ACTIVATION OF SIGNALLING PATHWAYS ASSOCIATED WITH ENDOCRINE DISRUPTION BY GAS- AND PARTICLE-PHASE AMBIENT AIR CONTAMINANTS

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Introduction

Poor ambient air quality has been linked to a variety of adverse health effects[1,2]. The chemical drivers of health impacts due to ambient air are unknown. Research into the health effects of organic air contaminants has emphasized the particle-phase of ambient air. A large body of evidence detailing mechanistic models and epidemiological associations of diesel exhaust particles (DEP) and diesel particulate matter (DPM) with human health exists. However, in reality, people are exposed to the highly complex mixture of both particle- and gas-phase organic contaminants in ambient air. Common approaches to predict the biological activity of a complex mixture, such as toxic equivalency factors, are of limited utility, partly because they do not account for interactions such as weak agonism at the cellular level[3].

Our study evaluated the relationship between the chemical composition and biological activity of ambient air contaminants by examining the interaction of both the gas- and particle-phases of ambient air with the estrogen receptor (ER) and aryl hydrocarbon receptor (AhR), both of which are implicated in endocrine disruption. The air samples were a comparative survey of ambient air along an urban to rural gradient, between the gas- and particle-phases, and between seasons.

Methods and Materials

Air samples were collected along an urban to rural transect between downtown Toronto and Egbert, Ontario, 75 km northeast of Toronto. High volume air samplers were used to collect particle- and gas-phase samples of ambient air. Details of the extraction and clean-up of these air samples is provided elsewhere[4,5]. PAH were quantified by capillary column gas chromatography/ low resolution mass spectrometry (GC-MS). PCDD/Fs were quantified by high resolution GC/MS in electron impact and selected ion monitoring modes. PCBs were quantified by GC with electron capture detection (ECD). Concentrations were not corrected for blanks as analyte concentrations in both method and field blanks were typically below 5% of those in samples.

Reporter Cell Lines. The AhR reporter cell line, H1L6.1c1, a murine hepatoma cell line[6], and the ER reporter cell line, BG1Luc4E2, a human ovarian carcinoma cell line[7], were kindly provided by M.S. Denison (UC Davis, California). Both are stably transfected cell lines in which luciferase expression is regulated by the AhR (H1L6.1c1 cells) or ER (BG1Luc4E2). Both cell lines were maintained as described elsewhere[8].

Treatment of H1L6.1c1 Cells. Cells were plated at a density of $6x10^4$ cells/well in 12-well plates. After 24 hours, cells were treated with growth medium (media control), growth medium plus 0.1% DMSO (solvent control), reference agonist (10^{-7} M βNF) and with various dilutions of the air samples. After a further 24 hours the cells were lysed and luciferase activity assessed (Promega luciferase assay kit). Luciferase activity was expressed as relative light units per mg cell lysate protein (8). Curve-fitting and calculation of EC_{50}s were done using GraphPad Prism software (Version 3.0, GraphPad Software, San Diego, CA).

Treatment of BG1Luc4E2 Cells. BG-1 cells were plated at a density of $6x10^4$ cells/well in 12-well plates. After 24 hours, the medium was changed to estrogen-reduced medium to minimise the basal expression of luciferase. After 48 hours, cells were treated with E2-reduced medium alone (media control), E2-reduced media plus 0.1% DMSO (solvent control), reference agonist (10^{-10} M E2) and various dilutions of the air samples. At 72 hours, the cells were harvested and luciferase activity assessed as described for the AhR reporter cells.

Results and Discussion

Chemical Analysis. Table 1 presents total PAH, N-PAH and PCB concentrations and the TEQ of each sample. PAH was the most abundant chemical class of those analysed, with concentrations typically an order of magnitude greater than those of PCBs and N-PAH, and two orders of magnitude greater than dioxins and furans. The PAH data were analysed by principal components analysis. Samples clustered as follows (results not shown): urban particle-phase samples from both summer and winter, rural and urban gas-phase samples, and rural particle-phase samples.

Activation of the AhR. The extracts of all samples, except extracts of fieldblanks, caused expression of luciferase in a concentration-dependent manner. The rank order of potency of samples, indicated by EC_{50}s, is presented in Table 1 below, along with total concentrations of PAH and N-PAH and TEQ (calculated on the basis of dioxin and furan concentrations alone). In general, extracts of urban particle-phase samples were more potent than gas-phase samples. The relatively high potency of the gas-phase March 2000 sample is difficult to explain, given that particle-phase PAH are more potent agonists of the AhR than gas-phase PAH[9], and the relatively low TEQ of that sample. The lack of correspondence between TEQs and EC_{50}s is illustrated in Fig. 1. The analysis by principal components suggested that the composition of the gas-phase March 2000 sample is similar to that of all other gas-phase samples (results not shown).

