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The Mutagenic Hazards of Polycyclic Aromatic Hydrocarbons (PAHs) in Settled House Dust

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The Mutagenic Hazards of Polycyclic Aromatic Hydrocarbons (PAHs) in Settled House Dust

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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Abstract

Settled house dust (SHD) may be a significant source of children's indoor exposure to hazardous substances including polycyclic aromatic hydrocarbons (PAHs). In this study, the extent to which mutagenic PAHs are present in SHD was examined, and the overall mutagenic hazards of SHD were evaluated using the *Salmonella* Mutagenicity Test. The results indicate a predominance of frameshift mutagens in the SHD, and a significant positive correlation was identified between mutagenicity and PAH concentration. Mutagenicity was empirically related to several household attributes including vacuuming habits and the number of inhabitants in the home. There is some indication that PAH concentration in SHD is related to an urban home location and the presence of smokers, but the effects are weak. A risk assessment to evaluate the excess lifetime cancer risks posed by preschoolers' non-dietary ingestion of PAHs in SHD revealed that such exposures may result in risks that exceed 1×10^{-5}.

Résumé

Chez les enfants, la poussière domestique accumulée en surface (PDAS) peut être une source importante d'exposition d'intérieur à des substances dangereuses comme les hydrocarbures aromatiques polycycliques (HAPs). Dans cette étude, la présence d'HAPs mutagéniques dans la PDAS a été quantifiée, et les dangers mutagéniques de la PDAS ont été évalués en utilisant l'essai de mutagénicité Salmonella. Les résultats indiquent que dans la PDAS, il y a une prédominance d'agents mutagènes qui provoquent des décalages du cadre de lecture, et une corrélation positive significative entre la mutagénicité et la concentration d'HAPs dans la poussière. La mutagénicité a été empiriquement liée à plusieurs attributs ménagers, incluant l'habitude de passer l'aspirateur au foyer et le nombre d'habitants dans la maison. Les résultats suggèrent que la concentration d'HAPs dans la PDAS est liée à l'emplacement des foyers urbains et à la présence de fumeurs dans ces foyers, mais ces effets sont faibles. Une évaluation des risques de cancer à vie résultant de l'ingestion non-diététique d'HAPs provenant de la PDAS chez les enfants d'âge préscolaire indique que de telles expositions peuvent avoir comme conséquence des risques qui excèdent 1×10^{-5}.

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List of Abbreviations

ACNP	acenaphthene
ACNPHY	acenaphthylene
ANOVA	analysis of variance
ANTH	anthracene
ASTM	American Society for Testing and Materials
BaA	benzo[*a*]anthracene
BaP	benzo[*a*]pyrene
BbkF	benzo[*b,k*]fluoranthene
BeP	benzo[*e*]pyrene
BghiP	benzo[*g,h,i*]perylene
°C	temperature in degree Celsius
CHRY	chrysene
cNR	classical nitroreductase
CORO	coronene
CPcdP	cyclopenta[*c,d*]pyrene
DA	dissemination area
DBahA	dibenz[*a,h*]anthracene
DCM	dichloromethane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
FLUORAN	fluoranthene
FLUOR	fluorene
HAA	heterocyclic aromatic amine
HCl	hydrochloric acid
HMW	high molecular weight
HVS3	high volume small surface sampler
I123cdP	indeno[1,2,3-*c,d*]pyrene
IARC	International Agency for Research on Cancer
K_{ow}	octanol-water partition coefficient

LMW	low molecular weight
M	molarity
MDL	method detection limit
NaOH	sodium hydroxide
NAPH	naphthalene
OAT	O-acetyltransferase
Pa	pressure measured in pascals
PAH	polycyclic aromatic hydrocarbon
PHEN	phenanthrene
PhIP	2-amino-1-methyl-6- phenylimidazo[4,5-b]-pyridine
PYR	pyrene
rev	revertants
rfa	deep rough mutation
SEM	standard error of the arithmetic mean
SHD	settled house dust
µg	microgram
µl	microlitre
USEPA	United States Environmental Protection Agency

General Introduction

Contaminants in the indoor environment

Much attention has been placed on researching, monitoring and regulating air pollution in the outdoor environment. As a result, there exists a general misconception that air pollution by chemical contaminants is an outdoor phenomenon. In reality, numerous studies have noted that indoor air can be many times more contaminated than outdoor air (USEPA 1987). Moreover, people spend the majority of their time indoors. For example, Canadians spend as much as 70% of their time at home and up to 90% of their time indoors (Health Canada 1989). These percentages are easily exceeded for mothers, children, the elderly, and the infirm. As a result, the health risks posed by contaminants in the indoor environment are of significant concern, and the potential hazards of indoor pollutants are now being more widely acknowledged. For instance, organizations such as the United States Environmental Protection Agency (USEPA) have now ranked indoor pollution as a high priority risk to human health (USEPA 1993).

Pollutants in the indoor environment can include radiation (e.g., radon gas), biological contaminants (e.g., bacteria, molds, viruses, dust mites), chemical contaminants (e.g., pesticides, metals, flame retardants, plasticizers), combustion products (e.g., environmental tobacco smoke, carbon monoxide, nitrogen dioxide), and others (Cooke 1991). Many of these contaminants adsorb to particulate matter suspended in indoor air that later settles out as house dust. Research investigating human exposures to priority pollutants have suggested that settled house dust (SHD) may be a significant source for indoor exposures (Roberts et al. 1992).

Exposure to these pollutants in the indoor environment has been associated with numerous adverse health effects including allergenic and immune system effects, respiratory effects, cardiovascular and nervous system effects, irritative effects of the skin and mucous membranes, cancer, and reproductive effects (Maroni et al. 1995).

Exposure to dust and its associated contaminant load may be of particular concern for children who tend to play or crawl on the floor and place objects in their mouths that have been in intimate contact with dusty floors or carpets (Lewis et al. 1994). Children may have also higher susceptibilities to the adverse effects of exposure to dust-contaminant mixtures owing to the immature state of their organs, nervous system and immune system (IPCS 1986).

Composition of Settled House Dust

The USEPA defines house dust as "a complex mixture of biologically-derived material (animal dander, fungal spores etc.), particulate matter deposited from the indoor aerosols, and soil particles brought in by foot traffic... The indoor abundance depends on the interplay of deposition from the airborne state, re-suspension due to activities, direct accumulation and infiltration" (USEPA 1997). The precise composition of a house dust sample is a function of numerous factors including environmental and seasonal factors, ventilation and air filtration, homeowner activities, and indoor and outdoor source activities. The penetration of outdoor particles into the indoor environment has been shown to be a significant source of indoor particles (Abt et al. 2000; Morawska et al. 2001; McKone et al. 2002; Riley et al. 2002). In the outdoor environment, natural sources of dust particles include pollen, soil, forest fire emissions and volcanic debris. Anthropogenic sources of outdoor particles include fossil fuel combustion (e.g., coal, oil), wood combustion, waste incineration, and a variety of industrial processes (e.g., iron founding, construction). In the indoor environment, dust sources include skin, hair, mites, fibres from clothing and furnishings, cooking emissions, heating emissions and cigarette smoke (Morawska and Salthammer 2003). This variety of indoor and outdoor sources yields a complex matrix that can be extremely heterogeneous in nature with temporal and spatial variability in particle size, particle shape, particle composition, and contaminant concentration. Consequently, the composition of SHD can differ considerably between rooms of a given house, as well as between houses, and among geographic locations in a study area (Lioy et al. 2002).

Most dust particles range from micrometers to millimetres in size and are generally classified as either fine or coarse particles. Although no standard exists, a common practice is to define fine particles as those less than 2.5 μm, while coarse particles are those greater than 2.5 μm (Morawska and Salthammer 2003). Dust particle size is of particular importance as it influences the deposition and re-suspension of dust in the indoor environment. Particles greater than 30 μm tend to fall and form SHD (Morawska and Salthammer 2003), while particles less than 30 μm tend to remain airborne and only constitute approximately 10% of SHD (Que Hee et al. 1985; Lewis et al. 1999; Molhave et al. 2000). The settling and re-suspension of dust is readily influenced by air flow patterns and activities taking place in the sampling area (Thatcher and Layton 1995; Ferro et al. 2004).

The physical-chemical characteristics and composition of house dust plays an important role in determining the types of contaminants that are associated with dust particles. The adsorption and adherence of chemical contaminants to particulate material depends on the type and size of the particles as well as the surface texture, polarity and lipophilicity (Butte and Heinzow 2002). Studies have revealed the presence of many chemical contaminants adsorbed to dust particles including: pesticides, smoke residues, PAHs, PCBs, flame retardants, plasticizers, heavy metals and asbestos (Butte and Heinzow 2002; Lioy et al. 2002; Greenpeace 2003; Rudel et al. 2003). Equilibrium concentrations on dust particles generally far exceed those in the gaseous portion of indoor air (Roberts and Dickey 1995), thus dust and its associated fine particulate matter tends to become a sink for semi-volatile organic compounds (Butte and Heinzow 2002). Furthermore, these compounds have the potential to persist and accumulate in indoor dust, as they are not subjected to the same degradation processes that occur outdoors. Indoors, compounds associated with dust particles are protected from sunlight, fluctuations in temperature and humidity, high rates of microbial degradation, and the overall effects of weathering (Paustenbach et al. 1997).

Some of the general characteristics of SHD are presented in Table 1. It should be noted however, that due to the complex nature of SHD and the numerous factors that influence its composition, actual values for specific dust characteristics (i.e. deposition rate, particle size distribution and loss on ignition) might vary considerably from the values shown in Table 1. A detailed overview of the sources and properties of SHD is provided by Morawska and Salthammer 2003.

Collection of Settled House Dust Samples

Researchers investigating dust contamination have devised a number of passive and active dust sampling techniques. Passive techniques may involve setting out stationary "dust fall" jars or non-electrostatic plates and simply letting dust accumulate for a given period of time. Active sampling techniques can include: surface wiping, press sampling, sweeping, or vacuuming. Each of these methods has been devised to measure specific parameters such as the total dust loading or dust available for dermal adsorption. No one sampling method can collect

dust equally well from all surfaces, and the optimal collection method will depend on the surface to be sampled and the goal of the study. A comprehensive review of the various sampling techniques is provided by Lioy et al. 2002.

Table 1. General characteristics of settled house dust (SHD).

Characteristic	**Typical Values**		**Reference**
Loading	0.6 - 1.3 g m^{-2}		(Roberts et al. 1991; Thatcher and Layton 1995; Roberts et al. 1999)
Deposition rate	0.0022 - 0.08 g m^{-2} d^{-1}		(Hawley 1985; Edwards et al. 1998; Seifert et al. 2000)
Particle size distribution	>125 μm	(40 %)	(Molhave et al. 2000)
	50-125 μm	(41 %)	
	25-50 μm	(18.3 %)	
	<10 μm	(0.6 %)	
	63 μm - 2 mm	(37.2 %)	(Salthammer 2003)
	<63 μm	(23.1 %)	
Loss on ignition[a]	63 μm - 2mm	(38.6%)	(Salthammer 2003)
	<63 μm	(58.6%)	

[a]A measure of organic matte content.

In an effort to obtain the most reliable information with the highest possible reproducibility, two standard methods for sampling SHD have been established; one by the American Society for Testing and Materials (ASTM), and the other by the German Association of Engineers (VDI). The ASTM method D 5438-00 makes use of the High Volume Small Surface Sampler (HVS3), a modified vacuum cleaner that collects particles greater than 5 μm using various cyclones (ASTM 2002). The VDI 4300 Part 8 guideline describes methods for a number of sampling techniques (e.g., commercial vacuum cleaners, surface wipes, deposition collection) in order to optimize sampling to the specific situation (VDI 2001). This guideline also distinguishes between "old dust" which is dust of unknown age, and "new dust" which is generally one to two weeks old. The collection methods employed in most published studies do not adhere to any rigid standards. This introduces variability (e.g., in particle size distribution), which complicates cross-study data analysis and interpretation.

Reviews by Butte and Heinzow 2002, Roberts and Dickey 1995, and Roberts et al. 1992 provide an overview of dust sampling studies to date. They also summarize the occurrence of various chemical contaminants in dust and assess potential exposure rates.

Exposure to Settled House Dust

Exposure to settled dust and associated contaminants may occur via dermal adsorption, inhalation, and non-dietary ingestion. Dermal absorption of dust may occur following contact with dust that has settled on furniture, floors or other objects. Dust particles less than 100-200 μm are most effectively retained by the skin (Lewis et al. 1999). It is estimated that approximately 28 mg of SHD per day adsorb to children's hands, while 51 mg adsorb to the hands of adults (Hawley 1985). In non-occupational settings, this route of exposure is thought to be less significant than inhalation and non-dietary ingestion (Chuang et al. 1999).

Inhalation of dust can occur when dust is suspended or re-suspended by activities such as vacuuming, cleaning, playing, or simply walking through a room (Thatcher and Layton 1995). It is estimated that young children inhale between 0.15 and 0.34 mg of dust per day, while adults inhale approximately 0.81 mg per day (Hawley 1985). Inhaled dust particles greater than 10 μm are generally trapped by the nose, throat or upper respiratory tract, whereas particles less than 2.5

μm have the ability to penetrate deep into the respiratory system where they are less likely to be eliminated (Morawska and Salthammer 2003). These finer particles, which often contain higher levels of contaminants (Lewis et al. 1999), can pose a toxic hazard to exposed individuals.

Non-dietary ingestion of settled dust generally occurs through accidental ingestion of particles that have adhered to food or skin. This route of exposure is thought to be of particular importance for children who frequently put their hands, toys and other objects into their mouths (Lewis et al. 1994). It is estimated that young children ingest between 10 and 100 mg of dust per day (Hawley 1985; Calabrese et al. 1989; Calabrese 2005) compared to adults who ingest an estimated 0.56 mg per day (Hawley 1985). A small percentage of children are known to exhibit pica behaviour, which involves the intentional eating of non-food items. These children may ingest up to 10 g of soil and dust per day (Calabrese and Stanek 1992).

It is clear that children are exposed to house dust and its associated contaminant load via inhalation, non-dietary ingestion and dermal adsorption. However, in order to determine risk posed by the dust mixture, both exposure and hazard need to be considered. At present, the inherent hazards posed by SHD are less clear.

Mutagenic Hazard

One of the endpoints that may be used to determine the hazards posed by house dust is mutagenic activity. Mutagenic activity can be defined as the extent to which a substance causes heritable changes in the sequence of an organism's genome (Friedberg et al. 1995). Evidence that chemical agents could exert mutagenic activity *in vivo* first came to light in the 1940s (Auerbach et al. 1947). These findings generated concern for human health, particularly in view of the somatic mutation theory of cancer - the dominant theory on carcinogenesis. This theory maintains that cancer is generated from a single somatic cell that has incurred multiple DNA mutations over time (Curtis 1965). Currently, it is generally accepted that most carcinogens are mutagens and that mutagenicity is frequently involved in carcinogenesis (Sarasin 2003). The discovery of chemical mutagens, which therefore had the potential to cause cancer in humans, led to numerous regulatory testing requirements. In the early 1970s, many short term *in vitro*

assays were created in effort to screen environmental chemicals for mutagenic and carcinogenic potential (MacGregor et al. 2000).

The *Salmonella* Mutagenicity Test: Background

The *Salmonella* Mutagenicity Test, also widely known as the Ames Test, was among the assays created to detect chemical carcinogens as mutagens (Ames et al. 1975). The test was designed to assess the ability of suspected mutagens to revert specially constructed strains of *Salmonella typhimurium* from histidine auxotrophy to histidine prototrophy (Ames et al. 1975). Extensive chemical testing in the mid 1970s revealed that 90% of known carcinogens tested with the *Salmonella* Mutagenicity Test also tested positive as mutagens (McCann and Ames 1976). These mutagenicity studies generated substantial interest, and it was considered highly probable that the *Salmonella* Mutagenicity Test could be used to predict carcinogenicity, and possibly replace *in vivo* cancer bioassays (Ames et al. 1975). However, the concordance of *Salmonella* Mutagenicity Test results with carcinogenicity results was reduced when later work revealed higher incidences of non-genotoxic carcinogens and genotoxic non-carcinogens, the former of which could not be detected by the this test system (Shelby and Stasiewicz 1984; Tennant et al. 1987). It was later acknowledged that, as an *in vitro* bacterial assay, the *Salmonella* Mutagenicity Test simply could not respond to all of the diverse mechanisms that lead to carcinogenicity *in vivo* (Douglas et al. 1988). Consequently, the predictivity of the assay was not quite as high as originally believed.

Today, although the *Salmonella* Mutagenicity Test has not replaced the need for animal cancer studies, the test has become an integral component of many regulatory test batteries used to identify genotoxic substances (Muller et al. 1999; MacGregor et al. 2000). As a bacterial test for gene mutation, it is used around the world as a rapid and economical assay to screen new test substances for mutagenicity and the potential for carcinogenicity (Mortelmans and Zeiger 2000).

The *Salmonella* Mutagenicity Test: Methodology and Bacterial Strains

The *Salmonella* Mutagenicity Test is a reversion assay that makes use of various strains of *Salmonella typhimurium.* All of the strains have been constructed with mutations in the histidine operon, which eliminates the ability of the bacteria to synthesize the histidine required

for normal colony growth. When the *Salmonella* strains are exposed to chemical mutagens, new mutations, which form at the site of pre-existing mutations or sites nearby, can restore the gene's function and allow the production of histidine. Mutagenicity is measured by reversion to histidine prototrophy and the formation of colonies (or "revertants") in a histidine-depleted environment.

The *Salmonella* Mutagenicity Test consists of combining the *Salmonella* tester strain, and the test substance with molten agar in a test tube (Figure 1). The contents are poured onto a glucose minimal agar plate and allowed to solidify. The plates are inverted and incubated at 37°C for 48-72 hours and the number of revertant colonies on the plates is then scored.

In many cases, a metabolic activation system (also known as "S9") is added to the test tube containing the agar, test strain and test substance. The metabolic activation system is generally derived from the liver homogenates of Aroclor 1254 induced rats and contains the cytochrome P450 metabolic enzymes which are lacking in the *Salmonella* bacteria. The addition of these mixed function oxidase enzymes mimics the metabolism that occurs in mammalian systems and allows indirect acting chemicals (e.g., PAHs) to be metabolized to their active form. Once metabolized, these chemicals can interact directly with DNA and are detected as mutagens by the assay.

A variety of *Salmonella* strains have been engineered with different types of mutations in different genes of the histidine operon. This enables detection of mutagens with different modes of action (e.g., base pair substitution, frameshift mutation). For example, the strain TA100 has been created with a mutation in the hisG46 allele, which codes for the first enzyme required in histidine biosynthesis. The mutation is an A:T→ G:C mutation which results in the substitution of a proline (GGG/CCC) where a leucine (GAG/CTC) normally exists in the wild-type. This mutation is reverted when the strain is exposed to mutagenic substances that cause base pair mutations at one of the GC pairs. TA98 has been created with a mutation in the hisD3052 allele. This mutation is caused by the deletion of one nucleotide (-1 frameshift mutation) that affects the C-G-C-G-C-G-C-G sequence. This mutation is reverted by mutagens which restore the reading frame (e.g., adding a base pair, taking out two base pairs, taking out five base pairs etc.).

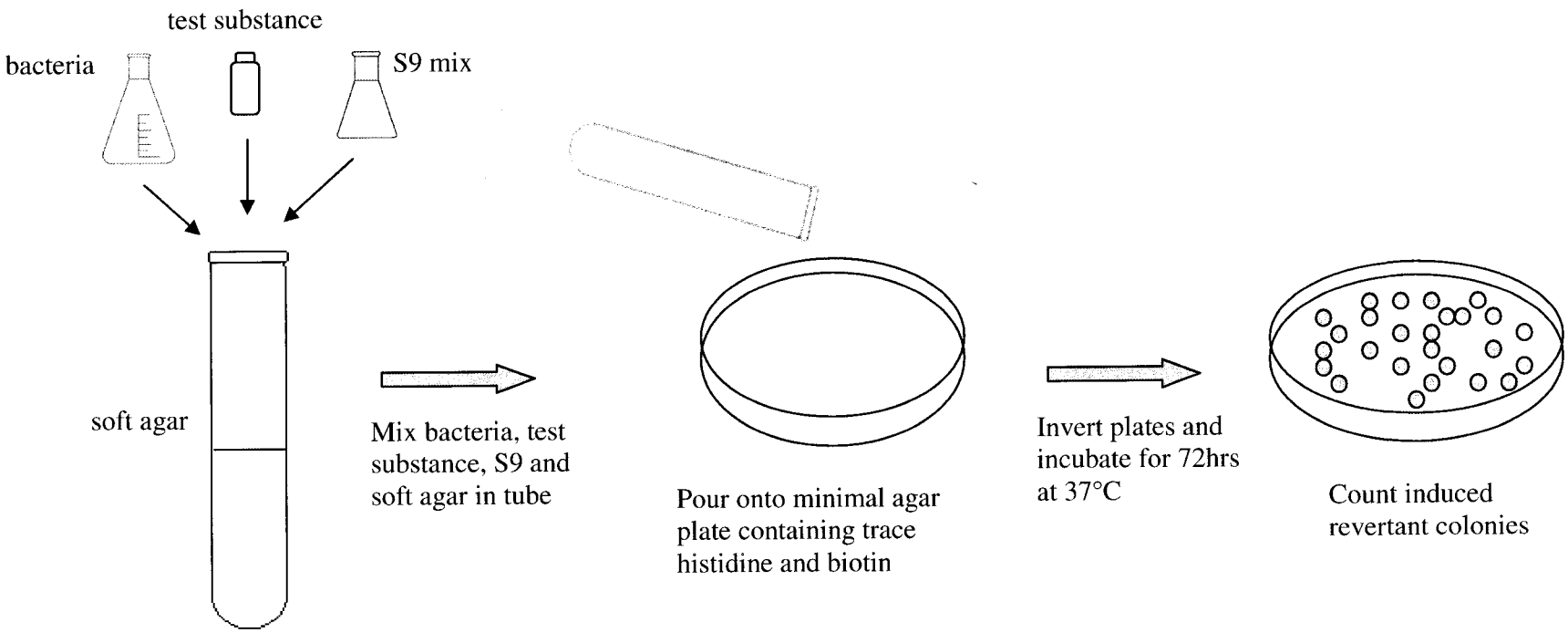


Figure 1. Illustration of the *Salmonella* Mutagenicity Test. Strains of *Salmonella* bacteria are combined with the test substance in a tube containing melted agar. Metabolic activation, usually in the form of rat liver enzyme (S9), may also be added to the tube if required. The contents of the tube are mixed and poured out on to a minimal agar plate containing trace amounts of histidine and biotin. Once solidified, the plates are inverted and incubated at 37°C for 3 days. The number of reverted colonies are then counted and compared to background spontaneous reversion rates.

In addition to the mutations in the histidine operon, most of the strains carry further mutations or genetic factors that increase their sensitivity to chemical mutagens. One of these mutations involves the deletion of the *uvrB* gene that eliminates the nucleotide-excision repair system and increases error-prone repair and mutagenesis. Another involves a mutation to the cell wall, called the deep rough mutation (*rfa*), which facilitates the movement of bulky chemicals across the cell wall. A third alteration is the addition of plasmids that enhance the bacteria's sensitivity to UV and chemical induced mutagenesis. The properties of several *Salmonella* strains are listed in Table 2.

Table 2. Properties of the *Salmonella typhimurium* strains used in the *Salmonella* Mutagenicity Test. The *rfa* mutation results in a loss of the distal portion of the lipopolysaccharide barrier that covers the bacterial surface, thereby enabling bulky chemicals to penetrate the cell wall. The *uvrB* mutation is a deletion through the *uvrB* gene, which removes the coding for DNA excision repair, and results in an increase in error prone repair. The plasmids enhance sensitivity to UV and chemical induced mutagenesis.

Strain	Mutation Location	DNA Target	Other Mutations	Plasmids	Use
TA98	hisD3052	GC	*rfa, uvrB*	pKM101	Detects frameshift mutagens.
YG1041	hisD3052	GC	*rfa, uvrB*	pKM101, pYG233	Derived from TA98. Sensitive to nitroarenes and aromatic amines.
TA100	hisG46	GC	*rfa, uvrB*	pKM101	Detects base pair mutagens.
YG1042	hisG46	GC	*rfa, uvrB*	pKM101, pYG233	Derived from TA100. Sensitive to nitroarenes and aromatic amines.
TA102	hisG428	AT	*rfa*	pAQ1, pKM101	Detects transitions and transversions. Sensitive to oxidative damage.

Mutagenicity of House Dust

To date, only one study has employed the *Salmonella* Mutagenicity Test to investigate the mutagenic hazards of SHD. In their study of 29 houses in Washington state, Roberts et al. examined the *Salmonella* TA98 mutagenicity of dust collected from homes in high and low pollution areas (Roberts et al. 1987). The results showed that 10 out of 29 dust extracts had significantly elevated levels of *Salmonella* mutagenicity in the absence of metabolic activation. In the presence of metabolic activation, 5 out of 29 samples showed significantly elevated levels of *Salmonella* mutagenicity. TA98 mutagenic potency values ranged from 1190 to 6570 revertants per gram without S9 and from 1340 to 4180 revertants per gram with S9. The tests also revealed an increase in the mutagenic activity with decreasing particle size.

The mutagenicity of SHD, as determined in the Roberts et al. study, can be evaluated against the mutagenicity of other particulate matrices. Comparisons reveal that settled dust tends to be more mutagenic than most outdoor soils, including those collected from contaminated industrial sites, but less mutagenic than suspended particulate matter collected from either indoor or outdoor air (Table 3). Geometric mean mutagenic potency values for contaminated soils from industrial sites tend to yield TA98 potency values in the 1000 revertants per gram range, although individual values for heavily contaminated soils can yield 10^5 revertants per gram (White and Claxton 2004). Although the potency of suspended particulate material collected in both indoor and outdoor environments can vary a great deal, organic extracts of these samples often yield potency values greater than 10^5 TA98 revertants per gram (Claxton et al. 2004). This relative ranking of mutagenic potency seems reasonable since settled dust contains deposited particulates from both indoor and outdoor air, as well as tracked-in soil particles (Thatcher and Layton 1995). The relatively low mutagenic potency of SHD in comparison to suspended particles in indoor or outdoor air is likely due to the dilution of SHD with large particles of inert material (e.g., sand grains), biologically-derived material (e.g., skin flakes), and textile fibers that are non-mutagenic. In a similar fashion, the lower levels of mutagenic potency of soil particles is almost certainly accounted for, at least in part, by the presence of large amounts of inert inorganic material of geological origin.

Table 3. *Salmonella* TA98 mutagenic potency[a] of dust, indoor air, outdoor air, and soil.

Media	Sampling Location	Areal/Volumetric Dust Concentrations[b]	Revertants g^{-1} -S9	Revertants g^{-1} +S9	Reference
Settled Dust	29 homes in high and low pollution areas	1,900,000 ± 300,000 μg m^{-2}	1,190 – 6,570 [c]	1,340 – 4,180 [d]	(Roberts et al. 1987)
Indoor Air	39 rural homes	37 – 210 μg m^{-3}	40,000 – 60,000	240,000 – 550,000	(van Houdt et al. 1984)
	1 home in a residential area	NA	70,000 – 460,000	130,000 – 370,000	(Lioy et al. 1985)
	24 rural homes	36 – 59 μg m^{-3}	120,000 – 260,000	280,000 - 450,000	(van Houdt et al. 1986)
	4 urban homes	50 – 110 μg m^{-3}	12,000 – 78,000	14,000 -187,000	(Nardini et al. 1994)
	1 rural home	30 – 140 μg m^{-3}	6,000 – 36,000	17,000 – 49,000	
Outdoor Air	1 industrial location	5.13 – 13.73 μg m^{-3}		26,000 – 87,330	(Massolo et al. 2002)
	2 urban locations	3.97 – 5.75 μg m^{-3}		17,920 – 50,910	
	1 industrial location	68.5 μg m^{-3}	520,000	577,000	(Monarca et al. 1997)
	1 industrial location	5.3 – 15.8 μg m^{-3}	1,000,000 – 1,537,974	867,924 – 1,649,425	(Cerna et al. 2000)
	1 agricultural location	3.6 – 8.2 μg m^{-3}	638,889 – 2,097,560	750,000 – 1,329,268	
Soil	Heavily contaminated sites	NA	770 ± 180	950 ± 170	(White and Claxton 2004)
	Urban/industrial sites	NA	430 ± 40	470 ± 50	(White and Claxton 2004)
	Remote/rural sites	NA	57 ± 6	60 ± 5	(White and Claxton 2004)

[a]Defined as the initial slope of the concentration-response curve (see (Bernstein et al. 1982) or similar).
[b]Mean value or range where available.
[c]Range for 14 positive samples.
[d]Range for 15 positive samples.

Sources of House Dust Mutagenicity: Polycyclic Aromatic Hydrocarbons (PAHs)

There are a number of substances that could potentially contribute to the mutagenicity of SHD. These could include a host of organic and inorganic compounds commonly associated with a variety of industrial products (e.g., textiles, paints, furniture) such as hexavalent chromium, nickel compounds, styrene, tetrachloroethylene, benzidine and vinyl chloride (IARC 1987; IARC 1990; IARC 1995; IARC 2002).

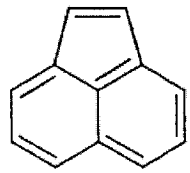
One group of chemicals that is suspected of contributing to the mutagenic activity of dust is the polycyclic aromatic hydrocarbons (PAHs) and related compounds (e.g., nitroarenes, heterocyclic amines). PAHs are a group of organic compounds made up of two or more fused benzene rings (Figure 2). There is a wide range of possible PAH isomers, the most thoroughly studied of which is benzo[*a*]pyrene.

Research on PAHs began indirectly in the early 1900s when investigators were attempting to elucidate the cause of high incidences of skin cancer rates in the paraffin refining, shale oil and coal tar industries (Phillips 1983). In 1915, researchers first replicated the skin tumours in rabbits by frequently painting their ears with coal tar (Phillips 1983). However, it was not until 1930 that researchers were able to identify the benzanthracene derivatives as the substances that were likely causing the tumours (Hieger 1930). Shortly thereafter, the PAH dibenz[*ah*]anthracene, a component of the coal tar, was confirmed to be carcinogenic when applied to the skin of mice (Kennaway and Hieger 1930). In 1933, a publication by Cook et al. positively identified benzo[*a*]pyrene as a carcinogenic component of coal tar (Cook et al. 1933).

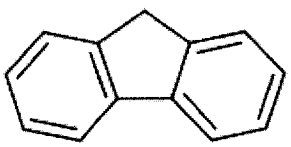
It was originally believed that the PAH parent molecules themselves were directly carcinogenic. It was not until the late 1960s, through *in vitro* testing with hepatic microsomal fractions, that researchers showed that the toxicity of PAHs was in fact dependent on the presence of metabolic activation and the formation of reactive intermediates which would bind to DNA (Gelboin 1969). Following this discovery, much research has been conducted in attempts to fully characterize PAH toxicity in cells, animals, and ultimately humans (IPCS 1998).

There exists a high potential for human exposure to PAHs due to their ubiquity in both the indoor and outdoor environments. The highest levels of PAHs are produced through anthropogenic combustion processes. Indoor sources of PAHs include cooking (Koo et al. 1994; Zhu and Wang 2003), heating (Moriske et al. 1996), smoking (Mitra and Ray 1995), wood burning (Rogge et al. 1998), candle burning (Lau et al. 1997) and incense burning (Koo et al. 1994; Lung and Hu 2003). Outdoor sources include vehicle exhaust (Dubowsky et al. 1999), and industrial processes such as aluminium smelting and coke production (Government of Canada 1994). In addition to these pyrogenic processes, PAHs may also be emitted to a lesser extent by petrogenic processes (Baumard et al. 1998). The presence of PAHs in petroleum is a result of diagenetic processes and environmental contamination that can result from events such as petroleum refining and transportation.

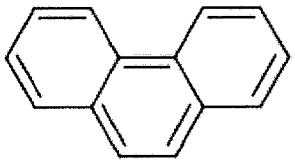
Most PAHs have low vapour pressures, low water solubilities, and high octanol-water partition coefficients (K_{ow}) (MacKay 2001). PAHs with fewer than four rings generally have higher vapour pressures and can be found either adsorbed to particulate matter or found in the gaseous state. PAHs with four or more rings have lower vapour pressures and higher K_{ow}s, and are generally expected to be adsorbed to particulate material (Hoff and Chan 1987). Table 4 includes a summary of the physical-chemical properties of several PAHs, and the calculated fraction of each PAH that would be expected to be adsorbed to indoor particulate matter at 25°C. This value, calculated using the level I fugacity models of MacKay 2001, reveals that, for PAHs with log K_{ow} values greater than 4.0 and vapour pressure values less than 1.0 Pa, almost all of the PAH will be adsorbed to particulate (aerosol) material. However, it should be noted that this calculation is based on a simplified indoor system composed only of air, water (humidity) and particulate material. Thus, it could not account for PAHs adsorbed to indoor surfaces (e.g., walls, furniture), since the fugacity capacities of these indoor surfaces are not known.



