MODULE 4 SPECIAL STUDIES

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4.1

PCB IN HATCHERY FISH-DEP/DIFW

PCB IN HATCHERY FISH-DEP/DIFW

A preliminary study in 2001 indicated slightly elevated concentrations of PCB in feed and fish from Maine hatcheries. The study needed to be repeated to confirm these results. The Maine Department of Inland Fisheries and Wildlife (DIFW) supplied landlocked salmon from 2 hatcheries. The ten salmon were combined into 2 composites of 5 fish each. We were also to collect feed and a sediment sample from the settling pond of each hatchery for PCB analysis, but due to an oversight no samples were collected. In order to determine any reductions in concentrations due to depuration and growth dilution, DIFW provided 20 landlocked salmon from each of 2 lakes that had been stocked with fish from 2 of the hatcheries we tested, but no brown trout were collected. The two lakes represented both slow and fast growing salmon.

The results showed that PCB concentrations in salmon from the hatcheries were lower than those in 2001 (Table 4.1). Contrary to expectations, concentrations in salmon that had been in the lakes for 2 years were not lower than those in fish directly from the hatcheries. In fact, salmon from Pleasant Pond in Casco seemed to be higher than those from the source hatchery at Casco, but sample size (n=2) of the hatchery fish was too small for meaningful statistical analysis.

Table 4.1. PCBs in fish from Maine hatcheries and stocked lakes (ug/kg)

		FISH ID					
WATER	**SPECIES**	**1**	**2**	**3**	**4**	**5**	**mean**
Casco Hatchery 2001	LL Salmon						55.3
Casco Hatchery 2002	LL Salmon	30.1	33.8				32.0
Grand L. Str Hatchery 2001	LL Salmon						39.1
Grand L. Str Hatchery 2002	LL Salmon	21	21.9				21.5
New Gloucester Hatchery	brown trout	19.7	14.2				17.0
Palermo Hatchery	brown trout	36	41.1				38.6
Pleasant P Casco	LL Salmon	82.1	71.9	84.8	68.8	113.2	
		83.4	38.9	81.3	61.1	45.6	
		70.5	84.9	77.8	57.3	56.6	
		76.7	63.8	54.4	58.7	55.5	71.9
West Grand Lake	LL Salmon	40.6	39.7	48.5	34.3	59.4	
		22.9	39.9	27.1	32.8	33	
		20.8	61.1	34.6	38.5	42.6	
		56.2	43.5	42.3	44.7	53.3	38.4

4.2

INVESTIGATION OF THE BIOLOGICAL EFFECTS OF AGROCHEMICALS

In vitro Endocrine Effects of Selected Agrochemicals

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Introduction:

Numerous toxicants of natural and anthropogenic origin have been released into the environment in quantities sufficient to disrupt developing endocrine and nervous systems in wildlife and humans (Oberdoster and Cheek, 2001; Damgaard *et al.*, 2002; Kirk, *et al.*, 2003). Many such toxicants have been identified as acute problems in Maine, including organophosphates and other pesticides, herbicides, organo-arsenic, organo-mercury, dioxins and polychlorinated biphenyls (PCBs). Consequences of endocrine disruption can be profound because of the pivotal role that hormones play in controlling development and reproduction (Colborn and Clement, 1992; Birnbaum, 1994). Since the endocrine system is enormously complex, a single chemical can induce alterations through multiple mechanisms.

Nineteen agricultural chemicals are currently registered for use in maintaining blueberry fields of Maine. The fish and shellfish resident in rivers of eastern Maine are potentially exposed to these chemicals through runoff into the watershed. Very little is known about the effects of these agrochemicals on aquatic populations. Four of the chemicals used on Maine blueberry fields (hexazinone, diazinon, malathion and methoxychlor) had previously been tested for estrogenicity using an *in vitro* E-SCREEN assay (Soto *et al.*, 1995). Of these four, only methoxychlor tested positive at a concentration of 10μM. There are no data available on the estrogenicity of the formulation actually applied to the fields. In addition, no data exist on the biological effects of the other eight active components of other herbicides/pesticides used in Maine (guthion, benomyl, phosmet, glyphosate, propiconazole, sethoxidim, clethodim and fluazifop-p-butyl). The degree of estragenicity of these twelve chemicals relative to 17 •-estradiol was determined using E-SCREEN (Soto *et al.*, 1995). Those with low estrogenic activity include diazinon, propiconizol, terbacil, sinbar, benomyl, and carbendazim.

These results suggested that the work should be expanded to include additional formulations and their active compounds and other endocrine effects. In addition to being able to screen individual chemicals, the E-SCREEN assay can also be used to test mixtures of chemicals. Soto *et al.* (1994) have shown that estrogenic chemicals may act in a cumulative fashion.

Relatively little work has been done to demonstrate androgenic activity of environmental contaminants. We tested the same battery of agrochemicals for their ability to act as anti-androgens using MCF7-AR1 cells that stably express a complete human androgen receptor. These cells still

proliferate when 17 β-estradiol is added to charcoal-dextran stripped serum media, but do not obtain the ability to proliferate when androgens were added to the same media. Therefore, androgenic potential can be detected by a decrease in cell proliferation when a test compound is added (Szelei, *et al*., 1997). Assays were run in parallel with the MCF-7 (estrogen-responsive) cell line.

Objectives

The Specific Aims of this project are:

(1). To complete the determination of estrogenic activity of herbicides, pesticides and mixtures using the E-SCREEN assay to measure proliferation of estrogen-responsive MCF-7 cells.

(2) To assess the ability of these compounds to act as androgens, using androgen-responsive cell lines and reporter genes.

Materials and Methods:

(1) E-SCREEN

The E-SCREEN assay is based on the observations that: (1) a protein inherent in serum specifically inhibits proliferation of human estrogen-sensitive MCF-7 cells; and (2) estrogens (or compounds that mimic estrogen) induce cell proliferation by overriding the inhibitory effect (Soto *et al*., 1995). Human breast cancer cells (MCF-7) and the protocols for maintaining cells and running the E-SCREEN were generously provided by Drs. Ana Soto and Carlos Sonnenschein (Tufts University, Boston, MA). The cells were maintained in Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT) in an atmosphere of 6.7% CO_2/93.3% air under saturating humidity, at 37°C. All agricultural chemicals were donated by Dr. David Yarborough (Extension Blueberry Specialist, University of Maine). The 17β-estradiol reference compound was purchased from Calbiochem (Richmond, CA).

MCF-7 cells were plated into Falcon 12-well plates at a concentration of 30,000-40,000 cells/well. The test compound was added directly to the medium, at three different concentrations (10 pM, 1 nM and 10 nM) and cells incubated at 37°C for 5 days. Scoring of the estrogenic effects of each xenobiotic was done by first measuring the proliferative effect (PE), which is the ratio between the highest cell yield counted with the test chemical to the yield of negative control cells (Soto *et al*., 1995). PE was then used to determine RPE, which is calculated as 100 times

the ratio of the highest cell yield from the chemical-exposed cells to cells exposed to 17β-estradiol (Soto *et al.*, 1995). Estradiol is assigned a RPE score of 100%, and all test xenobiotics compared to estradiol. A score of RPE of 100% or greater indicates a full xenoestrogen, while a RPE score between 20 and 50% indicates a partial xenoestrogen. A score of <20% indicates no estrogenic activity. These experiments were repeated up to five times. Assay results that deviated more than two standard deviations from average were not used in the RPE calculations. As of January, 2004, all assays were counted with using a Beckman Coulter Counter ViCell. Accuracy was verified using a hemacytometer.

In addition to being able to screen individual chemicals, the E-SCREEN was also be used to test mixtures of chemicals. Soto *et al.* (1994) have shown that estrogenic chemicals may act in a cumulative fashion. Mixtures of compounds were also tested, based on what we would expect to see applied to the fields. We also tested methoxychlor, Velpar and SuperBK 32 at higher concentrations (up to 10μM). These higher levels, although not considered environmentally relevant, allowed us to compare our data to values previously reported in the literature.

