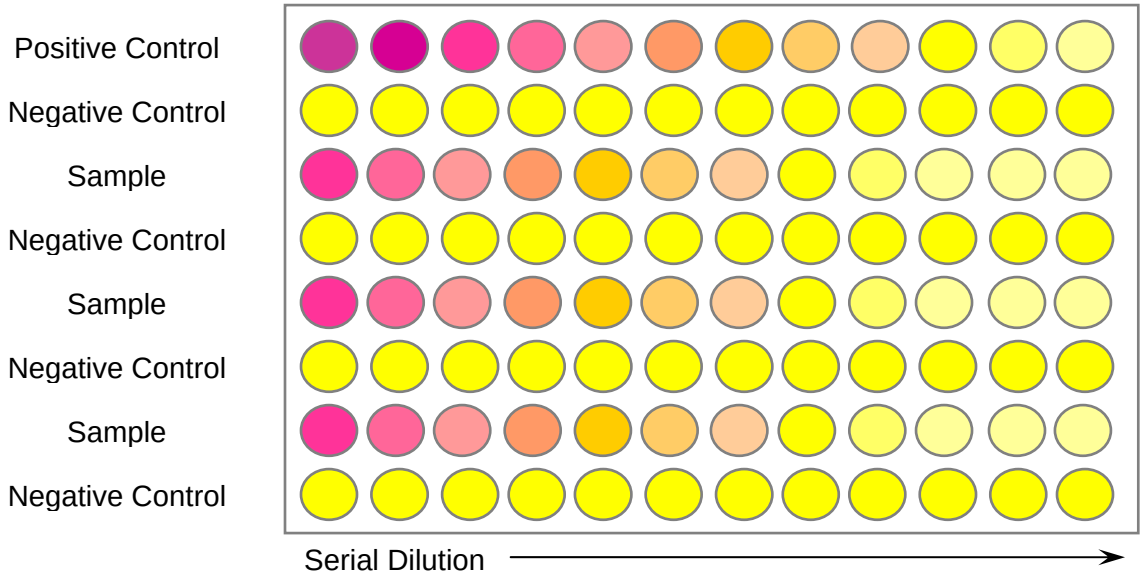
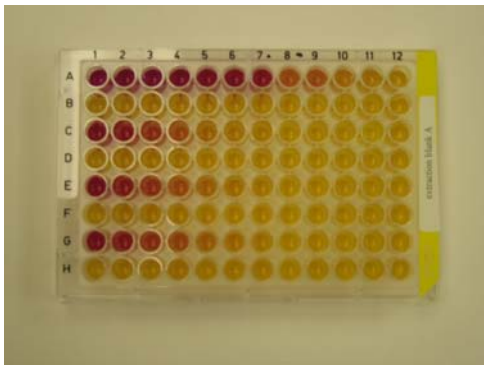
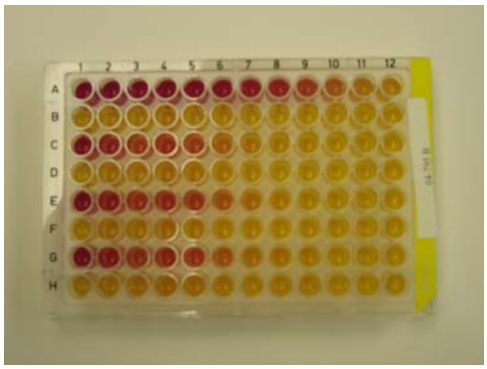


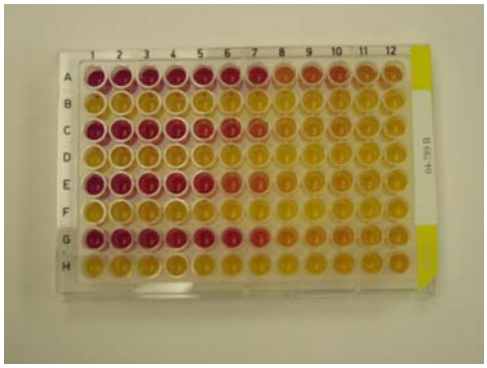
Figure 2. Photographs of Representative 96-well plates for the YES assay at 72 hr.