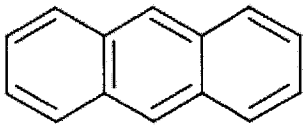
Acenaphthylene



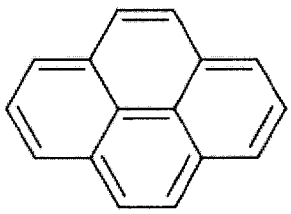
Fluorene



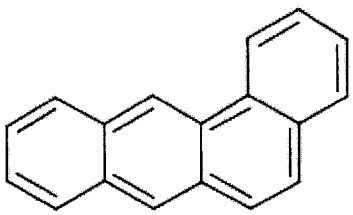
Phenanthrene



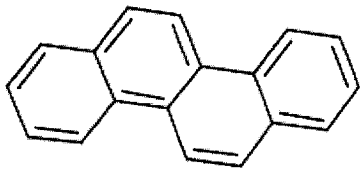
Anthracene



Pyrene



Benz[*a*]anthracene



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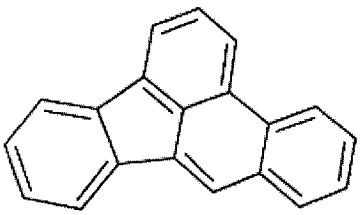
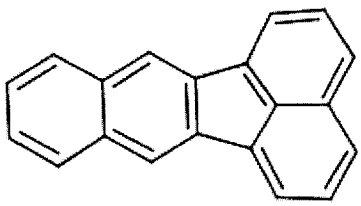
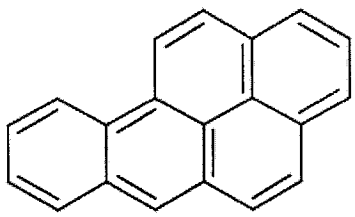


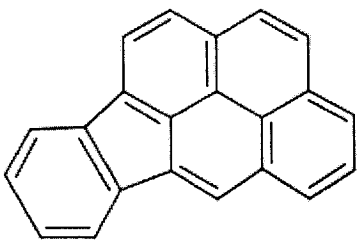
Benzo[*b*]fluoranthene



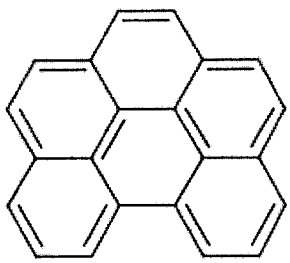
Benzo[*k*]fluoranthene



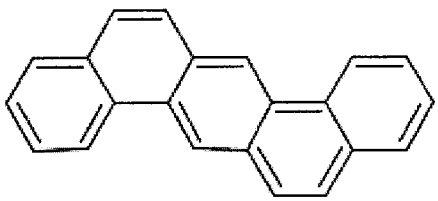
Benzo[*a*]pyrene



Indeno[1,2,3-*c,d*,]pyrene



Benzo[*g,h,i*]perylene



Dibenz[*a,h*]anthracene

Figure 2. Structures of 13 polycyclic aromatic hydrocarbons (PAHs) commonly measured in environmental samples.

Table 4. Physical-chemical properties of several PAHs[a].

Compound	Vapour Pressure at 25°C (Pa)	Log K_{ow}	Fraction Adsorbed to Household Dust at 25°C (%)[b]
Acenaphthene	8.93E-01	3.55	98.88
Acenaphthylene	3.78E-01	4.03	97.40
Anthracene	8.31E-04	4.54	99.96
Benz[*a*]anthracene	4.10E-06	5.91	99.99
Benzo[*a*]pyrene	7.00E-07	6.50	100.0
Benzo[*e*]pyrene	7.32E-07	6.44	100.0
Benzo[*b*]fluoranthene	6.67E-05	6.50	99.99
Benzo[*k*]fluoranthene	6.70E-05	6.20	99.99
Benzo[*g,h,i*]perylene	1.39E-08	7.10	100.0
Chrysene	4.00E-06	5.91	99.99
Coronene	1.95E-10	7.64	99.99
Cyclopenta[*c,d*]pyrene	NA[c]	NA	NA
Dibenz[*a,h*]anthracene	1.30E-08	6.20	100.0
Fluoranthene	6.42E-03	5.22	99.97
Fluorene	9.46E-02	4.18	99.56
Indeno[1,2,3-*c,d*]pyrene	NA	NA	NA
Naphthalene	1.08E+01	3.36	81.71
Phenanthrene	1.61E-02	4.57	99.95
Pyrene	6.00E-04	5.18	99.99

[a]Physical-chemical properties obtained from (MacKay et al. 1992).

[b]Level I fugacity calculations assuming a home has a volume of 295 m^3, containing 3.4 L of water (i.e., 50% relative humidity) and 8.3 L of particulate material (ρ = 1172.1 ± 359.5 kg m^{-3}).

[c]Data not available.

Exposure to PAHs in Settled House Dust

Only a small number of studies have assessed human exposure to PAHs in SHD (Chuang et al. 1999; Wilson et al. 2001; Wilson et al. 2003). These studies have examined exposures to PAHs in dust in comparison to exposures via other media such as air or food. These assessments show that dietary ingestion of PAHs in food is often the primary exposure pathway for children. This holds true when considering exposure to both the sum of all targeted PAHs, or only those PAHs considered to be carcinogenic. When considering only the carcinogenic PAHs, non-dietary ingestion of PAHs in dust and soil is the second most important exposure route for children, and more important than inhalation.

Assessment of PAH exposure in children due to non-dietary ingestion of SHD indicates that toddlers playing on the floor and exhibiting hand-to-mouth behaviour can ingest more than 2.5 times as much as adults (Roberts et al. 1991). Since a child's body weight is only about one-fifth that of an average adult, children's exposure to PAHs in dust, in mg per kg body weight, are far greater than those for adults. It is generally acknowledged that the higher surface area to body mass ratio, low body weight, and relatively high intake of food, water and air (per unit body weight) contribute to higher exposures of children to certain contaminants (USEPA 2005). Moreover, the early development stage of organ, immune and nervous systems in children are thought to contribute to an enhanced contaminant sensitivity (IPCS 1986). Therefore, the adverse health risks for children exposed to PAHs, especially via non-dietary ingestion, are generally believed to be considerably greater than for adults.

At present, there are no reference doses or tolerable daily intake values for PAHs in SHD. The German Federal Environmental Agency's Commission for Indoor Air Quality has established the only guideline that currently exists. It states that exposure to levels above 10 μg of benzo[*a*]pyrene per gram of household dust should be minimized in order to prevent unspecified adverse health effects (Heudorf and Angerer 2001).

Metabolism and Toxicity of PAHs

Human exposure to PAHs may occur via the lungs, gut or skin. As lipophilic substances, PAHs readily cross cellular membranes and, regardless of the route of exposure, PAHs can enter the blood stream and distribute widely in the body (WHO 2000).

The metabolism of PAHs is a complex process that involves a number of metabolic pathways and results in a number of reactive intermediates. Figure 3 shows the various pathways for benzo[*a*]pyrene metabolism as a model for PAH metabolism. In general, most of these reaction pathways are considered to be detoxification processes, and the PAHs are eliminated primarily via hepatobiliary excretion through the faeces (WHO 2000). However, the pathway leading to the formation of diol epoxides is considered to be an activation reaction. Of particular concern is the formation of the 7,8-diol-9,10-epoxides on PAHs, such as benzo[*a*]pyrene, that possess a "bay region". These "bay region" diol epoxides are resistant to further metabolism because of steric hindrance. Their electrophilic properties allow for interaction with DNA and the formation of adducts, primarily with the exocyclic N^2-amino group of deoxyguanosine residues (Cheng et al. 1989). This DNA interaction is implicated as the basis for the mutagenesis and carcinogenesis of PAHs (Brookes and Lawley 1964). The International Agency for Research on Cancer (IARC) has classified a number of PAHs as mutagens and/or animal carcinogens (IARC 1983), and a number of agencies have listed PAHs as carcinogenic and toxic substances (Table 5).

Other Polycyclic Compounds

In addition to the homocyclic, unsubstituted PAHs, there are a number of closely related polycyclic compounds that are also genotoxic, and may contribute to the mutagenic activity of house dust. Specifically, many nitroPAHs and aromatic amines (including heterocyclic amines) have been found in the indoor environment and identified as potent mutagens.

NitroPAHs are derivatives of PAHs and are formed either through atmospheric transformation, or through the nitration of PAHs during combustion (Arey 1998). Sources of nitroPAHs include diesel exhaust, and to a lesser extent, gasoline exhaust, emissions from

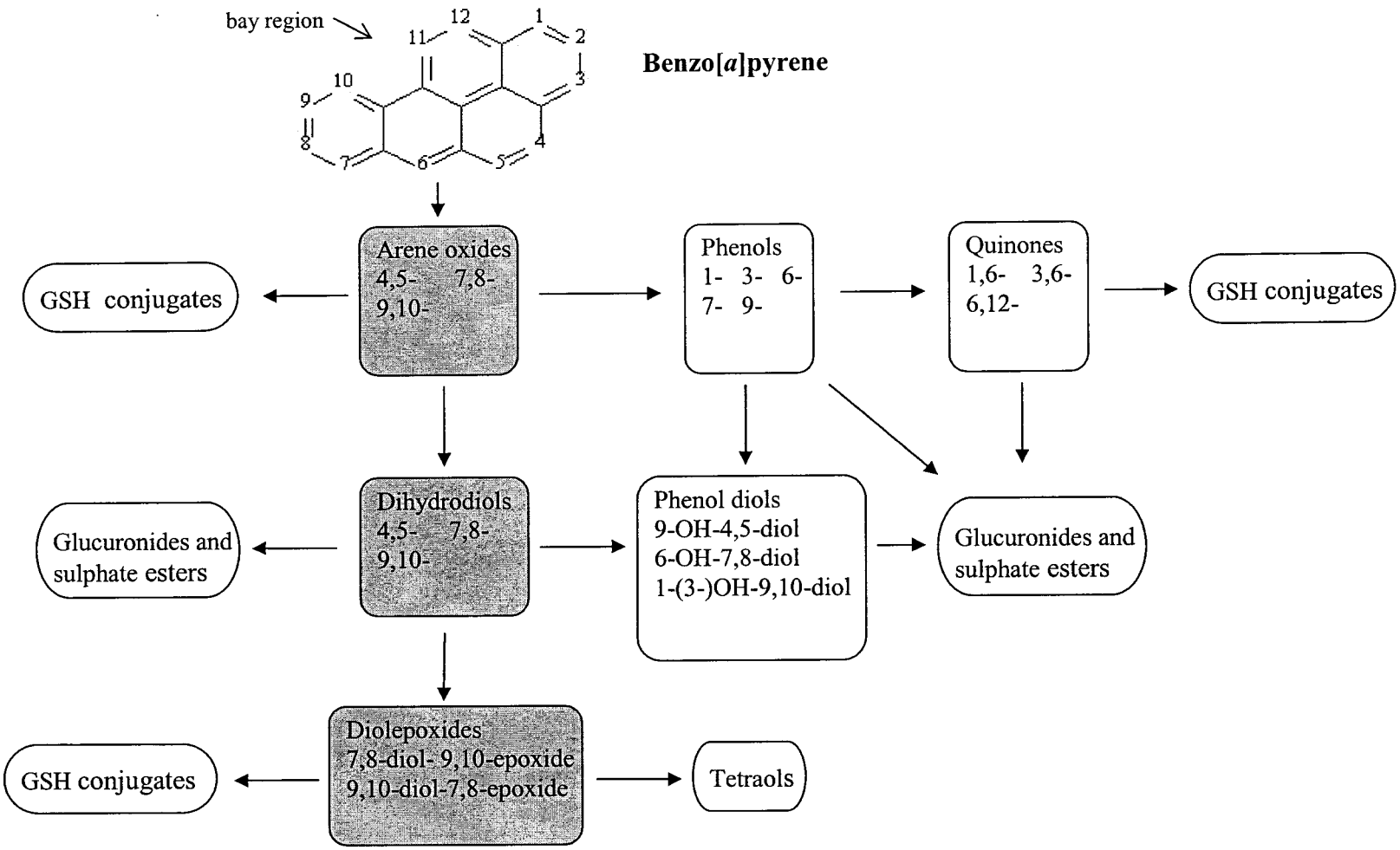


Figure 3. The metabolism of benzo[*a*]pyrene as a model of general PAH metabolism. While most metabolic processes result in detoxification, the highlighted pathway leading to the formation of the diol epoxides is considered to be an activation pathway. Due to steric hinderance, the diol epoxides resist further metabolism and their electrophilic properties allow for the formation of DNA adducts. This DNA interaction is implicated as the basis for the mutagenesis and carcinogenesis of PAHs. (Adapted from IARC 1983.)

Table 5. Mutagenicity and carcinogenicity of selected PAHs targeted for analysis in published studies of SHD.

PAH	CAS Registry Number	Mutagenicity[a]	Carcinogenicity (IARC)[b]	Carcinogenicity (IRIS)[c]	Toxic under CEPA 11(c)[d]
Acenaphthene	83-32-9	0	Not assessed	Not assessed	Not assessed
Acenaphthylene	208-96-8	No data	Not assessed	D	Not assessed
Anthracene	120-12-7	1[e]	3	D	Not assessed
Benz[*a*]anthracene	56-55-3	1,2,3	2A	B2	Not assessed
Benzo[*a*]pyrene	50-32-8	1,2,3	2A	B2	Yes
Benzo[*e*]pyrene	192-97-2	1,2	3	Not assessed	Not assessed
Benzo[*b,k*]fluoranthene	205-99-2, 207-08-9	1	2B	B2	Yes
Benzo[*g,h,i*]perylene	191-24-2	1	3	D	Not assessed
Chrysene	218-01-9	1,2	3	B2	Not assessed
Coronene	191-07-1	1	3	Not assessed	Not assessed
Cyclopenta[*c,d*]pyrene	27208-37-3	1,2	3	Not assessed	Not assessed
Dibenz[*a,h*]anthracene	53-70-3	1,2	2A	B2	Not assessed
Fluoranthene	206-44-0	1,2	3	D	Not assessed
Fluorene	86-73-7	0	3	D	Not assessed
Indeno[1,2,3-*c,d*]pyrene	193-39-5	0	2B	B2	Yes
Naphthalene	91-20-3	2[f]	2B[g]	D	Not assessed
Phenanthrene	85-01-8	1,2	3	D	Not assessed
Pyrene	129-00-0	1,2	3	D	Not assessed

[a]Based on information from IARC (IARC 1983), the Genetic Activity Profile database (Lohman and Lohman 2000) and the National Toxicology Program (2004). 0 - no evidence of mutagenicity, 1 - mutagenic in bacterial and/or fungal/yeast cells *in vitro*, 2 - mutagenic in plants or animal cells *in vitro*, 3 - mutagenic in the *Drosophila melanogaster* somatic mutation and recombination test, and/or sex-linked recessive lethal test, and/or transgenic rodent assays, and/or rodent dominant lethal test.

[b]Based on information from IARC. IARC classification: 1 - carcinogenic to humans, 2A - probably carcinogenic to humans, 2B - possibly carcinogenic to humans, 3 - inadequate or limited evidence of carcinogenicity in experimental animals (IARC 1983).

[c]Based on information from the Integrated Risk Information System (IRIS). USEPA classification: A - Human carcinogen, B1 - probable human carcinogen (limited human data) B2 - Probable human carcinogens (primarily on

the basis of animal data), C - Possible human carcinogen, D - Not classifiable as to human carcinogenicity (inadequate or no evidence), E - Non-carcinogen (USEPA 2003).

[d]May constitute a danger in Canada to human life or health, as defined under paragraph 11(c) of the Canadian Environmental Protection Act (Government of Canada 1994).

[e]IARC noted that anthracene failed to induce mutations in bacteria or yeast, and did not induce cytogenetic effect *in vitro* or *in vivo* (IARC 1983). A single, subsequent publication noted that anthracene can induce mutations in *Salmonella* TA100 in the presence of rat liver or hamster liver S9 (10% v/v in activation mixture) (Mortelmans et al. 1986).

[f]The NTP executive committee working group indicates that the majority of genotoxicity tests have shown that naphthalene is not genotoxic in most *in vitro* assays and cannot induce mutations in bacteria (National Toxicology Program 2002). An earlier IARC publication noted that naphthalene showed clastogenic activity in cultured CHO cells (IARC 2002).

[g]IARC (2002) and the National Toxicology Program (2000) respectively noted that there is *sufficient evidence* to support the carcinogenic activity of naphthalene in experimental animals, and *clear evidence* of carcinogenic activity in rats. However, the 2002 report of the NTP executive committee working group (National Toxicology Program 2002) could not arrive at a consensus regarding the carcinogenicity of naphthalene. The USEPA stated that naphthalene is not classifiable with respect to its carcinogenic activity (USEPA 2003).

kerosene heaters and liquid petroleum gas burners, and cooking oil fumes (Tokiwa and Ohnishi 1986; IPCS 2003). NitroPAHs are mutagenic in bacterial test systems (Tokiwa et al. 1981), and nitropyrenes in particular, are among the most mutagenic chemicals reported in the literature (Mermelstein et al. 1981). Like PAHs, nitroPAHs generally occur in complex mixtures and can be found in the vapour phase or adsorbed to particulate matter (IPCS 2003). Although it has yet to be investigated, it is suspected that nitroPAHs are present in house dust and contribute to its mutagenic activity.

Like PAHs and nitroPAHs, heterocyclic amines are also produced through combustion processes. They are found in materials such as diesel exhaust (Manabe et al. 1993), cigarette smoke (Manabe and Wada 1990; Kataoka et al. 1998) and most notably cooked foods (Felton and Knize 1991; Eisenbrand and Tang 1993). Heterocyclic amines have been shown to be mutagenic (de Meester 1989; Felton et al. 1994), and studies suggest that they are ubiquitous environmental pollutants (Manabe et al. 1992; Manabe et al. 1993; Kataoka et al. 1998). Again, although it has yet to be investigated, it is suspected that these compounds could also contribute to the mutagenic activity of house dust.

Risk Assessment

Exposure to mutagenic and/or carcinogenic substances in SHD may pose potential health risks to the individuals exposed. This risk of adverse effect is a function of various factors including: the inherent hazard (or toxicity) of the SHD and its associated chemicals, the frequency and magnitude of SHD exposure, and the characteristics or susceptibility of the exposed group of individuals (Kolluru 1996). In order to estimate the probability of adverse health effects following exposure to SHD and its associated contaminants, a quantitative risk assessment can be undertaken. Risk assessments generally include four main steps: selection of the chemical/substance of concern, a toxicity assessment, an exposure assessment, and a risk characterization that may include uncertainty and sensitivity analyses (Kolluru 1996). Although the practice of risk assessment is not an exact science, and frequently includes a number of assumptions and uncertainties, it can be a useful tool for predicting the likelihood of negative human health effects, and subsequently for initiating and prioritizing activities that mitigate harm.

Study Objectives

Given that SHD is a complex mixture likely containing numerous mutagens and carcinogens, and given that children are exposed to the dust-contaminant mixture, further investigations into the mutagenic and carcinogenic hazards and risks posed by SHD are warranted. This thesis addresses these issues in two main sections. The first section (Chapter 1) is a literature review on PAHs in SHD. The objectives of this review are as follows:

1. To compile published data on PAH levels in SHD, and analyze relationships between these levels and various attributes of the households (e.g., location, presence of smokers, type of flooring).
2. To assess the potential contributions of frequently measured PAHs to the overall mutagenic hazards of SHD.
3. To estimate the carcinogenic risks associated with preschool exposure to PAHs in SHD.

The material in this chapter has been published (along with material included in this General Introduction) as a review in Mutation Research 567(2004):401-426. Dr. Paul White made significant contributions to this work and is listed as third author. Ms. Jennifer Bailey initiated the collection of published of the PAH data, and is listed as second author. The Crown holds the copyright for this material and it was reproduced here with permission (see Appendix I).

The second section (Chapter 2) contains new experimental work that examines the mutagenic potency of SHD collected from Ottawa area homes, and evaluates the mutagenic responses in relation to possible sources of mutagens and numerous household attributes. The objectives of this section are as follows:

1. To assess the mutagenicity of house dust samples collected in Ottawa, ON.
2. To evaluate the extent to which PAHs (and PAH derivatives) contribute to the mutagenic activity of the Ottawa dust samples.
3. To examine the relationships between Ottawa dust mutagenicity, PAH levels in dust, and various household characteristics.
4. To estimate the excess lifetime cancer risks associated with preschool children's non-dietary ingestion of PAHs in dust from Ottawa homes.

Chapter 1

The Mutagenic Hazards of Polycyclic Aromatic Hydrocarbons (PAHs) in Settled House Dust: A Review

Introduction

Studies investigating the composition of settled house dust (SHD) have revealed the presence of numerous chemical contaminants in dust including pesticides, smoke residues, PCBs, flame retardants, plasticizers, heavy metals and asbestos (Butte and Heinzow 2002; Lioy et al. 2002; Greenpeace 2003; Rudel et al. 2003). When examining the health effects associated with exposure to these contaminants, researchers have often focused on lead (Vostal et al. 1974; Laxen et al. 1987; Davies et al. 1990; Lanphear et al. 1996; Lanphear et al. 1998; Meyer et al. 1999) and pesticides (Roberts et al. 1991; Lewis et al. 1994; Whitemore et al. 1994; Lewis et al. 2001; Nishioka et al. 2001; Pang et al. 2002). However, polycyclic aromatic hydrocarbons (PAHs) have also been detected in SHD and these substances could pose additional health concerns.

To date, only a small number of studies have examined the PAH content of SHD (Chuang et al. 1993; Camann and Buckley 1994; Chuang et al. 1994; Chuang et al. 1995; Chuang 1996; Chuang et al. 1997; Chuang et al. 1997; Mukerjee et al. 1997; Simrock 1998; Chuang et al. 1999; Dieckow et al. 1999; Lewis et al. 1999; Camann et al. 2001; Rudel et al. 2001; Wilson et al. 2001; Butte and Heinzow 2002; Camann et al. 2002; Lioy et al. 2002; Greenpeace 2003; Rudel et al. 2003; Wilson et al. 2003). Several of the important published findings are highlighted in Table 1. In order to gain additional information on the sources and hazards of PAHs in SHD, a thorough review and analysis of published data was undertaken. A complete dataset containing all of the available published data on PAHs in SHD was created and the pooled data were analyzed to identify empirical trends that were not apparent in the individual studies.

The data analyses focussed on three main areas. Firstly, the data were examined to determine the types and concentrations of PAHs commonly found in SHD. These data were then evaluated against information about the sampling site to determine whether household attributes (e.g., the presence of smokers, an urban location) are empirically related to PAH dust concentrations. Secondly, the extent to which commonly measured PAHs could account for

measured mutagenic potency of SHD was investigated. These analyses made use of the pooled PAH data, as well as additional published data on the mutagenicity of SHD, and the mutagenicity of individual PAHs. Lastly, information on the types and amounts of carcinogenic PAHs found in SHD was used to conduct a risk assessment. Specifically, the excess cancer risk attributable to non-dietary ingestion of PAHs in SHD during preschool years was assessed.

Table 1. Factors associated with the PAH composition of settled house dust (SHD).

Study Area	**Variables Investigated**	**Conclusion**	**Reference**
Ohio, North Carolina	Track-in soil	PAH concentrations in SHD are greater than that in outdoor soil	(Chuang 1996; Chuang et al. 1999)
North Carolina, Minnesota	Air (indoor and outdoor)	PAH concentrations in SHD are correlated with PAH concentrations in both indoor and outdoor air	(Wilson et al. 2000; Clayton 2003)
Texas	Season	PAH concentrations in SHD are higher in the spring than in the summer	(Mukerjee et al. 1997)
North Carolina	Location	PAH concentrations in SHD from urban areas are higher than from rural areas	(Chuang et al. 1999)
North Carolina	Income	The differences in PAH concentrations between SHD from low-income and middle-income houses are small	(Wilson et al. 2003)
Ohio	Smoking	Smoking is not the primary determinant of PAH levels in SHD	(Chuang et al. 1995)

Methods

Collection and Description of Published Data on PAHs in House Dust

PAH data in SHD were collected from 18 publications including several peer-reviewed journal articles and government reports. In cases where the original (i.e., raw) published data were difficult to locate, study authors were contacted and, where possible, original reports were acquired directly from the author. Only studies that provided the concentration ($\mu g\ g^{-1}$) of the PAHs in SHD, as opposed to surface loading ($g\ m^{-2}$), were included. All PAH concentrations in dust were expressed in $\mu g\ g^{-1}$. The 18 studies contained a combined total of 132 observations, which are summarized in Appendix II.

The majority (122) of the 132 observations recorded in Appendix II reflect the results of analyses conducted on samples collected from individual houses. The remaining ten observations represent studies that only provided mean or median values for a series of locations. Forty-eight percent of the 132 observations are from urban areas, 15% are from rural areas, and 1% from suburban areas. The remaining observations (36%) are from locations that were not adequately described. Most of the observations are from sites where smokers were not present (47%). Fewer observations were collected from sites with smokers (17%), and several studies did not provide information on the smoking habits of the inhabitants (36%). Information on the socio-economic status of the sampled households was not available for much of the dataset (59%). Where this information was provided, most of the samples came from low-income areas (40%), while a small number came from medium-income households (1%).

Unfortunately, few of the studies provided detailed information about the methods employed for sample collection, processing, and analysis. The majority of the SHD samples (69%) were collected using the High Volume Small Surface Sampler (HVS3). A smaller number of samples were collected using household vacuum cleaners (4%), and a smaller number still were collected using a combination of sampling techniques (1%) or unidentified methods (26%). The type of surface sampled included carpet only (42%), a combination of surfaces (21%), or unspecified surfaces (37%). In all cases, the dust particles selected for study were less than 150 μm in diameter, except for one study (Dieckow et al. 1999) in which the particle size

was not indicated. The majority of the SHD samples were extracted using hexane (75%) or diethyl ether in hexane (23%). One study (Simrock 1998) used acetone and cyclohexane, and one study (Dieckow et al. 1999) did not specify the extraction solvent. Where analytical instrumentation was specified, all studies employed gas chromatography/mass spectrometry for identification and quantification of PAHs. Eighteen PAHs were selected for analysis across the studies. However, the number and identity of the PAHs examined differed across the studies. The most commonly studied PAHs were benzo[*a*]pyrene and benz[*a*]anthracene, compounds which are also among the most mutagenic and carcinogenic (see Table 5 in the General Introduction).

Data Analysis

All analyses were performed using the SAS system version 8.02 for Windows (SAS Institute 2001). Analysis of variance (ANOVA) was employed to investigate relationships between PAH concentration values ($\mu g\ g^{-1}$) and various site attributes (e.g., urban, low-income). Following the notation of Gujarati (Gujarati 1988), the general model $Y_i = \alpha_1 + \alpha_2(D_2) + \alpha_3(D_3) + \alpha_n(D_n) + \mu_i$ was fit to the data. Y_i is the observed PAH concentration for observation i, and D_2 through D_n are dichotomous variables that indicate membership of observation i in a given group (e.g., urban sites, low-income sites). D_2 through D_n are set to 1 when the condition of group membership is satisfied and 0 when the condition is not satisfied. Where necessary, the data were log transformed to meet the normality assumptions of ANOVA. The residual error term μ_i was assumed to be independent and normally distributed. Normality was assessed using the Shapiro-Wilk statistic and visual examination of a normal probability plot (SAS Institute 1989). The absolute value of the residual error values (μ_i) was used to detect outliers and identify data entry errors. To identify significant outliers, externally studentized residuals (d*i**) were calculated for each validated residual error value (Neter et al. 1990). All analyses were conducted for total PAHs (i.e., sum of the 18 targeted PAHs), the total low molecular weight PAHs (i.e., those having two or three fused rings), the total high molecular weight PAHs (i.e., those with four or more fused rings) and the total carcinogenic PAHs (i.e., only those PAHs defined by the USEPA as B2 carcinogens).

Results and Discussion

PAH Content of Settled House Dust

Examination of the raw data (Appendix II) indicates that the PAH content of SHD is extremely variable. Concentrations spanned up to four orders of magnitude for a single PAH, and up to five orders of magnitude across different PAHs. The sum of the reported PAHs for each observation (i.e., total PAH) ranged from approximately 0.5 $\mu g\ g^{-1}$ to 500 $\mu g\ g^{-1}$. The minimum, maximum and mean PAH concentrations are summarized in Table 2. Overall, the PAHs that occurred in the lowest concentrations were acenaphthene, acenaphthylene and cyclopenta[*c,d*]pyrene. The low concentrations are likely in part due to the volatile and reactive nature of these PAHs (Chuang et al. 1993). The PAHs that occurred in the highest concentrations are benzo[*b,k*]fluoranthene, fluoranthene and pyrene. Mixtures of PAHs have been shown to have a relatively high abundance of pyrene (Jongeneelen 2001). Moreover, PAHs with molecular weights of 202, such as fluoranthene and pyrene, can be present in both a gaseous and particle-adsorbed state at room temperature, while PAHs with molecular weights greater than 228, such as benzo[*b,k*]fluoranthene, are predominantly associated with particulate matter (Jongeneelen 2001). This pattern is also consistent with the data shown in Table 4 in the General Introduction. Compounds such as acenaphthene, acenaphthylene have relatively high vapour pressure values (i.e., ~0.3 Pa) and low K_{ow} values (i.e., 10^4 range), whereas compounds such as benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and pyrene have far lower vapour pressure values (i.e., 10^{-4} to 10^{-3} range) and higher K_{ow} values (i.e., $10^5 - 10^6$ range). Pyrene and fluoranthene have been suggested as potential source markers for incineration, wood burning and oil combustion (Harrison et al. 1996), with the ratio of fluoranthene to pyrene providing information on PAH source. If the fluoranthene/pyrene index is greater than 1, the PAHs are considered to have been generated by pyrolytic processes, whereas if the index is less than 1, they are considered to be petrogenic in origin (Baumard et al. 1998). The mean ratio of fluoranthene to pyrene in the collected data is 1.25 ± 0.015 and 93% of the observations have a ratio greater than 1. Therefore, the PAHs detected in the SHD samples appear to be predominantly pyrolytic in origin.

Table 2. Minimum, maximum and mean PAH concentration values in SHD from 18 studies.

PAH	N	Minimum ($\mu g\ g^{-1}$)	Maximum ($\mu g\ g^{-1}$)	Arithemtic Mean ($\mu g\ g^{-1}$)	SEM[a]	Geometric Mean ($\mu g\ g^{-1}$)
Acenaphthene	115	0.001	1.900	0.115	0.029	0.032
Acenaphthylene	113	0.001	0.520	0.063	0.008	0.026
Anthracene	125	0.005	5.800	0.284	0.070	0.065
Benz[*a*]anthracene	130	0.017	40.000	1.476	0.421	0.241
Benzo[*a*]pyrene	131	0.015	54.000	2.110	0.597	0.285
Benzo[*e*]pyrene	122	0.015	41.000	1.733	0.503	0.286
Benzo[*b*, *k*]fluoranthene	127	0.030	108.000	4.005	1.270	0.570
Benzo[*g,h,i*]perylene	126	0.001	35.000	1.380	0.375	0.252
Chrysene	127	0.036	43.000	1.987	0.528	0.372
Coronene	124	0.001	7.200	0.359	0.076	0.095
Cyclopenta[*c,d*]pyrene	122	0.003	0.620	0.062	0.008	0.034
Dibenz[*a,h*]anthracene	128	0.003	9.000	0.410	0.103	0.082
Fluoranthene	124	0.047	90.000	4.058	1.194	0.588
Fluorene	123	0.004	3.000	0.196	0.045	0.054
Indeno[1,2,3-*c,d*]pyrene	126	0.002	41.000	1.593	0.445	0.255
Naphthalene	114	0.001	42.000	1.175	0.498	0.068
Phenanthrene	124	0.038	43.000	2.343	0.633	0.416
Pyrene	124	0.042	69.000	3.111	0.907	0.490
Total PAHs[b]	112	0.404	554.03	28.335	8.072	4.477
Total LMW[c]	112	0.067	65.94	4.377	1.106	0.768
Total HMW[d]	121	0.335	505.06	22.835	6.587	3.796
Total B2 Carcinogens[e]	126	0.141	268.000	11.673	3.358	1.902

[a]Standard error of the arithmetic mean.

[b]Total PAHs = the sum of the 18 PAHs. The number of observations included in Total PAHs refers to only those where all of the 18 PAHs were measured.

[c]LMW = low molecular weight PAHs having 2-3 rings (Myers et al. 1994).

[d]HMW = high molecular weight PAHs having 4 or more rings (Myers et al. 1994).

[e]PAHs classified as probable human carcinogens by the USEPA (USEPA 2003).

Relationships Between PAH Content and Household Characteristics

The large range in PAH content is likely related to numerous factors that are thought to influence the levels of PAHs in SHD. Household characteristics including cigarette smoking, site location, type of flooring, and socio-economic status were examined to determine their influence on the PAH content of SHD. A paucity of information on socio-economic status and flooring type prohibited statistical investigations of relationships between PAH content and these variables.

Two-way ANOVA (analysis of variance) revealed significant effects ($r^2 = 0.15 - 0.21$, $p<0.01$) of both smoking status and home location on PAH content (i.e., Total PAH, LMW, HMW, or Total B2 carcinogens), but failed to reveal a significant interaction between the home location and smoking status effects ($p>0.15$). A subsequent one-way ANOVA using all the data confirmed a significant relationship ($0.02>p<0.04$) between the PAH content and the presence of smokers. However, separate analyses of the rural and urban data revealed that the relationship is only significant ($0.02>p<0.03$) for urban observations (Figure 1). One-way ANOVA also confirmed a significant relationship ($0.0002>p<0.02$) between the PAH content and house location (i.e., urban or rural) (Figure 2). Details of the ANOVA results for both effects are summarized in Table 3.

The smoking effect (Figure 1) indicates that the PAH content of SHD from urban houses with smokers is 3.4 to 4 times higher than SHD samples from urban houses without smokers. The home location effect (Figure 2) shows that PAH content from houses in urban areas is 3-5 times higher than that collected in rural areas. Subsequent analyses of the deleted studentized residuals from each ANOVA revealed several significant outliers ($p<0.05$). These outliers, all of which had positive residuals, were observations from urban homes in Columbus, OH sampled in 1992 and 1993 (Chuang et al. 1993). The total PAH composition of SHD samples from these homes (287.3 to 554.0 $\mu g\ g^{-1}$) is far higher than the geometric mean total PAH concentration (4.5 $\mu g\ g^{-1}$), and one of the sites (H08) yielded the highest total PAH value in the dataset. The authors of this study commented on the extremely high PAH content of SHD from this home, and noted that the home is located only one-quarter of a mile from a freeway, and road construction was underway during one of the sample collection periods.