(2) A-SCREEN Assay

An MCF-7 AR-1 cell line and the protocols for maintaining the cells and running the A-SCREEN were kindly provided by Dr. Ana Soto (Tufts University, Boston, MA). The cells were maintained at 37°C in Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY) supplemented with 5% fetal bovine serum (GIBCO) in an atmosphere of 6.7% CO_2 under saturating humidity. Purified active ingredients were obtained from EPA repositories by Brian Perkins (University of Maine). All formulations applied in the field were provided by Dr. David Yarborough (Extension Blueberry Specialist, University of Maine). The 17 ß-estradiol was purchased from Sigma Chemical Co. (St. Louis, MO) and the synthetic androgen steroid, methyltrienolone (R1881), was purchased from NEN/Perkin Elmer.

Maintaining cell cultures - Cells were grown in 25cm^2 flasks with 5mL DMEM Dulbecco's Modified Eagle Medium) in 5% FBS with a media change every 3-4 days. Cells at 90% confluency (~every 6-7 days) were split (1:10) into 2 new flasks. Cells were passed 2-3 times prior to the assay.

A-SCREEN - MCF-7 AR1 cells were plated at a concentration of 45,000 cells/well. The MCF-7 androgen-transfected cells still proliferate in the presence of estrogen and 5% CDFBS/DMEM medium, but proliferation is inhibited when R1881

is added (see Fig. 10). When cells are dosed with R1881 (the synthetic androgen, methlytrienolone) and grown in 5% CDFBS/DMEM media supplemented with 1nM estradiol, proliferation is decreased. The next pesticides that will be tested for androgen activity will be Velpar, and 2,4D Acetic acid.

Dosing - Test media was added 24 hours (+/-3 hours) after subculturing cells. Growth media was removed, cells were rinsed and 1ml of CDFBS 5% experimental media was added to each well (DMEM without phenol red, with charcoal/dextran stripped FBS). Test chemicals were added, in three replicates, at 10nM, 1nM, 0.1nM, 10pM, 1pM. Cells were harvested on Day 5 after treatment by trypsinization and counted using a Beckman Coulter Counter ViCell. A standard curve of R1881 at the final concentrations of 0.1pM, 1pM, 10pM, 100pM, 1000pM in the presence of 1nM estradiol was run in parallel with test samples.

Work accomplished:

(1) E-SCREEN Assay

Growth curves – Completion of the ESCREEN assays required purchase of additional serum of a new lot number. Serum batches were pre-screened by Gibco for the best match. Growth of MCF-7 cells in new serum (lot # 1156246) was compared to growth in the previous lot # (1125122) over a period of five days. **Fig. 1** shows that growth of MCF-7 cells in both lots was not significantly different. Growth curves were also done to compare our laboratory stocks with the parent cultures from Tufts University. Under our conditions, the two cell subcultures exhibited the same growth characteristics (**Fig. 1**).

Sample stability - In an attempt to improve the reproducibility of the ESCREEN assays, we tested the stability of our pesticide/herbicide stock solutions. Most of the organophosphates, such as diazinon, malathion, and glyphosate were found to be less stable than other compounds we tested and new stocks are now diluted every few weeks. Stocks of phosmet and 2,4-dichlorophenoxyacetic acid (2,4-D), which were maintained at -20°C for over one year, were very stable, giving the same RPE values as freshly made stocks (**Figs. 2 & 3**). We modified the procedure to make formulation stock dilutions in water, rather than ethanol, which is more relevant to their use in the field. Each compound is tested up to five times.

(1) F

Formulations and their active chemical ingredients tested in this project period are listed in **Tables I & II**. A comprehensive summary of all the data collected to date is given in **Table III**. Compounds that tested positive for partial estrogen-like activity (RPEs greater than 20%) include: methoxychlor (10μM), Diazinon 50W (diazinon), propiconizole, terbacil, Sinbar, and carbendazim. Velpar and active compound hexazinone were marginally positive at 15.5% and 18% RPE, respectively. Stability of the compounds, such as Round Up/glyphosate and phosmet, may be contributing to the variability in some of the data. Compounds that were positive for partial estrogen-like activity at environmentally relevant levels were re-tested at the higher concentration of 10μM.

Mixtures - Two mixtures (0.5 ppm each compound) were tested, as part of ongoing *in vivo* studies on the effect on Atlantic salmon. The combination of Velpar, Orbit, 2,4-D gave an RPE of 27%. The mixture of Imidan

2.5EC, Sinbar and Orbit was negative at 15% RPE (see **Figs 4 & 5)**.

(2) A-SCREEN

A standard curve was completed to show that proliferation is inhibited when the MCF7-AR1 cells are dosed with R1881 (**Fig. 9**). A second standard curve was completed to show the decrease in proliferation when the cells are supplemented with 1nM 17β-Estradiol and dosed with R1881 (**Fig. 10**). Hexazinone was tested once using the A-SCREEN; no androgenicactivity was detected (**Fig. 10**). Hexazinone, Velpar and 2,4 D were tested at environmentally relevant levels as well as the higher levels.

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Table I Formulations and their active ingredients tested by E-SCREEN

Compound	Active ingredient
Benlate	Benomyl[1]
Diazinon 50W	Diazinon
Imidan 2.5EC	Phosmet
Orbit	Propiconizole[1]
Poast	Sethoxydim
Round Up	Glyphosate
Sinbar	Terbacil
Super BK32	2,4-D (acetic acid form)[2]
Velpar	Hexazinone
Carbendazim	Metabolite of Benomyl

[1]No longer used on blueberries.
[2] widely used historically in Maine; although still used extensively worldwide, it is not currently used on blueberry fields in Maine.

Mixtures tested (0.5ppm of each pesticide)

Velpar, Orbit, 2,4-D
Imidan 2.5EC, Sinbar, Orbit

Table II Summary RPEs of compounds tested in E-SCREEN Assay

Test Compound	N	RPE (Ave + SD)
Clethodim	3	18 ± 4
Diazinon	5	21 ± 8.8
Diazinon 50W	3	21 ± 10.6
Fluazifop p butyl	6	15.3 ± 6.7
Hexazinone	3	18 ± 11
Velpar	4	15.5 ± 7.6
Methoxychlor	3	18 ± 10.6
Phosmet	4	15 ± 7
Imidan 2.5EC	5	16.2 ± 6
Propiconizole	4	20.5 ± 5.2
Orbit	4	16.5 ± 6.2
Sethoxydim	2	12.5 ± 7.8
Poast	2	9
Terbacil	4	21 ± 9.6
Sinbar	3	33 ± 22.6
Benomyl	3	20.3 ± 11
Benlate	3	10.3 ±6.4
Glyphosate	4	15.2 ± 4
Round Up	5	17.4 ± 9.9
Carbendazim	4	23 ± 7.3
2,4 D acetic acid	4	13 ± 5.8
Mixture (Velpar, Orbit, 2,4D)	2	18.5 ± 12
Mixture (Imidan 2.5EC, Sinbar, Orbit)	2	14 ± 1.4

N = # of assays completed

Table III: Comparison of E-SCREEN Assays at high and low contaminant levels

Test Compound	N	range tested	RPE (Ave + SD)
2,4-D acetic acid	3	0.0001-1nM	13 ± 5.8
	1	0.01-10μM	12
Hexazinone	3	0.0001-1nM	18 ± 11
	1	0.01-10μM	32
Hexazinone[1]			
Methoxychlor[2]	3	0.0001-1nM	18 ± 10.6
	1	0.01-10μM	54
Sinbar	3	0.0001-1nM	33 ± 22.6
	1	0.01-10μM	4
Terbacil	4	0.0001-1nM	21 ± 9.6
	1	0.01-10μM	5
Propiconizole	4	0.0001-1nM	20.5 ± 5.2
	1	0.01-10μM	6
Round Up	5	0.0001-1nM	17.4 ± 9.9
	1	0.01-10μM	7
Carbendazim[1]	4	0.0001-1nM	23 ± 7.3
	1	0.01-10μM	9
Benomyl[1]	3	0.0001-1nM	20.3 ± 11
	1	0.01-10μM	5
Phosmet[1]	4	0.0001-1nM	15 ± 7
	1	0.01-10μM	6
Glyphosate[1]	4	0.0001-1nM	15.2 ± 4
	1	0.01-10μM	6

[1] 5/24/04 received new stocks of pesticides.
[2] used as positive control.