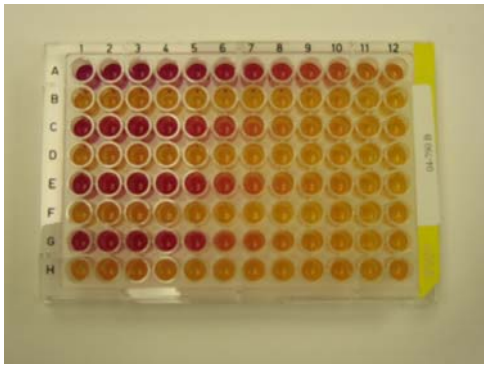
Extraction Blank



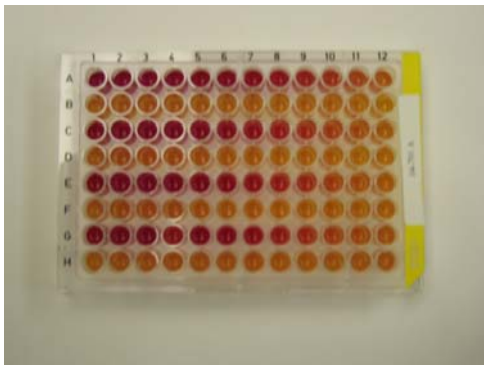
Trip Blank



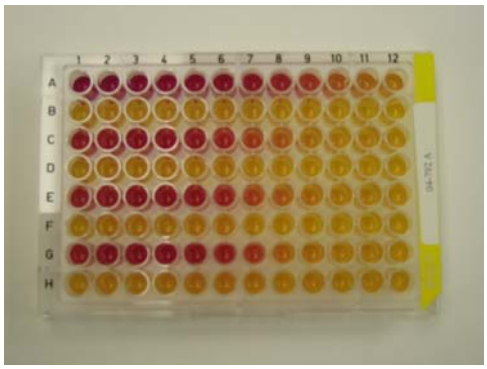
Site CFO4 B1



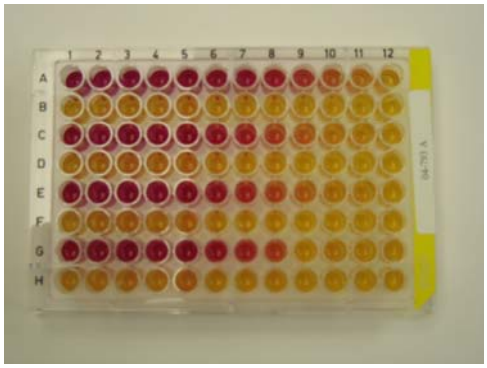
Site CFO4 I1



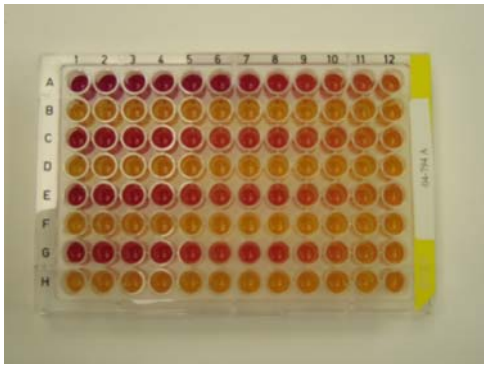
Site CFO4 E1



Site CFO4 M1

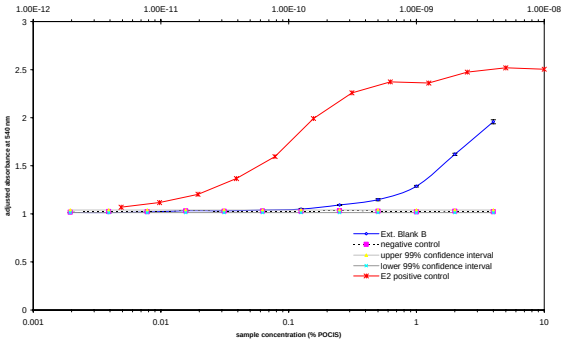


Site CFO4 W1

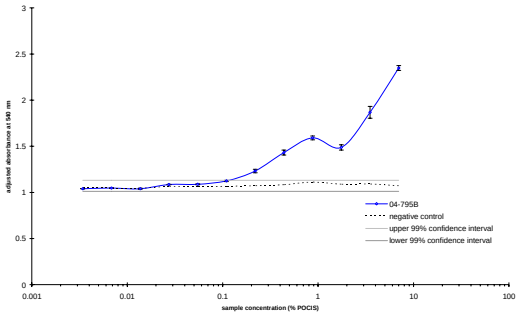


Site CFO4 W2

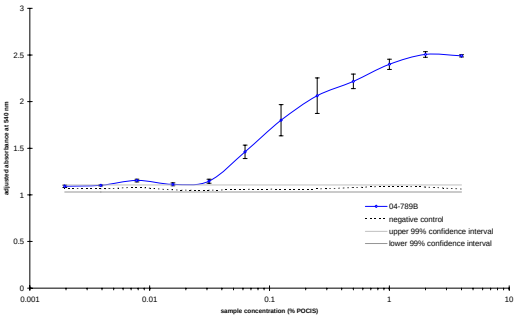
Figure 3. Representative Dose-Response Curves for the YES at 72 hr.



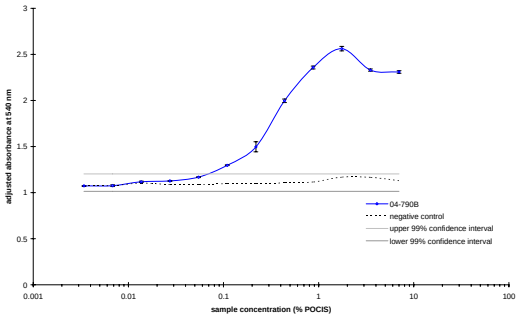
Extraction Blank with E2 Positive Control