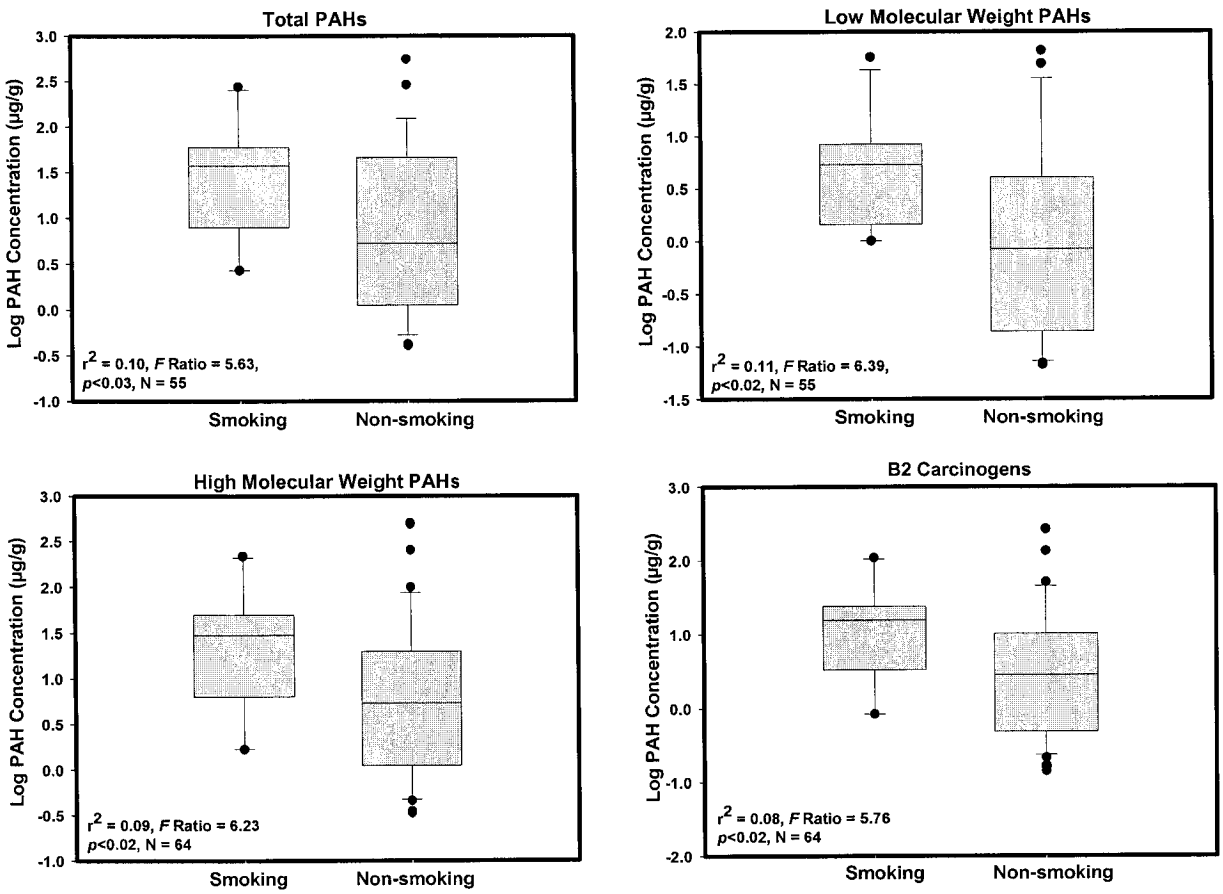


Figure 1. Box and whisker plots showing the effect of cigarette smoking in urban locations on the contamination of SHD with total PAHs, low molecular weight PAHs, high molecular weight PAHs, and total B2 PAH carcinogens.

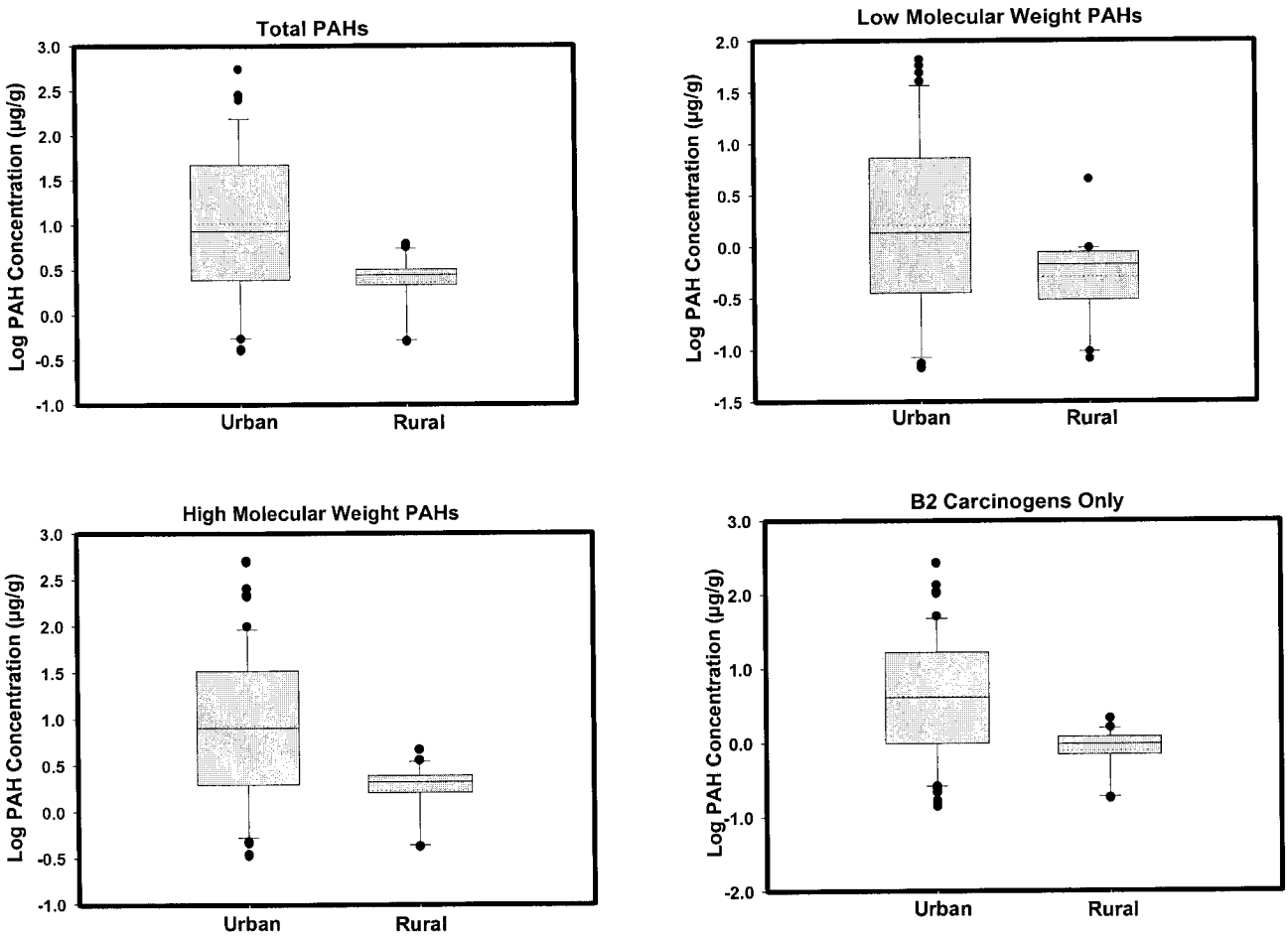


Figure 2. Box and whisker plots showing the effect of house location (urban versus rural) on the contamination of SHD with total PAHs, low molecular weight PAHs, high molecular weight PAHs, and total B2 PAH carcinogens.

Table 3. ANOVA results summarizing the effects of house location and cigarette smoking on the PAH content of settled house dust.

PAHs Considered	Effect	r^2	*F* Ratio	*p*
Total PAHs[a]	Smoking – urban	0.096	5.63	<0.03
Low Molecular Weight PAHs[b]	only (smokers	0.108	6.39	<0.02
High Molecular Weight PAHs[c]	present/not present)	0.090	6.23	<0.02
B2 Carcinogenic PAHs[d]		0.085	5.76	<0.02
Total PAHs[a]	Location	0.132	11.06	<0.002
Low Molecular Weight PAHs[b]	(urban/rural)	0.080	6.34	<0.02
High Molecular Weight PAHs[c]		0.149	14.34	<0.0003
B2 Carcinogenic PAHs[d]		0.156	15.14	<0.0002

[a]18 targeted PAHs (see Table 2 in Chapter 1).

[b]PAHs having 2-3 rings (Myers et al. 1994).

[c]PAHs having 4 or more rings (Myers et al. 1994).

[d]PAHs classified as probable human carcinogens by the USEPA 2003.

The low r^2 values shown in Table 3 (8-11% for the smoking effect and 8-16% for the location effect) indicate that these effects only account for a small proportion of the total variation in the PAH content of SHD. The slightly higher r^2 and least-square means associated with the location effect suggest that location may be more important than smoking in determining the PAH content of SHD. Other investigations of PAHs in SHD have also noted differences between rural and urban (inner city) houses, with SHD from urban homes having markedly higher PAH concentrations (Chuang et al. 1999). In terms of smoking, a pilot study of eight homes in Columbus, OH, noted that although the PAH content of SHD tended to be higher in the smokers' homes, smoking did not appear to be a primary determinant of PAH content in dust (Chuang et al. 1995).

The weak effects of both smoking and location suggest that the PAH content of SHD is largely accounted for by other factors. The inability to account for the effects of flooring type, and socio-economic status has already been noted. Some of the additional variability in PAH content is almost certainly attributable to a lack of a standardized dust collection protocol. For example, studies that employed the HVS3 sample collection method will likely contain the finer particles between 5 and 10 μm (Chuang et al. 1994) and this will certainly affect the measured PAH content of SHD (Lewis et al. 1999). Moreover, variations in the deposition time between the last cleaning and the sample collection time can introduce variations in the chemical and physical properties of SHD. For a given deposition rate (mg m^{-2} d^{-1}), the size of the collected SHD sample will depend on the interval between the last cleaning and the sampling time. This should not adversely affect PAH content measurements unless the concentrations drop below the analytical detection limit. However, the time interval between last cleaning and sampling could affect PAH determinations if contamination of the settling dust particles is temporally variable. Although several studies have shown that the concentration of suspended particulate matter in indoor air and the deposition rate of house dust is temporally variable and dependant on the magnitude, frequency and nature of household activities (e.g., cooking, cleaning, movement) (Thatcher and Layton 1995; Abt et al. 2000; Ferro et al. 2004), no studies have rigorously investigated temporal variability in the chemical content of SHD. Thus, for analyses of toxic substances, including mutagens and carcinogens, in SHD it is important to select a deposition period that provides sufficient sample for analysis, and integrates the temporal variability in

deposition rate and contamination. The method recently published by the German Society of Engineers (VDI 2001) recommends collection of 7-day time-integrated samples of settled dust (i.e., 7-day interval between thorough cleaning and sample collection).

Contribution of PAHs to the Mutagenicity of Settled House Dust

PAH concentration data and published mutagenic potency values for each PAH were employed to calculate the predicted contribution of measured PAHs to the *Salmonella* mutagenic potency of SHD. The calculation assumed that the final mutagenic hazard of a given SHD sample containing a PAH mixture is the sum of that expected from each of the identified PAHs (i.e., the effects are additive). This assumption is supported by several published studies (Pfeiffer 1975; Schmahl et al. 1975; Nesnow et al. 1998; White 2002). For example, White showed that when PAH concentrations are low (<1 μg per assay ml) the total genotoxicity of PAH mixtures is largely determined by the sum of the effects from each of the mixture components (i.e. they are additive) (White 2002). Of the 18 PAHs targeted in the published dust studies, 14 are known to exhibit *Salmonella* mutagenic activity. The *Salmonella* mutagenic potency for each PAH and the predicted mutagenic potency for SHD extracts are summarized in Table 4.

The results obtained reveal that the commonly measured PAHs listed in Table 4 can be expected to contribute approximately 230 revertants per gram of dust to *Salmonella* TA98 mutagenicity, and 301 revertants per gram of dust to *Salmonella* TA100 mutagenicity. These potency values, attributable only to the listed PAHs, can be compared to the study by Roberts et al. 1987. The predicted potency values (<300 revertants $gram^{-1}$) are far lower than the measured potency values for actual dust extracts (1000-7000 TA98 revertants $gram^{-1}$). Despite a paucity of mutagenicity measurements on actual samples of SHD, these results indicate that the measured PAHs are unable to account for more than 3-23% of the mutagenic hazard. Nevertheless, the presence of PAHs in SHD, and their carcinogenicity, is certainly cause for concern.

Table 4. Predicted *Salmonella* mutagenic potency of settled house dust based on the potency and mean concentration of selected PAHs.

PAH	Mutagenic Potency[a] +S9 (rev μg^{-1})		Dust PAH Concentration ($\mu g\ g^{-1}$)[h]	Predicted Mutagenic Potency (rev g^{-1})	
	TA98	TA100		TA98	TA100
Acenaphthene	NM	NM	0.032	-	-
Acenaphthylene	NA	NA	0.026	-	-
Anthracene	NM	0.243[b]	0.065	-	0.016
Benz[*a*]anthracene	56[c]	51.2	0.241	13.5	12.4
Benzo[*a*]pyrene	488	396	0.285	139	113
Benzo[*e*]pyrene	14.2	12.9	0.286	4.05	3.69
Benzo[*b,k*]fluoranthene[i]	60[c]	145[d]	0.570	34.2	82.4
Benzo[*g,h,i*]perylene	7.52	6.52	0.252	1.90	1.64
Chrysene	0.516	81.6	0.372	0.192	30.3
Coronene	33.1[e]	3.00[e]	0.095	3.15	0.286
Cyclopenta[*c,d*]pyrene	470[f]	523[f]	0.034	16.0	17.6
Dibenz[*a,h*]anthracene	39.0[c]	43.6	0.082	3.18	3.56
Fluoranthene	17.6	13.8	0.588	10.3	8.11
Fluorene	NM	NM	0.054	-	-
Indeno[1,2,3-*c,d*]pyrene	NA	79.1[g]	0.255	-	20.2
Naphthalene	NM	NM	0.068	-	-
Phenanthrene	1.38	1.20	0.416	0.576	0.499
Pyrene	8.32[e]	14.3[e]	0.490	4.07	7.01
			TOTAL:	230	301

NM = Not mutagenic in *Salmonella* mutagenicity assay. NA= Data not available.

[a]Defined as the initial slope of the dose-response curve. Potency data taken from (White and Rasmussen 1996) except where indicated: [b](Mortelmans et al. 1986) [c](Nagai et al. 2002), [d](Kubo et al. 2002), [e](Sakai et al. 1985), [f](Eisenstadt and Gold 1978), [g](Rice et al. 1985). All values were taken directly from the publications except for those marked [e] and [g] which were calculated by plotting the concentration response data and conducting linear regression analysis of the linear portion of the dose-response curve.

[h]Geometric means of PAH data from 18 studies (see Table 2 in Chapter 1 and Appendix II).

[i]The potency value for benzo[*b,k*]fluoranthene was derived from the mean of the individual potency values for benzo[*b*]fluoranthene and benzo[*k*]fluoranthene.

Cancer Risk Assessment of PAHs in Settled House Dust

The previously compiled PAH concentration data were used to assess excess lifetime cancer risk in preschool aged children from non-dietary exposure to carcinogenic PAHs in SHD. The following equations were used to calculate excess lifetime cancer risk (Masters 1991). Equation (1) assesses the average lifetime daily dose.

(1) $$\textbf{Lifetime Average Daily Exposure Dose} = \frac{\textbf{C x IR x EF}}{\textbf{BW}}$$

Where:

C = Concentration of carcinogenic PAHs in the dust, in mg g^{-1}. The exposure calculations used the 5th percentile, geometric mean, median, and 95th percentile of the collected dust concentration values (see Table 5).

IR = Ingestion Rate: The amount of dust consumed via non-dietary ingestion in grams per day. The value used was 0.1 g d^{-1} (Hawley 1985).

EF = Exposure Factor: The fraction of an average person's lifetime that is occupied by the exposure period. These analyses investigated a range of exposure factors representing various weekly exposure periods up to the age of 5 (i.e., preschool years between birth and 5th birthday).

BW = The average body weight, in kg. A standard value of 13 kg was used (Health Canada 1995).

Equation (2) uses the average lifetime daily dose, the cancer slope factor and potency equivalency factors to calculate the excess lifetime cancer risk from exposure to carcinogenic PAHs in SHD.

(2) $$\textbf{Lifetime Cancer Risk} = \sum_{i=1}^{n} \textbf{Lifetime Average Daily Exposure Dose}_i\ \textbf{(mg kg}^{-1}\ \textbf{d}^{-1}\textbf{) x Slope Factor (mg kg}^{-1}\ \textbf{d}^{-1}\textbf{)}^{-1}\ \textbf{x Potency Equivalency Factor}_i$$

for B2 PAHs 1 through n

Where:

The Slope Factor is the estimate of the probability of a response occurring per unit intake of the PAH over a lifetime. For these analyses, an oral slope factor for benzo[*a*]pyrene of 7.3 was used (USEPA 2003). The Potency Equivalency Factors are conversion factors used to express the potency of various PAHs in terms of benzo[*a*]pyrene equivalents (see Table 5).

Table 5. PAH concentrations and PAH Potency Equivalency Factors (PEF) used to calculate the excess lifetime cancer risks associated with non-dietary ingestion of carcinogenic PAHs in house dust by preschool aged children.

Carcinogenic PAH	**PAH Concentration in Dust (μg g^{-1})**				**PEF**[†]
	5th Percentile	**Geometric Mean**	**Median**	**95th Percentile**	
Benzo[*a*]pyrene	0.023	0.285	0.195	13.000	1
Benz[*a*]anthracene	0.026	0.241	0.172	5.100	0.1
Benzo[*b,k*]fluroanthene	0.046	0.570	0.402	15.000	0.1
Chrysene	0.046	0.372	0.270	7.200	0.01
Indeno[1,2,3-*c,d*]pyrene	0.032	0.255	0.162	6.900	0.1
Dibenz[*a,h*]anthracene	0.012	0.082	0.058	1.800	5
Total carcinogenic PAHs	0.244	1.902	1.302	44.000	

[†] Potency Equivalency Factors taken from (Collins et al. 1998) except for dibenz[*a,h*]anthracene, which was obtained from (Nisbet and LaGoy 1992).

With the exception of chrysene, only PAHs classified as IARC 2A or 2B carcinogens were included in the analyses. Although chrysene is classified as a class 3 carcinogen by IARC (limited evidence of carcinogenicity), it has been classified by the USEPA as a probable carcinogen (i.e., B2) and was therefore included. Naphthalene was excluded from the risk assessment calculations due to the lack of consensus regarding carcinogenic hazard by an NTP working group, and the designation of *not classifiable* by the USEPA (National Toxicology Program 2002; USEPA 2003) (see Table 5 in the General Introduction). Table 6 contains a summary of the assumptions for the risk assessment calculations.

Table 6. Assumptions for assessment of excess cancer risk due to non-dietary exposure of PAHs in settled house dust by preschool aged children.

Parameter	**Assumptions and Limitations**
Dust Contamination	The observed PAH concentrations in SHD are representative of the dust to which children are routinely exposed
	The PAH concentration is constant across time and space
Exposure	Exposures occur only before the age of 5
	Exposures occurs only in the home
	Ingestion of dust occurs only during waking hours
	The daily ingestion rate is constant across time, space and subject
	The collected dust samples are representative of the ingested material
Bioavailability	The PAHs in dust samples are absorbed and bioavailable to the same extent as in food (i.e., the slope factors are the same for dietary and non-dietary ingestion routes)

Figure 3 illustrates the relationship between the calculated excess lifetime cancer risk and the exposure rate (hrs/week) for several levels of PAH content derived from the collected data (i.e., 5th percentile, median, etc.). The results indicate that for the 5th percentile and median contamination levels even very high exposure rates do not yield excess cancer risk values that are appreciably above 1 x 10^{-6}. Only the extreme PAH levels denoted by the 95th percentile yield excess cancer risks appreciably above 1 x 10^{-6} for any exposure rate. At an exposure rate of 50 hours per week, the calculated excess cancer risk is approximately 28 x 10^{-6}. This value rises to more than 50 x 10^{-6} at exposure rates greater than 88 hours per week. However, exposures of 88 hours per week may not be likely since that would equate to more than 12 waking hours per day of SHD exposure. Closer inspection of the data confirmed that the highest risk values correspond to exposures to the highly contaminated SHD samples (i.e., total PAH >250 μg g^{-1}) collected from homes located in highly urban areas in Columbus, OH (Chuang et al. 1993).

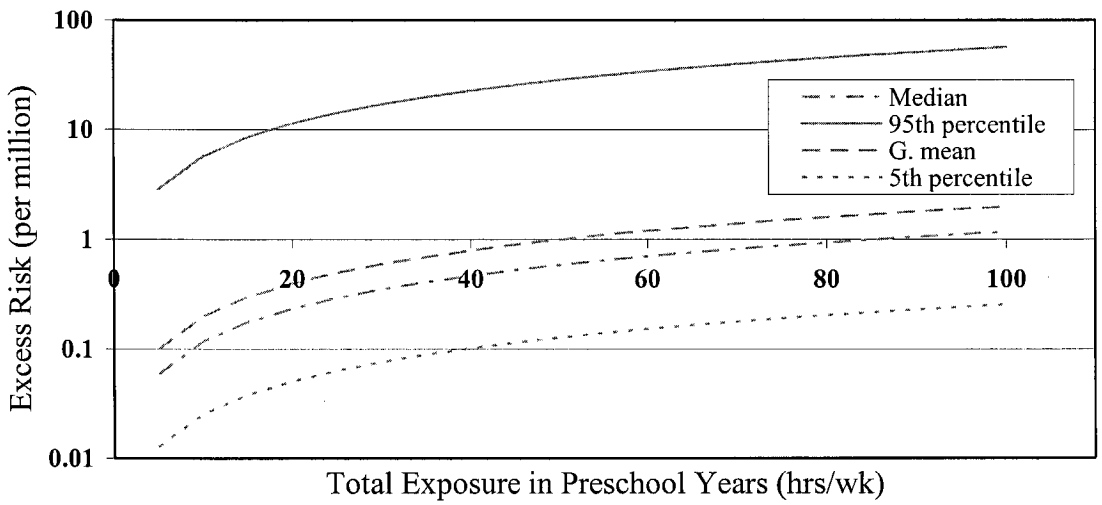


Figure 3. Excess cancer risk due to non-dietary ingestion of B2 PAHs in house dust during preschool years. Correction for enhanced risk due to childhood exposures was not applied.

It should be noted that the risk estimates did not attempt to account for enhanced risk that may be associated with exposures that occur early in life (i.e., younger than 15 years of age). Factors such as incomplete immune system development, rapid growth, enhanced rates of cell division, and hormonal fluctuations are thought to contribute to an enhanced lifetime risk of cancer from early life stage (i.e., childhood) exposures (Anderson et al. 2000; Birnbaum and Fenton 2003). In a recent comprehensive guidance document currently being considered by a review panel of the EPA Science Advisory Board (USEPA 2004), the USEPA has proposed that, for exposures to carcinogens with mutagenic modes of action, risk estimates should be adjusted by a factor of 10 for exposures that occur before the age of 2, and a factor of 3 for exposures that occur between the ages of 2 and 15. In the context of the risk assessment conducted here, the safety factors being considered in the USEPA guidance document would translate into a 10-fold increase in excess lifetime risk for 40% of the 5 year exposure period (i.e., birth to 2nd birthday), and a 3-fold increase in risk for 60% of the exposure period (i.e., 2nd to 5th birthday). This would result in a composite weighting factor for the preschool years of 5.8. If this adjustment is applied to the aforementioned risk estimates, the final values associated with exposures to 95th percentile PAH levels correspond to risk estimates of 146 x 10^{-6} for 50 hours per week (~7 hrs per day) and 260 x 10^{-6} for 88 hours per week (~12.5 hrs per day). Based on USEPA current risk assessment guidelines (USEPA 1990), these values would be approximately 1.5- to 2.6-fold times the range of acceptable excess lifetime cancer risk (i.e., 10^{-6} to 10^{-4}). In terms of Canadian standards, these values would be up to 26 times the maximum acceptable level (i.e., 1 x 10^{-5}) (Health and Welfare Canada 1989).

Thus, the risk assessment calculations indicate that, with the exception of circumstances whereby PAH concentrations are exceptionally high and adjustments are made for early life exposure, regulatory agencies such as the USEPA and Health Canada would deem the excess lifetime cancer risk from preschool, non-dietary ingestion of carcinogenic PAHs in SHD as acceptable.

Numerous investigators have suggested a number of simple precautionary measures to reduce exposures to PAHs and other contaminants in SHD, and consequently reduce the risk of adverse effects. These measures include use of a door mat to reduce the quantity of particle-

bound pollutants tracked in from outside, removal of shoes inside the home, appropriate ventilation, installation of air-filters, as well as frequent and appropriate cleaning of carpets and floors (Lewis et al. 1995).

Conclusions

House dust is a complex mixture of particulate materials of both natural and anthropogenic origin. Although it is commonly seen as a simple nuisance, dust can also play an important role in the exposure of humans to toxic contaminants. Dust can act as a reservoir for semi-volatile organic compounds such as polycyclic aromatic hydrocarbons (PAHs) that adsorb to suspended particulates and are deposited on indoor surfaces (e.g., furniture, floors). The chemical composition, physical structure, and potential hazard of settled house dust is dependent on a wide range of factors that govern the penetration of particulate material from outdoor environments (e.g., soil and suspended particulates), the magnitude and nature of indoor activities that can generate toxic substances (e.g., combustion, cooking), the magnitude and nature of indoor activities (e.g., movement and cleaning), and the magnitude and frequency of dust exposure (e.g., ingestion, inhalation).

Studies available to date indicate that SHD contains a variety of substances that are known to be mutagenic (e.g., PAHs and related compounds). Despite the presence of these mutagenic PAHs, it appears that less than 25% of the mutagenic activity measured on the *Salmonella* reversion assay can be accounted for by the mutagenic PAHs selected for chemical analysis. However, it is readily apparent that there is a paucity of information on the sources, hazards and fate of potential mutagens in SHD. The portion of the S9-activated mutagenicity that cannot be accounted for by the frequently measured PAHs, are potentially attributable to the presence of non-target PAHs, heterocyclic compounds, and PAH derivatives that are not measured during routine chemical analyses.

Additional analyses of published data showed that an urban location, and the presence of cigarette smokers, increases the PAH content of SHD. However, the detected empirical effects of home location and cigarette smoking are weak, and the identification of other factors (e.g., flooring type, season, deposition rate, ventilation, socio-economic status) that may affect the PAH content of SHD seems a promising area for further research. Moreover, further research investigating the temporal variability of dust contamination, the sources and fate of PAHs in

SHD, and the size spectrum of resuspended particles, would improve the ability to collect representative samples.

The estimated excess lifetime cancer risks from non-dietary ingestion of PAHs in settled house dust by preschool aged children appears to be predominantly in the range that is acknowledged as acceptable by regulatory agencies such as the USEPA (i.e., $10^{-6} - 10^{-4}$) and Health Canada (i.e., $10^{-6} - 10^{-5}$). Substantially elevated risk estimates in the range $1.5 - 2.6 \times 10^{-4}$ correspond only to situations where the PAH content is at or beyond the 95^{th} percentile, and the risk estimates are adjusted for enhanced susceptibility at early life stages. More detailed investigations of high PAH SHD samples from inner city homes; including source apportionment of carcinogenic PAHs, is an area that requires further attention. Moreover, additional research should continue to address issues of early life stage susceptibility and rates of childhood exposure to carcinogens in SHD. More precise assessments of childhood exposure rates via non-dietary ingestion, inhalation and dermal adsorption, as well as information on adsorption rates, would permit more accurate and thorough characterizations of the carcinogenic hazards posed by PAHs and other substances in SHD.

Chapter 2

The Mutagenic Hazards of Polycyclic Aromatic Hydrocarbons (PAHs) in Settled House Dust from Ottawa Homes

Introduction

Although it is well known that dust contains many chemical contaminants, the hazards (i.e., toxicity) associated with dust as a complex mixture are less well understood. Preliminary data suggests that SHD can pose a mutagenic hazard (Roberts et al. 1987), however the extent of the mutagenicity and the nature of the mutagens in SHD are currently unknown. Although PAHs are likely responsible for a portion of the mutagenic hazard of the dust, compounds such as nitroarenes and aromatic amines are also suspected of contributing to the mutagenicity. Nitroarenes and aromatic amines both occur in complex mixtures, and as products of combustion, they are considered to be ubiquitous in the environment (Felton and Knize 1991; IPCS 2003). Many of these compounds are potent mutagens (Mermelstein et al. 1981; Felton et al. 1994).

The health effects associated with exposure to SHD and its associated contaminant load are difficult to assess. Far less work has been done to characterize exposure to contaminants in SHD as compared to air, water or food. However, researchers have highlighted the importance of including dust as an exposure pathway in risk assessments. Moreover, it has been emphasized that risks to health will likely be underestimated unless exposure to SHD is included in assessments (Roberts et al. 1991). The importance of integrating dust ingestion into health assessments is demonstrated by Davies et al. who observed that the surface loading of lead in carpet dust is the single best predictor of the concentration of lead in children's blood (Davies et al. 1990).

In this study, the *Salmonella* Mutagenicity Test is used to test the mutagenic hazard of SHD collected from homes in Ottawa (Canada). Several diagnostic test strains are used to characterize the mutagenic activity of the dust extracts. TA98 is used to detect frameshift mutations while TA100 and TA102 are used to detect base pair mutations, with TA102 being particularly sensitive to oxidative damage (Isono and Yourno 1974; Barnes et al. 1982; Levin et al. 1982). The newer diagnostic strains YG1041 and YG1042 are also used. Based on TA98 and TA100 respectively, these newer strains have enhanced sensitivities towards nitroarenes and

aromatic amines (including N-containing heterocyclics) (Hagiwara et al. 1993). This enhanced sensitivity is due to the presence of plasmids which carry the O-acetyltransferase (i.e., OAT) and classical nitroreductase (i.e., cNR) genes. These enzymes are responsible for metabolizing nitroarenes and aromatic amines to their mutagenic active form. Thus, a higher revertant rate on YG1041 and YG1042 indicates the presence of aromatic amines and nitroarenes in the tested samples.

The dust extracts are also evaluated for PAH content, as well as the content of various heterocyclic amines. Empirical analyses are undertaken to identify determinants of dust contamination and/or mutagenic hazard. Finally, a risk assessment is conducted to evaluate the excess lifetime cancer risks associated with non-dietary ingestion of PAHs in SHD during childhood.

Materials and Methods

Dust Sample Collection

Between November 2002 and March 2003, Health Canada conducted an indoor air quality study of homes located in Ottawa, the capital city of Canada (Zhu et al. 2005). Located in south-eastern Ontario with a population of 774,072, Ottawa's largest industry sectors are public administration, scientific and technical services, retail trade, and health care (Statistics Canada 2002). A two stage stratified sampling process was used to randomly select homes for participation in the study. In the first stage, the city of Ottawa was divided into three geographical areas based on 2001 census data from Statistics Canada (Statistics Canada 2002): urban core, urban fringe and rural fringe. In each of these geographical areas, smaller geographic units known as dissemination areas (DA) were selected by simple random sample. Thirty-one DAs were selected in the urban area, 4 in the urban fringe and 3 in the rural fringe, representing approximately 3-4% of the possible DAs for each area.

In the second stage, each DA was visited and characterized in terms of the number of houses and apartments it contained. Letters were sent to 306 randomly selected dwellings (196 homes and 110 apartments). Seventy-five homeowners agreed to participate in the study resulting in a response rate of 38%. However, none of the apartment dwellers contacted agreed to participate, reducing the overall response rate to 25%. Although not part of the random selection process, one additional home (house code = A001) was also included in the study.

During the indoor air quality study, each study participant was asked for his or her vacuum cleaner bag. The bags were removed from the vacuum cleaner, placed in zip-seal plastic bags (Fisher Scientific, Ottawa, CAN) and transported to the laboratory. In addition, all participants answered an intensive questionnaire that was designed to collect information on the household and activities that might affect chemical loading. The survey data relevant to the present study are listed in Appendix III.

Dust Preparation

Following collection and transportation to the lab, the vacuum cleaner bags were stored in the freezer at -20°C. Prior to sieving, the bags were taken out of the freezer and allowed to defrost overnight in a fume hood. The dust was removed from the vacuum cleaner bags using large forceps and placed into a USA Standard Testing Sieve, ASTM E-11 Specification, with a 150 μm opening. The dust was then shaken through the sieve using an AS200 Digit Analytical Sieve Shaker (Retsch GmbH & Co. KG, Haan, Germany). The shaker was run at an amplitude of 80% (20mm) for 10 minutes. After allowing the dust to settle, the collection pan was removed and a new pan was put underneath the sieve. The sieve was placed back on the shaker for an additional 5 minutes of sieving. Any visible hairs were removed from the first collection pan using tweezers and/or a paint brush. The dust was then transferred to a crystallizing dish. The particles that did not pass through the sieve (>150 μm) were collected in a plastic bag. The dust from the crystallizing dish was then resieved on the vibratory sieve shaker for an additional 3 minutes. Any dust particles that did not pass through the sieve were added to the plastic bag. The sieved dust was transferred into a glass jar and the weight of the sieved dust recorded. The jars were sealed with Teflon tape and stored at –20°C. Of the 75 dust samples that were collected, 52 samples contained sufficient dust for chemical and mutagenic analyses.

Extraction

Approximately 3 g of each of the 52 dust samples were extracted with dichloromethane (DCM) and hexane (ACS grade, Fisher Scientific, Ottawa, ON, CAN) (1:1) using an ASE 200 Accelerated Solvent Extractor (ASE) (Dionex, Oakville, ON, CAN). The ASE was run at 175°C and 1500 PSI with a preheat time of 7 minutes, a heat time of 5 minutes and a static extract time of 10 minutes. The extracts were collected in vials containing approximately 5 g of sodium sulphate (ACS grade, EMD Chemicals, Gibbstown, NJ). Empty cells were included with each run as extract blanks.

Following extraction, the samples were filtered through 0.45 μm Whatman syringe filters (Fisher Scientific Ltd.) and transferred to TurboVap® tubes. The samples were reduced under nitrogen to 0.5 ml using a TurboVap® solvent evaporator at 30°C and 10 psi ultra-pure nitrogen gas. The sample volume was then brought up to 2 ml with DCM and transferred to 4 ml glass

vials. Gel permeation chromatography (GPC), using a Waters Autopurification system with tandem Waters Envirogel™ GPC columns (19x300 mm and 19x150 mm, styrene/divinylbenzene,) (Waters, Mississauga, ON, CAN), was used to remove high molecular weight compounds. The GPC was performed and calibrated according to EPA Method 3640a (USEPA 1986). Eluted samples were reduced under nitrogen to 0.5 ml and then brought up to 2 ml in dimethylsulfoxide (DMSO, ACS grade, Sigma-Aldrich Canada Ltd., Oakville, ON, CAN). Five fold and 50 fold dilutions were made of each extract prior to mutagenicity testing.

Fractionation

Following ASE extraction and GPC clean up, four of the dust extracts were fractionated into bases and base/neutral compounds, using acid-base liquid-liquid extraction. To separate the basic compounds, each sample in DCM was combined with 50 ml of 6N HCl in a separatory funnel and gently shaken. The organic portion was removed and washed two more times with 50 ml HCl. The three aqueous portions were then combined in a separatory funnel and the pH was adjusted to 10 with 10N NaOH. 50 ml of DCM was added to the aqueous portion in the separatory funnel, and gently shaken. The organic portion was removed and the aqueous portion was washed two more times with 50 ml DCM. The organic portions were combined and evaporated to 0.5 ml under nitrogen. The final extract was then brought up to 2 ml in DMSO.