ASCREENS:

Test Compound	N	range tested	Result
2,4D Acetic acid see graph	1	0.0001-1nM	non androgenic,
2,4D Acetic acid see graph	1	1nM-10uM	non androgenic,
Hexazinone[1]	1	1nM-10uM	non androgenic
Hexazinone[1]	2	0.0001-1nM	non androgenic

[1] 5/24/04 received new stocks of pesticides.

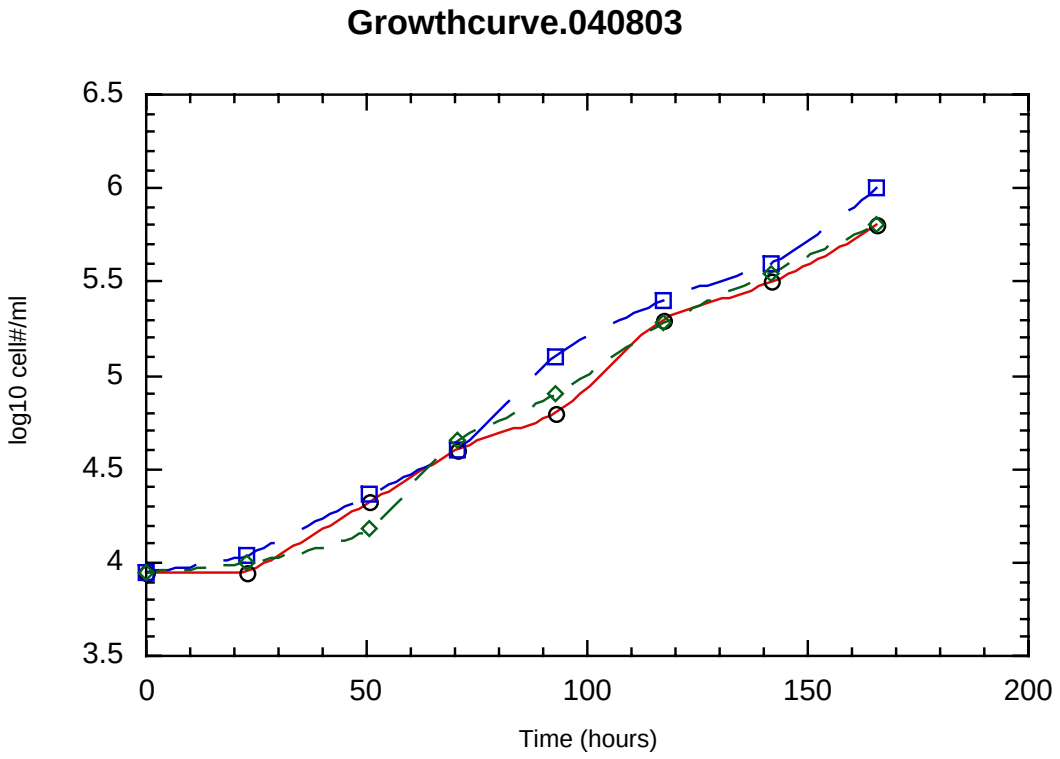

Fig. 1 E-SCREEN Assay: (a) Comparison of University of Maine cultures of MCF-7 cells grown in two different lots of serum [serum lot #1156246 (—●—) and serum lot #1125122 (—●—)] showed very similar growth characteristics. (b) Inter-laboratory comparison of Tufts University parental stocks. (—¤—) to both University of Maine cultures tested at the University of Maine showed no differences in growth.

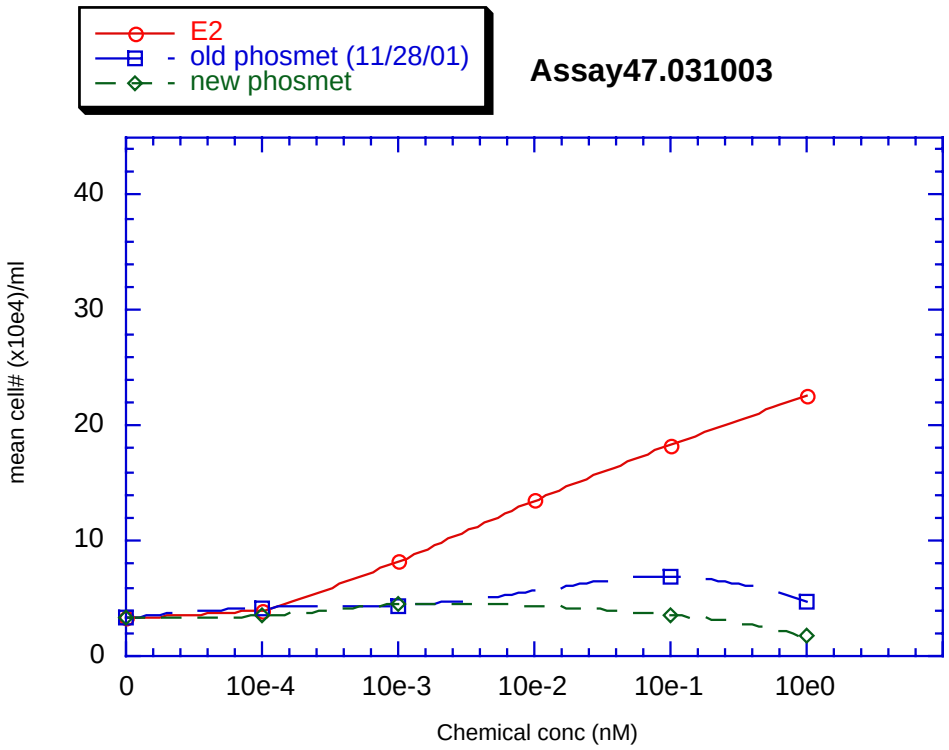

Fig. 2 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—o—) to cells exposed to phosmet to compare stability of stock #1 [>1.5 years old, maintained at -20°C, (—•—)] to stock #2 [made fresh on day of testing, (—•—)].

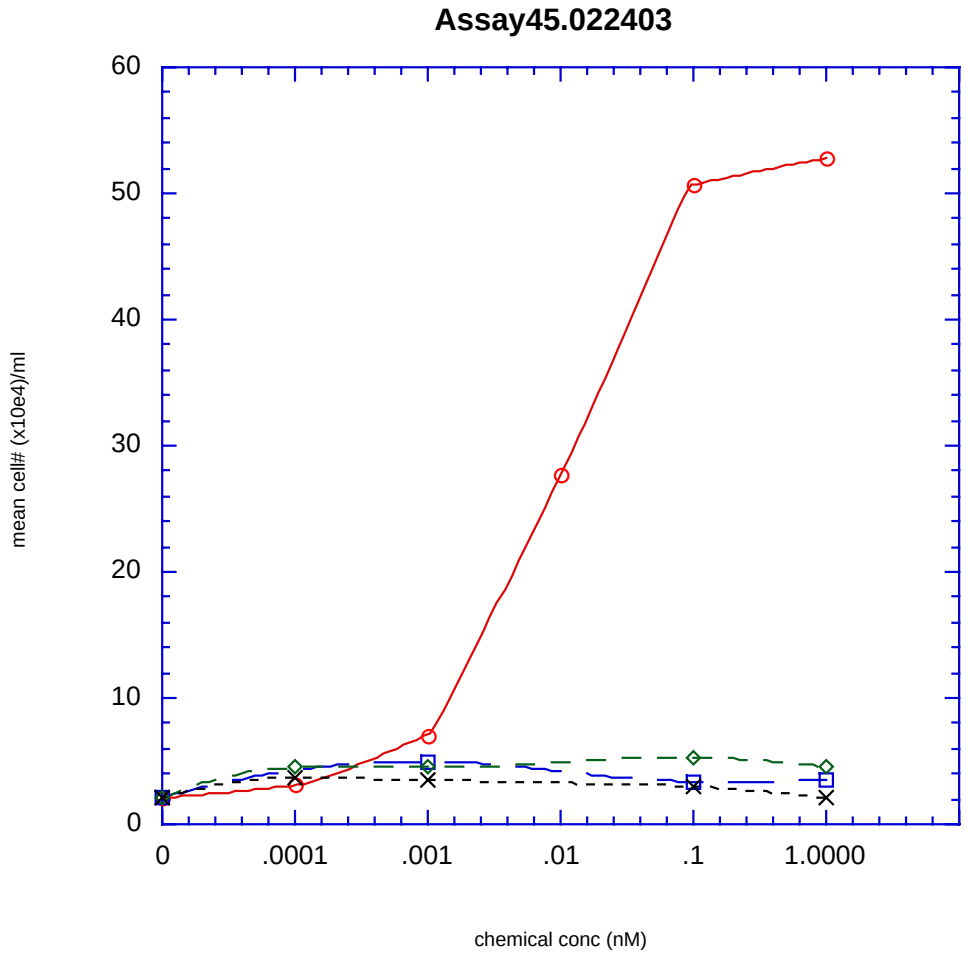

Fig. 3 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—○—)to cells exposed to stock #1 of 2,4-dichlorophenoxyacetic acid (2,4-D) [> one year old, maintained at -20°C] (—●—) to stock #2 [made day of testing] of 2,4-D
(—●—) or the Super BK32 formulation (---**x**---).