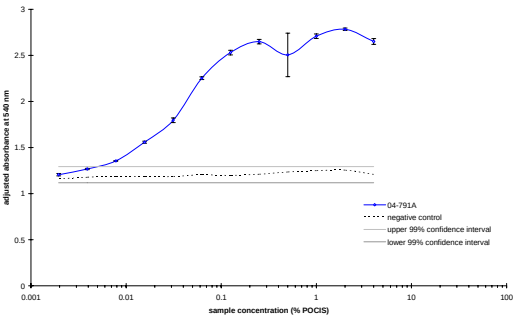
Trip Blank



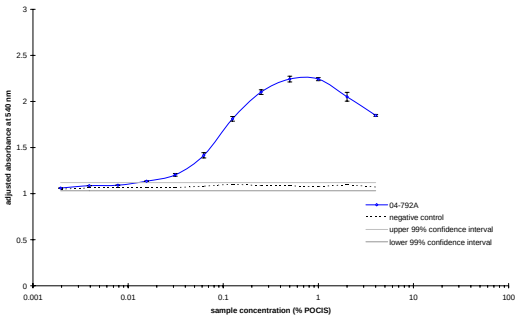
Site CFO4 B1



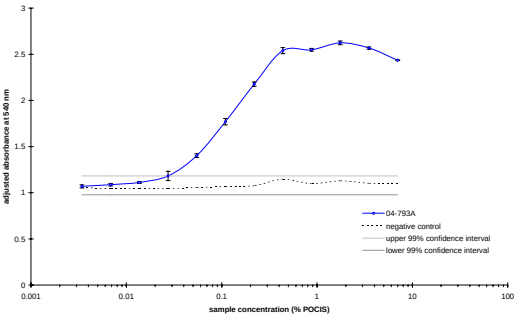
Site CFO4 I1



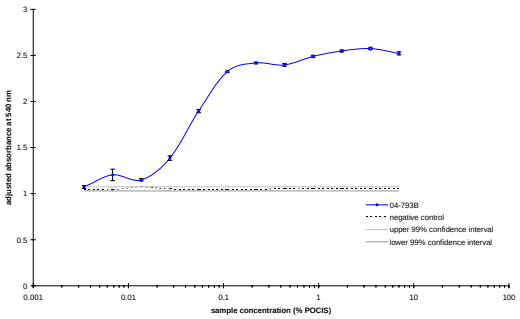
Site CFO4 E1



Site CFO4 M1

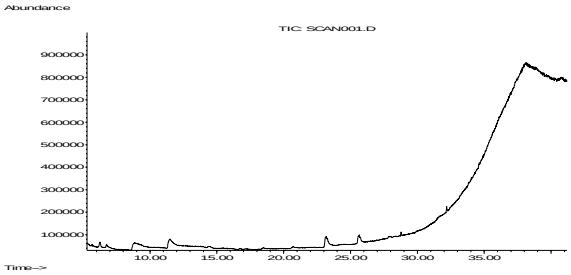


Site CFO4 W1

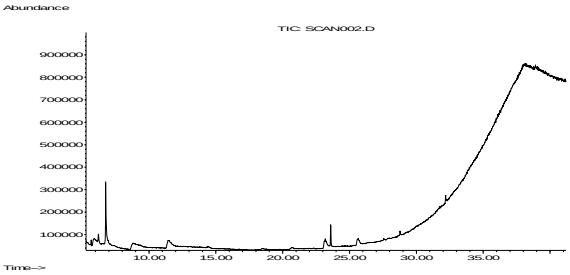


Site CFO4 W2

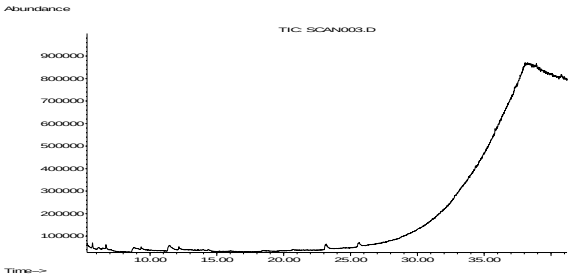
Figure 4. Representative GC-MSD Total Ion Chromatograms



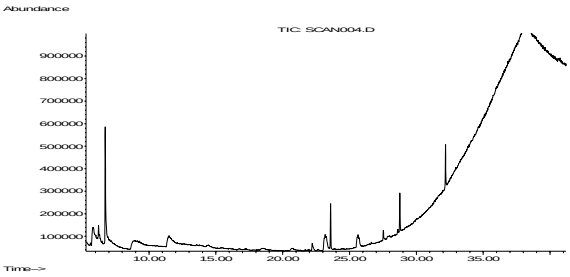
Extraction Blank - Pharmaceutical



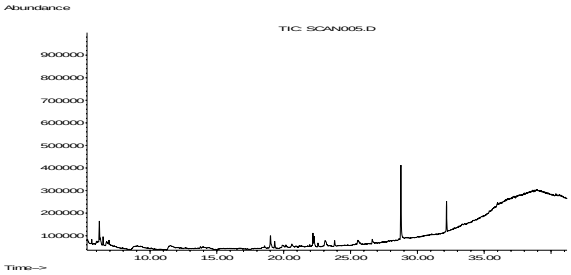