To isolate the base/neutral compounds, each sample was combined with 50 ml of 5N NaOH in a separatory funnel and gently shaken. The organic portion was removed and washed two more times with 50 ml NaOH. The organic portions were then combined in a round bottom flask and approximately 100 g of sodium sulfate was added to remove any remaining aqueous components. The extracts were then evaporated to 0.5 ml under nitrogen and then brought up to 2 ml in DMSO.

Mutagenicity Testing

Chemicals, Reagents, Laboratory equipment

Reagents for the *Salmonella* Mutagenicity Test were prepared according to Standard Operating Procedures defined by the Environmental Carcinogenesis Division of the USEPA in

Research Triangle Park, North Carolina. All water used for making the reagents was purified using a Milli-Q Ultrapure Water Purification System (Fisher Scientific Ltd.).

The 50X Vogel-Bonner Medium E (VBME) salt solution consisted of 10 g magnesium sulphate (0.04M), 100 g citric acid monohydrate (0.5M), 500 g potassium phosphate dibasic anhydrous (2.9M) and 175 g sodium ammonium phosphate (0.8M) per litre of water. The histidine/biotin solution consisted of 97.6 mg d-biotin (0.4 mM) and 52.5 mg of L-histidine-HCl (0.2 mM) per litre of water. The dextrose solution consisted of 300 g dextrose per litre of water (30% w/v). The chemicals used for making the VBME, histidine/biotin solution, and dextrose were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, CAN.) The VBME solution and dextrose were sterilized by autoclaving for 20 minutes at 121°C, while the histidine/biotin solution was sterilized by filtration through a 0.2 μm Nalgene 50 mm bottle top filter (Fisher Scientific Ltd.).

Minimal glucose plates consisted of 45 g of agar (4.5% w/v) (Fisher Scientific Ltd.), 200 ml of dextrose (20% w/v), 60 ml of 50X VBME (6% v/v), and 30 ml of histidine/biotin (3% v/v) per litre of water. The agar and water were combined and sterilized at 121°C for 20 minutes using an Integra Bioscience Mediaclave (Mandel Scientific Company Inc., Guelph, ON, CAN). Following cooling to 50°C, the dextrose, histidine/biotin and VBME were added and the solution was mixed. Plates were poured using Technomat Line plate pourer (Mandel Scientific Company Inc.) set at a volume of 26 ml per plate and a speed of 75%.

Top agar consisted of 6 g agar (0.6% w/v) (Fisher Scientific Ltd.) and 5 g of NaCl (0.5% w/v) per litre of water. The top agar was dissolved and transferred to 10 ml borosilicate glass culture tubes using a Calibrex 20 bottle top dispenser (VWR, Mississauga, ON, CAN). The tubes were autoclaved for 20 minutes at 121°C and then placed in a heating block set at 53°C for the duration of the experiment.

The S9 metabolic activation mixture consisted of 2 ml (2% v/v) of microsomal salt solution (0.4 M magnesium chloride and 1.65 M potassium chloride (Sigma-Aldrich Canada Ltd.)), 141 mg (4.1M) of glucose-6-phosphate (monosodium salt, Sigma-Aldrich Canada Ltd.),

306 mg (3.9M) of nicotinamide adenine dinucleotide phosphate disodium salt (NADP, Roche Diagnostics, Laval, QC, CAN), 50 ml (50% v/v) of 0.2 M phosphate buffer pH 7.4, 43 ml (43% v/v) of water, and 5 ml (5% v/v) of Aroclor 1254 induced rat liver S9 (protein levels 35.7- 43.5 mg ml^{-1}, Moltox Inc., Boone, NC, USA) per 100 ml of mixture.

The Salmonella *Mutagenicity Test*

The *Salmonella* Mutagenicity Test was carried out according to the plate incorporation test methods specified in Maron and Ames (1983) and Mortelmans and Zeiger 2001 (see also Figure 1 in the General Introduction). Minor modifications to the methods included adding the histidine and biotin to the bottom agar, as opposed to the top agar, and using nutrient agar for the master plates.

Five strains of *Salmonella typhimurium* were used to test the dust samples. The strain TA98 was used to detect frameshift mutations, while TA100 and TA102 were used to detect base pair mutations, with TA102 being particularly sensitive to oxidative damage (Isono and Yourno 1974; Barnes et al. 1982; Levin et al. 1982). Aside from these standard tester strains, the newer diagnostic strains YG1041 and YG1042 which have enhanced sensitivity to nitroarenes and aromatic amines were also used (Hagiwara et al. 1993). TA98, TA100 and TA102 were either purchased from Moltox Inc. (Boone, NC, USA) or donated by Dr. Iain Lambert (Carleton University, Ottawa, ON, CAN). YG1041 and YG1042 were donated by Dr. Takehiko Nohmi (National Institute of Health Sciences, Tokyo, Japan). Frozen permanent cultures were made for each strain by combining 150 μl of culture with 850 μl of glycerol in 1 ml cryogenic vials. The vials were stored at -80°C. Master plates were made for TA100, TA102 and YG1042 by streaking cultures onto nutrient agar plates supplemented with the following antibiotics: 25 μg ml^{-1} ampicillin (TA100), 2 μg ml^{-1} tetracycline (TA102), and 25 μg ml^{-1} each ampicillin and kanamycin (YG1042). All plates were incubated at 37°C for 24-48 hours. The TA100 and YG1042 master plates were stored for up to one month in the refrigerator, while the TA102 master plates were stored for two weeks.

For each experiment, tester strains were grown overnight in 50 ml flasks containing 20 ml of Oxoid nutrient broth No. 2 (Oxoid Inc., Ottawa, ON, CAN), and either 100 μl of culture from thawed frozen permanents (TA98 and YG1041), or a single colony from a master plate (TA100, TA102, YG1042). The flasks were then placed in a Gyrotory Water Bath Shaker (Model G76, New Brunswick Scientific, Edison, NJ, USA) at 37°C for 16 hours at 200 RPM.

The mutagenicity testing was carried out in a Microzone BM6-2B-49 laminar flow hood. A negative (solvent) control and positive control were included in all testing. Positive controls consisted of 2-aminoanthracene (CAS# 613-13-8), mitomycin C (CAS# 50-07-7), 2-nitrofluorene (CAS# 607-57-8), daunomycin (CAS# 20830-81-3), and methyl methanesulfonate (CAS# 66-27-3) (Moltox Inc., Boone, NC, USA). Each positive control chemical was prepared as needed and stored at 4°C between experiments. Five concentrations of each dust extract, ranging from 0.5-50 mg equivalent of dust per plate, were tested with each *Salmonella* strain. Each dose was tested in triplicate. All dust extract samples were tested with and without S9 metabolic activation (hereafter referred to as S9).

Following testing, all plates were inverted and placed in an incubator at 37 °C for 72 hours. Revertant colonies were counted using a Protocol RGB Colony Counter (Synbiosis, Frederick, MD, USA). Each plate was counted twice and the results were averaged. Test results were considered to be positive if a dose-related increase in the number of revertants was observed and the number of revertants was at least double the background rate for two test concentrations. The mutagenic potency of the dust was calculated from the initial slope of the linear portion of the dose-response curve using least squares linear regression analyses using Microsoft Excel 2002.

Chemical Analyses

Heterocyclic Aromatic Amine (HAA) Analyses

Nine dust extracts and one blank were sent to Lawrence Livermore National Laboratory (LLNL) in Livermore, California for the analyses of HAAs. These samples were selected based on their range of mutagenic activity demonstrated during the *Salmonella* mutagenicity testing. The dust extracts were shipped in 1 ml volumes of 30% acetone and 70% hexane. Following

receipt, the samples were placed in a 50°C water bath and the carrier solvents were evaporated under nitrogen. The dried samples were combined with a 50:50 mixture of methanol and HPLC mobile phase (95% water and 5% acetonitrile) and then filtered. Extracts were analyzed by reverse phase chromatography using a Waters HPLC equipped with a Tosoh BioScience ODS-80TM TSK GEL column (4.6 mm x 25 cm with 5 μm particle size), and photodiode array UV and fluorescence detectors. Each sample was analyzed for 17 mutagenic heterocyclic amines including DMIP, Glu-P-2, IQ, IQx, 1,5,6-TMIP, MeIQ, Glu-P-1, 8-MeIQx, 4-MeIQx, IFP, 7,8-DiMeIQx, 4,8-DiMeIQx, Tryp-P-2, PhIP, Tryp-P-1, AaC and MeAaC, and the 2 co-mutagens harman and norharman (Table 1). Prior to analyzing the dust extracts, standards of each of the heterocyclic amines were run individually to determine retention times and to establish a library of UV spectra characteristics. For each dust sample, data were collected over a 40 minute time period. Between samples, the column was allowed to re-equilibrate for 10 minutes. Following each sample run, the chromatogram peaks were compared with the retention times and characteristic spectral shapes in the standards' library. The detection limit was approximately 2.5 ng g^{-1}.

Polycyclic Aromatic Hydrocarbon (PAH) Analyses

Three grams of each dust sample were extracted, and forwarded, along with a blank, to the Chemistry Research Division, Safe Environments Program at Health Canada (Ottawa) where the PAH analyses were carried out. The dust extracts were analyzed for 13 PAHs including acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, indeno[1,2,3-*c,d*]pyrene, dibenz[*a,h*]anthracene, and benzo[*g,h,i*]perylene (see Figure 2 in the General Introduction). The samples were analyzed on a Hewlett-Packard 5890 gas chromatograph (GC) equipped with a HP5972 mass selective (MS) detector (Agilent Technologies, Palo Alto, CA, USA), and fitted with a HP-5MS capillary column (30 m x 0.25 mm x 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). The GC temperature conditions were as follows: the initial temperature was held at 50°C for 2 minutes, then ramped 8°C/minute to 300°C for 12 minutes; injection temperature was 270°C; detector temperature was 280°C. One microlitre volumes of the samples or standard solutions were injected in splitless mode. The purge time was 2 minutes. The MS

Table 1. Structures and abbreviated names for 19 heterocyclic aromatic amines commonly measured in environmental samples.

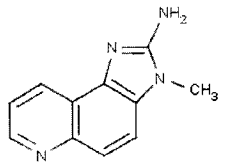
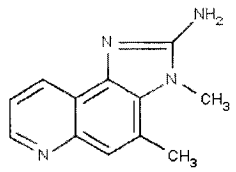
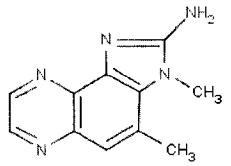
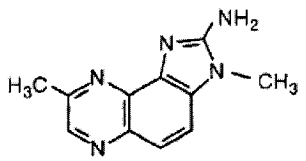
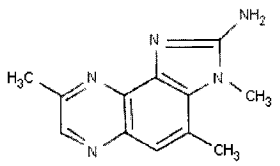
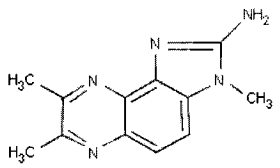
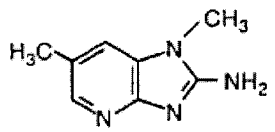
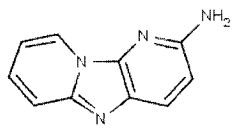
Abbreviation	Full Name	Structure
IQ	2-amino-3-methylimidazo [4,5-f]quinoline	
MeIQ	2-amino-3,4-dimethylimidazo[4,5-f]quinoline	
4-MeIQx	2-amino-3,4-dimethylimidazo[4,5-f]-quinoxaline	
8-MeIQx	2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline	
4,8-DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-f]-quinoxaline	
7,8-DiMeIQx	2-amino-3,7,8-trimethylimidazo[4,5-f]-quinoxaline	
DMIP	2-amino-1,6-dimethyimidazo[4,5-b]pyridine	
Glu-P-2	2-aminodipyrido-[1,2-a:3',2'-d]-imidazole	

Table 1. (continued)

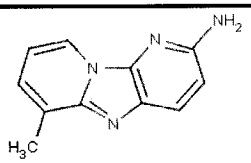
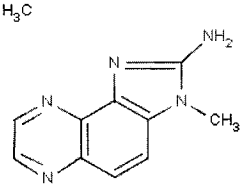
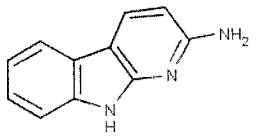
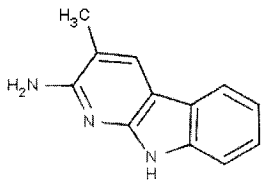
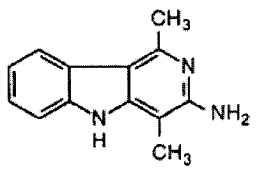
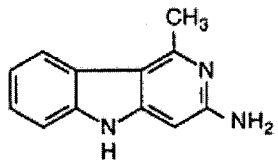
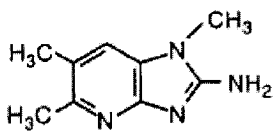
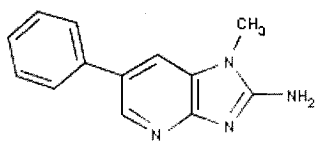
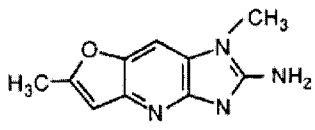
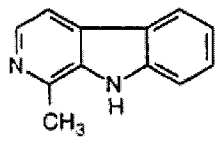
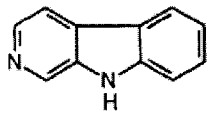
Abbreviation	Full Name	Structure
Glu-P-1	2-amino-6methyldipyrido[1,2-a:3′,2′-d]-imidazole	
IQx	2-amino-3-methylimidazo[4,5-f]-quinoxaline	
AaC	2-amino-9H-pyrido-[2,3-b]-indole	
MeAaC	2-amino-3-methyl-9H-pyrido[2,3-b]-indole	
Trp-P-1	3-amino-1,4-dimethyl-5H-pyrido[4,3-b]-indole	
Trp-P-2	3-amino-1-methyl-5H-pyrido[4,3-b]-indole	
1,5,6-TMIP	2-amino-1,5,6-trimethylimidazo[4,5-b]-pyridine	
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine	
IFP	2-amino-1,6-dimethylfuro[3,2e]imidazo[4,5b]pyridine	

Table 1. (continued)

Abbreviation	Full Name	Structure
Harman	1-methyl-9H-pyrido[4,3-b]-indole	
Norharman	9H-pyrido-[4,3-b]-indole	

was operated in the selected ion monitoring mode. The charge to mass ratios of the molecular ions and fragment ions monitored are listed in Table 2. The first ion (the molecular ion) listed in the table for each PAH was used as the quantification ion.

A calibration standard consisting of a standard solution of 13 PAHs (EPA 525 PAH Mix A, Supelco, PA, USA) and two deuterated PAHs (acenaphthene d_{10} and benzo[*a*]pyrene d_{12}) was run with each batch of samples. Each sample was also spiked with the two deuterated standards (acenaphthene d_{10} and benzo[*a*]pyrene d_{12}). Identification of the PAHs was based on their retention time relative to the calibration standard solution. Quantification of the PAHs was based on the internal standards. MSD Productivity ChemStation (Agilent Technologies 2001) was used to calculate the areas under the peaks and convert the measurements into concentrations. The method detection limit (MDL) and recovery efficiencies for each of the PAHs are listed in Table 3.

Table 2. Mass to charge ratio (m/z) of molecular ions and fragment ions of 13 PAHs and one deuterated PAH targeted for quantification by GC/MS.

PAH	**Mass to Charge Ratio (m/z) of Molecular Ions and Fragments**
Acenaphthylene	152, 151, 76
Fluorene	166, 164, 82
Phenanthrene	178, 176, 89
Anthracene	178, 176, 89
Pyrene	202, 101, 100
Benzo[*a*]anthracene	228, 114, 101
Chrysene	228, 114, 101
Benzo[*b*]fluoranthene	252, 126, 113
Benzo[*k*]fluoranthene	252, 126, 113
Benzo[*a*]pyrene	252, 126, 113
Benzo[*g,h,i*]perylene	276, 138, 137
Indeno[*1,2,3-c,d*]pyrene	276, 138, 137
Dibenz[a,h]anthracene	278, 139, 138
Acenaphthene d_{10}	164,162
Benzo[*a*]pyrene d_{12}	264,132

Table 3. Method detection limits (MDL) and recovery efficiencies for the quantification of 13 PAHs in settled house dust by GC/MS.

PAH	**MDL ($\mu g\ g^{-1}$)**	**Recovery Efficiency (%)**	**Corrected MDL ($\mu g\ g^{-1}$)[a]**
Acenaphthylene	0.006	57.120	0.011
Fluorene	0.009	65.827	0.013
Phenanthrene	0.008	70.065	0.012
Anthracene	0.007	62.697	0.011
Pyrene	0.009	74.662	0.012
Benz[*a*]anthracene	0.015	72.437	0.021
Chrysene	0.019	75.666	0.025
Benzo[*b*]fluoranthene	0.014	72.145	0.019
Benzo[*k*]fluoranthene	0.026	74.345	0.034
Benzo[*a*]pyrene	0.029	57.142	0.051
Indeno[1,2,3-*c,d*]pyrene	0.027	68.140	0.039
Dibenz[*a,h*]anthracene	0.038	70.568	0.054
Benzo[*g,h,i*]perylene	0.023	69.681	0.034

[a] Corrected MDL = MDL/recovery efficiency

Cancer Risk Assessment of PAHs in Settled House Dust

Following quantification of the PAHs, a risk assessment was conducted to evaluate the potential adverse health effects associated with exposure to these contaminants. Specifically, the excess lifetime cancer risks associated with non-dietary ingestion of PAHs in SHD during preschool years were assessed. The same assumptions included in the risk assessment in Chapter 1 were included in this assessment (see Table 6 in Chapter 1). The following equation was used to estimate lifetime risk (Masters 1991):

$$\textbf{(3) Lifetime Cancer Risk} = \sum_{i=1}^{n}\left(\frac{(Ci \times PEFi) \times IR \times EF \times SF \times AF}{BW \times 1000}\right),$$

for B2 PAHs 1 through n

Where:

C = Concentration of each carcinogenic PAH in the SHD samples in $\mu g\ g^{-1}$. The PAHs included in this assessment were: benzo[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), chrysene (CHRY), dibenz[*a,h*]anthracene (DBahA), and indeno[1,2,3-*c,d*]pyrene (I123cdP). All of these PAHs are categorized as probable human carcinogens (B2) based on USEPA classifications (USEPA 2003).

PEF = Potency equivalency factor. These factors are applied to the individual PAH concentrations to express the potency of each PAH in terms of a single PAH, namely benzo[*a*]pyrene. The PEFs were as follows: BaA = 0.1, BbF = 0.1, BkF = 0.1, CHRY = 0.001, I123cdP = 0.1, DBahA = 5. All PEFs were taken from Collins et al. 1998 except for that for DBahA which was taken from Nisbet and Lagoy 1992.

IR = Daily ingestion rate of dust expressed in grams per day. Three ingestion rates were considered in this risk assessment: 0.01, 0.05 and 0.1 $g\ d^{-1}$. Investigators estimate that children ingest between 0.05 and 0.1 grams of dust per day depending on the season and the amount of time spent indoors (Hawley 1985). Both 0.05 and 0.1 $g\ d^{-1}$ are considered to be conservative estimates, which err on the side of greater exposure. Based on studies with tracer elements, other

researchers have suggested that children likely ingest closer to 0.04 g d^{-1} of soil and dust combined (Calabrese et al. 1989). Furthermore, dust is estimated to account for only a quarter of this value (Calabrese et al. 1989; Calabrese 2005). Consequently, a lower ingestion rate of 0.01 g d^{-1} was also used.

EF = Exposure factor. The average proportion of their lifetime that children are exposed to dust via non-dietary ingestion, based on a seventy-year lifespan. Various exposure factors were considered. Seven hours per day was considered an average exposure rate based on the fact that children spend approximately 19-20 hours per day indoors (USEPA 1997; USEPA 2002) and sleep for approximately 12-13 hours per day (USEPA 1997; Iglowstein et al. 2003). It was assumed that preschool aged children would be exposed from birth up to the fifth birthday.

BW = Average body weight, in kg. A standard value of 13 kg was used (Health Canada 1995).

SF = Slope factor expressed in $(mg\ kg^{-1}\ d^{-1})^{-1}$. This is the estimate of the probability of a response occurring per unit intake of the PAH over a lifetime. For these analyses, an oral slope factor for benzo[*a*]pyrene of 7.3 was used (USEPA 2003). Slope factors represent the upper-bound estimate of risk per unit dose for an average population (USEPA 2005).

AF = Adjustment factor. This factor accounts for the fact that exposures are taking place during early life stages when children are more susceptible to the effects of chemical toxins (IPCS 1986). For exposure to carcinogens with a mutagenic mode of action, the USEPA recommends an adjustment factor of 10 for children less than 2 years of age, and an adjustment factor of 3 for children between 2 and 15 years of age (USEPA 2005). Therefore, a composite adjustment factor of 5.8 was used for this risk assessment where exposures occur from birth to 5 years of age.

Data Analyses

All analyses were performed using the SAS System version 8.2 for Windows (SAS Institute 2001). Data analyses were conducted using three sets of data: the mutagenic potency data, the PAH concentration data, and the information contained in the homeowner survey.

Descriptive statistics (e.g., sample size, minimum, maximum, mean) were calculated for each of these data sets individually. Ordinary least-squares linear regression, Pearson correlations, multiple linear regression (stepwise regression), one-way analysis of variance (ANOVA) and discriminant function analyses were employed to analyze these three data sets.

The residual error terms for the linear regression, discriminant functional analysis, and ANOVA models were assumed to be normally distributed, independent, and to have a constant variance (Glantz and Slinker 2001). All mutagenic potency and PAH concentration data were log transformed prior to the data analyses in order to equalize the variance across the range of observations and meet the normality assumptions contained in the statistical models. Similarly, the data for two variables contained in the homeowner survey (vacuum frequency and the number of people living in the house) were log transformed. The Shapiro-Wilk statistic and inspection of normal probability plots were used to assess normality and, by extension, the constant variance of the residuals. In a small number of cases, the residuals were not normally distributed despite log transformation. Significant outliers were identified by calculating the studentized deleted residual (also referred to as the externally studentized residual) for each residual error value (Neter et al. 1990). For data analysis purposes half the method detection limit was substituted for observations where PAH concentrations were below detection (USEPA 1991; PMRA 2003).

Results

Summary of Household Attributes

Fifty-two households from the Ottawa area were included in this study. Each homeowner answered a series of questions aimed at characterizing the attributes of their household (Appendix III). The majority of household occupants classified their home as being located in a quiet residential area (61%). Fewer homes were located in a main residential area (29%), while an even lower number of homes were located in a main commercial (6%) or rural area (4%). Twenty-four percent of all the homes had garages which were attached and had direct entry into the home. Ten percent had attached garages with no direct entry, while 18% did not have a garage or did not describe it in the survey. Most of the households were characterized as non-smoking households (85%). Only 15% of the households contained occupants who smoked, and the median number of cigarettes smoked per day was eight. The primary heating source in most of the homes was natural gas (83%), while oil and electric heat were less common (11% and 4% respectively), and one home did not describe their heating source. Other relevant household characteristics are summarized in Table 4.

Table 4. Summary of the characteristics of the 52 Ottawa homes included in the settled house dust study.

Household Attribute	Range (median)
Number of people living in the home	1 – 9 (3)
Number of cats/dogs inside the home	0 – 3 (0)
Age of the home (years)	0.04 – 101 (29.5)
Percentage of home carpeted	0 – 100 (50)
Vacuum frequency (times per month)	1 – 30 (4)
Carpet shampoo frequency (times per year)	0.2 – 2 (1)

Collection of Household Dust Samples

Dust was collected by volunteer homeowners using ordinary household vacuum cleaners. This collection method provided a convenient means for obtaining time-integrated samples. The weights of the dust in each vacuum cleaner bag were variable, ranging between 109 and 1,158 grams in weight (Figure 1A). Following sieving to obtain particles less than 150 μm in diameter, the samples ranged in weight from 21 to 182 grams (Figure 1B). The sieved weights were, on average, 15% of the full bag weights. The distribution of the weights of the vacuum cleaner bags were positively skewed with more samples being lighter than heavier in weight. Values for skewness were 0.97 and 1.02 for the full bag weight and the sieved bag weight respectively.

Although the sieved and unsieved weights of the vacuum cleaner bags were compared with information in the homeowner's survey, there did not appear to be any correlations between the dust weights and parameters such as the vacuum frequency, the extent of carpeting in the home, the number of people in the home, or the number of cats and dogs in the home. There was, however, a weak significant difference in the total dust weight from homes that had recently used wood fireplaces compared to those that had not (Figure 2). Homes in which the fireplace had recently been used had vacuum cleaner bags that weighed more than 1.5 times the bags from homes where a fireplace had not been used. This suggests that a small portion (13%) of the differences in dust weights may be attributed to increased particulate matter generated by the wood combustion.

Salmonella Mutagenicity Testing of Dust Samples

Negative and Positive Controls

Positive and negative controls were routinely employed to ensure acceptable bioassay function. The spontaneous reversion rates (i.e., the negative control) for each test strain, calculated as the average from all test runs, are listed in Table 5. The number of revertants observed for each strain's positive control, calculated as the average from all test runs, is listed in Table 6.

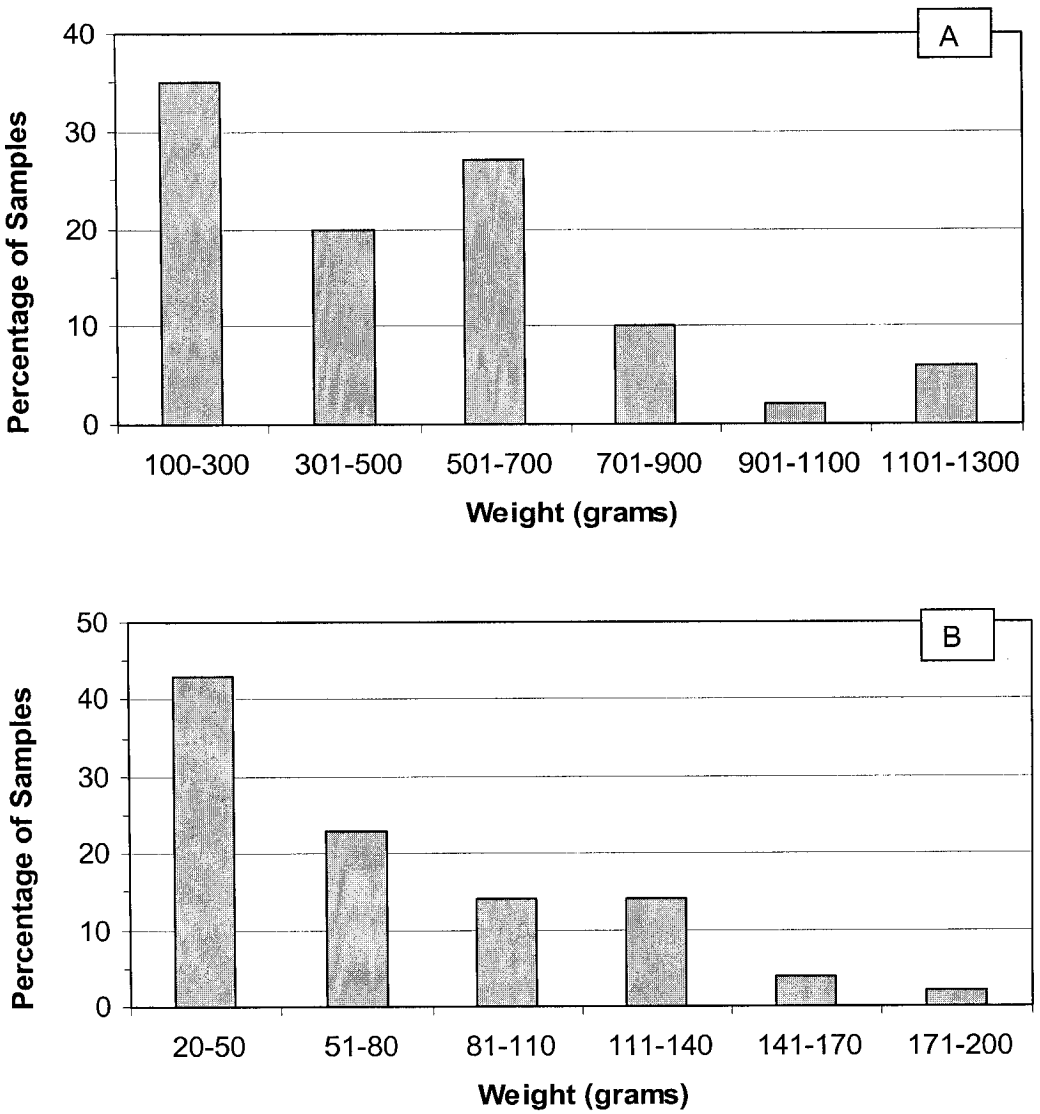


Figure 1. Relative frequency distribution of dust weights in vacuum cleaner bags collected from 51 homes in the Ottawa area. A) Total dust weight before sieving. B) Total dust weight after sieving to particles less than 150 μm in diameter.

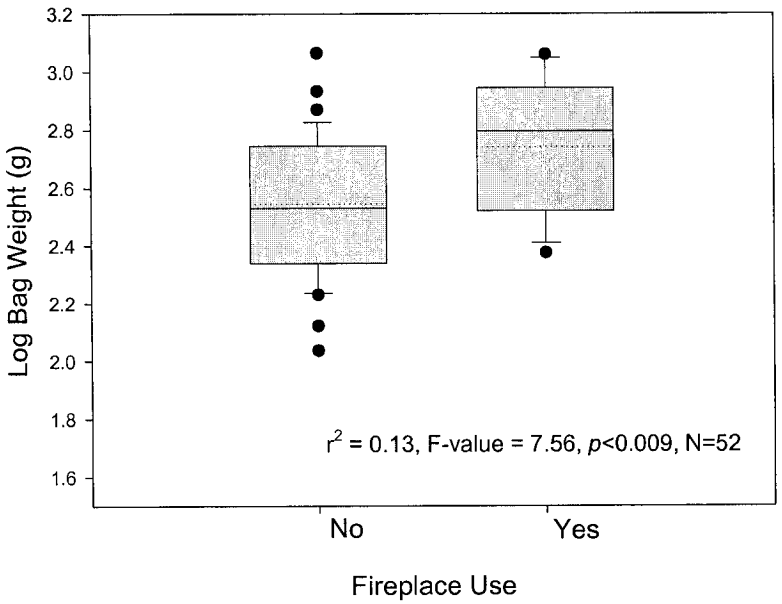


Figure 2. Box and whisker plot showing a significant difference in the total weight of vacuum cleaner bags taken from homes that had recently used wood fireplaces compared to those that had not. The solid line dividing the boxes represents the median weight of the bags, while the dotted line represents the mean weight. The first and third quartiles (i.e., the 25th and 75th percentiles) are represented by the bottom and top horizontal lines of the box respectively. Whisker caps indicate 10th and 90th percentiles, with dots noting suspected outliers.

Table 5. Mean spontaneous reversion rates for five *Salmonella typhimurium* strains used in the *Salmonella* Mutagenicity test.

Strain	**Metabolic Activation**	**Mean Number of Revertants Per Plate (± S.E.M.[a])**
TA98	-S9	24 ±1
	+S9	35 ± 1
TA100	-S9	117 ± 4
	+S9	138 ± 4
TA102	-S9	223 ± 10
	+S9	296 ± 4
YG1041	-S9	32 ± 1
	+S9	49 ± 1
YG1042	-S9	86 ± 2
	+S9	168 ± 8

[a] standard error of the mean

Table 6. Responses of five *Salmonella typhimurium* strains to various positive controls used in the *Salmonella* Mutagenicity Test.

Strain	**Metabolic Activation**	**Positive Control**	**Dose (μg plate^{-1})**	**Mean Number of Revertants Per Plate (± S.E.M.[a])**
TA98	-S9	daunomycin	0.5	747 ± 41
	+S9	2-aminoanthracene	0.5	434 ± 7
TA100	-S9	methyl methanesulfonate	1μl plate^{-1}	1735 ± 57
	+S9	2-aminoanthracene	0.5	573 ± 13
TA102	-S9	mitomycin C	0.5	1012 ± 33
	+S9	2-aminoanthracene	5	1454 ± 20
YG1041	-S9	2-nitrofluorene	0.1	250 ± 14
	+S9	2-aminoanthracene	0.1	1106 ± 30
YG1042	-S9	2-nitrofluorene	3	1251 ± 16
	+S9	2-aminoanthracene	0.5	664 ± 23

[a] standard error of the mean

Mutagenicity Testing of the Whole Dust Extracts

Three grams of each dust sample were extracted and tested for mutagenicity using five different bacterial strains (TA98, TA100, TA102, YG1041, YG1042) in the *Salmonella* Mutagenicity Test. The number of extracts that tested positive is shown in Figure 3 as a percentage of the total number of extracts tested. During the initial phase of testing, all dust extracts were tested with the five *Salmonella* strains. However, TA100 and TA102 were excluded from further testing once it became apparent that the dust extracts were not testing positive with these strains. The number of dust extracts that were tested with the remaining three strains ranged from 41-52, depending on the amount of extract available.

Positive responses were more frequently observed with the frameshift mutation strains (i.e., TA98 and YG1041) as opposed to the base pair mutation strains (i.e., TA100 and YG1042). The YG1041 strain elicited the highest number of positive responses with all but one extract testing positive when metabolic activation was added. A fewer number of extracts tested positive with the strain TA98. More than twice as many extracts tested positive with TA98 +S9 as compared with TA98 -S9. Less than a quarter of the dust extracts tested positive with YG1042. Unlike TA98 and YG1041, testing with YG1042 without S9 revealed more positive responses than with YG1042 with S9.

For each dust extract that tested positive, the mutagenic potency was calculated from the linear portion of the dose-response curve. The individual mutagenic potencies for each dust sample are shown in Appendix IV, and a summary of the mutagenic potencies is shown in Table 7. Dust extracts tested with the enhanced YG strains tended to show higher levels of mutagenic potency than those tested with TA98, suggesting the presence of aromatic amines and/or nitroarenes in the sample. Although only a small percentage of samples tested positive with YG1042 +S9, these samples showed some of the highest levels of mutagenic potency overall. In general, mutagenic potencies tended to be greater when the extracts were tested with metabolic activation than without.