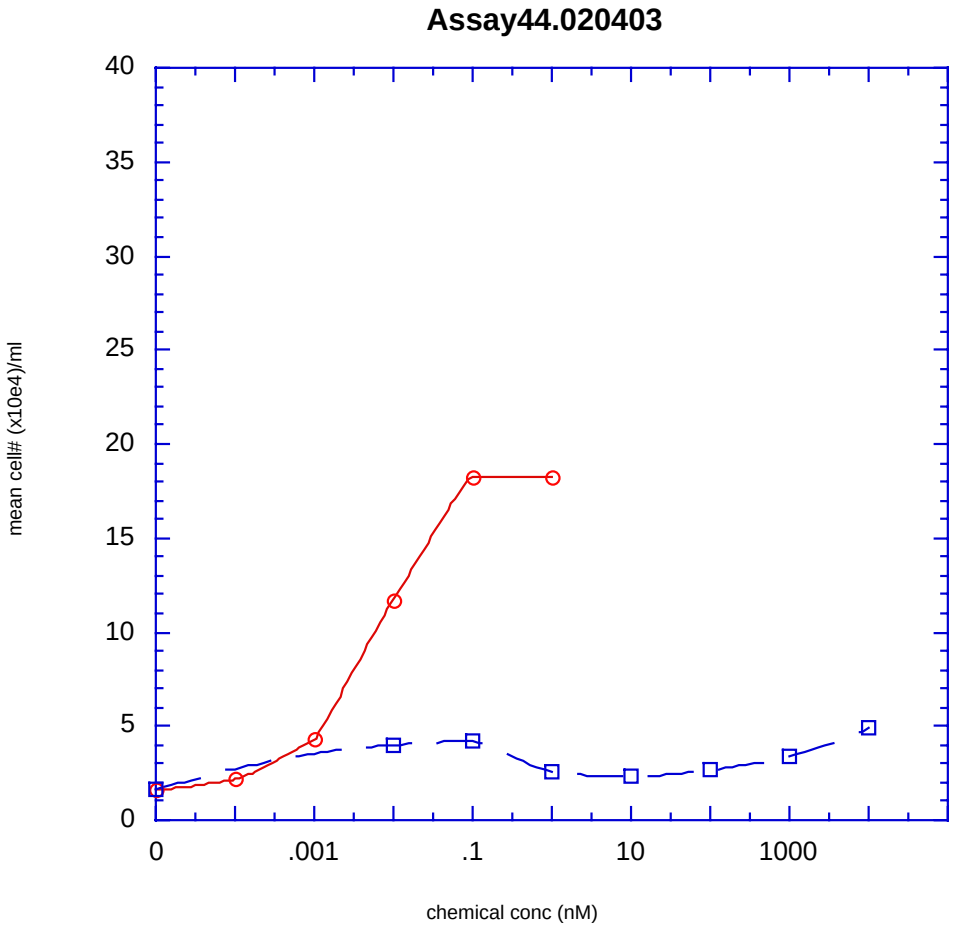

Fig. 4 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—○—) to cells exposed to a mixture of 2,4-D, Velpar and Orbit (—●—).

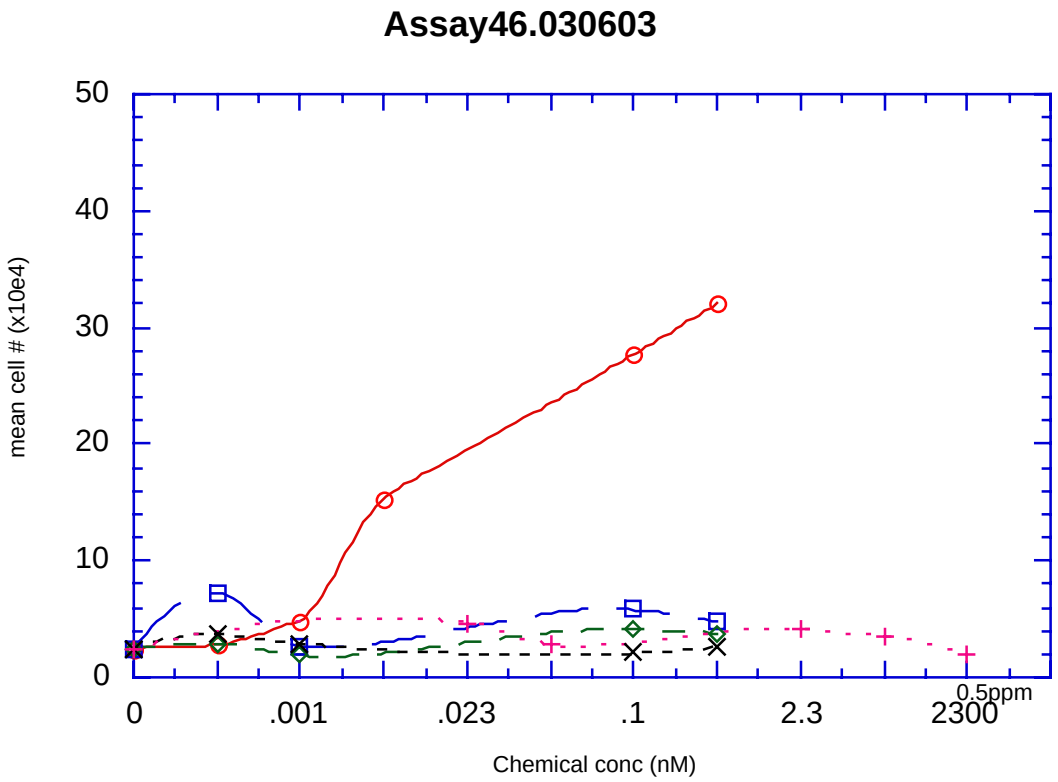

Fig. 5 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—o—) to cells exposed to Imidan (—•—), Orbit (—•—), Sinbar or (---X---), or a mixture of the three (---+---).