Table 1: Chemical Composition and Rank Order of Potency in AhR Reporter Cells

Samples in Descending Rank Order of Potency	**EC_{50},** m^3 air/well	**TEQ** *PCDD/Fs only*	**Σ PAH, ng/m^3**	**Σ N-PAH, pg/m^3**	**Σ PCB pg/m^3**
urban PUF March 2000	0.011	2.6	16	40	n.a.*
urban filter March 2000	0.063	26	3.6	65	n.a.
urban filter July 2000	0.13	5.8	0.80	31	1.4
urban filter March 2001	0.20	13	1.9	54	76
urban PUF July 2000	0.39	13	19	106	240
rural filter July 2000	0.68	0.73	0.082	4.3	n.d.*[1]
rural PUF July 2000	0.71	4.9	1.7	21	18
urban PUF March 2001	2.4	1.5	10	15	1000

* not available *[1] non-detect

Activation of the ER. Extracts of all samples activated the ER in a concentration-dependent fashion. Comparisons between samples are expressed as the concentration causing 50% of maximum activity in the cell line due to 17-β-estradiol, and are given in Table 2 along with total PAH, total PCB, and 2,3,7,8-TCDF concentrations. Compared to the AhR, fewer and weaker known agonists of the ER have been identified in ambient air. Both the concentrations of total PAH and those of 2,3,7,8-TCDF in the air extracts are moderately linearly correlated with 50% E2 activity concentrations (r=-0.79 and r=-0.77 respectively). PAHs are weak ER agonists that have been shown to act additively in other *in vitro* assays[10], but 2,3,7,8-TCDF is not known to be an ER

agonist. There was no significant correlation between 50% E2 activity concentrations and scores on the first two principal components for PAH. Surprisingly, there were no obvious trends in potency in the ER cell line due to either the phase, location or season sampled (Fig. 2).

Table 2: Chemical Composition and Rank Order of Potency in ER Reporter Cells

Samples in Descending Rank Order of Potency	50% E2 Activity Concentrations, m^3 air/well	Σ PAH, ng/m^3	2,3,7,8-TCDF, fg/m^3	Σ PCB, pg/m^3
urban PUF July 2000	2.5	19	38	240
urban PUF March 2000	4.0	16	25	–
rural filter July 2000	9.9	0.082	n.d.	n.d.
urban filter March 2001	13	1.9	15	76
urban filter March 2000	13	3.6	18	–
rural PUF July 2000	13	1.7	10	18
urban PUF March 2001	15	10	10	1000
urban filter July 2000	18	0.80	4	1.4

This study confirms that urban samples of ambient air tend to be more potent activators of the AhR than rural samples. However, TEQs calculated on the basis of dioxins and furans alone were not able to predict the potency of AhR activity caused by ambient air contaminants, suggesting that AhR activation due to complex mixtures may not be primarily controlled by any one class of contaminants. To our knowledge, this is the first study that has examined an urban-rural transect for activation of the ER. Gas-phase extracts were as capable as particle-phase extracts of activating both the AhR and ER. Total PAH and 2,3,7,8-TCDF concentrations were moderately correlated with ER activity, although neither are strong ER agonists and PAH, compared to organochlorine pesticides, have received relatively less scientific scrutiny as xeno-estrogens.

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Figure 1: EC_{50}s in AhR reporter cell line and TEQs of ambient air samples

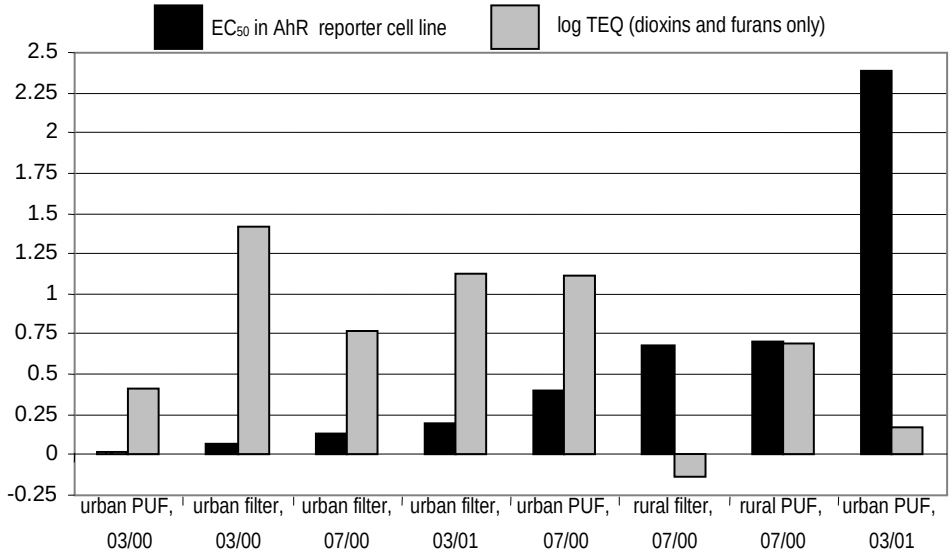

Figure 2: 50% E2 Equivalents and ΣPAH concentration, ng/m^3