As expected, since the strain YG1041 is derived from TA98, the mutagenic responses obtained through testing with these two strains were significantly related (Figure 4). The

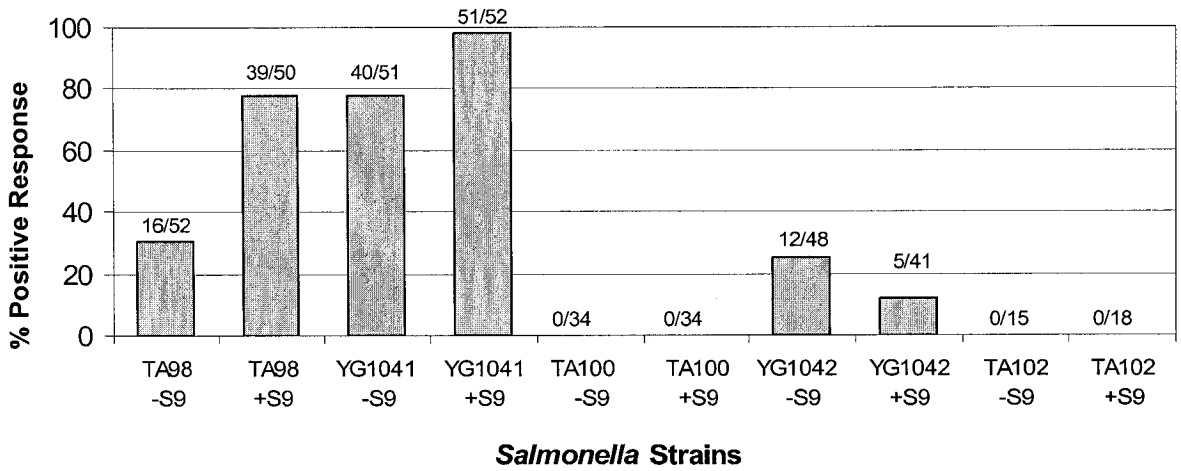


Figure 3. Frequency distribution of the mutagenic responses of dust extracts in the *Salmonella* Mutagenicity Test. The number of positive responses compared to the number of dust samples tested is shown above the bars.

Table 7. Minimum, maximum, and mean *Salmonella typhimurium* mutagenic potencies (revertants gram^{-1}) of house dust extracts.

Strain	Metabolic Activation	Percent Positive[a]	Minimum	Maximum	Arithmetic Mean	S.E.M.[b]	Geometric Mean
TA98	+S9	78	1,620	14,452	4,319	427	3,768
	-S9	31	780	43,283	5,099	2,657	2,293
YG1041	+S9	98	2,622	37,965	15,408	1,203	13,268
	-S9	78	1,287	188,429	11,430	4,803	5,132
YG1042	+S9	12	10,390	55,624	27,264	7,587	23,593
	-S9	25	1,922	16,300	8,429	1,333	7,156

[a] Percentage of samples tested that were considered to be mutagenic.

[b] Standard error of the arithmetic mean.

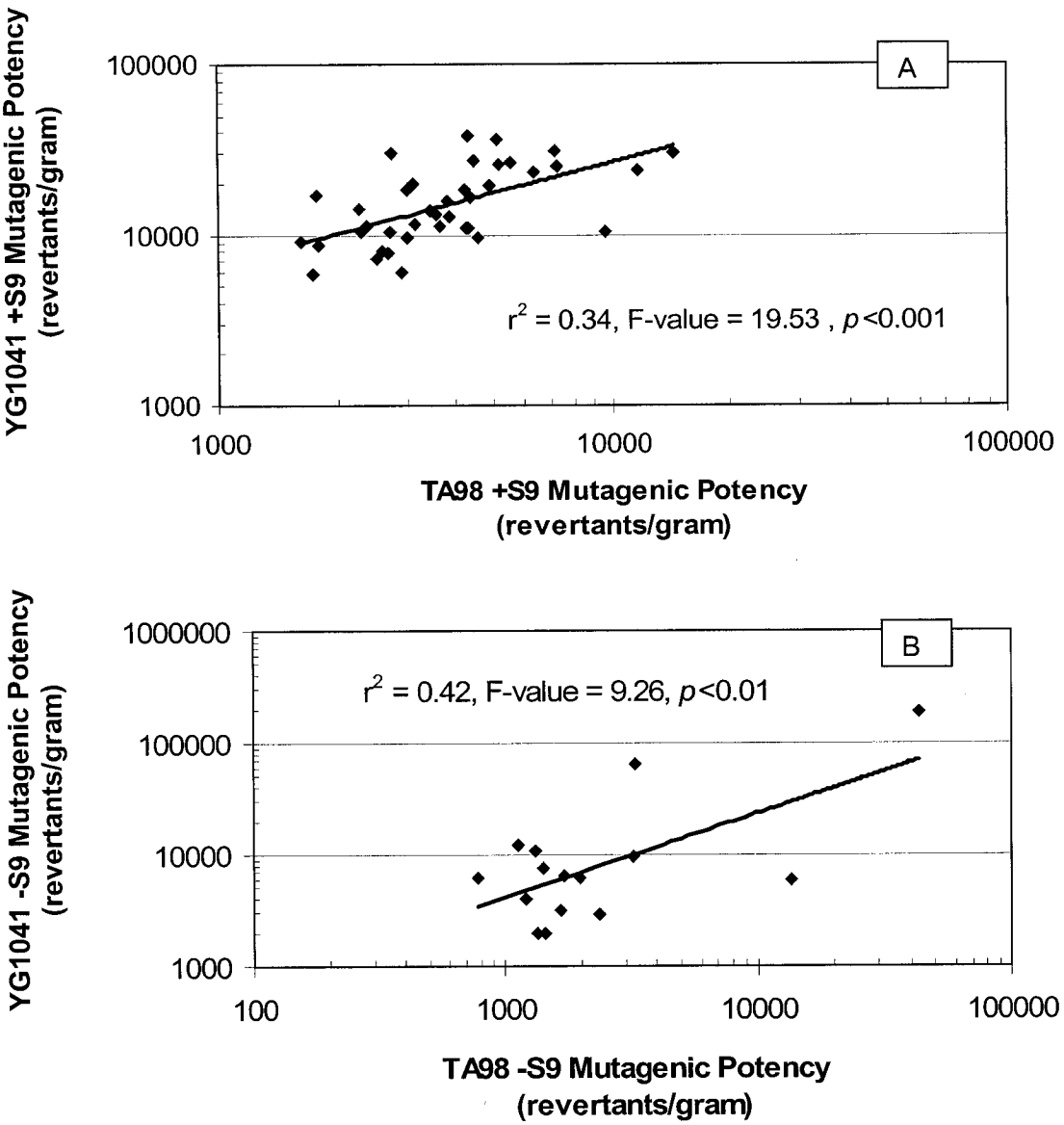


Figure 4. Empirical relationships between the *Salmonella* TA98 and YG1041 mutagenic potencies of settled house dust samples. A) Mutagenic potency values for dust extracts tested with the addition of S9 metabolic activation. B) Mutagenic potency values for dust extracts tested without the addition of metabolic activation.

coefficients of determination indicate a good relationship between the responses obtained with the two strains. Moreover, the results displayed in Figure 4 indicate that YG1041 potency values are, on average, 4-5 fold greater than corresponding TA98 values. This may indicate responses to similar environmental mutagens whose mutagenicity can be increased on metabolically enhanced strains such as YG1041.

Mutagenicity Testing of Fractionated Dust Extracts

In order to acquire additional information on the physical-chemical properties of the putative mutagens contributing to the observed mutagenicity, four dust extracts were fractionated into basic compounds and base/neutral compounds. These fractions were tested with TA98 and YG1041, both with and without metabolic activation. Testing with YG1042 was excluded due to the low response rate previously observed when testing the whole dust extract. The mutagenic potency of each dust fraction is shown in Table 8.

Based on the results with these few fractions, it appears that the base/neutral fractions may be more mutagenic than the basic fractions alone. This appears to be true for all test scenarios (i.e., with TA98 and YG1041, with and without S9) indicating that neutral compounds may be important contributors to the mutagenicity of the dust samples. When examining only the basic fractions or only the base/neutral fractions, the addition of S9 does not appear to result in any discernable trends (i.e. an increase or decrease in mutagenicity). In addition, no trends are evident when evaluating the mutagenicity of the fractions as a percentage of the mutagenicity of the whole dust extract. Furthermore, the sum of the mutagenicity of basic fractions and the base/neutral fractions is substantially less than the mutagenicity of the whole dust extract. This suggests that a large component of the mutagenic activity of the dust may due to acids or components that were lost in the extraction.

Chemical Analyses of the Dust Samples

The dust extracts were chemically analyzed for two groups of compounds that were suspected of contributing towards the mutagenic activity of the samples. Specifically, nine of the dust extracts were analyzed for heterocyclic aromatic amines, and all of the dust extracts were analyzed for polycyclic aromatic hydrocarbons.

Table 8. *Salmonella* mutagenic potencies (revertants gram^{-1}) of the basic fraction (B), base/neutral fraction (B&N), and whole extract (W) from four selected house dust samples. - indicates a negative result, and (+) indicates that the sample was marginally positive but could not be verified due to an insufficient quantity of the sample.

	TA98 -S9			TA98 +S9			YG1041 -S9			YG1041 +S9		
	B	B&N	W	B	B&N	W	B	B&N	W	B	B&N	W
C003	-	11,820	43,283	831	6,904	4,299	3,492	24,518	188,429	(+)	15,014	37,965
C012	-	-	1,116	-	3,954	5,127	-	583[a]	12,093	2,953	12,612	35,816
E009	-	-	1,947	-	5,983	2,762	-	2,192	(+)	3,148	12,507	30,283
S007	-	-	1,306	2,819	4,785	7,213	-	2,520	10,750	3,764	10,076	31,387

[a] Testing with this sample resulted in a response where only one test dose was over double the background.

Heterocyclic Aromatic Amines (HAAs)

Nine dust extracts including C003, C012, S007, E007, C016, C001, C011, A001, C005, and one solvent blank were analyzed for HAA content. Each sample was evaluated for 17 mutagenic HAAs and 2 co-mutagens (see Table 1). The analyses revealed that none of the 17 mutagenic HAAs or the co-mutagen harman could be positively identified in any of the dust samples or the blank. Three of the samples (C012, E009, S007) did however tested positive for norharman. Although it could not be positively identified, three of the dust extracts (C005, C001, C011) also yielded UV and fluorescence peaks in the vicinity of that expected for 2-amino-1-methyl-6- phenylimidazo[4,5-b]-pyridine (PhIP).

Polycyclic Aromatic Hydrocarbons (PAHs)

All of the dust extracts were evaluated for the presence of 13 targeted PAHs (see Figure 2, General Introduction). Of these 13 measured PAHs, only 3 of the lightest and most volatile PAHs (acenaphthylene, fluorene, anthracene) (Harvey 1991) were not detected in all of the samples. Acenaphthylene was not detected in 59% of the samples, fluorene was not detected in 14% of the samples, and anthracene was not detected in 4% of the samples. In those cases where the PAHs were not detected, a value of one half the method detection limit was substituted into the dataset for use in the statistical analyses (USEPA 1991; PMRA 2003).

The individual concentrations of the 13 PAHs measured in each dust sample are listed in Appendix V. The concentrations spanned between 2 and 3 orders of magnitude both for a single PAH and between different PAHs. The minimum, maximum and mean concentrations for each of the 13 targeted PAHs are summarized in Table 9. The PAH detected in the lowest concentration was acenaphthylene, while the PAH that occurred in the highest concentration was benzo[*b*]fluoranthene. No particular trends were observed in terms of the concentration of the PAH based on the number of rings contained in its structure. The sum of the 13 targeted PAHs, referred to hereafter as total PAHs, ranged between 1.5 – 325 $\mu g\ g^{-1}$. The distribution of the total PAHs was positively skewed (skewness = 4.00) with the concentrations in the majority of samples being less than 30 $\mu g\ g^{-1}$ (Figure 5). The sum of the 7 PAHs classified as probable human carcinogens by the USEPA 2003, referred to hereafter as the B2 PAHs, accounted for approximately 60% of the total PAHs measured.

Table 9. Minimum, maximum, and mean values of 13 PAHs measured in settled house dust collected from homes in Ottawa, ON.

PAH	N	MDL[a] ($\mu g\ g^{-1}$)	Number of Samples Below MDL	Minimum ($\mu g\ g^{-1}$)	Maximum ($\mu g\ g^{-1}$)	Median ($\mu g\ g^{-1}$)	Arithmetic Mean ($\mu g\ g^{-1}$)	S.E.M.[b]	Geometric Mean ($\mu g\ g^{-1}$)
Acenaphthylene	51	0.011	30	0.005	0.171	0.005	0.039	0.007	0.015
Fluorene	51	0.013	7	0.007	1.369	0.093	0.170	0.032	0.084
Phenanthrene	51	0.012	0	0.149	20.954	1.482	2.782	0.558	1.532
Anthracene	51	0.011	2	0.006	6.624	0.196	0.485	0.136	0.222
Pyrene	51	0.012	0	0.207	46.020	1.456	4.365	1.146	1.911
Benz[*a*]anthracene	51	0.021	0	0.105	32.073	0.696	2.382	0.707	0.956
Chrysene	51	0.025	0	0.150	35.116	1.188	3.290	0.858	1.455
Benzo[*b*]fluoranthene	51	0.019	0	0.160	53.950	1.663	4.871	1.312	2.010
Benzo[*k*]fluoranthene	51	0.034	0	0.049	19.027	0.532	1.595	0.442	0.674
Benzo[*a*]pyrene	51	0.051	0	0.040	38.750	0.803	2.909	0.899	0.963
Indeno[1,2,3-*c,d*]pyrene	51	0.039	0	0.100	33.546	0.911	3.069	0.819	1.286
Dibenz[*a,h*]anthracene	51	0.054	0	0.022	6.266	0.185	0.549	0.148	0.250
Benzo[*g,h,i*]perylene	51	0.034	0	0.118	31.408	0.793	2.790	0.764	1.131
Total PAHs[c]				1.501	325.269	9.526	29.297	7.775	12.920
B2 PAHs[d]				0.656	218.727	6.055	18.666	5.170	7.679

[a] Method detection limit. Samples below the MDL were assigned a value of ½ the MDL.

[b] Standard error of the arithmetic mean.

[c] Sum of the 13 targeted PAHs.

[d] Sum of the PAHs classified as probable human carcinogens by the USEPA (USEPA 2003).

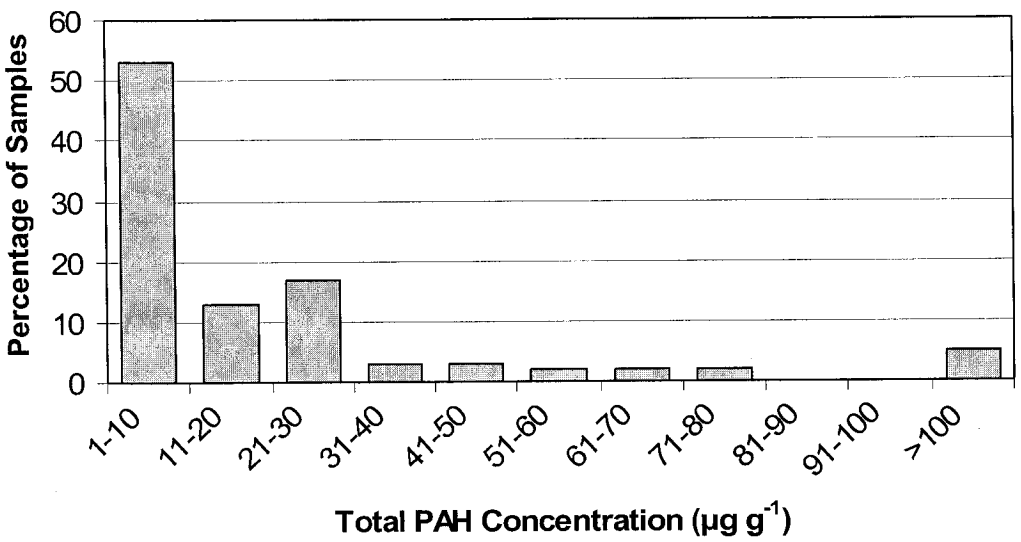


Figure 5. Relative frequency distribution of the total PAH concentrations measured in dust samples extracted from the vacuum cleaner bags of 51 homes.

Empirical Analyses of Settled House Dust Mutagenic Activity, PAH Contamination and Household Attributes

Survey information collected from the homeowner volunteers provided details about household attributes (e.g., home location and age, heating system, flooring) that have been previously highlighted as noteworthy determinants of PAH contamination in house dust (see Introduction and Table 1, Chapter 1). PAHs include several known mutagens and were suspected of contributing to the mutagenic activity of the dust samples. Therefore, the correlations between dust mutagenicity and PAH content, PAH content and household attributes, and dust mutagenicity and household attributes, were each examined. Scatter plots were visually inspected to confirm the linearity of the relationships. All significant correlations are presented in the sections below.

Relationships Between Dust Mutagenicity and PAH Content

Since the presence of PAHs was suspected of contributing to the mutagenic activity of the dust samples, analyses were undertaken to evaluate the empirical relationship between these two variables. Firstly, discriminant function analyses were undertaken to determine if the PAHs could predict whether a dust sample would test positive or negative for mutagenicity with each of the *Salmonella* strains. Acenaphthylene was found to be the only predictor of a response on TA98 -S9 (r^2= 0.12, F-value = 6.63, p<0.02). With an error rate of 34%, it was predicted that when the concentration of acenaphthylene was large, the response on TA98 -S9 would be positive. None of the other PAHs were significant predictors of the type of response on other strains.

Linear regression analyses were conducted to determine the relationship between PAH concentration and the extent of the positive responses on the *Salmonella* strains. No significant correlations were evident between the PAHs and the response on YG1042 +S9 or any of the strains in the absence of metabolic activation. Significantly positive relationships were noted between each of the PAHs and the TA98 +S9 and YG1041 +S9 mutagenic responses. Figure 6 illustrates the correlations between the total PAHs and the TA98 +S9 and YG1041 +S9 mutagenic potencies (A), and between the B2 PAHs and the TA98 +S9 and YG1041 +S9 mutagenic potencies (B). The coefficients of determination indicate that the PAHs account for

between 22 and 43% of the variation in the mutagenic activity of the dust samples. Therefore, although other environmental mutagens are likely present in the dust samples, PAHs can account for a substantial portion of the variability in the mutagenic activity.

Analysis of the residuals from the relationships between PAH concentration and mutagenicity identified several significant outliers. In the majority of cases, these were positive outliers indicating that the observed mutagenicity values were higher than expected for the given PAH concentration. Although the properties of each of these outliers were examined individually, the reasons for their atypical mutagenicity values were not readily apparent.

Stepwise multiple linear regression was undertaken to determine which individual PAH could best account for the variability in the mutagenic potency of the dust samples. Benzo[*b*]fluoranthene, which was generally present in the highest concentrations in the dust samples, was determined to be the best predictor for the mutagenic activity of a sample when tested with TA98 +S9 ($R^2 = 0.44$, DF = 37, F value = 29.1, $p<0.0001$). Phenanthrene was the best predictor for mutagenic activity when the sample was tested with YG1041 +S9 ($R^2 = 0.28$, DF = 49, F value = 18.5, $p<0.0001$). No PAHs met the 0.05 significance level for entry into the model for TA98 or YG1041 without S9, or YG1042 with and without S9.

Additional calculations were conducted to further investigate the extent to which PAHs might contribute to the mutagenic activity of dust. Nine of the 13 PAHs measured in this study were considered to be mutagenic in the *Salmonella* mutagenicity test. The *Salmonella* mutagenic potencies of each of these PAHs, as determined in previous studies, are listed in Table 10. The published potency value for each PAH and its actual concentration in each dust sample were used to establish a set of predicted mutagenicity values. The predicted values for each PAH were then summed to obtain a predicted mutagenic potency value for each dust sample. These predicted mutagenicity values based only on PAH contamination alone, were then compared with the observed mutagenic potencies for each dust sample. Analyses were limited to data with TA98, as very little published data is available on the mutagenicity of individual PAHs as tested with YG1041 and YG1042. The results of these calculations are listed in Table 11.

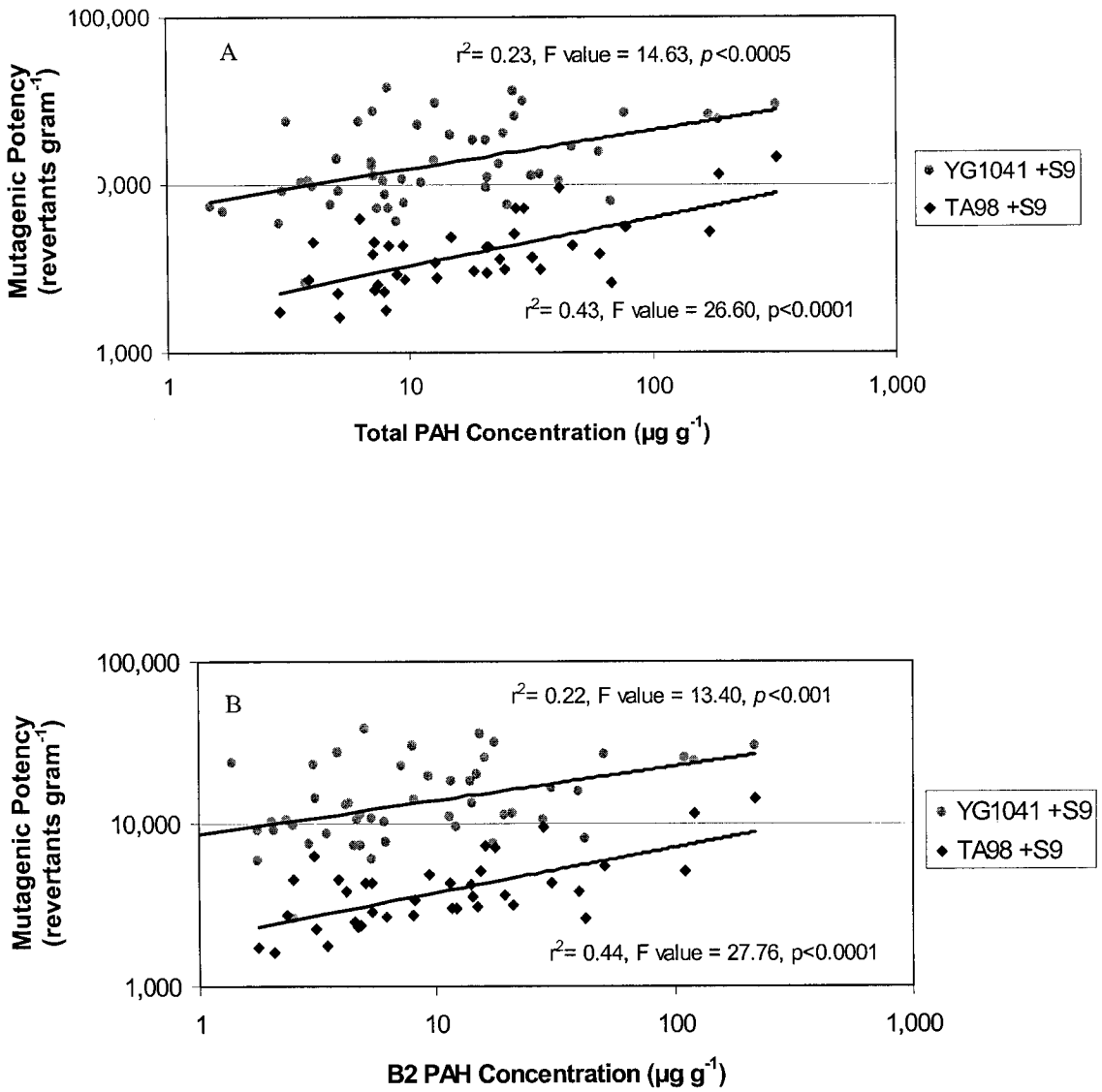


Figure 6. Relationship between the PAH concentration and the TA98 +S9 and YG1041 +S9 *Salmonella* mutagenic potency of dust samples collected from 51 homes in Ottawa, ON. A) Total PAHs B) B2 PAHs

Table 10. Published *Salmonella* mutagenic potency values for nine PAHs.

PAH	TA98 +S9 Mutagenic Potency (revertants μg^{-1})	Reference
Phenanthrene	1.38	White and Rasmussen (1996)
Pyrene	8.32	Sakai et al. (1985)
Benz[*a*]anthracene	56.0	Kubo et al. (2002)
Chrysene	0.516	White and Rasmussen (1996)
Benzo[*b*]fluoranthene	61.0	Nagai et al. (2002)
Benzo[*k*]fluoranthene	59.0	Nagai et al. (2002)
Benzo[*a*]pyrene	488	White and Rasmussen (1996)
Benzo[*g,h,i*]perylene	7.52	White and Rasmussen (1996)
Dibenz[*a,h*]anthracene	39.0	Nagai et al. (2002)

Table 11. Observed and predicted *Salmonella* mutagenic potency values for settled house dust samples collected in Ottawa, ON. Predicted mutagenic potency values were based on PAH content only and were calculated by multiplying the published mutagenic potency value of the PAH by its concentration in the dust sample.

House Code	Observed TA98 +S9 Mutagenic Potency (revertants gram^{-1})	Predicted TA98 +S9 Mutagenic Potency (revertants gram^{-1})	Percent Predicted/Observed Mutagenic Potency
A001	1,737	161	9.3
C001	5,540	5,767	104.1
C002	3,120	1,403	45.0
C003	4,299	489	11.4
C005	2,899	391	13.5
C007	3,641	1,752	48.1
C012	5,127	1,634	31.9
C014	4,277	1,224	28.6
C015	3,577	1,434	40.1
C016	4,486	274	6.1
C019	2,272	237	10.4
C022	2,306	419	18.2
E001	4,903	1,040	21.2
E002	4,347	3,056	70.3
E004	3,023	1,253	41.4
E006	2,516	476	18.9
E008	9,566	2,979	31.1
E009	2,762	662	24.0
E012	4,317	417	9.7
E013	2,731	228	8.4
E016	6,313	263	4.2
E017	3,432	814	23.7
E018	5,164	12,612	244.2

Table 11. (continued)

House Code	Observed TA98 +S9 Mutagenic Potency (revertants gram^{-1})	Predicted TA98 +S9 Mutagenic Potency (revertants gram^{-1})	Percent Predicted/Observed Mutagenic Potency
E024	3,836	4,076	106.2
E027	3,000	1,406	46.9
E028	11,596	13,448	116.0
E029	2,617	4,833	184.7
E031	4,220	1,456	34.5
S003	3,877	404	10.4
S005	3,149	2,158	68.5
S007	7,213	1,812	25.1
S010	2,700	650	24.1
S011	14,452	26,030	180.1
S013	1,620	198	12.2
W001	2,377	543	22.8
W002	4,583	229	5.0
W005	1,789	295	16.5
W006	7,285	1,830	25.1

The predicted mutagenic potencies of the dust samples, based on PAH content only, ranged from 160 to 26,000 revertants $gram^{-1}$, and accounted for between 4 and 244% of the observed mutagenic potencies. The median value was approximately 25%, indicating that in general, the mutagenic PAHs evaluated may account for approximately a quarter of the mutagenic activity of the dust samples.

Most of the predicted mutagenic potencies were lower than the observed potencies, likely indicating the presence of mutagens other than the measured PAHs. However, for 6 of the dust samples, the predicted mutagenic potencies were higher than the observed. These 6 dust samples also contained the highest levels of PAHs of all the dust samples.

Empirical Relationships Between Dust Mutagenicity and Household Attributes

Discriminant function analyses were undertaken to determine if any of the household attributes could predict whether a dust sample would test positive or negative for mutagenicity with each of the *Salmonella* strains. The analyses revealed statistically significant results for YG1042 -S9 only. The age of the home, the vacuum frequency and the extent of carpeting in the home were significant predictors of whether the dust sample from a home would test positive on YG1042 -S9. Stepwise discriminant function analysis showed age to be best overall predictor of the response (R^2=0.21, F-value= 11.05, $p< 0.002$). The model predicted with a 30% error rate that when the house was older in age, the dust samples would give a positive response on YG1042 -S9

Linear regression revealed that the mutagenic potencies of the dust samples that tested positive with TA98 -S9, YG1041 +S9, YG1042 +S9 and YG1042 -S9 were significantly correlated with the number of people in the household (Figure 7). Each of these correlations was significantly positive, except for YG1042 +S9, which was negative. Although the correlation between YG1042 +S9 and the number of people in the household was highly significant, only 5 data points were available for inclusion in the regression. Hence, it is possible that the relationship might have been different had more data had been available for analysis.

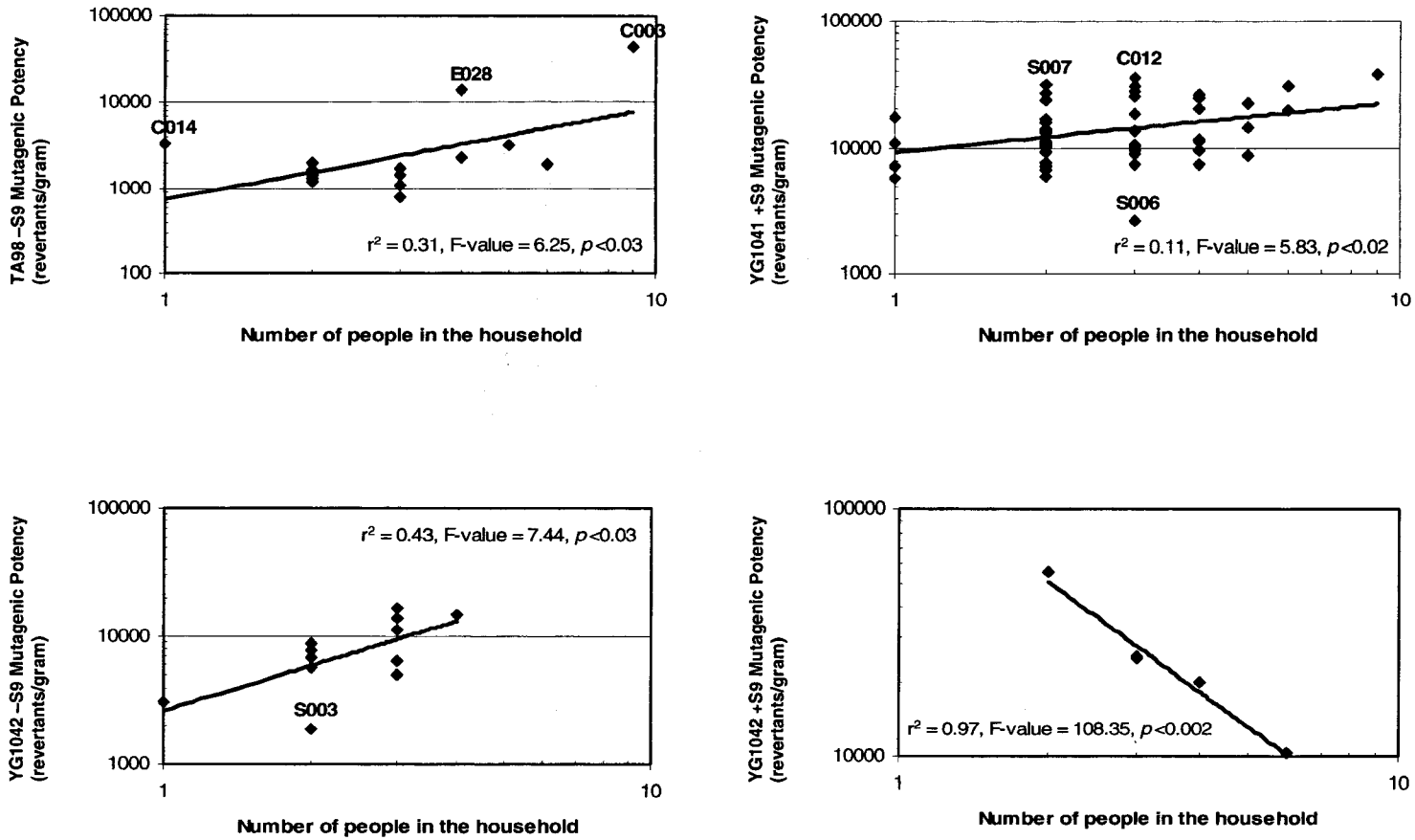


Figure 7. Relationships between the number of inhabitants in the sampled household and the mutagenic potency of the dust samples. Observations with accompanying house codes were identified as significant outliers.

Linear regression also identified a weak significant positive relationship between the mutagenic potency on TA98 +S9 and the time since the last vacuuming took place (N =38, r^2 =0.11, F-value = 4.48, p<0.05).

One-way ANOVAs revealed that the mutagenicity of the dust samples were significantly related to three household activities that involve combustion (Table 12). The YG1042 -S9 mutagenic potency was significantly lower in houses where occupants burned incense in comparison with those that did not. Similarly, the TA98 +S9 mutagenic potency was significantly lower for houses in which candles had recently been burned in comparison with those where they had not. Lastly, a significant decrease was noted in the YG1041 -S9 mutagenic potency between houses that used a secondary heating source and those that did not.

Table 12. Results of one-way ANOVAs investigating the effects of household activities on the mutagenicity of dust samples.

Mutagenicity Tester Strain	**Household activity**	**N**	**r**	**F-ratio**	***p***
YG1041 -S9	Use of secondary heating sources	40	-0.31	4.17	<0.05
YG1042 -S9	Incense burning	12	-0.61	6.04	<0.04
TA98 +S9	Candle burning	39	-0.52	13.96	<0.0007

Empirical Relationships Between Dust PAH Content and Household Attributes

Analyses were conducted to determine if the PAH concentrations in the dust samples could be linked to household activities recorded in the homeowner survey. It was expected that the PAH concentrations would be related to PAH source events taking place in the home (e.g., cigarette smoking, fireplace use). However, the results revealed that there were no significant differences in the total PAH or B2 PAH concentrations between homes with specific PAH source events and those without.

The relationships between PAH concentrations and parameters other than PAH source events were also examined. Weak significant negative relationships (r = -0.29 to -0.32) were found between the concentrations of various PAHs (in particular PAHs with four or more rings) and the frequency of vacuuming within the home (Table 13).

Statistical analyses also revealed a significant relationship with one other household attribute. The number of pets in the home was found to be negatively correlated with the concentration of fluorene in the dust samples ($N = 51$, $r^2 = 0.15$, $p<0.005$). The number of pets was also negatively correlated with the concentration of phenanthrene, pyrene, benzo[*b*]fluoranthene, and total PAHs at a level that was marginally significant (i.e., $p<0.06$).