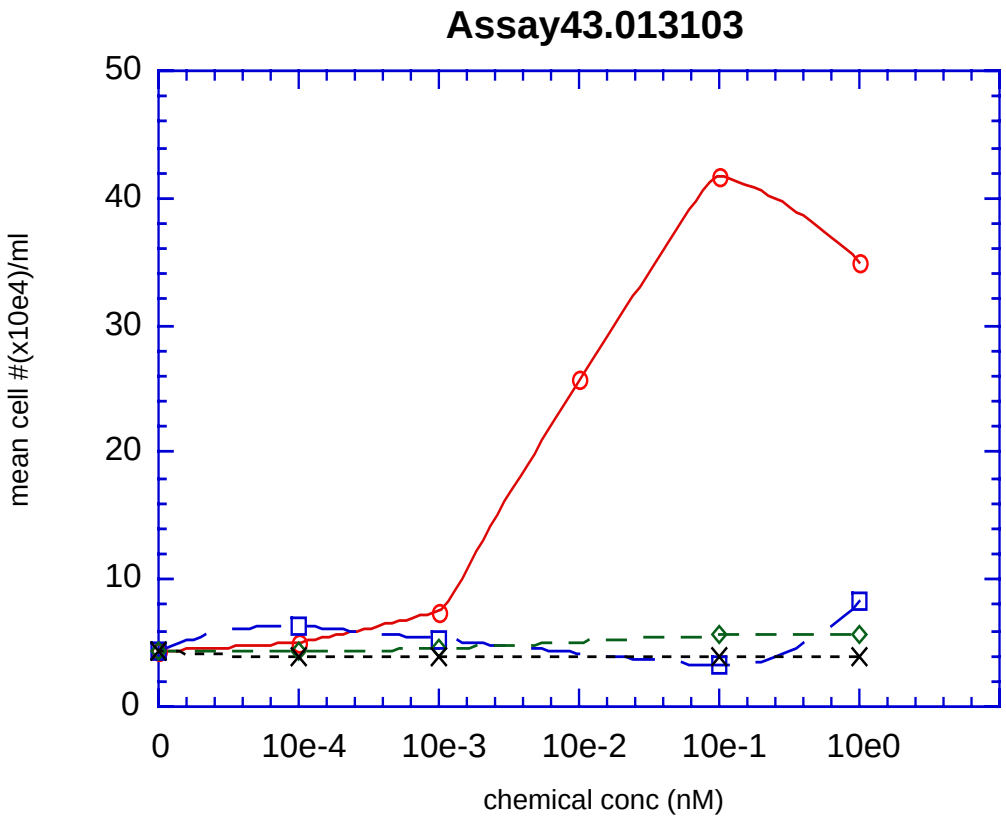

Fig. 6 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—o—) to cells exposed to 2,4- Dichlorophenoxyacetic acid (—●—), Velpar (—●—) or Orbit (---X---).

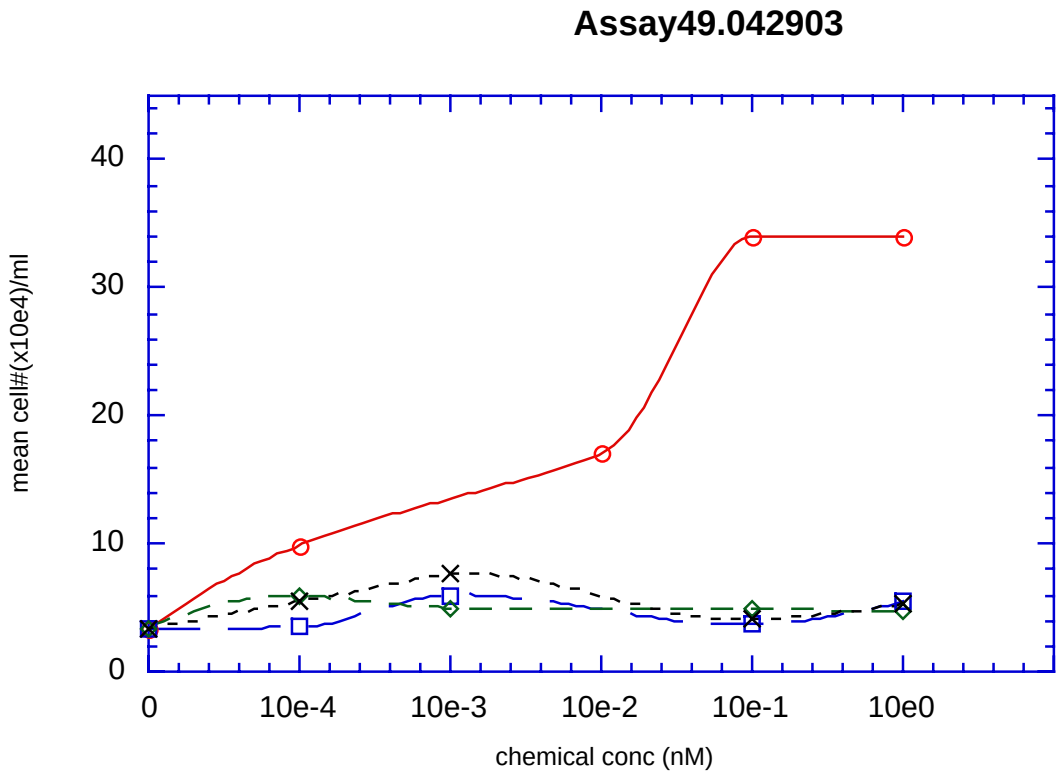

Fig. 7 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—○—) to cells exposed to Clethodim (—●—), Diazinon (—●—) or Diazinon 50W (---X---).

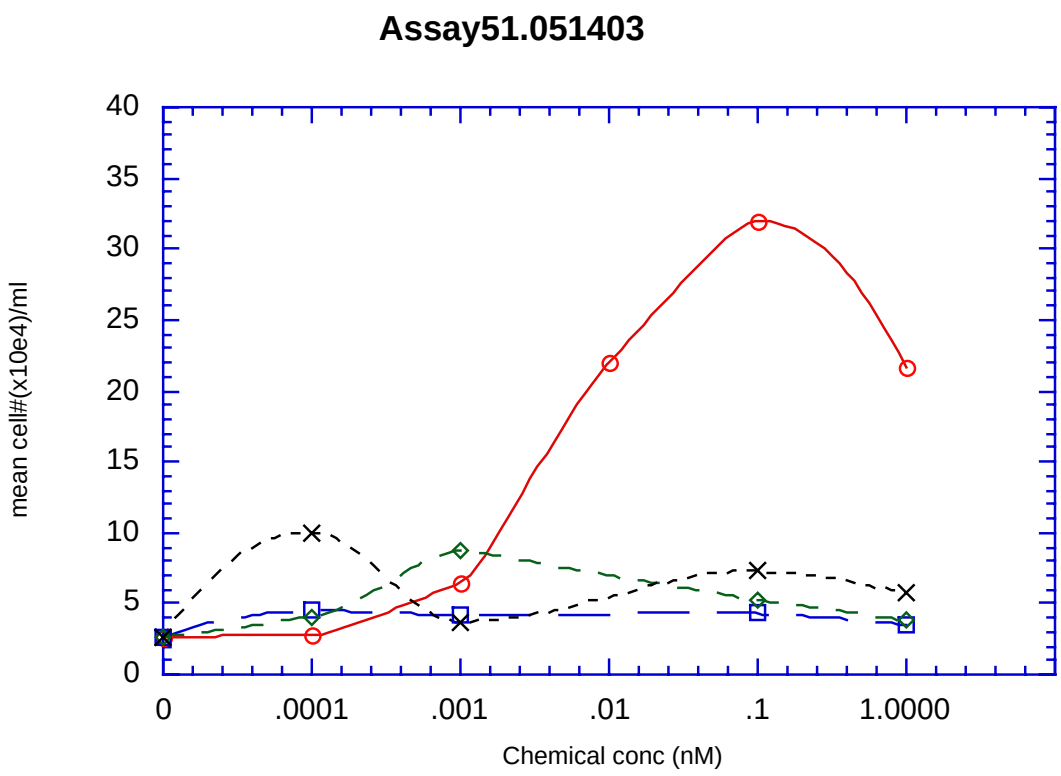

Fig. 8 E-SCREEN assay: Comparison of MCF-7 cell growth in media containing 17β-estradiol (—o—) to cells exposed to Fluazifop (—•—), Velpar (—•—) or Hexazinone (---X---).