Extraction Blank - Pesticide



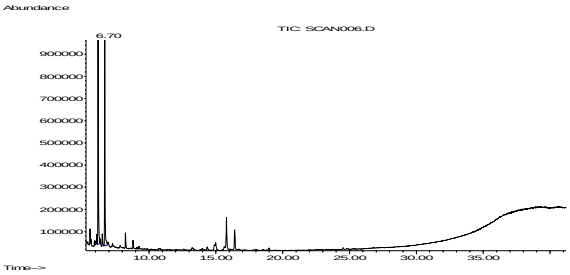
Trip Blank - Pharmaceutical



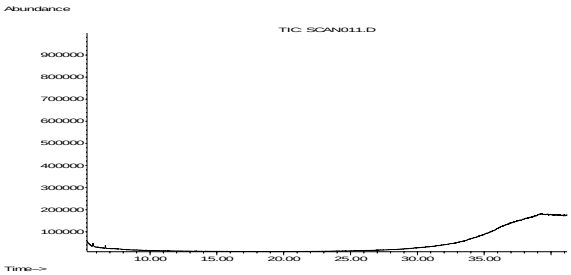
Trip Blank - Pesticide



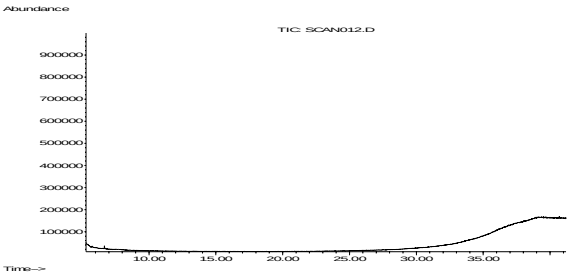
CFO4 B1 - Pharmaceutical



CFO4 B1 - Pesticide



CFO4 M1 - Pharmaceutical



CFO4 M1 - Pesticide

See Table 4 for peak identifications.
Total Ion Chromatograms (TICs) for CFO4 E1, CFO4 I1, CFO4 W1 and CFO4 W2 were similar to those shown for CFO4 M1.
Many of the small peaks observed in the TICs were identified as siloxane compounds traced to the silicone septa used in the GC vials.