Table 13. Pearson Correlation Coefficients for the relationship between individual PAH concentrations in settled house dust samples and the frequency of vacuuming in the home.

PAH	**N**	**r**	***p***
Acenaphthylene	47	0.06	<0.69
Fluorene	47	-0.17	<0.25
Phenanthrene	47	-0.12	<0.41
Anthracene	47	-0.24	<0.11
Pyrene	47	-0.30*	<0.05
Benz[*a*]anthracene	47	-0.29*	<0.05
Chrysene	47	-0.30*	<0.05
Benzo[*b*]fluoranthene	47	-0.32*	<0.03
Benzo[*k*]luoranthene	47	-0.31*	<0.04
Benzo[*a*]pyrene	47	-0.33*	<0.03
Indeno[1,2,3-*c,d*]pyrene	47	-0.32*	<0.04
Dibenz[*a,h*]anthracene	47	-0.28	<0.06
Benzo[*g,h,i*]perylene	47	-0.31*	<0.04
Total PAHs	47	-0.28	<0.07
B2 PAHs	47	-0.31*	<0.04

* Significant at the 0.05 level.

Cancer Risk Assessment of PAHs in Settled House Dust

Exposure to carcinogenic PAHs in house dust can increase the likelihood of deleterious health effects (e.g., cancer). In order to assess the potential consequences associated with this exposure, a risk assessment was conducted using the B2 PAH concentration data. Specifically, this risk assessment evaluated the excess lifetime cancer risk resulting from non-dietary ingestion of carcinogenic PAHs in SHD during preschool years. Excess lifetime cancer risk was calculated using equation 3 described in the Materials and Methods.

The results indicate that exposure to carcinogenic PAHs at levels found at most of the sampled households (i.e., less than 40 $\mu g\ g^{-1}$) results in excess cancer risks that are generally between 1×10^{-6} and 1×10^{-4}. Five of the house dust samples contained levels of carcinogenic PAHs that were higher than 40 $\mu g\ g^{-1}$. At the lowest dust ingestion rate, exposure to these levels still resulted in risks below 1×10^{-4}. However, at the highest dust ingestion rate, exposure to these levels resulted in excess risks that were as high as 5.5×10^{-4}. Only exposures to PAHs at concentrations lower than approximately 4 $\mu g\ g^{-1}$, at the lowest ingestion rate, resulted in risk levels below 1×10^{-6}.

Figure 8 shows the excess cancer risk resulting from non-dietary ingestion of PAHs during preschool years plotted against the concentration of B2 PAHs that were found in each house dust sample. Risk curves are shown for three different dust ingestion rates including 0.01, 0.05, and 0.1 $g\ d^{-1}$.

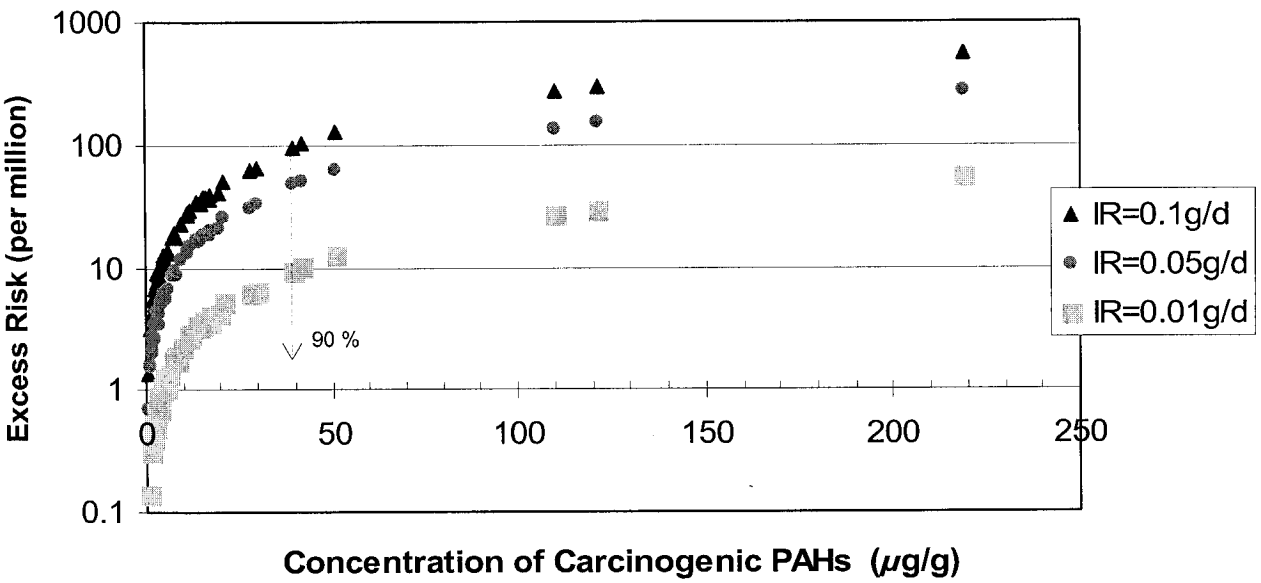


Figure 8. Excess cancer rates due to non-dietary ingestion of B2 PAHs in SHD during preschool years. Arrow denotes the 90th percentile of the B2 PAH concentrations.

Discussion

Salmonella Mutagenicity Testing of Dust Samples

Whole Dust Extracts

All of the house dust extracts tested in this study were shown to be mutagenic with at least one of three *Salmonella* strains (i.e., TA98, YG1041 and YG1042). Since a higher number of samples tested positive with TA98 and YG1041 as opposed to YG1042, it appears that SHD extracts contain predominantly frameshift mutagens, as opposed to base pair mutagens. No positive responses were seen with TA102, indicating that the samples likely do not contain oxidative mutagens that cause transversion or transition mutations. Similarly, none of the dust samples tested positive with the base pair detecting strain TA100. Although no positive results were seen with TA100 (i.e., a doubling of the background number of revertants for two or more concentrations), a number of dust samples did show a dose-response pattern with increasing reversion frequency at higher concentrations. However, when higher concentrations were tested, many of the samples displayed evidence of toxicity before a doubling could occur. These results may indicate that base pair mutagens were present in the samples but only at low concentrations.

The results of the present study can be compared in part to a previous study which also examined the mutagenicity of house dust. Roberts et al. also obtained positive responses when testing dust samples with the frameshift detecting strain TA98 (Roberts et al. 1987). Nineteen percent of their samples tested positive with TA98 in the presence of S9, and 34% tested positive without S9. When compared with the present study, a similar percentage of the samples (31%) tested positive without S9. However, a much larger percentage (78%) of the Ottawa samples tested positive with S9. Since dust is a complex mixture containing numerous chemical contaminants (Butte and Heinzow 2002; Greenpeace 2003; Rudel et al. 2003), it would not be surprising to detect both direct acting and indirect acting frameshift mutagens in dust samples (i.e., positive responses with or without S9). However, further investigations would be necessary to determine whether indirect or direct acting mutagens are in fact more prevalent in SHD. It is also important to note that in the present study, the dust samples were cleaned up with GPC prior to mutagenicity testing. In contrast, no cleanup was undertaken in the Roberts et al.

study. It is possible that the GPC cleanup, which removes high molecular weight material, may have removed substances that interfere with S9 activation, and consequently allowed for a higher level of response in the present study. In addition, researchers have previously noted that samples that were not originally mutagenic, displayed mutagenic activity after fractionation, possibly due to the separation of toxic components from the mutagenic components (Austin et al. 1985).

In the study by Roberts et al., the TA98 mutagenic potency of the dust samples ranged from 1,340 - 4,180 revertants gram^{-1} with S9 and 1,190 - 6,570 revertants gram^{-1}without S9. In comparison, although minimum potencies were similar, much higher maximum potencies were reached in the present study with values ranging from 1,620 - 11,596 revertants gram^{-1} with S9 and 780 - 43,283 revertants gram^{-1} without S9. This could indicate a higher concentration of mutagens, or more potent mutagens, in the extracts evaluated in the present study. The presence of more, or more potent, mutagens may be due to a difference in the mutagenic substances that make up the original dust samples. Alternatively, it is possible that the difference in mutagenicity results from the different extraction techniques used. Roberts et al. extracted their samples by sonication for 30 minutes at 5 watts in 3 volumes of DMSO. They state that this extraction technique was "designed to allow consistent comparisons between samples rather than exhaustive extraction of mutagens". It is possible that the ASE extraction used in the present study was a more effective technique for extracting mutagens and resulted in higher potency levels.

Dust samples in the Roberts et al. study were tested with TA98 only. In the present study, comparison of the responses between different *Salmonella* strains showed a more frequent and stronger response with the metabolically enhanced YG strains than with TA98. The stronger responses with YG1041 and YG1042 (metabolically enhanced versions of TA98 and TA100 respectively) indicate contributions from mutagenic nitroarenes and/or aromatic amines (Hagiwara et al. 1993).

Nitroarenes and aromatic amines are both ubiquitous environmental compounds that yield strong positive responses in the *Salmonella* Mutagenicity Test (Tokiwa and Ohnishi 1986; Turesky 2002). Although nitroarenes and aromatic amines can act as either frameshift or base

pair mutagens (McCann et al. 1975; IPCS 2003), in this study, the highest number of positive responses was seen with the frameshift detecting strain YG1041. This frameshift activity may be attributed to heterocyclic amines in the dust. Heterocyclic amines tend to show a stronger response on frameshift strains as opposed to base pair strains (Sugimura and Sato 1983; Hatch et al. 1991). Heterocyclic amines are only weakly active in TA100, and virtually inactive in TA102 (Felton and Knize 1990).

Mutagenic responses were observed both with and without the addition of S9. This was not unexpected since, although both nitroarenes and aromatic amines require metabolic activation, some compounds can be metabolized to their active form by endogenous *Salmonella* enzymes (e.g., nitroreductase) Others require the addition of an exogenous enzyme system (e.g., rat liver S9) (Bartsch 1982; IPCS 2003). For example, the *Salmonella* mutagenicity of nitroarenes generally depends on the reduction of the nitro group (Rosenkranz and Mermelstein 1983) by a rate limiting nitroreductase (Watanabe et al. 1989). Most nitroarenes, including the highly mutagenic dinitropyrenes, can readily be reduced by the *Salmonella* nitroreductases (IPCS 2003). To a lesser extent, other nitroarenes, undergo nitroreduction or ring oxidation, which requires the mixed-function oxidase enzymes present in the S9 metabolic activation (Anderson et al. 1987). Therefore, although the addition of S9 may sometimes enhance mutagenicity, most nitroarenes exhibit potent mutagenicity without the addition of exogenous S9 (Fu et al. 1988). In contrast to the nitroarenes, aromatic amines are metabolized (e.g., oxidized) (Bartsch 1982) by components in an exogenous metabolic activation system (Hatch et al. 1991). Therefore, in the *Salmonella* Mutagenicity Test, aromatic amines generally require S9 to exert their mutagenic effects. In the present study, although positive results were observed both with and without S9, the highest frequency of positive responses was observed with YG1041 +S9. Therefore, it is likely that the predominant mutagens in the dust extracts are frameshift aromatic amines and, perhaps to a lesser extent, frameshift nitroarenes that require exogenous metabolic activation.

Though the highest number of mutagenic responses was seen with YG1041 +S9, it is worthwhile to note that some of the most potent responses were observed in the few dust extracts that tested positive with YG1042 +S9. This could point toward the presence of additional base

pair aromatic amines (IARC 1982; IARC 1993). However, the literature on the base pair mutagenicity of aromatic amines is sparse and the reason for the higher potencies is not clear.

Fractionated Dust Extracts

The whole extracts from four dust samples were fractionated into basic and base/neutral compounds in order to obtain further information on the putative mutagens in the dust. The fractions were tested with TA98 and YG1041 only, since previous testing with the whole extracts showed a clear predominance of frameshift mutagens. When the basic fractions were tested, it was found that the majority of these fractions were not in fact mutagenic, except when tested with YG1041 +S9. This finding again points to the likely presence of aromatic amines that are readily detected by the YG strains and require S9 for mutagenic activity.

When comparing the mutagenic activity of the basic fractions to the combined base/neutral fractions, the results showed that the base/neutral fractions were clearly more mutagenic. Although these results are based on a very small number of samples, this suggests that neutral compounds are important in eliciting the observed mutagenic activity. Alternatively, it is also possible that components in the base/neutral fractions are able to interact producing mutagenic activity that would not occur in either of the fractions alone. Confirmation of interactions would require additional analyses.

In two cases, the mutagenic activity of the whole dust extract was observed to be less than the mutagenic activity of the combined fractions. This may be due to normal day to day variability in the assay. Alternatively, since the whole dust extract and the fractions are different mixtures, it is possible that components of these mixtures are interacting differently (e.g., exhibiting antagonistic interactions in the whole mixture and/or synergisms in the fractions).

In the majority of cases, the mutagenic activity of the combined base/neutral compounds was less than the activity of the whole dust extracts. Assuming additivity of the fractions, this suggests that another source of the dust mutagenicity was the acid fraction, or alternatively, components that were lost in the fractionation process. Although the acid fraction was not tested for mutagenic activity in this study, previous studies have highlighted the importance of the

organic acid fraction in the mutagenicity of complex mixtures such as urban air (Lewtas et al. 1990). Hydroxylated nitroaromatics are among the compounds suspected of contributing to the mutagenicity of the acid fraction (Nishioka and Lewtas 1992).

Although the mutagenicity testing of the whole dust extracts shows that aromatic amines are likely important mutagens in the dust, the fractionation suggests that the neutral compounds (e.g., PAHs and nitroarenes) are equally if not more important. Furthermore, although a portion of the dust mutagenicity appeared to stem from components other than the basic or base/neutral fractions, this could not be confirmed without further testing.

Chemical Analyses of the Dust Samples

In attempts to identify the putative mutagens in the dust, the dust extracts were chemically analyzed for two groups of compounds: heterocyclic aromatic amines (HAAs), which are basic compounds, and polycyclic aromatic hydrocarbons (PAHs), which are neutral compounds.

Heterocyclic Aromatic Amines (HAAs)

Nine of the dust samples were analyzed for the presence of 17 mutagenic and 2 co-mutagenic heterocyclic aromatic amines (HAAs). These chemical compounds are ubiquitous environmental pollutants (Manabe et al. 1992; Manabe et al. 1993; Kataoka et al. 1998) and are associated with particulate matter in indoor air (Manabe and Wada 1990; Manabe et al. 1992). They are also potent indirect acting mutagens which preferentially induce frameshift mutations (Sugimura and Sato 1983; Fuscoe et al. 1988). The mutagenic properties of HAAs coincide with the pattern of mutagenicity previously noted for the dust extracts.

Contrary to what was expected, the analyses of the nine dust samples failed to reveal the presence of any of the 17 mutagenic HAAs, or the co-mutagen harman. Three of the dust samples (C005, C001, C011) did have UV and fluorescence peaks very close to the correct retention time for PhIP, however, the spectral pattern could not provide positive confirmation of PhIP. Although it is possible that these 17 HAAs were not present in the samples, it is also possible that they may have been present at levels below the detection limit of 2.5 ng g^{-1}.

Previous studies have shown HAAs to be generally present at low concentrations in various media. In cooked foods, these compounds are present at levels ranging from less than 0.1 to 50 ng g^{-1} (Felton et al. 1994). In indoor air they have been found at levels between 0.44-0.76 pg m^{-3}, in outdoor air at 0.95-27.8 ng g^{-1} (Manabe et al. 1992; Manabe et al. 1993), and in soil at levels between 4.4-9.3 pg g^{-1} (Manabe et al. 1992). Despite their low environmental concentrations, these HAAs are potent mutagens with some compounds having TA98 +S9 *Salmonella* mutagenic potencies as high as 661 revertants ng^{-1} (Sugimura and Sato 1983). Consequently, it is possible that these mutagenic HAAs were present in the dust samples at levels that could not be detected using chemical methods, but that still contribute to the mutagenic activity of the dust. Alternatively, it is also possible that heterocyclic amines other than those specifically targeted in these analyses were present in the dust samples and were responsible for the mutagenic activity.

Although the 17 mutagenic HAAs targeted were not found in the dust samples, the co-mutagen norharman was positively identified in three of the samples. The three samples in which norharman was detected (C012, E009, S007) also had among the highest levels of YG1041 +S9 mutagenicity. Norharman is considered a co-mutagen in the presence of other heterocyclic amines (Totsuka et al. 1999), and has also been shown to enhance the mutagenicity of PAHs such as benzo[*a*]pyrene (Fujino et al. 1978). Therefore, it is possible that the presence of norharman in these three samples enhanced the mutagenicity of HAAs that were present at levels below the detection limit, or that were not measured in these analyses. Similarly, norharman could have enhanced the mutagenicity of PAHs present in the samples.

Polycyclic Aromatic Hydrocarbons (PAHs)

Like the heterocyclic amines, PAHs are considered to be ubiquitous environmental pollutants (Menzie 1992) and are suspected of contributing to the mutagenic activity of the dust. The dust extracts were analyzed for the presence of 13 PAHs, and all 13 PAHs were detected in most of the dust samples. The concentrations of the total PAHs ranged between 1.5 – 325 μg g^{-1}, with a geometric mean of 12.9 μg g^{-1}. These values are quite similar to the concentrations found in the analyses of the pooled dataset in Chapter 1 where the total PAHs ranged between 0.4 – 544 μg g^{-1} and the geometric mean was 4.5 μg g^{-1} (see Table 2, Chapter 1).

In the present study, the sum of the carcinogenic B2 PAHs was approximately 60% of the total PAHs. Similar results were observed in other studies where the concentrations of the B2 PAHs were found to equal approximately one half of the total PAH concentrations (Chuang 1996; Chuang et al. 1999). The slight difference in percentages likely reflects the fact that 18 PAHs made up the total PAHs in the previous studies, and 13 PAHs were used in the present study.

In terms of individual PAHs, concentrations spanned between two and three orders of magnitude both within a single PAH and between different PAHs. This variation in PAH concentration is lower than in the pooled data set in Chapter 1 (Appendix II). The larger variation in the results from Chapter 1 likely reflects the more diverse sampling locations, the different sampling methods, and the variation in analytical techniques used to quantify the PAHs.

The PAH detected in the lowest concentration was acenaphthylene, and the PAH detected in the highest concentration was benzo[*b*]fluoranthene. With a molecular weight of 152.2 and a vapour pressure of 0.378 Pa (Harvey 1991), acenaphthylene is more readily volatilized and has been commonly found only at low levels in dust samples (Chuang et al. 1995; Chuang et al. 1997; Simrock 1998; Wilson et al. 2001). In contrast, benzo[*b*]fluoranthene is a heavier and less volatile molecule, and has previously been detected in the highest concentrations in dust samples (Chuang et al. 1995; Lewis et al. 1999).

The house with the lowest total PAH level (1.5 $\mu g\ g^{-1}$) was E022, a newly constructed home whose owners moved in shortly before the sampling took place. In contrast, the house with the highest total PAH level (325 $\mu g\ g^{-1}$) was S011. The only distinguishing feature of this house was that it is 90% carpeted. Since contaminants have the potential to accumulate significantly in carpet dust (Lewis et al. 1994), the high percentage of carpeting in this home may contribute to high PAH levels.

The German Federal Environmental Agency's Commission for Indoor Air Quality has established the only guideline for PAHs in dust that currently exists. It states that exposure to levels above 10 μg of benzo[*a*]pyrene per gram of household dust should be minimized in order

to prevent unspecified adverse health effects (Heudorf and Angerer 2001). In the present study, three samples contained concentrations of benzo[*a*]pyrene that were above 10 μg g^{-1} (ppm), with maximum values reaching 39 μg g^{-1}. Although there are currently no Canadian guidelines for PAH contaminants in SHD, there do exist guidelines for PAHs in residential soils, a comparable particulate matrix. The 2003 Canadian Environmental Quality Guideline for benzo[*a*]pyrene in residential soil is 0.7 μg g^{-1} (ppm) (CCME 2003). More than half of the SHD samples examined in this study contained concentrations above this value. Similarly, the Environmental Quality Guideline for benzo[*b*]fluoranthene in residential soils is 1 μg g^{-1}. The geometric mean value for benzo[*b*]fluoranthene in SHD was 2 μg g^{-1} and the maximum value was 54 μg g^{-1}. Comparable trends were observed for other individual PAHs. Previous studies have noted similar observations. For example, Camann and Buckley 1994 found that median concentrations of benzo[*a*]pyrene in indoor dust were over four times higher than the USA Superfund limit of 0.26 ppm for outdoor soil (Camann and Buckley 1994). It should be noted that residential soils are diluted by coarse inorganic material such as sand. In contrast, this study assessed the PAH concentration in fine settled house dust that could pass through a 150 μm sieve. The PAH levels in indoor dust clearly have the potential to exceed the German Federal Environmental Agency's guideline for dust, as well as the Canadian Environmental Quality Guidelines for outdoor residential soil, both of which were established with the aim of protecting human health.

Empirical Analyses of Settled House Dust Mutagenic Activity, PAH Contamination and Household Attributes

Empirical Relationships Between Dust Mutagenicity and PAH Content

Of the 13 PAHs targeted for analyses in this study, nine (i.e., phenanthrene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, dibenz[*a,h*]anthracene) have previously been shown to be mutagenic with both TA98 and TA100 in the *Salmonella* Mutagenicity Test (see Table 10 and IARC 1983; IPCS 1998). Even though the dust samples in this study contained PAHs that are known to be mutagenic with TA100, none of the tested dust extracts gave a positive response with TA100 (Figure 3). A dose-response was, however, seen with a small number of samples. Although the reasons for a lack of a positive response are unclear, several explanations are possible.

Firstly, it is possible that the PAH concentrations in the dust were too low to show a positive response (i.e., a dose-response *and* a doubling of spontaneous reversion rates for two or more doses), but were high enough to show a dose-response. For example, previous studies show pyrene begins eliciting notable mutagenic responses above approximately 5 $\mu g\ plate^{-1}$ (Bos et al. 1988). However, the median level of pyrene in this study is only 1.5 $\mu g\ g^{-1}$ with resulting test concentrations close to 0.1 $\mu g\ plate^{-1}$. Furthermore, testing of the same compounds on the *Salmonella* Mutagenicity Test may yield different results for different laboratories (Claxton et al. 1992). Recent testing to confirm the mutagenic potency of selected PAHs showed that, in contrast to previously published data, a number of PAHs exhibited a dose response on TA100, but did not elicit twice background, even at higher doses (Gagnon 2005).

It is also possible that chemical interactions, including antagonistic interactions, reduced the TA100 responses. Specifically, other compounds present in the mixture may compete for the same metabolic enzymes as PAHs (Levin et al. 1982). If there are not sufficient enzymes to metabolize the PAHs to their active form, their mutagenic effects will be masked. The occurrence of antagonistic interactions in simple mixtures of PAHs has previously been noted, particularly for TA100 (Hass et al. 1981).

Although none of the dust samples tested positive on TA100, several positive responses were obtained for TA98, YG1041 and YG1042. Discriminant function analyses were undertaken to determine if the PAHs could predict whether a dust sample would test positive or negative for mutagenicity with these strains. Only the concentration of acenaphthylene in a dust sample was found to be able to predict the response on TA98 -S9, with higher concentrations of acenaphthylene increasing the probability of a positive response on TA98 –S9. Since acenaphthylene is not a mutagen in *Salmonella*, it is possible that its presence and concentration is indicative of other direct acting mutagens with similar physical properties.

Relationships between the PAHs and the mutagenic response observed with each of the *Salmonella* strains were also examined. The results confirmed a relationship between PAH concentrations and mutagenic responses on TA98 +S9 and YG1041 +S9 (Figure 6). Interestingly, no significant correlations were found between PAH concentrations and the

YG1042 +S9 mutagenic activity. This may be the result of a limited number of observations for this analysis (N=5). As expected, since PAHs require metabolic activation to exhibit mutagenicity (Gelboin 1969), no significant correlations were evident between the PAHs and the mutagenicity as determined with any of the strains in the absence of S9.

Linear regression analyses with the strain TA98 revealed that PAHs account for 43-44% of the variation in TA98 +S9 mutagenic potency of the dust. Similar regression analyses showed that PAHs likely account for 23-25% of the variation in YG1041 +S9 mutagenic potency. As expected, the PAHs were less well correlated with the YG1041 strain, which is more sensitive to other polycyclic compounds such as nitroarenes and aromatic amines (Hagiwara et al. 1993).

Calculations of the predicted dust mutagenicity based on PAH content only (Table 11), indicated that PAHs probably account for 25% (median) of the TA98 +S9 mutagenic activity. Calculations in Chapter 1 with a pooled dataset suggested that PAHs likely account for less than a quarter of the TA98 +S9 mutagenic activity. This is comparable to what has been found for other particulate matrices. Cerna et al. found that PAHs account for 23% or less of the TA98 +S9 mutagenic activity of outdoor air (Cerna et al. 2000). Similarly, White and Claxton found that 17-25% of the variation in the TA98 +S9 mutagenic potency of soil could be explained by PAH content (White and Claxton 2004).

Although calculations of the dust mutagenicity based on PAH concentration tend to be consistent with other findings, they are only an estimation. The calculations assume additivity, which is a reasonable assumption for mixtures involving unsubstituted, homocyclic PAHs (White 2002). However, the calculations do not take into account the effects of interactions with PAH derivatives or other compounds. The occurrence of chemical interactions in the dust samples is highly likely and is particularly evidenced by the fact that six of the dust samples had calculated PAH-related mutagenic potencies that were higher than the observed potencies. Assuming that the quantification of the PAHs and their published mutagenicity values were correct, this finding suggests that less than additive or antagonistic interactions were taking place in the dust mixtures. Furthermore, because these six samples had the highest PAH levels, it may suggest the saturation of metabolic pathways at high contaminant concentrations (Levin et al.

1982). Although other studies have also noted less than additive effects in PAH mixtures, the results have not been consistent or conclusive. For a review of these studies see The U.S. Department of Health and Human Services (1995) and Table 5 in White (2002).

Although PAHs likely account for a substantial proportion of the mutagenic activity of the dust extracts, other mutagenic substances are undoubtedly present in the dust mixtures. It is difficult to speculate about the identity of these compounds, particularly since many of the contaminants commonly measured in dust (e.g., lead, phthalates, pesticides) are not mutagenic in the *Salmonella* Mutagenicity Test (USEPA 2003). Mutagenic substances that may be found in whole dust samples could include a host of organic and inorganic compounds commonly associated with a variety of industrial products (e.g., textiles, paints, furniture) such as hexavalent chromium, nickel compounds, styrene, tetrachloroethylene, benzidine and vinyl chloride (IARC 1987; IARC 1990; IARC 1995; IARC 2002). However, the extraction methodology employed in this study makes it unlikely that the samples examined contain metals and/or volatile substances such as hexvalent chromium and tetrachloroethylene.

In addition to chemical compounds, biological contaminants found in house dust may account for some of the mutagenic activity of the dust. For example, *Aspergillus versicolor* is a species of mould that frequently occurs in damp houses and has recently been detected in carpet dust (Engelhart et al. 2002). *A. versicolor* is known to produce the mycotoxin sterigmatocystin, which tests positive with TA98 and TA100 in the *Salmonella* Mutagenicity Test (McCann et al. 1975). Sterigmatocystin is a potent mutagen that is at least three times as potent as benzo[*a*]pyrene. Similarly, the mould *Alternaria alternata* has been detected in carpet dust (Verhoeff et al. 1994), and a number of its metabolites have shown mutagenic responses with TA98 and TA100 (Stack and Prival 1986; Davis and Stack 1991).

Empirical Relationships Between Dust Mutagenicity and Household Attributes

In addition to examining the relationships between dust mutagenicity and PAH concentration, it is also worthwhile to look at the relationships between dust mutagenicity and the attributes of the homes in which the dust samples were collected. The household attributes

may provide an indirect measure of activities and lifestyle factors that contribute to contamination, and therefore may help to explain sources of the dust mutagenicity.

Discriminant function analyses were conducted to determine if any of the household attributes could predict whether a dust sample would test positive or negative for mutagenicity with each of the *Salmonella* strains. Significant predictors were found only for YG1042 –S9. The age of the home was ultimately the best predictor for mutagenic response with YG1042 –S9, with older houses being more likely to elicit a positive response on this strain. Previous investigations have noted that older homes are associated with increasing chemical contaminant concentrations in SHD (Ilacqua et al. 2003). Therefore it would not be unreasonable to expect that older homes may have higher levels of chemical contaminants which are also mutagenic and give a positive response in the *Salmonella* Test.

Linear regression analyses were conducted to examine relationships between the various household characteristics and the degree of mutagenic response on the various *Salmonella* strains. The analyses revealed that the TA98 -S9, YG1041 +S9, and YG1042 -S9 mutagenic potencies of the dust samples were positively correlated with the number of people in the household (Figure 7). These significant relationships suggest that the more people there are in a household, the greater the potential for mutagen generating events, including those events not recorded in the household survey. In other words, it appears that household occupancy is an important factor in determining sample mutagenic hazard. Since responses were seen both with and without metabolic activation, and on both frameshift and base pair mutagen detecting strains, the correlations do not give any indication as to the specific type of mutagens that may be generated by the household occupants. Possible mutagen generating events not captured in the survey that may be affected by the number of people in the house include automobile use, cooking time, and greater use of consumer products.

The TA98 +S9 mutagenic activity of the SHD extracts was also positively correlated with the time since the last vacuuming took place, suggesting that regular vacuuming can reduce mutagenicity. Although it is not immediately clear why cleaning frequency should affect dust contamination or mutagenic hazard, the relationship may indicate that residents who have most

recently cleaned have other "clean" habits that restrict the accumulation of mutagens in the dust. For example, previous studies have noted that the use of a high quality doormat reduces the track-in of particle-bound pollutants (Lewis et al. 1995).

The mutagenic activity of the dust extracts was also inversely related to three combustion events (Table 12). Specifically, the YG1042 -S9 and the TA98 +S9 mutagenic potencies were significantly lower in houses whose occupants had recently burned incense or candles respectively, compared to homes where these items had not been burned. Similarly, the YG1041 -S9 mutagenic potency was significantly lower in houses that had recently used a secondary heating source, compared to those that had not. These findings were contrary to what was expected since combustion events are thought to increase levels of contaminants such as PAHs (Lau et al. 1997; Lung and Hu 2003) and correspondingly, the mutagenic activity of the dust. Other investigators have suggested that such inverse relationships may be a result of compensatory activities. Combustion events leading to obvious indoor pollution (e.g., cigarette smoking, stir frying) may elicit responses such as increased ventilation (Koo et al. 1994). These compensatory actions could reduce ambient levels of contaminants and consequently levels of mutagenic activity. If increased ventilation accompanied the incense and candle burning and/or the use of secondary heating sources, this may have contributed to reduced levels of contaminants and mutagenicity.

In their study, Roberts et al. also examined the relationships between SHD mutagenicity and the characteristics of the household in which the dust was collected (Roberts et al. 1987). Only one significant association was observed; the TA98 +S9 mutagenic potency was significantly correlated with household income. Weak associations, though not statistically significant, were also found between the TA98 +S9 mutagenic potency and the age of carpet, between TA98 -S9 and nearby traffic, and TA98 -S9 and smoking in the home.

Interestingly, none of the household attributes that were related to mutagenicity were similar between the Roberts et al. study and the present study. This may be due to differences in the type of information that was collected in the homeowner surveys. However, it may also be

indicative of differences in house dust composition depending on the sample location and factors therein that influence the composition.

Empirical Relationships Between Dust PAH Content and Household Attributes

Previous studies have shown PAH concentrations in indoor air to be related to PAH source events such as smoking (Mitra and Ray 1995; Chuang et al. 1999), cooking (Zhu and Wang 2003), and fireplace and woodstove use (Alfheim and Ramdahl 1984; Daisey et al. 1989). Furthermore, analyses of the pooled dataset in Chapter 1 showed PAH levels in SHD to be related to home location and smoking habits (see Table 3, Chapter 1). Although it was expected that similar relationships would be found in the present analyses, no significant relationships were observed between the PAH concentrations in dust and PAH source events identified in the homeowner survey.

One reason for the lack of associations might be that the present study and the corresponding homeowner survey were not specifically designed to evaluate the relationship between PAH concentration and likely PAH sources. For example, urban locations are often associated with PAH sources such as high density traffic, proximal industrial activity, and greater environmental tobacco smoke (Chuang et al. 1997). Therefore, differences were expected in the PAH content of the dust depending on location. However these differences were not observed, and closer inspection of the classifications used for home location reveals four categories that are not in fact very distinct from each other. Only 6% of houses were located in a main commercial area (i.e., urban area) and 4% in a rural area, while the vast majority (90%) of houses were categorized as being in either main residential or quiet residential areas. Moreover, notes in the homeowner survey explain that some of the home locations are immediately adjacent to areas that would fall under a different location category, introducing uncertainty into the classifications. Had the study been designed to have a more even number of homes in urban, suburban and rural areas, and the questionnaire designed to accurately reflect these classifications, it is possible that significant differences in PAHs may have been observed between locations. The same may have held true for PAHs and other source events such as smoking or cooking.

Although the present analyses could not relate PAH concentration to categories in the homeowner survey that were reflective of combustion source events, an evaluation of PAH source markers reveals that the PAHs were likely a result of combustion activities. The ratio of phenanthrene to anthracene (P/A) gives an indication of the possible source events that contribute to the presence of the PAHs (Baumard et al. 1998). Although phenanthrene and anthracene are structural isomers, anthracene degrades more quickly in petroleum source events than combustion source events (Soclo et al. 2000). Therefore, when the P/A ratio is high (greater than 10), it is likely that the PAHs were generated petrogenically as opposed to pyrogenically. In this study, the mean P/A ratio in the dust samples was 6.7 ± 2.60. Of the 49 dust samples in which both phenanthrene and anthracene were detected, only 5 samples had P/A values that were over 10. Hence, it is likely that the PAHs in the majority of the dust samples were produced by combustion.

Relationships were also evaluated between PAH concentrations in the dust and parameters in the survey that were not combustion related. It was found that the concentration of PAHs with four rings or more was negatively related to the frequency of vacuuming within the home. This finding suggests that the cleaning habits of the inhabitants in some way influences the PAH concentration of the dust. It is possible that homeowners who vacuum more frequently do so because their homes are noticeably dustier. The dustier homes may create a diluting effect on the PAH content of the dust. Alternatively, the frequency of vacuum cleaning may be an indicator of the general cleanliness of a home's residents. Again, it is possible that these "cleaner" residents also have other habits that lead to lower PAH concentrations (e.g., removal of shoes in the home, more frequent ventilation during PAH source events).