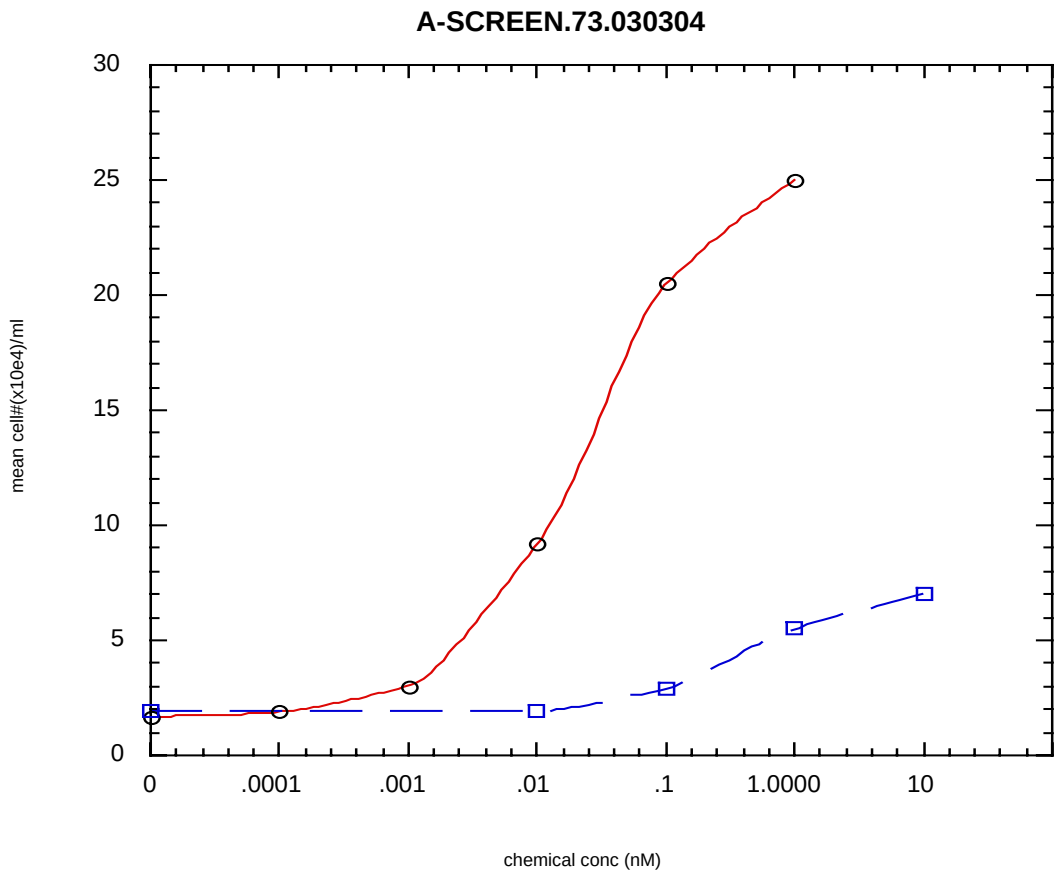

Fig. 9 A-SCREEN assay: Comparison of MCF7-AR1 cells grown with 17β-Estradiol (---o--) and with R1881 (--□--).

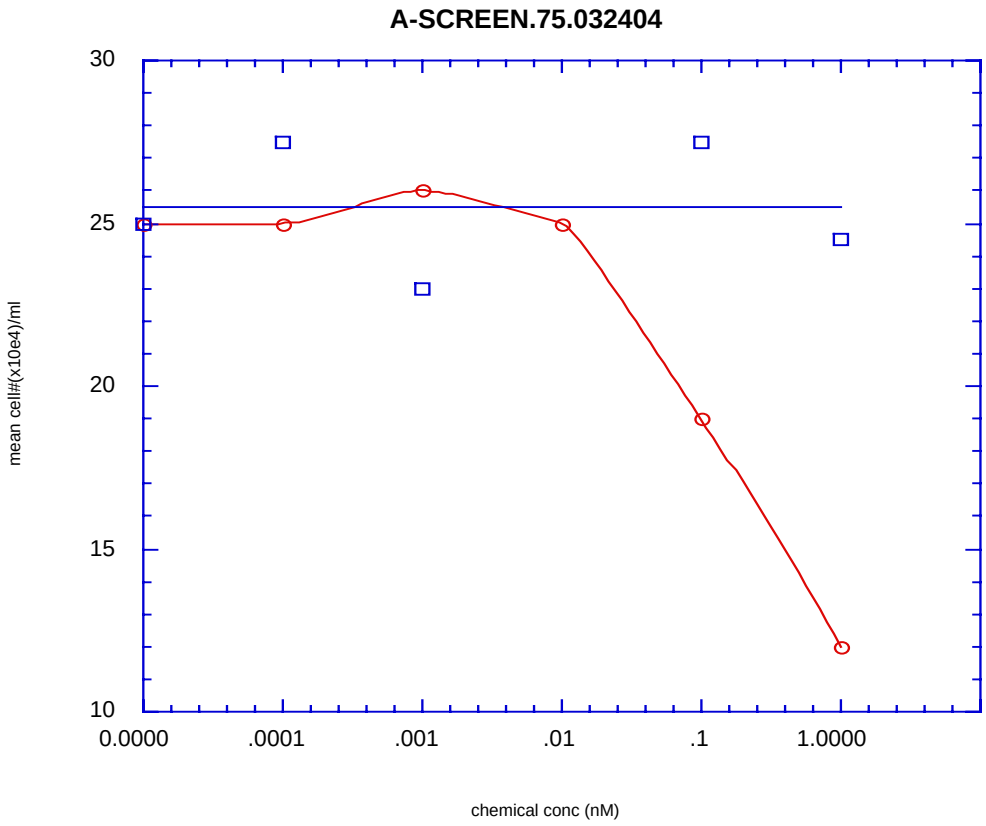

Fig. 10 A-SCREEN assay: Standard curve showing the decrease in proliferation when the cells are supplemented with 1nM 17β-Estradiol and dosed with the synthetic androgen R1881 (--o--). Hexazinone was non-androgenic (--□--).

4.3

EVALUATION OF BROMINATED ORGANIC COMPOUNDS IN THE PENOBSCOT WATERSHED, MAINE

EVALUATION OF BROMINATED ORGANIC COMPOUNDS IN THE PENOBSCOT WATERSHED, MAINE

Therese Anderson, EES Ph.D Candidate, University of Maine (advisor Dr. Jean MacRae)

Brominated Flame Retardants on the Penobscot River.

In recent years, concerns have been rising about the global presence of brominated flame retardants (BFRs) in all areas of the environment. In contrast to the declining levels of polychlorinated biphenyls (PCBs), dioxins and DDT in the environment, levels of BFRs have increased exponentially (1). These compounds are highly lipophilic and readily bioaccumulate in the food chain in a manner similar to dioxins and PCBs.

One class of BFRs is the Brominated Diphenylethers (BDPEs). Different degrees of bromination on the diphenyl ether backbone can result in 209 possible congeners, however only a limited number are actually formed due to the chemical directing properties of the ether group.They are commercially produced in mixtures, similar to the Aroclor mixes associated with PCBs. The mixes of concern are the Penta, Octa and Deca formulations. The State of Maine has recently banned the use of the Penta and Octa mixes. The Penta mix is comprised of two major congeners, BDE-47 and BDE-99. These account for over 70% of the total product by weight. BDE-100, BDE-153 and BDE-154 make up the majority of the remaining 30% of the mix. Trace amounts of BDE-17 and BDE-28 are also present. The Octa mix contains predominately BDE-183. BDE-153 and several additional octa and nona substituted BDE are found in minor amounts. The commercial Deca mix is 97% deca with the remainder being nona substituted BDEs. The Deca congener is more difficult to separate and analyze and was not specifically looked for in this study. Trace amounts were found in two wastewater samples.

While the toxicity of these compounds is currently being extensively studied, preliminary work has shown that the pentaBDE mixtures exhibit both dioxin-like Ah receptor mediation and competition with thyroid hormones (T3 andT4) for the transport protein, transthyretin, which could disrupt normal thyroid activity (2, 3). While these hormone effects appear to be lower than exhibited by coplanar PCBs, PBDEs background levels are correspondingly higher and are rising exponentially in North America (1, 4). Many textiles and foams treated with BFRs end up in the solid waste stream and are landfilled or incinerated along with other materials.

The predominant PBDE levels were examined in fish tissue procured for the SWAT/DMP project on the Penobscot River. Separate extractions were performed and the extracts cleaned to maximize the detection of these compounds. Wastewater and sludge samples from Orono Wastewater District were obtained and analyzed. Analysis was performed with low resolution mass spectrometry

instead of the high resolution technique outlined in the proposal because the instrument was not available. Also due to the increased costs associated with the low resolution method, the scale of the testing had to be reduced.

Fish samples from sites PBM, PBC, PBV and PBO were analyzed for predominate BFR congeners. Small mouth bass from PBM, PBC and PBM, white suckers from PBV and PBC and eels from PBO were sampled. Wastewater influent and effluent 24-hr composite samples and grab samples of activated sludge were obtained from the Orono wastewater treatment facility. Dewatered biosolids were also obtained and are in process at the time of this report. Results are presented in Table 1. (Concentrations range from noon-detect to 80 ppb in SMB fillets, wet weight, depending on the congener and from non-detect to 500 ppb in whole suckers, wet weight. Wastewater samples ranged from non-detect to 2 ppb. Fish data are in •g/Kg wet weight and wastewater samples are reported on a volume basis. Values lower than the stated detection limits are not reported. Table 2 reports the fish data in •g/g.

The results for the samples mirror the penta mix composition with BDE-47 and BDE-99 predominating. Totals for some of the congeners decrease as we move down the river but this does not account for all the BDEs found. Since all point sources have yet to be identified this type of analysis cannot be applied to this data set.

These data are consistent with values obtained in previous studies done in both the United States and Europe. Values obtained from the Great Lakes show concentrations for fillets ranging from non-detect to 80 ppb wet weight for congeners other then deca-BDE. Congeners BDE-47 and BDE-99 are the major peaks found after deca. Influent and effluent samples from the Netherlands show concentrations from non-detect to 10 ppb for BDE-47. (5, 6) A target dose for unlimited consumption based on EPA's reference dose for the most toxic mixture, PeBDE is 530 ug/kg. Future work includes looking at the fate of BDEs in sludge disposal and attempting to map the major potential point sources in the Penobscot watershed.

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Table 4.3 Poly-Brominated Diphenyl Ethers in Fish and Wastewater Treatment Plants

FISH

Congener	Detection limits	PBV-SMB -1	PBV-SMB -3	PBV-SMB -6	PBV-SMB -7	PBV-SMB -8
Initial weight/volume	grams	20.57	20.76	20.85	20.68	20.66
	µg/Kg	µg/Kg	µg/Kg	µg/Kg	µg/Kg	µg/Kg
TriDPE - 17	1.00					
TriDPE - 28	1.00					
TetraDPE - 47	1.00	63.7	47.7	44.1	35.3	18.4
TetraDPE - 71	0.50	1.46	2.89	3.84	2.90	1.45
PentaDPE - 100	0.50	16.5	16.9	6.24	4.84	9.68
PentaDPE - 99	0.50	80.2	62.1	23.5	13.1	43.6
PentaDPE - 85	0.50	0.49				
HexaDPE - 154	0.50	5.83	5.30	2.40	1.93	2.42
HexaDPE - 153	0.50	5.35	5.78	1.92	1.93	1.94
HexaDPE - 138	0.50					
HeptaDPE - 183	5.00					
HeptaDPE - 191	5.00					
DecaDPE - 209	25.00					
TOTAL		**173.6**	**140.7**	**82.0**	**60.0**	**77.4**

Congener	Detection limits	PBC-SMB -8	PBC-SMB -9	PBC-SMB -10	PBC-SMB -11	PBC-SMB -12
Initial weight/volume	grams	20.22	20.93	20.55	19.96	20.4
	µg/Kg	µg/Kg	µg/Kg	µg/Kg	µg/Kg	µg/Kg
TriDPE - 17	1.00					
TriDPE - 28	1.00					
TetraDPE - 47	1.00	48.0	72.1	10.2	20.5	3.92
TetraDPE - 71	0.50	1.98	4.30	0.97	1.00	
PentaDPE - 100	0.50	9.40	15.8	6.33	2.51	5.39
PentaDPE - 99	0.50	12.9	33.9	28.7	10.0	5.88
PentaDPE - 85	0.50					
HexaDPE - 154	0.50	5.93	10.5	2.43	6.51	0.98
HexaDPE - 153	0.50	1.48	2.39	2.92	4.01	
HexaDPE - 138	0.50	0.49	1.91		2.51	
HeptaDPE - 183	5.00					
HeptaDPE - 191	5.00					
DecaDPE - 209	25.00					
TOTAL		80.1	140.9	51.6	47.1	16.2

Table 4.3 Poly-Brominated Diphenyl Ethers in Fish and Wastewater Treatment Plants

FISH

Congener	Detection limits	PBC-WHS -C	PBV-WHS -C	PBM-SMB -7	PBM-SMB -9	PBM-SMB -10
Initial weight/volume	grams	19.99	19.76	20.06	20.17	20.2
	µg/Kg	µg/Kg	µg/Kg	µg/Kg	µg/Kg	µg/Kg
TriDPE - 17	1.00	1.50	4.55			
TriDPE - 28	1.00	35.5	64.3			
TetraDPE - 47	1.00	430	597	3.49	1.98	
TetraDPE - 71	0.50	4.00	4.55	2.99		
PentaDPE - 100	0.50	279	116	4.99	0.50	1.49
PentaDPE - 99	0.50	3.00	126	12.0	4.46	9.90
PentaDPE - 85	0.50		13.7			
HexaDPE - 154	0.50	60.0	22.3	1.00	0.50	1.98
HexaDPE - 153	0.50	21.5	10.1			1.49
HexaDPE - 138	0.50					3.96
HeptaDPE - 183	5.00					
HeptaDPE - 191	5.00					
DecaDPE - 209	25.00					
TOTAL		**834.9**	**959.0**	**24.4**	**7.4**	**18.8**

Congener	Detection limits	PBO-EEL -C1	PBO-EEL -C1	BLK 1 fish		BLK water
Initial weight/volume	grams	19.99	20.34	20.00		1.00
	µg/Kg	µg/Kg	µg/Kg	µg/Kg		µg/L
TriDPE - 17	1.00					
TriDPE - 28	1.00					
TetraDPE - 47	1.00	114	29.0	0.50		0.08
TetraDPE - 71	0.50	2.50	1.97			
PentaDPE - 100	0.50	58.5	33.4			
PentaDPE - 99	0.50	2.00	4.92			
PentaDPE - 85	0.50	39.5	18.7			
HexaDPE - 154	0.50	1.00	1.97			
HexaDPE - 153	0.50		0.98			
HexaDPE - 138	0.50					
HeptaDPE - 183	5.00					
HeptaDPE - 191	5.00					
DecaDPE - 209	25.00					
TOTAL		217.1	91.0	0.5		0.1

WATER

Congener	detection limits	effluent	effluent	Influent	influent	activated sludge	activated sludge
Initial weight/volume	liters	1.00	1.00	1.00	1.00	1.00	1.00
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
TriDPE - 17	0.10						
TriDPE - 28	0.10						
TetraDPE - 47	0.10	0.09		1.55	1.73	0.98	0.47
TetraDPE - 71	0.05			0.06	0.08	0.06	0.04
PentaDPE - 100	0.05			0.46	0.42	0.28	0.22
PentaDPE - 99	0.05		0.11	1.59	1.61	0.52	1.15
PentaDPE - 85	0.05					1.33	
HexaDPE - 154	0.05			0.13	0.10		0.10
HexaDPE - 153	0.05			0.12	0.09	0.33	0.14
HexaDPE - 138	0.05						
HeptaDPE - 183	0.50						
HeptaDPE - 191	0.50						
DecaDPE - 209	2.50	3.03			2.66		
TOTAL		**3.1**	**0.1**	**3.9**	**6.7**	**3.5**	**2.1**

4.4

DIOXIN INTERLAB COMPARISON

DIOXIN INTERLAB COMPARISON

This study was developed during discussion of the Dioxin Monitoring Program at the 2002 SWAT TAG meeting on June 14, 2002. Because the intial splits of 12 fish from the Androscoggin River at Rumford and Lisbon showed descrepancies of an order of magnitude between Midwest Research Institute (MRI) and the University of Maine's Environmental Chemistry Lab (ECL), DEP had queried both labs and the USF&WS lab in Columbia Mo about reasons. MRI used a new automated FMS cleanup and a confirmation column for furans that ECL did not. When ECL reran some of the samples on the confirmation column, furan levels were closer, but still higher than those from MRI. In the discussion it also became known that the samples for the two labs were handled differently. Those that went to MRI had been frozen and thawed at least once more than those used by ECL.

In this study, 10 samples of suckers (5 whole and 5 fileted) were handled the same way and analyzed by ECL and Alta Analytical Perspectives using similar methods. Samples were run with and without the confirmation column to see if there are any differences. There were 2 blind duplicates. The results were to shared with the TAG and then a decision made about use of confirmation column for 2002 samples.