A negative relationship was also found between the concentrations of fluorene in the dust samples and the number of pets in the home. A marginally significant negative relationship was also found between the concentration of phenanthrene, pyrene, benzo[*b*]fluoranthene, total PAHs and the number of pets in the home. A possible explanation for this relationship is that pets contribute fine dander to the dust that may in effect be diluting the PAH concentrations.

Cancer Risk Assessment of PAHs in Settled House Dust

Knowing that exposure to PAHs can cause deleterious health effects, a risk assessment was conducted to evaluate the excess lifetime cancer risk resulting from non-dietary ingestion of carcinogenic PAHs in SHD during preschool years. The risk was evaluated for low (0.01 grams per day), medium (0.05 grams per day) and high (0.1 grams per day) dust ingestion rate scenarios. To interpret the outcome of the assessment, the results are evaluated against values of "acceptable risk", where the cancer risks are deemed to be essentially negligible.

One cancer case per million people in the general population (i.e., one in one million or 10^{-6}) is commonly used as a baseline level of acceptable risk. Depending on exposure scenarios, agencies involved with risk assessment frequently build on this level and adopt ranges of acceptable risk. For example, the USEPA often uses the range of $10^{-4} - 10^{-6}$ (USEPA 1990), while Health Canada has established $10^{-5} - 10^{-6}$ as an acceptable range, particularly for carcinogens in drinking water (Health and Welfare Canada 1989). Other Canadian agencies have adopted 10^{-5} as the acceptable level of risk for exposures to carcinogens in other matrices including contaminated soils (Health Canada 2004). Further discussion on the development and definition of acceptable risk is available in Barnard (1990) and Health Canada (2004).

In this study, the risk assessment calculations revealed that exposure to PAH levels found at the majority of households (i.e., less than 30 $\mu g\ g^{-1}$) resulted in risks that were between 1×10^{-6} and 1×10^{-4} (Figure 8, Chapter 2). Five of the houses contained levels of carcinogenic PAHs that were higher than 40 $\mu g\ g^{-1}$ and resulted in risks above 1×10^{-4}. By USEPA standards, the levels of risk presented by PAHs in the majority of house dust samples were considered to be acceptable. For the five houses with the highest PAH concentrations, however, the maximum acceptable level of risk was exceeded, specifically when the middle or highest ingestion rates were considered. The five homes with the highest levels of PAHs were E029, C001, E018, E028 and S011. Examination of the household survey results did not reveal any distinguishing characteristics that were common to all of these homes and which may have accounted for the higher PAH levels.

If the risk assessment outcomes are compared to the Canadian maximum acceptable level of risk (i.e., 1 x 10^{-5}), the results vary substantially according to the ingestion rate considered. If the lowest ingestion rate is considered, only 5 homes (i.e., the same 5 homes as above) contain PAH levels that result in unacceptable risk rates. If the middle ingestion rate is considered, 21 homes (41%) would contain levels of carcinogenic PAHs above 8 μg g^{-1}, resulting in unacceptable risk rates. Similarly, at the highest ingestion rate, 34 homes (67%) would contain levels of carcinogenic PAHs above 4.2 μg g^{-1}, again resulting in unacceptable levels of risks.

If the risk assessment outcomes are compared against the most conservative interpretation of acceptable risk (i.e., 1 x 10^{-6}), the vast majority of houses in the study contain levels of carcinogenic PAHs in SHD that are unacceptable. Only exposures to PAHs at concentrations lower than approximately 4.2 μg g^{-1} (i.e. 33% of homes), at the lowest ingestion rate, result in acceptable levels of risk.

Table 14 provides a summary of the interpretation of the risk assessment results with reference to the dust ingestion rates and the levels of acceptable risk.

Table 14. Percentage of Ottawa homes sampled that contain levels of carcinogenic PAHs which exceed acceptable levels for excess lifetime cancer risks. (Based on non-dietary ingestion of the PAHs in settled house dust by preschool aged children.)

Dust Ingestion Rate (g d^{-1})	**Percentage of Homes Exceeding Levels of Acceptable Risk**		
	> 1 x 10^{-6}	**> 1 x 10^{-5}**	**> 1 x 10^{-4}**
0.01	67%	10%	0%
0.05	98%	41%	0%
0.1	100%	67%	10%

The calculation of risk involves a number of assumptions and uncertainties that have the potential to influence the outcome of the assessment. In addition to the assumptions listed in Table 6 in Chapter 1, other elements may have introduced bias in the risk assessment. For example, the use of potency equivalency factors (PEFs) may not accurately reflect the true toxicity of PAHs in mixture. Reeves et al. (2001) conducted a series of rapid bioassays to test the toxicity of PAH mixtures. They observed that the predicted potencies derived from chemical analyses using toxic equivalency factors were often in poor agreement with the assay derived potencies. Schneider et al. (2002) also found that PEFs do not adequately describe the potency of PAH mixtures. Depending on exposure pathway and the type of cancer considered, the use of PEFs often result in an underestimation of the true potency of PAH mixtures. Better information on the interaction of PAHs and other chemicals in mixtures, as well as more precise assessments of dust ingestion rates, PAH exposure levels, and daily exposure times would all help to improve the characterization of risk.

Accepting that, as with all risk assessment, there is uncertainty in the present risk calculations, the results can still be compared to the results of previously conducted risk assessments. Roberts et al. also evaluated the lifetime cancer risks associated with the ingestion of various PAH concentrations in SHD (Roberts et al. 1992). The exact number, type and proportion of individual PAHs that made up the total PAH concentration in the Roberts et al. study are unknown. However, general comparisons can still be made with houses in the present study that had similar levels of total carcinogenic PAHs. The results in Table 15 show that although the levels of excess cancer risk are comparable, the risk values calculated in the Roberts et al. study are consistently higher (approximately six times higher) than in the present study. The higher values may be partially accounted for by the use of a higher slope factor (i.e., 11.3 instead of 7.3). However, very little other information on their risk calculations is available to help explain the differences.

Table 15. Comparisons of levels of excess cancer risk resulting from the non-dietary ingestion of PAHs in settled house dust.

	Ingestion Rate = 0.05 g d^{-1}		Ingestion Rate = 0.1 g d^{-1}	
PAH Concentration	Roberts et al.[a]	Present Study	Roberts et al.[a]	Present Study
0.97 μg g^{-1}	7.8 x 10^{-6}	1.6 x 10^{-6}	1.6 x 10^{-5}	3.2 x 10^{-6}
4.2 μg g^{-1}	3.4 x 10^{-5}	5.1 x 10^{-6}	6.8 x 10^{-5}	1.0 x 10^{-5}
21 μg g^{-1}	1.7 x 10^{-4}	2.6 x 10^{-5}	3.4 x 10^{-4}	5.1 x 10^{-5}

[a] Values taken from (Roberts et al. 1992).

The levels of excess cancer resulting from children's non-dietary ingestion of PAHs in SHD can also be compared to the overall lifetime risks of developing cancer in general. In Canada, the lifetime probability of developing any type of cancer is 43.1% (1 in 2.3) for men and 38.4% (1 in 2.6) for women (National Cancer Institute 2004). The probability of developing stomach cancer (i.e., the type of cancer most similar to that upon which the benzo[*a*]pyrene oral slope factor is based) is 1.4% (1 in 71) for men and 0.8% (1 in 118) for women (National Cancer Institute 2004). In the present study, the highest calculated level of excess risk due to non-dietary PAH ingestion during preschool years was 5.49 x 10^{-4}. Therefore, based on the risk calculations, children's exposure to PAHs at levels in this home would increase their lifetime probability of cancer by approximately 0.055%. Consequently, the lifetime probability of developing stomach cancer would increase from 1.4% to 1.455% for men, and 0.8% to 0.855% for women. For the city of Ottawa, with a population of approximately 800,000 (Statistics Canada 2002), this would translate into an additional 440 cancer cases on top of the 6,800 - 11,250 stomach cancer cases that would otherwise be expected. Similarly, if the lowest and median levels of excess risk due to non-dietary PAH ingestion are considered (i.e., 1.38 x 10^{-7} and 6.68 x10^{-6}), this would translate into an additional 0.1 and 5.3 cancer cases respectively for the population of the city of Ottawa.

In terms of epidemiological studies, very little work has been done to evaluate whether cancer incidences can be linked to exposure to contaminants in carpet dust. However, a recent population-based case-control study conducted across four areas in the United States has noted

significant associations between the detection of organochlorines in carpet dust and the incidence of non-Hodgkin lymphoma in adults (Colt et al. 2005). Specifically, the authors noted that the detection of PCBs in carpet dust was associated with elevated risks for non-Hodgkin lymphoma (odds ratio = 1.5; 95% confidence interval = 1.2-2.0). The detection of *p,p*'-dichlorodiphenyldichloroethylene (DDE) in carpet dust (1.3; 1.0-1.7) was also associated with increased risks for non-Hodgkin lymphoma, but only in males. Even though PAHs were not evaluated in this study, the results provide further evidence that exposure to contaminants in carpet dust may be associated with adverse health effects, including cancer.

Conclusions

Based on a limited number of samples, the results of this study show that SHD is a potential source of mutagenic hazard. The mean mutagenic potency of the SHD extracts ranged from 2,300 to 23,600 revertants $gram^{-1}$, depending on the strain used, and a predominance of frameshift mutagenic activity was detected in the samples. Testing of the whole dust samples as well as the basic and base/neutral fractions with various *Salmonella* strains revealed that both aromatic amines and neutral compounds likely play an important role in the mutagenicity of the dust samples. Although the identity of these compounds is still largely unknown, chemical analyses confirmed that PAHs were present in the dust samples. The PAH levels ranged from 1.5 – 325 $\mu g\ g^{-1}$ and were comparable to those observed in the data analysis in Chapter 1. Although there is some variability, calculations indicate that overall, PAHs can account for approximately 25% of the mutagenic activity of the dust samples.

The detection of PAHs in the dust samples led to the expectation that PAH levels would be positively correlated with PAH source activities such as smoking or an urban environment, as was observed in the data analyses from Chapter 1. However, no significant associations were observed with these parameters, possibly due to a limited data set or inadequate information in the home survey.

Dust mutagenicity was positively correlated with household attributes such as the number of inhabitants in the home, and cleaning habits such as the time since the last vacuuming. Furthermore, contrary to what was expected, dust mutagenicity was negatively correlated with the burning of incense and candles, and the use of a secondary heating source. The factors responsible for these associations were not clear, and identification of the parameters that influence SHD mutagenicity would be a promising area for future research.

The risk assessment calculations conducted in this study give a preliminary indication of the level of adverse effects that might be expected from children's (i.e., preschool) non-dietary ingestion of PAHs in house dust. The calculations revealed that the risks range from a low of 1.4×10^{-7} to a high of 5.5×10^{-4}. The interpretation of the outcome of this assessment is dependent

on the level of risk that is considered to be acceptable. Based on Canadian standards, high levels of risk (i.e., $> 1 \times 10^{-5}$) were found at 41% of the homes where levels of carcinogenic PAHs were greater than 8 $\mu g\ g^{-1}$ and the ingestion rate was assumed to be 0.05 $g\ d^{-1}$.

Future estimates of the risks associated with exposure to contaminants in SHD would benefit from more accurate, age-specific, estimates of dust ingestion rates. Current values routinely employed for risk assessment are based on a very limited number of critical investigations (e.g., Calabrese et al.1989). Videotaping has proved valuable in relating pesticide hand loading to children's activities (Freeman et al. 2005), and may be a useful tool in deriving better estimates of hand dust loading and ingestion rates based on age and mouthing behaviour. Future risk assessments would also benefit from further investigations into the hazards of PAHs in mixtures. Since human exposure to compounds such as PAHs occurs in mixture, information on the cumulative (geno)toxicity of compounds in real mixtures could provide a more accurate indication of actual risk.

General Conclusions

This thesis confirms that organic components in settled house dust from homes in Ottawa can elicit a positive mutagenic response in the *Salmonella* Mutagenicity Test. It also confirms the presence of mutagenic PAHs in settled house dust samples, and points to aromatic amines as another likely source of mutagenic activity. Although the mutagenicity of the dust samples was demonstrated only in a bacterial test system, these positive results provide sufficient cause for concern and motivation for future analyses in other test systems. Knowing that many *Salmonella* mutagens, including several PAHs, are also animal cell mutagens and known carcinogens, testing in other systems would help further characterize the hazards of SHD. Understanding the hazards posed by dust as a complex mixture is important for protecting human health, and in particular, the health of those most vulnerable and most exposed to contaminants in dust, i.e., children. In addition, identifying the hazardous components in dust that may affect child health is of significant value since contaminant control and/or containment requires knowledge of the identity of the putative compound(s). The importance of such knowledge has been clearly illustrated by the considerable progress made in identifying household sources of lead, and reducing children's exposure to lead in house dust.

The risk assessment calculations conducted in this study reveal that the ingestion of PAHs in house dust during childhood years can result in risks that exceed national guidelines for levels of "acceptable risk". Even though the interpretation of risk, and what constitutes "acceptable risk", is subjective in nature, risk assessment is an important tool for predicting the likelihood of health effects and initiating activities that mitigate harm. The risks associated with exposure to contaminants in house dust are not likely to be uniformly distributed. The highest levels of excess risk are expected to be manifested in those homes with uncommonly higher levels of PAHs (which may also have higher levels of mutagenic activity). Although likely sources of PAHs in the household environment are generally known, the factors associated with the accumulation of excessive PAH concentrations in dust are not clear. There is some indication from the analyses in Chapter 1 that the home location and the presence of smokers in the home may play a role, but this has not been shown definitively and the effects appear to be weak. The identification and subsequent manipulation of factors associated with higher PAH concentrations in dust would be beneficial in reducing PAH levels and consequently lowering the risk for excess cancer cases. This is particularly important since a number of the homes

examined in both Chapters 1 and 2 of this study had PAH levels that exceeded the German Environmental Agency's guideline for PAHs in dust, as well as the Canadian Environmental Quality guideline for PAHs in soil.

The knowledge that dust is mutagenic and that chemicals such as PAHs are found in house dust is somewhat discouraging as it points to yet another environmental compartment that contains potentially hazardous pollutants. At the same time, however, it is encouraging to recognize that these substances occur where individuals generally have the most control over their environment, i.e., in their homes. Although methods of reducing the mutagenicity or PAH content of dust have not yet been investigated, researchers have evaluated ways to reduce the overall dust content of the home, which could therefore result in reduced contaminant levels. Roberts et al. showed that proper vacuuming with an effective vacuum cleaner can remove deep carpet dust and reduce lead levels (Roberts et al. 2005). More importantly, these investigators also developed methods that provide families with quick low-cost estimates of how much deep dust is present in their carpets, how much time is actually required to remove the deep dust (a timeframe that is often underestimated), and an indication of when the carpet is actually clean.

Although science is critical in identifying the hazards and risks associated with environmental contaminants, initiatives that develop and communicate practical ways to improve the general condition of spaces where children spend most of their time, should be pursued with equal vigour.

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Appendices

Appendix II. Polycyclic aromatic hydrocarbon (PAH) concentrations ($\mu g\ g^{-1}$) in settled house dust samples from 18 published studies.

Ref	Sample Area[a]	Site	Collection Method	Location	Smoking	Income	ACNP[b]	ACNPHY	ANTH	BaA	BaP	BeP	BbkF	BghiP	CHRY	CORO	CPcdP	DBahA	FLUORAN	FLUOR	I123 cdP	NAPH	PHEN	PYR
(Rudel et al. 2003)	ME	Mean	Vacuum	unknown	unknown	unknown			0.107	0.954	1.58													3.12
(Wilson et al. 2003)	NC	D03_1	HVS3	rural	no	low	0.084	0.01	0.092	0.345	0.403	0.319	0.423	0.368	0.424	0.104	0.11	0.144	0.908	0.042	0.396	0.046	0.703	0.721
	NC	D03_2	HVS3	rural	no	low	0.068	0.011	0.071	0.264	0.291	0.243	0.317	0.29	0.324	0.088	0.085	0.111	0.699	0.031	0.303	0.041	0.533	0.57
	NC	DO9_1	HVS3	urban	no	unknown	0.011	0.004	0.011	0.031	0.045	0.048	0.054	0.059	0.056	0.025	0.013	0.023	0.093	0.016	0.055	0.013	0.078	0.081
	NC	DO9_2	HVS3	urban	no	unknown	0.005	0.003	0.005	0.022	0.023	0.027	0.03	0.036	0.046	0.017	0.01	0.015	0.049	0.01	0.032	0.008	0.038	0.042
	NC	HA3	HVS3	rural	no	low	0.005	0.004	0.011	0.019	0.026	0.034	0.037	0.041	0.05	0.02	0.01	0.016	0.074	0.007	0.035	0.006	0.064	0.057
	NC	HB3	HVS3	rural	no	low	0.004	0.002	0.008	0.025	0.024	0.031	0.036	0.04	0.037	0.022	0.013	0.02	0.076	0.006	0.036	0.006	0.056	0.062
	NC	HC3	HVS3	rural	yes	low	0.01	0.003	0.015	0.059	0.07	0.075	0.096	0.075	0.086	0.026	0.022	0.032	0.185	0.009	0.076	0.007	0.108	0.142
	NC	HD3	HVS3	rural	no	low	0.008	0.003	0.012	0.033	0.042	0.045	0.055	0.058	0.061	0.023	0.014	0.027	0.093	0.009	0.053	0.006	0.065	0.073
	NC	HE9	HVS3	urban	no	unknown	0.004	0.003	0.006	0.023	0.037	0.038	0.046	0.051	0.041	0.026	0.01	0.02	0.077	0.005	0.048	0.005	0.044	0.061
	NC	HF9	HVS3	urban	no	unknown	0.019	0.023	0.066	0.519	0.768	0.809	0.967	0.961	0.838	0.32	0.172	0.294	1.555	0.028	0.963	0.035	0.596	1.202
	NC	HG9	HVS3	urban	no	unknown	0.007	0.006	0.013	0.054	0.086	0.092	0.108	0.116	0.225	0.065	0.026	0.044	0.239	0.012	0.108	0.001	0.143	0.181
	NC	HH9	HVS3	urban	no	unknown	0.008	0.007	0.012	0.042	0.084	0.095	0.11	0.112	0.09	0.047	0.024	0.044	0.203	0.01	0.112	0.006	0.128	0.155
	NC	HI9	HVS3	urban	no	unknown	0.005	0.005	0.011	0.04	0.07	0.076	0.089	0.101	0.097	0.056	0.022	0.041	0.168	0.01	0.094	0.015	0.093	0.129
(Camann et al. 2002)	MI, IA, LA, WA	Median	Vacuum	unknown	unknown	unknown				0.136	0.154		0.409		0.27			0.036			0.161			
(Camann et al. 2001)	NY	Median	HVS3	unknown	unknown	unknown				1.14	1.46							0.29						
(Rudel et al. 2001)	MA	Mean	Vacuum	unknown	unknown	unknown				2.91	2.9													
(Wilson et al. 2001)	NC	D01	HVS3	rural	no	low	0.022	0.003	0.038	0.155	0.208	0.128	0.15	0.099	0.206	0.011	0.045	0.031	0.305	0.019	0.095	0.015	0.875	0.258

al. 2001)																						
	NC	D02	HVS3	urban	no	low	0.02	0.018	0.076	0.491	0.688	0.627	0.762	0.497	1.213	0.136	0.145	0.109	1.827	0.019	0.48	0.015	0.637	1.457
	NC	D03	HVS3	rural	no	low	0.05	0.005	0.053	0.258	0.203	0.132	0.159	0.112	0.318	0.015	0.072	0.034	0.536	0.019	0.119	0.026	0.343	0.45
	NC	D04	HVS3	urban	no	unknown	0.006	0.006	0.014	0.052	0.143	0.064	0.078	0.04	0.102	0.004	0.018	0.014	0.178	0.006	0.04	0.006	0.092	0.14
	NC	D05	HVS3	urban	no	unknown	0.009	0.002	0.007	0.031	0.117	0.046	0.037	0.003	0.046	0.001	0.014	0.01	0.082	0.006	0.003	0.009	0.06	0.066
	NC	D06	HVS3	urban	no	low	0.009	0.002	0.008	0.036	0.098	0.042	0.042	0.015	0.066	0.001	0.016	0.008	0.077	0.004	0.013	0.003	0.049	0.069
	NC	D07	HVS3	urban	no	unknown	0.003	0.001	0.009	0.031	0.034	0.025	0.032	0.006	0.036	0.001	0.011	0.003	0.084	0.004	0.005	0.001	0.051	0.067
	NC	D08	HVS3	urban	no	unknown	0.011	0.005	0.013	0.075	0.131	0.085	0.1	0.043	0.132	0.002	0.03	0.012	0.18	0.007	0.035	0.005	0.098	0.149
	NC	D09	HVS3	urban	no	unknown	0.006	0.001	0.007	0.038	0.015	0.015	0.061	0.001	0.039	0.001	0.016	0.003	0.085	0.007	0.002	0.002	0.05	0.066
	NC	D10	HVS3	urban	no	unknown	0.001	0.01	0.019	0.562	0.82	0.662	0.861	0.725	1.19	0.2	0.146	0.207	1.141	0.009	0.786	0.008	0.312	0.898
(Chuang et al. 1999)	NC	pilot1	HVS3	urban	no	low	0.139	0.167	0.277	0.694	0.586	0.746	1.341	0.607	2.406	0.065	0.220	0.257	1.889	1.220	0.699	0.090	2.149	1.646
	NC	pilot2	HVS3	urban	yes	low	0.011	0.059	0.101	0.234	0.169	0.351	0.590	0.300	0.770	0.055	0.119	0.108	0.714	0.080	0.299	0.044	0.839	0.738
	NC	wrns1	HVS3	rural	no	low	0.049	0.104	0.042	0.126	0.139	0.143	0.346	0.197	0.217	0.204	0.061	0.086	0.445	0.051	0.172	0.085	0.284	0.337
	NC	wrns2	HVS3	rural	no	low	0.040	0.043	0.167	0.099	0.070	0.149	0.237	0.169	0.141	0.501	0.060	0.072	0.245	0.106	0.103	0.293	0.288	0.236
	NC	wrns3	HVS3	rural	no	low	0.012	0.008	0.021	0.103	0.104	0.115	0.343	0.147	0.139	0.073	0.049	0.060	0.333	0.053	0.110	0.056	0.142	0.205
	NC	wrns4	HVS3	rural	no	low	0.009	0.017	0.015	0.061	0.112	0.569	0.325	0.108	0.100	0.037	0.027	0.022	0.155	0.028	0.072	4.299	0.133	0.115
	NC	wuns1	HVS3	urban	no	low	0.177	0.090	0.027	0.337	0.286	0.361	0.745	0.240	0.360	0.062	0.126	0.088	0.764	0.144	0.218	0.315	0.425	0.754
	NC	wuns2	HVS3	urban	no	low	0.004	0.023	0.070	0.039	0.086	0.050	0.171	0.120	0.046	0.064	0.060	0.068	0.092	0.024	0.047	0.017	0.206	0.059
	NC	wuns3	HVS3	urban	no	low	0.040	0.075	0.083	0.358	0.254	0.252	0.515	0.224	0.293	0.062	0.079	0.089	0.442	0.075	0.224	0.076	0.416	0.335
	NC	wuns4	HVS3	urban	no	low	0.026	0.027	0.039	0.152	0.151	0.290	1.065	0.560	0.308	0.219	0.125	0.213	0.478	0.157	0.562	0.041	0.582	0.314
	NC	wuns5	HVS3	urban	no	low	0.146	0.089	0.032	0.340	0.285	0.381	0.763	0.245	0.301	0.086	0.106	0.115	0.682	0.135	0.235	0.315	0.376	0.612
	NC	suns1	HVS3	urban	no	low	0.043	0.272	0.161	0.602	0.633	0.420	1.052	0.583	0.780	0.260	0.209	0.414	0.969	0.105	0.620	0.316	0.483	0.725
	NC	suns2	HVS3	urban	no	low	0.038	0.168	0.046	0.095	0.069	0.108	0.243	0.091	0.187	0.097	0.038	0.039	0.300	0.049	0.084	0.155	0.342	0.303
	NC	suns3	HVS3	urban	no	low	0.039	0.074	0.096	0.167	0.289	0.221	0.516	0.286	0.355	0.151	0.072	0.073	0.532	0.069	0.227	0.136	0.394	0.396
	NC	suns4	HVS3	urban	no	low	0.051	0.053	0.063	0.218	0.236	0.258	0.665	0.364	0.351	0.214	0.072	0.077	0.501	0.081	0.278	0.121	0.515	0.378
	NC	suns5	HVS3	urban	no	low	0.035	0.064	0.068	0.171	0.281	0.272	0.587	0.297	0.405	0.160	0.049	0.088	0.500	0.057	0.254	0.280	0.377	0.439
	NC	srns1	HVS3	rural	no	low	0.051	0.051	0.131	0.172	0.208	0.186	0.424	0.214	0.323	0.062	0.036	0.050	0.489	0.072	0.182	0.242	0.388	0.339
	NC	srns2	HVS3	rural	no	low	0.020	0.018	0.028	0.111	0.186	0.153	0.366	0.158	0.237	0.068	0.036	0.048	0.359	0.038	0.139	0.060	0.195	0.268
	NC	srns3	HVS3	rural	no	low	0.023	0.034	0.040	0.414	0.141	0.144	0.453	0.166	0.204	0.061	0.065	0.054	0.280	0.056	0.146	0.262	0.259	0.209

	NC	srns4	HVS3	rural	no	low	0.035	0.023	0.058	0.14	0.229	0.176	0.408	0.169	0.279	0.054	0.074	0.055	0.495	0.048	0.151	0.127	0.368	0.349
	NC	4suns1	HVS3	urban	yes	low	0.055	0.033	0.748	0.452	0.483	0.429	1.118	0.477	0.644	0.231	0.145	0.143	0.967	0.063	0.496	0.211	0.44	0.756
	NC	4suns2	HVS3	urban	yes	low	0.055	0.116	0.282	0.07	0.24	0.106	0.258	0.101	0.167	0.127	0.025	0.027	0.251	0.091	0.075	0.152	0.312	0.236
	NC	4srs	HVS3	rural	yes	low	0.03	0.128	0.053	0.108	0.195	0.099	0.208	0.079	0.148	0.039	0.035	0.015	0.221	0.081	0.072	0.233	0.238	0.38
	NC	4srs2	HVS3	rural	yes	low	0.034	0.071	0.124	0.112	0.162	0.144	0.384	0.139	0.272	0.056	0.027	0.037	0.378	0.057	0.126	0.085	0.307	0.292
(Dieckow et al. 1999)	Germany	Median 1	Vacuum, sweeping, wiping	various	various	various					0.3													
(Lewis et al. 1999)	NC	Composite	Vacuum	suburban	unknown	middle				1.82	1.76		5.52	2.73	3.41	1.42		0.48			2.33			
(Simrock 1998)	Germany	Median	Vacuum	unknown	unknown	unknown																	0.3	
(Chuang et al. 1997)	NC	F	HVS3	urban	yes	low	0.055	0.033	0.75	0.45	0.48	0.43	1.1	0.48	0.64	0.23	0.14	0.14	0.97	0.063	0.5	0.21	0.44	0.76
	NC	G	HVS3	urban	yes	low	0.055	0.12	0.28	0.07	0.24	0.11	0.26	0.1	0.17	0.13	0.025	0.027	0.25	0.091	0.075	0.15	0.31	0.24
	NC	K	HVS3	rural	yes	low	0.03	0.13	0.053	0.11	0.2	0.099	0.21	0.079	0.15	0.039	0.035	0.015	0.22	0.081	0.072	0.23	0.24	0.38
	NC	M	HVS3	rural	yes	low	0.034	0.071	0.12	0.11	0.16	0.14	0.38	0.14	0.27	0.056	0.027	0.037	0.38	0.057	0.13	0.085	0.31	0.29
(Chuang et al. 1997)	NC	A-HD-X	No data	unknown	unknown	low	0.202	0.131	0.116	0.36	0.138	0.207	0.473	0.122	0.367	0.031	0.1	0.032	1.025	0.814	0.117	0.212	0.741	0.769
	NC	B-HD-X	No data	unknown	unknown	low	0.015	0.022	0.021	0.087	0.037	0.284	0.242	0.07	0.132	0.03	0.02	0.018	0.299	0.029	0.076	0.077	0.197	0.228
	NC	C-HD-X	No data	unknown	unknown	low	0.019	0.016	0.025	0.041	0.022	0.066	0.116	0.035	0.049	0.016	0.008	0.012	0.14	0.033	0.025	0.032	0.143	0.095
	NC	D-HD-X	No data	unknown	unknown	low	0.032	0.023	0.038	0.117	0.063	0.123	0.255	0.056	0.128	0.044	0.018	0.014	0.208	0.058	0.065	0.044	0.237	0.141
	NC	E-HD-X	No data	unknown	unknown	low	0.022	0.03	0.025	0.144	0.073	0.128	0.239	0.101	0.162	0.065	0.036	0.017	0.2	0.033	0.106	0.082	0.202	0.157
	NC	F-HD-X	No data	unknown	unknown	low	0.185	0.063	0.331	1.465	0.931	0.907	2.452	0.817	1.052	0.283	0.404	0.24	2.148	0.19	0.879	0.136	1.316	1.571
	NC	G-HD-X	No data	unknown	unknown	low	0.028	0.042	0.025	0.117	0.04	0.137	0.146	0.069	0.166	0.032	0.025	0.014	0.237	0.049	0.052	0.039	0.173	0.184

	NC	H-HD-X	No data	unknown	unknown	low	0.043	0.026	0.036	0.323	0.126	0.149	0.402	0.124	0.43	0.039	0.065	0.036	0.627	0.05	0.143	0.155	0.318	0.407
	NC	I-HD-X	No data	unknown	unknown	low	0.007	0.007	0.007	0.064	0.03	0.04	0.105	0.046	0.067	0.035	0.013	0.016	0.129					
	NC	J-HD-X	No data	unknown	unknown	low	0.019	0.015	0.019	0.09	0.062	0.101	0.215	0.083	0.106	0.038	0.026	0.02	0.249	0.029	0.088	0.061	0.137	0.169
	NC	K-HD-X	No data	unknown	unknown	low	0.055	0.023	0.029	0.187	0.09	0.241	0.365	0.122	0.171	0.037	0.045	0.031	0.491	0.027	0.134	0.026	0.183	0.334
	NC	L-HD-X	No data	unknown	unknown	low	0.009	0.01	0.036	0.209	0.293	0.043	0.765	0.322	0.225	0.12	0.061	0.076	0.498	0.035	0.338	0.018	0.237	0.347
	NC	M-HD-X	No data	unknown	unknown	low	0.037	0.046	0.022	0.108	0.044	0.11	0.205	0.069	0.126	0.039	0.022	0.019	0.272	0.069	0.074	0.05	0.225	0.185
(Lebowitz 1995; Chuang et al. 1997)	AZ	514125	No data	unknown	unknown	unknown	0.024	0.013	0.024	0.04	0.042	0.06	0.101	0.058	0.124	0.034	0.01	0.013	0.127	0.029	0.058	0.053	0.099	0.102
	AZ	181831	No data	unknown	unknown	unknown	0.015	0.007	0.017	0.111	0.143	0.148	0.357	0.148	0.146	0.054	0.024	0.036	0.282	0.02	0.164	0.041	0.17	0.239
	AZ	314275	No data	unknown	unknown	unknown	0.027	0.031	0.04	0.253	0.421	0.457	1.13	0.484	0.47	0.187	0.071	0.117	0.68	0.026	0.532	0.041	0.244	0.569
	AZ	314985	No data	unknown	unknown	unknown	0.017	0.014	0.021	0.054	0.067	0.107	0.254	0.105	0.183	0.05	0.012	0.029	0.202	0.025	0.113	0.044	0.156	0.164
	AZ	318479	No data	unknown	unknown	unknown	0.015	0.014	0.019	0.056	0.069	0.103	0.228	0.083	0.129	0.034	0.011	0.021	0.148	0.019	0.088	0.039	0.084	0.138
	AZ	523527	No data	unknown	unknown	unknown	0.017	0.013	0.048	0.033	0.063	0.11	0.211	0.091	0.121	0.056	0.008	0.026	0.143	0.025	0.104	0.025	0.074	0.123
	AZ	313546	No data	unknown	unknown	unknown	0.027	0.041	0.022	0.044	0.059	0.089	0.179	0.102	0.093	0.096	0.011	0.026	0.13	0.028	0.102	0.035	0.099	0.114
	AZ	181815	No data	unknown	unknown	unknown	0.014	0.001	0.024	0.072	0.072	0.126	0.295	0.098	0.101	0.062	0.013	0.018	0.225	0.026	0.106	0.032	0.125	0.188
	AZ	312413	No data	unknown	unknown	unknown	0.005	0.012	0.05	0.033	0.06	0.13	0.199	0.094	0.075	0.087	0.011	0.023	0.11	0.025	0.1	0.047	0.063	0.091
	AZ	513278	No data	unknown	unknown	unknown	0.017	0.013	0.038	0.468	0.68	0.578	1.578	0.636	0.685	0.215	0.148	0.158	0.746	0.019	0.727	0.02	0.237	0.72
	AZ	312572	No data	unknown	unknown	unknown	0.023	0.008	0.012	0.032	0.023	0.051	0.075	0.032	0.074	0.071	0.007	0.027	0.1	0.009	0.032	0.058	0.216	0.092
	AZ	317968	No data	unknown	unknown	unknown	0.014	0.007	0.014	0.022	0.018	0.126	0.145	0.047	0.074	0.025	0.003	0.012	0.102	0.018	0.049	0.029	0.082	0.081
	AZ	323619	No data	unknown	unknown	unknown	0.013	0.023	0.022	0.044	0.063	0.088	0.2	0.101	0.101	0.074	0.012	0.024	0.162	0.049	0.102	0.064	0.19	0.146
	AZ	321583	No data	unknown	unknown	unknown	0.039	0.023	0.028	0.106	0.112	0.193	0.666	0.199	0.186	0.16	0.023	0.05	0.281	0.054	0.222	0.095	0.172	0.231
	AZ	315148	No data	unknown	unknown	unknown	0.032	0.018	0.021	0.11	0.131	0.12	0.358	0.173	0.195	0.109	0.027	0.055	0.313	0.04	0.205	0.09	0.218	0.277
	AZ	323895	No data	unknown	unknown	unknown	0.009	0.015	0.13	0.039	0.035	0.054	0.113	0.061	0.078	0.055	0.004	0.036	0.081	0.02	0.066	0.082	0.078	0.085