The results showed very good correspondence between the two labs. All samples were within the 30% relative percent difference (RPD) goal and the average RPD was low and random for TCDD. For TCDF the average RPD was higher and positively biased at ECL. The data were validated by an outside reviewer, Joe Palusky, formerly dioxin analyst of Midwest Research Institute. Following is an except from the validated report:

"Window defining and isomer specificity requirements
Resolution criteria for 2378TCDD was met, a valley of 25% or less was demonstrated between 2378 TCDD and the non-toxic isomers. An isomer specificity solution for 2378 TCDF was analyzed for this batch of samples; there is a demonstration of baseline separation between 2378 TCDF and its closest eluter. Based on available literature for the DB-5ms column, no confirmatory column is required, as there is adequate separation between the toxic tetra PCDD/PCDF and their non-toxic isomers."

SUMMARY split sample analysis of sucker samples by UM Environmental Chemistry Lab (ECL) and Alta Analytical Perspectives (AAP)

SAMPLE	TCDD ECL	TCDD AAP	TCDD % RPD	TCDF ECL	TCDF AAP	TCDF % RPD
	May-03	Aug-02				
WHS01	0.249	0.296	-17.2	8.25	8.25	0.0
WHS02	0.148	0.192	-25.9	4.80	4.53	5.8
WHS03	0.145	0.151	-4.1	4.59	3.88	16.8
WHS04	0.121	0.121	0.0	3.35	2.53	27.9
WHS05	0.13	0.163	-22.5	4.2	4.20	0.0
WHS06	0.213	0.165	25.4	7.05	4.61	41.9
WHS07	0.289	0.200	36.4	8.32	5.11	47.8
WHS08	0.162	0.170	-4.8	5.96	4.93	18.9
MEAN	**0.182**	**0.182**	**-1.6**	**5.82**	**4.76**	**19.9**
STDEV	0.061	0.052	22.2	1.89	1.62	18.2
Ftest p (homogeniety of variance)			0.68			0.70
Lillefors p (normality)	0.144	0.203		0.488	0.049	
Mann Whitney p			**0.563**			**0.293**
t-test p			1.00			

4.5

DATABASE DEVELOPMENT

DATABASE DEVELOPMENT - DEP

All of the SWAT data and dioxin data are in spreadsheets by year and by contaminant. This makes it difficult for others to efficiently analyze the data in various ways. There is currently no easy way to download data for use in evaluating time trends, comparing data sets from location to location, comparing across species, or easily comparing various parameters (e.g., length, weight, percent lipid, contaminant concentration). This severely limits the value of the data.

The Department has begun development of a comprehensive database to house all surface water quality data including the SWAT and Dioxin data. The project will be comprised of the following 4 phases:

Phase I Business Analysis
Phase II Systems Analysis will begin in winter and last 4-6 months.
Phase III System Design or Purchase depends on recommendations from Phase II.
Phase IV System Install and Testing

Phase I is nearing completion and Phase II will begin soon.

4.6

PCB METHODS COMPARISON STUDY

PCB METHODS COMPARISON STUDY

PCBs are a class of 209 compounds that were sold as proprietary mixtures. Unfortunately, as those mixtures biodegrade and bioaccumulate, the relative concentrations of the individual congeners change. For the purposes of advisories, the Bureau of Health (BOH) is interested in the total amount of PCBs that someone is potentially exposed to. Additionally, the BOH also evaluates congener profiles – both for an evaluation of the consistency of the data, as well as for fingerprint analysis. Historically, the University of Maine Environmental Chemistry Lab (ECL) has provided the data based on chemical classes (homologue analysis), which, is an effective measure of total PCBs. Additionally, approximately 20 congeners were provided and used for both some congener analysis and for fingerprinting. In part, homologue analysis was chosen as a cost effective as well as accurate way of measuring total PCBs. However, the new managers at the lab suggest that the cost difference between congener analysis and homologue analysis has decreased. Additionally, they recommend congener analysis providing more detailed congener data as well as a more informative measure of total PCBs. The BOH and DEP have agreed and plan to switch to congener specific methods. To calibrate our thinking about past homologue data, we propose to analyze several samples using both methods to directly compare.

Specifically, we analyzed fish from 6 locations using both the congener method and the homologue method. At each location there were 5 individual fish analyzed for a total of 30 samples. Our objective was to analyze fish from a range of concentrations and characteristics. For example, we chose some fish with high levels of contaminants compared, as well as fish with lower levels of contaminants. We used 2002 samples that have not yet been analyzed.

The samples were analyzed by Texas A & M University's Geological and Enrvironmental Research Group (GERG) using GERG method 2005 for all 2009 congeners and EPA method 680 for homologue groups. The results showed that both methods gave similar results (Table 4.6). Average relative percent difference was within the acceptable range (30%) and neither method had a dominant bias. The homologue method was less expensive ($400 per sample) compared to the congener specific method ($500). The congener specific method provides more information and is the choice for many new investigations.

Figure 4.6 TOTAL PCBS IN FISH BY TWO METHODS

Client Sample ID	Total PCBs (ng/g) dw		RPD	Higher	% solid	Total PCBs (ng/g) ww	
	EPA 680	GERG 0205		Total		EPA 680	GERG 0205
ARB-STB-01	1285.35	906.0	34.6	EPA 680	25.4	326.5	230.2
ARB-STB-02	1144.12	911.7	22.6	EPA 680	22.9	261.8	208.6
ARB-STB-03	1594.43	1281.9	21.7	EPA 680	25.9	413.8	332.7
ARB-STB-04	1025.74	880.3	15.3	EPA 680	24.1	247.1	212.0
ARB-STB-05	1390.25	1240.7	11.4	EPA 680	24.5	340.0	303.4
KSD-STB-01	585.38	541.3	7.8	EPA 680	23.6	138.2	127.8
KSD-STB-02	220.11	165.8	28.2	EPA 680	21.5	47.3	35.6
KSD-STB-03	443.28	453.9	2.4	GERG 0205	24.3	107.6	110.1
KSD-STB-04	478.93	542.0	12.4	GERG 0205	24.2	115.9	131.2
KSD-STB-05	268.82	256.6	4.7	EPA 680	22.6	60.7	57.9
KAG-SMB-01	616.81	811.1	27.2	GERG 0205	22.1	136.4	179.4
KAG-SMB-02	392.49	502.5	24.6	GERG 0205	22.6	88.6	113.5
KAG-SMB-03	506.53	620.7	20.2	GERG 0205	22.4	113.6	139.2
KAG-SMB-04	491.71	531.0	7.7	GERG 0205	21.1	103.8	112.1
KAG-SMB-05	246.31	281.7	13.4	GERG 0205	23.0	56.7	64.9
SFB-SMB-01	1133.63	1496.2	27.6	GERG 0205	20.1	227.4	300.1
SFB-SMB-02	349.25	351.3	0.6	GERG 0205	22.5	78.6	79.1
SFB-SMB-03	379.34	357.4	6.0	EPA 680	21.5	81.6	76.9
SFB-SMB-04	283.06	291.0	2.8	GERG 0205	20.4	57.8	59.4
SFB-SMB-05	321.87	364.0	12.3	GERG 0205	19.7	63.6	71.9
ALV-SMB-04	54.68	29.4	60.1	EPA 680	19.5	10.7	5.7
ALV-SMB-05	88.45	98.6	10.8	GERG 0205	23.1	20.4	22.8
ALV-SMB-07	73.37	62.8	15.5	EPA 680	20.9	15.3	13.1
ALV-SMB-09	82.93	70.3	16.5	EPA 680	21.4	17.8	15.0
ALV-SMB-10	133.91	146.6	9.1	GERG 0205	22.4	30.0	32.9
KFF-BNT-01	56.30	41.3	30.8	EPA 680	27.7	15.6	11.4
KFF-BNT-02	46.21	38.7	17.6	EPA 680	27.0	12.5	10.5
KFF-BNT-03	39.47	34.3	14.1	EPA 680	26.9	10.6	9.2
KFF-BNT-04	33.04	26.5	21.9	EPA 680	27.5	9.1	7.3
KFF-BNT-05	28.20	25.6	9.6	EPA 680	28.5	8.0	7.3
NIST 2978	850.59	659.1	25.4	EPA 680			
NIST 2978	480.08	662.4	31.9	GERG 0205			
		Average RPD	17.7				