	AZ	315845	No data	unknown	unknown	unknown	0.011	0.007	0.015	0.037	0.051	0.031	0.093	0.045	0.053	0.032	0.018	0.013	0.072	0.01	0.049	0.017	0.041	0.06
	AZ	324319	No data	unknown	unknown	unknown	0.017	0.01	0.026	0.057	0.067	0.081	0.252	0.106	0.096	0.065	0.015	0.039	0.139	0.021	0.12	0.045	0.073	0.12
	AZ	513656	No data	unknown	unknown	unknown	0.013	0.008	0.014	0.026	0.02	0.123	0.127	0.033	0.082	0.015	0.006	0.013	0.098	0.017	0.043	0.037	0.063	0.075
	AZ	323521	No data	unknown	unknown	unknown	0.009	0.005	0.005	0.017	0.021	0.062	0.126	0.043	0.055	0.035	0.005	0.009	0.047	0.013	0.05	0.022	0.04	0.043
	AZ	181525	No data	unknown	unknown	unknown	0.009	0.004	0.008	0.142	0.093	0.049	0.09	0.04	0.048	0.021	0.003	0.01	0.079	0.011	0.045	0.006	0.071	0.063
	AZ	319179	No data	unknown	unknown	unknown	0.011	0.005	0.009	0.211	0.04	0.069	0.138	0.072	0.101	0.04	0.008	0.016	0.101	0.012	0.078	0.01	0.044	0.086
(Mukerjee et al. 1997)	TX	Mean1	HVS3	various	various	low	0.0100		0.1820	0.1030	0.1280		0.2700	0.1300	0.1930			0.0321	0.2750	0.0216	0.1400	0.1880	0.1790	0.2420
	TX	Mean2	HVS3	various	various	low	0.0182		0.0278	0.0824	0.1108		0.2000	0.1038	0.1455			0.0258	0.2375	0.0169	0.1090	0.3000	0.2495	0.1962
(Chuang 1996)	KY	HD31	HVS3	unknown	unknown	unknown	0.011	0.055	0.582	0.223	0.189	0.227	0.522	0.228	0.356	0.13	0.073	0.098	0.325	0.069	0.21	0.093	0.25	0.27
	KY	HD32	HVS3	unknown	unknown	unknown	0.011	0.041	0.042	0.18	0.177	0.2	0.492	0.186	0.276	0.061	0.064	0.061	0.324	0.035	0.184	0.027	0.2	0.249
	KY	HD33	HVS3	unknown	unknown	unknown	0.043	0.191	0.051	0.145	0.131	0.114	0.321	0.151	0.155	0.085	0.051	0.06	0.293	0.061	0.149	0.065	0.287	0.212
(Camann and Buckley 1994)	IL, IN, IA, MI, MN, NJ, OH, PA, WI	Median	HVS3	unknown	unknown	unknown				0.71	1.12		2.34	0.71	1.26	0.28		0.36			0.72			
(Chuang et al. 1994)	WA	HD1	HVS3	urban	no	unknown			0.062	0.32	0.49	0.51	1	0.44	0.53	0.14	0.01	0.15	0.75	0.047	0.35		0.6	0.66
	WA	HD2	HVS3	urban	no	unknown			0.09	0.41	0.6	0.54	1.1	0.5	0.7	0.11	0.01	0.093	1.1	0.062	0.41		0.75	0.93
	WA	HD3	HVS3	urban	no	unknown			0.10	0.68	0.76	0.87	2.2	0.94	1.2	0.22	0.01	0.26	1.5	0.12	0.77		1.4	1.4
	WA	HD7	HVS3	urban	no	unknown			0.11	0.44	0.52	0.68	1.6	0.68	0.86	0.48	0.01	0.21	1.2	0.06	0.59		0.91	1.2
	WA	HD9	HVS3	urban	no	unknown			0.084	0.32	0.49	0.41	0.88	0.36	0.54	0.065	0.01	0.073	1	0.053	0.33		1	0.91
	WA	HD10	HVS3	urban	no	unknown			0.091	0.42	0.61	0.67	1.2	0.66	0.95	0.37	0.06	0.51	0.86	0.039	0.65		0.76	0.97
	WA	HD13	HVS3	urban	no	unknown			0.063	0.25	0.46	0.38	0.77	0.37	0.52	0.11	0.01	0.16	0.76	0.053	0.37		1	0.68
	WA	HD14	HVS3	urban	no	unknown			0.40	1.5	1.7	1.5	3.5	1.3	2.4	0.14	0.01	0.32	3.9	0.28	1.4		3.1	3
	WA	HD15	HVS3	urban	no	unknown			0.10	0.54	0.92	0.74	1.6	0.83	0.98	0.14	0.05	0.15	1.6	0.089	0.81		1.1	1.4
(Chuang et al. 1993)	OH	H01DS_Jun	HVS3	urban	yes	unknown	0.28	0.2	0.73	3.3	13	3.6	10	4.6	5.7	2.2	0.21	1.8	12	0.48	5.5	0.01	6.8	9.4

OH	H01DS_Oct	HVS3	urban	yes	unknown	0.24	0.21	0.65	3.3	3.7	3.1	7.8	3.1	5.1	0.81	0.07	0.92	10	0.48	3.5	3	6.2	7.9
OH	H01DS_Apr	HVS3	urban	yes	unknown	0.15	0.12	0.44	2	3	2.5	5.7	2.4	3	0.25	0.022	0.5	6.7	0.31	2.7	0.03	4.3	4.8
OH	H02DN_Jun	HVS3	urban	no	unknown	0.09	0.12	0.25	1.3	21	1.7	3.7	2.4	2.2	1.4	0.17	0.96	3.7	0.15	2.7	0.16	2.1	2.9
OH	H02DN_Oct	HVS3	urban	no	unknown	0.08	0.03	0.16	1.5	1.5	1.5	3.5	1.2	2.1	0.3	0.03	0.44	3.1	0.18	1.3	32	2.1	2.6
OH	H02DN_Apr	HVS3	urban	no	unknown	0.13	0.036	0.29	1.6	2.1	1.9	4.4	1.6	2.5	0.11	0.01	0.28	5	0.23	1.8	0.068	3.3	3.7
OH	H03DN_Jun	HVS3	urban	no	unknown	0.07	0.04	0.16	1	1.8	1.6	3.5	1.5	1.6	0.7	0.04	0.67	3.2	0.11	1.8	0.09	2.1	2.5
OH	H03DN_Oct	HVS3	urban	no	unknown	0.11	0.07	0.28	1.8	2.4	2.1	5.8	2.5	2.7	0.52	0.02	0.81	6.1	0.2	2.6	9.2	3.7	4.8
OH	H03DN_Apr	HVS3	urban	no	unknown	0.1	0.17	0.27	1.9	4.2	3.1	8.1	3.7	2.2	2.1	0.62	1.8	5.2	0.17	5.4	0.066	2.7	4.1
OH	H04DS_Jun	HVS3	urban	yes	unknown	0.05	0.04	0.11	0.48	0.7	11	1.6	1	1	0.5	0.06	0.37	1.8	0.08	1.3	0.24	0.98	1.3
OH	H04DS_Oct	HVS3	urban	yes	unknown	0.11	0.04	0.25	1.5	2.5	2.4	5.5	2	2.6	1.6	0.14	1.2	4.7	0.23	2.4	3.6	3.1	3.5
OH	H04DS_Apr	HVS3	urban	yes	unknown	0.11	0.026	0.27	1.3	1.8	1.6	1.6	1.4	2.2	0.016	0.01	0.22	3.9	0.17	1.6	0.044	2.4	2.8
OH	H05aDS_Jun	HVS3	urban	yes	unknown	0.05	0.02	0.12	0.7	3.9	0.25	2.6	0.72	1.1	0.21	0.05	0.21	2.2	0.09	0.91	0.01	1.1	1.5
OH	H05SHD_Oct	HVS3	urban	yes	unknown	1.6	0.29	2.70	17	18	16	38	11	20	1.8	0.01	3.9	46	2.8	12	17	33	34
OH	H05SHD_Apr	HVS3	urban	yes	unknown	1.9	0.27	3.30	14	17	13	33	13	21	1.6	0.046	2.7	45	2.6	16	0.34	32	32
OH	H06DS_Jun	HVS3	urban	yes	unknown	0.15	0.36	0.69	4.1	2	2.4	5.6	3.2	6.2	2.1	0.19	1.8	11	0.55	4.1	0.23	6.3	8
OH	H06DS	HVS3	urban	yes	unknown	0.17	0.19	0.33	3.9	3	4.4	9.8	2.7	3.2	0.64	0.19	0.69	4.9	0.36	2.8	1.6	4.5	5.4

	_Oct																					
OH	H06DS_Apr	HVS3	urban	yes	unknown	0.18	0.14	0.45	2.8	3.5	2.9	7	2.4	4	0.29	0.026	0.53	7.4	0.33	2.8	0.074	4.5	5.5
OH	H07DN_Jun	HVS3	urban	no	unknown	0.24	0.1	1.10	5.1	7.7	6.6	15	5.3	7.2	1.9	0.23	2.1	15	0.46	6.9	0.06	7.5	11
OH	H07DN_Oct	HVS3	urban	no	unknown	0.19	0.1	0.57	5	4.6	4.7	9.9	2.9	5.4	0.99	0.08	0.98	12	0.36	2.9	42	6	7.1
OH	H07DN_Apr	HVS3	urban	no	unknown	0.33	0.11	1.20	6.4	9.1	7.1	17	6.4	9.8	0.88	0.036	1.3	20	0.56	7.5	0.048	10	14
OH	H08D_Jun	HVS3	urban	no	unknown	1.1	0.52	3.90	24	54	41	103	35	34	7.2	0.36	7.5	90	2.1	41	0.35	41	68
OH	H08D_Oct	HVS3	urban	no	unknown	1.8	0.34	5.80	40	41	37	108	22	43	3.5	0.06	9	88	3	27	12	43	69
OH	H08D_Apr	HVS3	urban	no	unknown	1.1	0.24	3.50	20	23	17	42	16	27	2.1	0.056	3.4	48	1.7	19	0.2	26	37

[a]AZ - Arizona IL - Illinois IN - Indiana IA - Iowa KY - Kentucky LA - Los Angeles
MA - Massachusetts ME - Maine MI - Michigan MN - Minnesota NJ - New Jersey NY - New York
NC - North Carolina OH - Ohio PA - Pennsylvania TX - Texas WI - Wisconsin WA - Washington

[b]ACNP – acenaphthene ACNPHY- acenaphthylene ANTH – anthracene BaA - benzo[*a*]anthracene
BaP - benzo[*a*]pyrene BeP - benzo[*e*]pyrene BbkF - benzo[*b,k*]fluoranthene BghiP - benzo[*g,h,i*]perylene
CHRY – chysene CORO – coronene CPcdP - cyclopenta[*c,d*]pyrene DBahA - dibenz[*a,h*]anthracene
FLUORAN – fluoranthene FLUOR - fluorene I123cdP- indeno[1,2,3-c,d]pyrene NAPH - naphthalene
PHEN – phenanthrene PYR - pyrene

Appendix III.

Health Canada Indoor Air Study Questionnaire November 2002 - March 2003. Survey results were abbreviated to include only those questions and responses relevant to settled house dust. Blank fields indicate that either the survey question was not applicable to the household, or that the question was answered with a response that did not fit the form required and the information was removed.

	A001[a]	C001	C002	C003	C005	C007	C011	C012	C013	C014	C015	C016	C017	C019
Number of People Living in Home	1	2	4	9	2	4	1	3	1	1	2	3	1	5
Number of Cats/Dogs Inside Home	0	1	1	0	1	0	2	0	0	0	0	0	0	3
Age of Home (years)	8	55	80	77	90	55	90	101	100	80	80	85	24	10
Main Heating Source[b]	ng	fo	ng	ng	ng	ng	ng	ng	ng	ng	ng	fo	ng	ng
Secondary Heating Source Operated[c]	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
Fireplace or Woodstove Operated[c]	no	no	no	no	no	no	yes	yes	no	no	yes	no	yes	no
% of Home Carpeted	90	25	20	30	10	5	5	15	0	5	15	30	65	100
Type of Garage[d]	ade													ade
Home Location[e]	mr	qr	qr	mr	qr	mc	qr	qr	mr	qr	mc	mr	mr	mr
Smokers Present in Household	no	no	no	no	no	no	no	no	no	no	no	no	yes	no
Tobacco Smoked[f]	no	no	no	no	no	no	yes	no	no	no	no	yes	yes	no
Number Cigarettes Smoked Per Day[g]							2						8	
Incense Burned[c]	no	no	no	no	no	no	yes	yes	no	yes	no	no	no	no
Candles Burned[c]	yes	no	yes	yes	yes	yes	no	yes	yes	yes	no	yes	yes	no
Indoor BBQ[c]	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Cooking Oil[c]	yes	yes	yes	yes	yes	yes	no	yes	no	no	yes	yes	yes	yes
Vacuum Frequency (times per month)	4	4	2	4	4	10	1.65	4	2	1.65	4	4	4	4
Time Since Last Vacuum (days)	7	4	14	1	3	3	7	7	1	4	1	1	2	1
Carpet Shampoo Frequency (times per year)		1	2	1							1	0	0	1
Time Since Last Carpet Shampoo (months)	24	8	6	12							9	36	24	
Vacuum Cleaner Type[h]	pt	pt	pt	cv	pt	pt	pt	pt	pt	pt	pt	pt	pt	pt

	C022	C024	E001	E002	E004	E006	E008	E009	E011	E012	E013	E015	E016	E017
Number of People Living in Home	2	3	6	2	3	2	3	6	3	2	2	2	2	2
Number of Cats/Dogs Inside Home	0	1	0	0	2	0	0	0	0	0	1	1	0	0
Age of Home (years)	74	50	12	33	16	17	29	12	30	35	17	33	24	25
Main Heating Source[b]	e	ng	ng	ng	ng	ng	ng	ng	ng	ng	ng		ng	ng
Secondary Heating Source Operated[c]	no	no	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes
Fireplace or Woodstove Operated[c]	no	no	yes	no	yes	no	no	yes	no	yes	no	no	no	yes
% of Home Carpeted	5	13	50	90	30	90	13	70	75	75	50	40	60	
Type of Garage[d]			ade	ade		ade	ade	ade		ade	ade	ade	ade	ade
Home Location[e]	qr	mr	mr	mr	qr	qr	mc	qr	qr	qr	qr	qr	ql	qr
Smokers Present in Household	no	no	no	no	yes	no	yes	no	no	no	no	no	no	no
Tobacco Smoked[f]	no	no	no	no	yes	no	yes	no	yes	no	yes	no	no	no
Number Cigarettes Smoked Per Day[g]					61		8				6			
Incense Burned[c]	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Candles Burned[c]	no	no	yes	no	yes	yes	no	yes	yes	yes	yes	yes	no	yes
Indoor BBQ[c]	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Cooking Oil[c]	yes	no	no	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	no
Vacuum Frequency (times per month)	1	4	4	1	4	4	2	4	4	4	2	4	4	1
Time Since Last Vacuum (days)	1	14		7	1	5	1	7	1	4	5	2	7	1
Carpet Shampoo Frequency (times per year)		0	1	1	1			1		2	1		1	
Time Since Last Carpet Shampoo (months)		24	8	24	6	60		6		2	12	6	12	
Vacuum Cleaner Type[h]	pt	pt	pt	pt		pt	pt	cv	pt	pt	pt	pt	cv	cv

	E018	E019	E021	E022	E024	E027	E028	E029	E031	S003	S005	S006	S007	S010
Number of People Living in Home	4	4		3	2	4	4		3	2	4	3	2	2
Number of Cats/Dogs Inside Home	0	1	0	0	0	1	0	0	0	1	2	2	0	2
Age of Home (years)	12	17	33	0.04	33	17	14	23	15	15	18	50	39	35
Main Heating Source[b]	ng	ng	fo	ng	ng	ng	ng	ng	ng	ng	ng	fo	ng	ng
Secondary Heating Source Operated[c]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Fireplace or Woodstove Operated[c]	no	no	no	no	yes	yes	no	yes	no	no	yes	no	no	no
% of Home Carpeted	75	40	30	30	25	50	50	80	95	30	40	80	25	60
Type of Garage[d]	ade	ade	ade	ade	ade	ande	ande	ade	ade	ade	ade	ande		ade
Home Location[e]	mr	qr	qr	qr	qr	qr	qr	mr	qr	mr	qr	qr	mr	qr
Smokers Present in Household	no	no	no	no	no	no	yes	no	no	no	yes	no	no	yes
Tobacco Smoked[f]	no	no	yes	yes	no	no	yes	yes	no	no	yes	no	no	yes
Number Cigarettes Smoked Per Day[g]			1				3				28			25
Incense Burned[c]	yes	no	no	no	no	no	no	no	no	yes	no	no	yes	yes
Candles Burned[c]	yes	no	yes	yes	no	yes	no	yes	no	no	yes	no	no	yes
Indoor BBQ[c]	no	no	no	no	no	no	no	no	no	no	yes	no	yes	no
Cooking Oil[c]	yes	no	yes	yes	yes	no	yes	yes	yes	no	yes	yes	yes	yes
Vacuum Frequency (times per month)	4	4	2	15	4	30	4	2	4	1	4	4		4
Time Since Last Vacuum (days)	6	1	7	2	2	1	6	1	7	7	7	7	7	1
Carpet Shampoo Frequency (times per year)			0				2	1	1	1		1		
Time Since Last Carpet Shampoo (months)	36		12		36	24	6		24			6	48	
Vacuum Cleaner Type[h]	pt	cv	cv	cv	pt	pt	pt	pt	pt	pt	pt	pt	pt	pt

	S011	S012	S013	S015	S016	W001	W002	W003	W005	W006
Number of People Living in Home	3	2	3	2	5	2	3	2	5	3
Number of Cats/Dogs Inside Home	0	1	2	3	1	0	0	0	0	1
Age of Home (years)	18	40	40	19	16	25	17	17	35	33
Main Heating Source[b]	ng	ng	ng	ng	ng	ng	ng	e	fo	fo
Secondary Heating Source Operated[c]	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Fireplace or Woodstove Operated[c]	no	no	yes	no	no	yes	no	no	no	yes
% of Home Carpeted	90	60	50	0	80	70	90	75	90	40
Type of Garage[d]	ande	ande		ade	ade	ande	ande	ande	ande	ande
Home Location[e]	qr	qr	qr	qr	qr	qr	mr	ql	qr	mr
Smokers Present in Household	no	no	no	no	no	yes	no	yes	no	no
Tobacco Smoked[f]	no	no	no	no	yes	yes	no	yes	yes	no
Number Cigarettes Smoked Per Day[g]						10				
Incense Burned[c]	no	no	no	no	yes	yes	no	no	no	no
Candles Burned[c]	no	no	yes	yes	yes	yes	yes	no	yes	no
Indoor BBQ[c]	no	no	no	no	no	no	no	no	no	no
Cooking Oil[c]	yes	no	yes	no	yes	yes	yes	yes	yes	yes
Vacuum Frequency (times per month)	4	30	15	4	4	15	4	8	15	2
Time Since Last Vacuum (days)	8	3	2	1	5	1	7	4	1	5
Carpet Shampoo Frequency (times per year)	1	1	1				1	2		1
Time Since Last Carpet Shampoo (months)	18	6	10				8	4		3
Vacuum Cleaner Type[h]	cv	pt	pt	cv	cv	pt	pt	pt	pt	pt

[a] House was added to the study and was not part of the random selection process.

[b] ng = natural gas, e = electric, fo = fuel oil

[c] Materials used during two weeks prior to survey.

[d] ande = attached with no direct entry to house, ade = attached with direct entry to house

[e] mc = main commercial, mr = main residential, qr = quiet residential, ql = quiet rural

[f] Tobacco smoked inside or outside during week proceeding survey.

[g] Tobacco smoked inside during week proceeding survey.

[h] c = central vacuum, pv = portable vacuum

Appendix IV. *Salmonella* mutagenic potencies (revertants gram^{-1}) of house dust extracts tested with the strains TA98, YG1041 and YG1042, both with and without S9 metabolic activation.

		Mutagenic Potency (revertants gram^{-1}) ± S.E.M.				
House	**TA98 +S9**	**TA98 -S9**	**YG1041 +S9**	**YG1041 -S9**	**YG1042 +S9**	**YG1042 -S9**
A001[a]	1,737 ± 260	-	5,853 ± 307	6,574 ± 1,734	-	-
C001	5,540 ± 542	1,643 ± 240	26,856 ± 978	3,140 ± 287	-	8,619 ± 1,154
C002	3,120 ± 304	-	20,137 ± 713	10,747 ± 1,049	19,967 ± 998	14,682 ± 1,461
C003	4,299 ± 159	43,283 ± 4,091	37,965 ± 714	188,429 ± 20,943	-	-
C005	2,899 ± 252	-	6,054 ± 326	5,476 ± 766	-	-
C007	3,641 ± 344	-	11,280 ± 758	12,626 ± 1,136	-	-
C011	-	-	-	1,495 ± 302	-	-
C012	5,127 ± 507	1,116 ± 157	35,816 ± 2,396	12,093 ± 990	NT	4,981 ± 1,027
C013	1,784 ± 139	-	17,105 ± 1,330	2,910 ± 216	-	3,119 ± 495
C014	4,277 ± 498	3,250 ± 395	10,924 ± 562	64,433 ± 3,670	NT	NT
C015	3,577 ± 313	-	13,276 ± 340	4,219 ± 274	-	6,829 ± 549
C016	4,486 ± 277	-	27,386 ± 945	8,682 ± 1,230	24,928 ± 1,789	16,300 ± 1,566
C017	-	-	7,299 ± 477	-	-	-

House	Mutagenic Potency (revertants gram^{-1}) ± S.E.M.					
	TA98 +S9	TA98 -S9	YG1041 +S9	YG1041 -S9	YG1042 +S9	YG1042 -S9
C019	2,272 ± 483	3,200 ± 1,283	14,269 ± 1,308	9,533 ± 2,767	-	-
C022	2,306 ± 272	-	10,481 ± 780	2,510 ± 360	NT	-
C024	-	-	10,282 ± 380	5,017 ± 521	-	6,365 ± 918
E001	4,903 ± 677	-	19,609 ± 959	4,027 ± 434	10,390 ± 783	-
E002	4,347 ± 463	-	16,564 ± 783	2,770 ± 355	NT	5,680 ± 503
E004	3,023 ± 486	1,726 ± 312	18,226 ± 1,249	6,367 ± 1,263	-	13,800 ± 1,151
E006	2,516 ± 260	-	7,290 ± 988	-	-	-
E008	9,566 ± 1,360	1,430 ± 188	10,437 ± 785	1,953 ± 185	-	-
E009	2,762 ± 341	1,947 ± 245	30,283 ± 1,485	I	-	-
E011	-	-	13,399 ±654	1,793 ±248	NT	-
E012	4,317 ±663	-	10,827 ±628	3,961 ±541	-	-
E013	2,731 ±325	1,962 ±671	10,457 ±1,348	6,308 ±788	-	7,707 ±1,439
E015	-	-	10,305 ±408	-	-	-
E016	6,313 ±440	-	23,381 ±694	-	55,624 ±3,289	-

House	Mutagenic Potency (revertants gram^{-1}) ± S.E.M.					
	TA98 +S9	TA98 -S9	YG1041 +S9	YG1041 -S9	YG1042 +S9	YG1042 -S9
E017	3,432 ±349	-	13,892 ±778	6,647 ±783	-	I
E018	5,164 ±782	2,337 ±457	25,720 ±998	2,954 ±732	-	-
E019	-	-	7,530 ±414	1,287 ±132	-	-
E021	-	-	7,567 ±541	-	-	-
E022	I	780 ±106	7,458 ±924	6,125 ±272	NT	-
E024	3,836 ±497	1,417 ±370	15,611 ±1,148	7,583 ±717	-	-
E027	3,000 ±385	-	9,556 ±728	1,295 ±100	NT	-
E028	11,596 ±1,149	13,633 ±926	24,062 ±2,555	5,957 ±841	-	-
E029	2,617 ±1,050	-	8,011 ±721	-	-	-
E031	4,220 ±300	-	18,340 ±764	4,333 ±521	NT	-
S003	3,877 ±433	-	12,890 ±454	-	-	1,922 ±470
S005	3,149 ±248	-	11,564 ±726	-	-	-
S006	NT	-	2,622 ±331	-	NT	NT
S007	7,213 ±469	1,306 ±320	31,387 ±1,053	10,750 ±708	-	-

House	Mutagenic Potency (revertants gram^{-1}) ± S.E.M.					
	TA98 +S9	TA98 -S9	YG1041 +S9	YG1041 -S9	YG1042 +S9	YG1042 -S9
S010	2,700 ±477	-	7,767 ±546	-	-	-
S011	14,452 ±1,505	-	30,077 ±1,998	10,200 ±841	NT	NT
S012	-	1,345 ±206	23,436 ±6,193	1,988 ±399	-	-
S013	1,620 ±218	-	9,094 ±483	-	-	-
S015[b]	-	1,214 ±208	9,158 ±646	4,000 ±544	-	-
S016	-	I	22,577 ±840	3,263 ±377	-	-
W001	2,377 ±262	-	11,169 ±802	3,218 ±278	-	-
W002	4,583 ±642	-	9,687 ±741	3,949 ±859	-	-
W003	-	-	6,826 ±513	2,608 ±493	-	-
W005	1,789 ±153	-	8,633 ±605	1,842 ±373	NT	-
W006	7,285 ±337	-	25,427 ±1,607	14,133 ±1,624	25,410 ±1,701	11,142 ±704

- : samples were not mutagenic at the doses tested

NT: samples were not tested because of insufficient sample

I: testing resulted in inconclusive results

[a] House was added to the study and was not part of the random selection process.

[b] S015 was a very large bag. Only 1/3 of the bag was sieved instead of the entire bag.

Appendix V. Concentrations ($\mu g\ g^{-1}$) of 13 targeted PAHs measured in house dust samples collected from Ottawa, ON. Values are corrected for recovery efficiencies.

House Code	ACENPHY	FLUOR	PHEN	ANTH	PYR	BaA	CHRY	BbF	BkF	BAP	I123cdP	DBahA	BGHIP	Total PAHS	B2 PAHS
A001[a]	0.005[b]	0.034	0.302	0.006[b]	0.451	0.200	0.305	0.515	0.146	0.205	0.345	0.066	0.320	2.902	1.783
C001	0.064	0.287	5.709	1.624	11.306	7.427	8.512	12.259	3.974	8.499	8.856	1.497	7.470	77.484	51.024
C002	0.076	0.248	3.380	0.514	3.655	2.270	3.039	3.928	1.273	1.829	2.149	0.472	1.790	24.624	14.960
C003	0.053	0.106	0.995	0.156	1.175	0.718	0.917	1.330	0.419	0.653	0.833	0.185	0.705	8.245	5.055
C005	0.061	0.007[b]	1.255	0.239	1.241	0.584	1.393	1.373	0.440	0.458	0.911	0.169	0.709	8.840	5.327
C007	0.005[b]	0.208	4.022	0.526	5.050	2.329	4.094	5.219	1.831	2.260	3.178	0.559	2.757	32.039	19.470
C011	0.005[b]	0.065	0.824	0.147	0.868	0.548	0.654	1.013	0.366	0.451	0.687	0.119	0.565	6.314	3.839
C012	0.146	0.264	3.877	0.754	4.495	2.443	2.973	3.711	1.313	2.287	2.210	0.483	1.875	26.831	15.419
C014	0.168	0.194	4.052	0.604	3.111	1.738	2.042	3.034	0.834	1.712	1.767	0.325	1.535	21.117	11.453
C015	0.153	0.233	2.733	0.525	3.645	2.423	2.696	3.544	1.314	1.924	1.928	0.423	1.846	23.388	14.252
C016	0.005[b]	0.107	1.460	0.188	1.167	0.631	1.084	1.005	0.282	0.290	0.470	0.106	0.375	7.169	3.868
C017	0.026	0.107	1.345	0.158	1.216	0.691	0.885	1.316	0.388	0.631	0.685	0.178	0.588	8.214	4.774
C019	0.005[b]	0.007[b]	0.533	0.162	0.746	0.368	0.666	0.663	0.371	0.279	0.577	0.175	0.481	5.033	3.098
C022	0.005[b]	0.077	0.911	0.163	1.176	0.583	0.917	1.163	0.416	0.545	0.851	0.190	0.790	7.787	4.665
C024	0.005[b]	0.155	2.447	0.266	1.574	0.842	1.219	1.663	0.538	0.765	0.851	0.177	0.755	11.257	6.055
E001	0.005[b]	0.086	1.566	0.257	2.281	1.137	1.551	2.613	0.810	1.488	1.495	0.282	1.348	14.921	9.377
E002	0.049	0.312	3.816	0.281	7.478	2.949	6.013	8.690	2.627	4.248	4.889	0.768	4.315	46.436	30.185
E004	0.005[b]	0.125	1.613	0.283	3.122	1.488	2.070	2.832	1.030	1.804	1.972	0.340	1.737	18.421	11.536
E006	0.005[b]	0.059	0.960	0.165	1.105	0.547	0.769	1.235	0.390	0.668	0.733	0.135	0.638	7.410	4.477

E008	0.042	0.084	1.315	0.248	5.662	2.735	4.406	8.318	2.387	4.215	5.504	0.675	5.686	41.278	28.240
E009	0.087	0.118	1.482	0.193	1.887	0.696	1.963	2.039	0.692	0.856	1.407	0.309	1.264	12.993	7.961
E011	0.005 [b]	0.055	0.750	0.172	0.948	0.431	0.753	1.073	0.446	0.523	0.908	0.154	0.829	7.048	4.289
E012	0.005 [b]	0.145	1.609	0.154	1.362	0.599	1.186	1.474	0.532	0.482	0.865	0.177	0.763	9.353	5.315
E013	0.005 [b]	0.007 [b]	0.371	0.128	0.572	0.252	0.415	0.525	0.230	0.318	0.487	0.113	0.411	3.835	2.340
E015	0.005 [b]	0.007 [b]	0.529	0.127	0.552	0.228	0.362	0.574	0.195	0.239	0.363	0.090	0.322	3.593	2.051
E016	0.005 [b]	0.077	1.525	0.331	0.806	0.403	0.690	0.799	0.218	0.330	0.484	0.127	0.396	6.191	3.051
E017	0.005 [b]	0.086	1.226	0.191	2.153	0.989	1.523	2.296	0.794	1.097	1.180	0.208	1.033	12.780	8.086
E018	0.171	0.632	13.453	1.138	27.183	12.040	18.619	28.394	9.191	18.744	19.786	3.053	19.015	171.420	109.828
E019	0.019	0.041	0.517	0.066	0.743	0.350	0.524	0.805	0.312	0.376	0.440	0.093	0.436	4.722	2.899
E021	0.005 [b]	0.090	1.675	0.231	3.350	1.800	3.358	5.068	1.519	1.958	3.090	0.491	2.534	25.170	17.284
E022	0.005 [b]	0.007 [b]	0.149	0.056	0.207	0.134	0.150	0.209	0.087	0.114	0.184	0.062	0.135	1.501	0.941
E024	0.005 [b]	0.364	6.006	0.933	8.111	4.582	6.519	10.319	3.804	5.725	7.379	1.238	5.852	60.837	39.566
E027	0.072	0.170	2.857	0.382	3.700	1.635	2.261	3.194	1.015	2.050	1.662	0.333	1.401	20.733	12.151
E028	0.135	0.726	16.445	2.145	29.297	14.428	22.368	33.112	10.042	19.431	18.537	3.660	16.594	186.919	121.577
E029	0.055	0.478	7.114	1.021	10.148	5.166	7.160	11.427	3.236	7.108	6.814	1.136	5.942	66.806	42.047
E031	0.005 [b]	0.093	1.482	0.230	2.644	1.409	2.254	3.780	1.217	2.077	2.784	0.439	2.429	20.844	13.959
S003	0.005 [b]	0.068	0.895	0.196	1.092	0.510	0.716	1.088	0.356	0.548	0.794	0.138	0.637	7.043	4.150
S005	0.005 [b]	0.253	3.918	1.217	4.633	3.000	3.436	5.188	1.801	3.012	3.681	0.693	3.329	34.167	20.811
S006	0.005 [b]	0.023	0.187	0.048	0.638	0.351	0.510	0.708	0.251	0.241	0.370	0.072	0.312	3.716	2.504
S007	0.022	0.167	2.845	0.331	4.015	1.913	2.908	5.050	1.575	2.485	3.163	0.484	4.481	29.438	17.577
S010	0.005 [b]	0.075	0.945	0.106	1.456	0.661	1.168	1.670	0.488	0.936	1.069	0.153	0.793	9.526	6.145
S011	0.166	1.369	20.954	6.624	46.020	32.073	35.116	53.950	19.027	38.750	33.546	6.266	31.408	325.269	218.727
S012	0.161	0.009	0.911	0.093	0.337	0.150	0.350	0.372	0.122	0.040	0.277	0.069	0.233	3.125	1.380
S013	0.005 [b]	0.140	1.884	0.135	0.502	0.240	0.417	0.518	0.210	0.260	0.341	0.089	0.409	5.151	2.075

S015[c]	0.005[b]	0.007[b]	0.444	0.052	0.476	0.236	0.376	0.440	0.176	0.170	0.304	0.064	0.258	3.007	1.766
S016	0.005[b]	0.007[b]	0.862	0.126	1.492	0.776	1.188	1.888	0.595	1.066	1.421	0.231	1.220	10.877	7.165
W001	0.005[b]	0.056	0.629	0.006[b]	1.035	0.589	0.813	1.156	0.453	0.803	0.795	0.162	0.631	7.133	4.771
W002	0.005[b]	0.048	0.452	0.079	0.575	0.298	0.387	0.689	0.267	0.295	0.473	0.071	0.345	3.983	2.480
W003	0.021	0.032	0.447	0.063	0.366	0.105	0.159	0.160	0.049	0.060	0.100	0.022	0.118	1.702	0.656
W005	0.005[b]	0.208	2.425	0.205	1.209	0.441	0.896	0.871	0.261	0.371	0.555	0.091	0.477	8.016	3.485
W006	0.082	0.365	3.754	0.675	4.582	2.365	3.065	4.140	1.045	2.679	2.375	0.408	2.007	27.542	16.077

[a] House was added to the study and was not part of the random selection process.

[b] Values of ½ the MDL were substituted for non-detects.

[c] S015 was a very large bag. Only 1/3 of the bag was sieved instead of the entire bag.